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WELCOME ADDRESS BY THE CHIEF HOST

Welcome Address by Prof. Mohammed Nasiru Maiturare Vice Chancellor, Ibrahim Badamasi Babangida University, Lapai At the First Faculty of Natural Sciences Annual Conference (FONSAC 2019) 0n 7th May, 2019

PROTOCOLS, GREETINGS……

Universities and other higher institutions of learning are involved in knowledge creation, development and exchange. It is essential that fountain of new ideas, technologies and innovations flow from these institutions to the market place. Commercialization of academic research results is, thus, the avenue through which ideas, knowledge, and innovation generated through research are converted to tangible assets that would benefit the researcher, the economy and satisfy societal need. Universities and research institutes in Nigeria have over the years generated ideas, knowledge and innovations, unfortunately most of these potential trademarks are just placed on the shelves lying fallow in our libraries wasting away fantastic economic opportunities.

Several factors have been identified as being responsible for our inability to convert our research outputs to tangible assets in Nigeria. At IBB University Lapai, we have identified some of these to include research funding, the private sector not investing in R & D, the absence of research-industries-linkages as well as the near absence of the relevant engineering infrastructure required to translate prototypes to actual commercial models. IBB University is addressing some of these identified challenges by supporting researches in our institution with funding and creating an enabling environment for the synergy between the gown and the town in the research design of our faculty members.

However, one critical challenge that serves as a limiting factor to all our efforts is unreliable power supply. No meaningful research endeavor can flourish under an atmosphere of darkness and intense heat. In fact I would like to proffer that Universities must prioritize provision of uninterrupted power supply from the national grid over all other concerns. This is the only way that Nigerian Universities would play their expected leading role of being the subterfuge of national innovation and technological progress.

Indeed, the Federal Government of Nigeria has posited through the ministry of Science and Technology that science, technology and innovation are the primary driving force of development and globalization,' adding that 'deployment of science and technology apparatus will enhance the pace of socio-economic development of the country. The ministry has therefore tasked the scientists on the need to promote problem-solving and market driven research and to ensure maximum benefit of research outputs to the states, local government areas and even individual homes and lives.

It is in response to this desire of government and the need to diversify our economy through innovative researches with market potential that the Faculty of Natural Sciences, IBB University Lapai, have put together this conference to curate research ideas and outputs that can ignite the industrial revolution we so much desire in this country. Indeed, IBB University is well placed to drive this initiative as our research profile is rising to enviable heights. Our research efforts has attracted over a N billion inflow to

the University in the last 2-3 years alone. IBBUL is today a recipient of a multi-million naira Professorial Chair in Basinal Studies, the first of its kind in Nigeria.

Furthermore, in addition to being the only University in the North Central, apart from UNILORIN, to win the highly competitive National Research Fund grant a few years ago, we have this year won two additional grants of the National research Fund. Indeed, IBBUL we are well endowed to undertake cutting-edge research as we have invested heavily in acquiring the latest most modern research equipment.

In 1954 three researchers at Bell Laboratories published the results of their discovery of the world's first practical photovoltaic cell, which was capable of converting sunlight into electricity. In 1959 Sharp Corporation bought over the research idea, with mass production starting in 1963. Some of us are using these photovoltaic cells (solar panels) in our houses today as alternative source of power. That is how research can change the society. It is my expectation that at the end of this conference, we would have at least one of such revolutionary research output curated and ready for commercialization.

I therefore wish you impactful presentations and fruitful deliberations at the technical session. Welcome to Lapai, try to also embrace the serenity of the town and let it inspire you to more groundbreaking research ideas.

May God bless you all and grant you journey mercies at the end of the conference. Thank you for coming.

WELCOME ADDRESS BY THE CHAIRMAN, CONFERENCE ORGANIZATION COMMITTEE

HISTORIC MILESTONE

Being a speech delivered by Musa Achimugu Dickson, PhD At the First Faculty of Natural Sciences Annual Conference (FONSAC 2019) At the University Auditorium, Ibrahim Badamasi Babangida University, Lapai, 0n 7th May, 2019

PROTOCOLS/ GREETINGS

It gives me great pleasure to extend to you all a very warm welcome on behalf of the Dean, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University, Lapai, the Faculty Standing Committee on Conferences and Seminar and the Conference Organizing Committee of FONSAC 2019 to the opening ceremony of the maiden Faculty of Natural Sciences (IBBUL) Annual Conference, 2019.

The theme of our maiden conference is **Commercialization of Research Output for National Development**". This is borne out of genuine desire for the economic revolution of Nigeria through knowledge generation. We believe strongly that the role of applied scientific research in national development cannot be overemphasized, and as our humble contribution to national development and in support of the economic diversification programme of the government, we have put together a platform to showcase and curate researches in Institution of higher learning and research institutes in Nigeria that can be commercialized and drive the economic growth of the nation.

We are delighted to have in our midst today, researchers from all over the country. I am so pleased to see many professors, researchers, scientists and colleagues here today. It is gratifying to note that the technical session has topics that covers a wide range of very interesting items relating to the theme and subthemes of this conference. Your faith in us, strong support and active participation has greatly encouraged us to do more. By the time we would be having the second edition, we would have corrected some lapses that may have been witnessed in this maiden edition.

Thank you COC members.

Thank you all once again. Welcome to Lapai and Ibrahim Badamasi Babangida University. Enjoy the ambience of the environment. Network with one another. Have fruitful deliberations. God bless you.

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Commercialization of Research Outputs for National Development

Prof. S.A. Thomas

DG/CEO Sheda Science and Technology Complex, Abuja. Nigeria.

INTRODUCTION: KNOWLEDGE-BASED ECONOMY

Knowledge is said to the most important resource and learning, the most important process (Lundvall, 1992). Therefore, to enhance productive learning processes of innovation systems, there is a need for better synergy between Industry, government agencies, universities and formal or Informal organizations (Kemmis, 2008).

Nowadays, knowledge, technology, innovation and human capital are generally understood as central and key drivers for generating sustainable economic growth and competitiveness. They represent key explanations for significant and persistent divergences in economic growth and development between countries and regions but not natural resources or exports based on cheap Labour (Howells, 2005). Knowledge-based economy is depended on high investment in education and training, research and development (R&D), the presence of high-quality scientific research institutions, extensive relationships between governments, academia, and industry and the protection of intellectual property (Lowe, 2005; World Economic Forum, 2010/2011).

To stay ahead in this era of incredibly rapid change and stiff global completion, industries have to pursue access to exclusive resources, which include new knowledge. As a consequence of this, industry/university collaboration has emerged as a sustainable enabling solution for technology transfer and commercialization as well as a fertile breeding ground for new inventions (Abbate and Cesaroni, 2017, Galati et al 2017, Munari, et al 2017, Bigliardi, et al 2015).

No doubt, Universities have important roles to play in the creation of knowledge and technology either at the economic or social levels of any nation. University-industry collaboration may begin in response to an industry need for new technology, or conversely, when new knowledge generated within a scientific field yields practical applications (Etzkowitz, 2004). By means of this collaboration, it is possible to make a bridge for the exchange of new knowledge and technologies generated within the university, and the real-world practice and marketing experience of particular organizations (Rothaermel *et al*., 2006).

Various Studies have argued that university-industry collaborations, in particular where these are formed within science and technology-based industrial sectors, should be recognized as an important impetus for National Development (Cohen et al 2002). Absorbing new knowledge and technology from universities as well as maximizing their capacity to use that knowledge for performance gains is critical for any industry survival through the utilization of open innovation (Yun, *et al*., 2015; Han *et al.,* 2016). Not surprisingly, university activities such as technology transfer and commercialization have received much attention in recent years.

It is very important to note that the more experience accumulated in let say biotechnology with university partners, the greater the performance impact of these university-industry collaborations. Industries located in proximity to universities are likely to grow faster thanks to university-industry collaboration which has grown from collaborative experience. In particular, these collaborations have the potential to act as a bridge in transforming academic discoveries into commercially successful innovations.

We can say that there has been misguided conception on what research and development (R & D) really means. The former been a back-end of the exercise where the researcher is in most cases not seen except at data collection stage of the exercise. Development on the other hand is the front end of the exercise and this is where the activities that have occurred in the back-end are brought to the fore for either display or transformation into more explicit products. At the developmental stage, the critical/ main activity here is where commercialization lies. Since the fulcrum to success and survival of any nation or organization lies in its knowledge and capability to commercialize its innovations, understanding of the basic concept of the subject is thus vital for tangible impact and transformational results.

Policy makers in recent times have accepted the academic research and knowledge generated from both private and public funded research entities as a mean for productivity and economic growth via their commercialization (Ndonzuau et al, 2002; Mowery et al, 2001). It is well taken that for nations to have increased economic development, there is a strong need to drastically produce and utilize scientific knowledge and this new ideas are key fundamental for the economic development because it enhances job creation which in turn boosts the competitiveness of industries (Youtie and Shapira, 2008). This growing acceptability of the commercialization of academic research activities has now placed enormous burden on universities since they are the birthplaces of innovations.

Notwithstanding that the Universities are the created to educate and turn out researchers, Universities have been forced to take up wider missions from their traditional ones of Research and Training, which is to contribute directly to the socioeconomic development of their catchment areas. According to Etzkowitz (2003), these new missions which universities have taken up, marks the beginning of the "second academic revolution" and this has changed the traditional mission of universities from teaching and research to contributing to the development of enterprises.

The combination of the traditional and the new third mission has placed lots of challenges on university and their entrepreneurial activities. The entrepreneur mission of universities has been embraced worldwide because it is believed that business activities can drive economic growth and reduce unemployment.

Universities have reliably contributed to society by providing high level education, but in recent times they are now expected to establish direct contact with industries in the economy. There is an increasing demand for universities to transfer the knowledge they generate further than their academic community to reach other users like industries (Mansfield and Lee 1996). As a result, many universities have embraced their third mission of contributing to society through the active conversion of new scientific knowledge into commercial values through spinoff opportunities (Harrison and Leitch 2005).

The establishment of spin-off companies has been the best way of commercializing knowledge generated by the academic institutions (Landry et.al, 2006). Many universities have supported the commercialization drive by creating specialized supporting structures which can either be technology transfer offices (TTOs), incubators and science parks within or in close proximity to their campuses.

EARLY COMMERCIALIZATION OF UNIVERSITY RESEARCH

The process of commercialization of research projects must be chosen from scratch, not only technically but also business perspective has the highest potential. So, given the cost and time required for the commercialization of research, commercialization of the project, before the potential of the exact the allocation of time and resources is essential. Supply of high quality value for industries that are expected to be purchased and converted to the innovation of scientific research team includes key elements. Researchers can work together in order to achieve the goals set out at the time as one of the requirements should be scrutinized.

Furthermore, for a long standing process of commercialization of new innovations can be activated by Patenting via industry-university collaboration. In fact, what is 'commercialization' from the standpoint of the Industry, becomes 'knowledge transfer' from the point of view of the university. Hence, In order to implement the process of commercialization, there are numerous difficulties and hurdles to be overcome. Patents covering the output of innovation can be used as a tool not only for protection in innovation management but also for incentive creation, collaboration, negotiation and licensing, etc. (Nicholas, 2011).

The commercialization of academic activities and spin off formation has been very successful in the United States of America with the renowned "Silicon Valley" and "Route 128" been established in close proximity to recognized and leading spin off creation universities such as Stanford and Massachusetts Institute of Technology (MIT). How far has this gone in other places in the world, Nigeria inclusive?

COMMERCIALIZING OF RESEARCH OUTPUTS FOR NATIONAL DEVELOPMENT

As the global economy recovers from the economic met down, it is constantly looking at developing economies for full recovery (Gurria, 2007). It is highly likely that in the next two decades, emerging economies will represent almost two-thirds of global output. The main reason for this is innovation which can be derived from any place. The global economy is highly interdependent, and more so than ever before as is evidenced by ever-increasing exchanges of goods, services, ideas, values, expertise in various fields, and in the manner in which solutions are sought to solve global problems. Companies in developing nations are called upon to conceive and develop revolutionary and progressive products and processes since this is the way they will be able to retain a competitive advantage.

Consequently, business investment in R&D is in a sense nonnegotiable. Top-notch scientific research institutions are undoubtedly an essential partner in innovation and there is also a need for far greater collaboration in research promotion between industrial role-players and universities. By enhancing research and boosting innovation, economic growth is bolstered and developing nations are empowered to create new and very often, unique products and services (Agrawal, 2002).

Agrawal, (2002); Martino, (2009) have reported positive correlations between the amount of R&D and productivity growth as well as R&D positive impacts on total factor productivity. With this, Human capital improves and inflows of foreign direct investment (FDI) tend to soar. As we create, disseminate and apply knowledge we boost economic expansion. Innovation is a fundamental starting place of effective competition and economic development. It is critical to transforming society.

Economic growth across sub-Saharan Africa is expected to be on the increase in recent times, and this is attributed to the role which innovation is expected to play especially for nations that have reached what is termed the "high-tech frontier", as this is the only self-sustaining driver of growth.

This necessitates an environment that is favorable to innovation and one in which both the public and the private sectors are key players. Innovation may assume a variety of types and may be a process, product, service, or anything that assists companies and nations to achieve more. Innovation is within the potential of anyone but necessitates a critical view and inquisitiveness that enables one to explores perceived limitations and develop new designs and initiatives. It is innovation that has created global business giants such as Microsoft, Apple, GEC, Siemens, Sony, Nike, Adidas, Virgin and many others.

What can be said to be the drivers of Economic growth in Nigeria? Do companies have comparatively high level of innovation? What's the level of innovation costs which is the scope for development. For positive national development, there should be a considerable positive link between R&D and innovation. If innovation is utilized in a practical manner it will undoubtedly serve as a major catalyst for development and growth in of any economy. As academics publish more research globally, what is learned in one nation serves as the basis for improvements in other developing nations. Developing nations can perk up their productivity levels by taking up existing technologies or by making gradual improvements in a wide range of other areas.

However, once the innovation stage of national development is arrived at, more needs to be done to grow productivity. National innovation policies ought to seek to cultivate an environment which promotes entrepreneurship and innovation right across the entire economic spectrum. In this regard, universities in developing nations have decisive roles to play in developing sufficiently distinguishable and effective academic structures. They also need to empower their nations to link with the global knowledge society and compete in the sophisticated knowledge economy (Altbach, 2013). In the current scenario, innovation needs to be rethought as it is posed fresh challenges. In the globalized knowledge based economy what establishes an innovative performance in the corporate sector has to an extent changed as a result of IT and communication technologies.

ROLE OF THE UNIVERSITY INNOVATION IN NATIONAL DEVELOPMENT.

The key driver of economic development is innovation based on R&D and this must be the product of collaboration between universities and corporations. Entrepreneurship and innovation are critical since the national economy can only be built with novel ideas, as much as with capital provision by both the public and private sectors and effective labour. Innovation, ventures and intellectual assets are what ultimately drive economic growth and lead to increases in the standards of living. Innovation plays a huge role in job creation and provides higher incomes which promote societal development. Where

R&D from universities should lead to greater innovation, investment opportunities arise, especially from abroad which allow a nation to develop at a greater pace and be positioned to solve more of the problems which plague society.

This is why it is imperative for the government to further incentivize R&D and innovation and guide the process for the protection of intellectual property rights (IPR). The latter play a crucial role in further driving innovation and intensifying information and they also directly impact economic performance and generate economic growth through increased productivity, increased trade and greater levels of investment. Where market failures exist these are to an extent founded on the inability of individuals and companies, to thwart others from making use of the new knowledge they have created. Where there are effective IPR protection measures these serve as a strong incentive which permit companies to invest in generating new technology in a number of sectors without fear that an invention, for example, will be easily imitated.

It is argued that research represents about 25% of the price tag of commercializing a new technology or system and exclusive rights granted to a patent holder for a limited time provide a huge incentive for promoting greater economic support. This permits the development of ideas to generate a marketable product or technology. IPRs also promote private and public ventures to transfer technology through the development of innovative approaches, direct investment both foreign and local, greater technology sales and distribution and possible other cooperative undertakings leading to a more competitive economy. A competitive economy is one which is more likely to develop faster in the medium to long-term.

An important development by most universities in systematizing commercialization is the creation of a Technology Transfer offices (TTO), as one-stop service whose role embraces some or all of the following activities:

1. Educating and creating awareness of intellectual property processes and requirements amongst researchers;
2. Assisting researchers with their intellectual property and patent protection;
3. Assessing market potential;
4. Identifying potential industry partners and collaborators;
5. Negotiating license agreements;
6. Forming start-up companies; and
7. Finding investors and industry partners.

Nigeria universities have not, until recently, systematically sought to exploit the outputs of science research through commercialization, recent example is the University of Lagos which has created companies from some of their innovative researches.

CHALLENGES FOR COMMERCIALIZATION OF UNIVERSITY RESEARCH

Notwithstanding the potential roles which University R and D plays in nation building, they are still not deviled of challenges, such challenges could hamper the progress of R and D results for commercialization

Challenges 1: Timeliness: A commercialization activity is a long process over time. It requires initial investment before making profit. Initial investments are normally intellectual property filing, company registration, hiring qualified entrepreneur and marketing of products. It is said to be from the university's perspective: a money-losing operation. The fees that universities get do not cover the expenses involved in licensing and marketing the inventions. That's because only a very small fraction of licenses actually generate much revenue. This discourages universities to venture into such as such innovations end up only in published articles in Journals.

Challenges 2: Incentives and Rewards: Another problem in the commercialization activity is the lack of proper incentives and rewards system. Although there is an ongoing debate about reward and incentive, it is imperative that universities have a proper rewards system this is consistent with a long research stream on pay and performance. On the other hand, royalties' incentive effect might seem surprising, given the norms of open science and free dissemination of knowledge under which universities are expected to operate (Agrawal, 2006).

In as much as some other researchers have different views about this (Markman, el al, 2005), suggesting that monetary incentives given to university scientists are negatively related to the number of equity licenses in young ventures and to the number of start-ups; similarly, sharing revenues with scientists' departments is negatively related to the number of incubators. Friedman and Silberman, (2003) proposes that royalties grant to faculty inventors have positive effect on the number of licenses, while royalties granted to the inventors' departments have a negative effect

Challenges 3: University-industry Interactions: Some school of thoughts feel that because Industry/companies fund research in universities, they may be undue influence by them as well as the potential abuse by faculty and university staff due to conflicts of interest, triggered by the lure of readily available money and conflicts of commitment. This can seriously hamper an unbiased research results if findings show that the negative contrary to what the funding company might want.

SCIENCE AND TECHNOLOGY ADVANCEMENT, THE NIGERIAN PERSPECTIVE

I will not fail to address the role which the Nigeria has played in Science and Technology Development and to also highlight the key R and D which agencies and research institutes under the dynamic leadership of the Minster of Science and technology Dr. Ogbonnaya Onu has in making Nigerian R and D Commercializable.

Nigeria, knowing the strategic importance of R&D to its development, in the 1970s created institutions for the coordination and promotion of R&D activities in Science and Technology (S&T) in the country. The first effort was the establishment in 1970 of the National Council for Science and Technology (NCST) responsible for ordering national priorities in scientific research and coordinating and supervising both

basic and applied research activities. In 1971 the Agricultural Research Council and the Industrial Research Council were established, and followed by the

Medical Research Council and the Natural Science Research Council in 1972 and 1973 respectively. In January 1977, NCST was replaced with the National Science and Technology Development Agency (NSTDA) with a revised mandate for the promotion and development of S&T including initiation of policy in relation to scientific research and technology. The response to the general call to make scientific research relevant to economic development in

Nigeria led to the establishment of a full-fledged Federal Ministry of Science and Technology (FMST) in 1980 to take over the responsibilities of NSTDA. In 1984, FMST was merged with Federal Ministry of Education. It regained its autonomy in 1985 and was again merged with the Federal Ministry of Industry in 1992. FMST was however reactivated in 1993 with mandates to, among others, promote basic science research; scientific and technological research for agricultural, industrial, medical, and energy applications; administration of technology transfer programmes; coordination and issuance of policy guidelines to all S&T research institutes in Nigeria; advising the President on S&T matters.

R&D IN NIGERIA SCIENCE AND TECHNOLOGY AGENCIES

The key to developing a vibrant Science and Technology ministry is to first understand the imperatives of R&D while aligning it to meet both the immediate and future needs of the country for a competitive economy capable of ensuring wealth and job creation, enterprise development, and reducing mortality rate of its population due to common diseases typical of the region.

The higher educational institutions (Universities), research institutes (RIs), private research establishments, government agencies with R&D mandates are the key players in R&D in Nigeria (Siyanbola et al 2011). To support the development of R&D in Nigeria, government has made concerted effort over the years to fast track economic empowerment through the adoption of policies to guide and direct research in its public institutions and agencies.

Fourteen (14) of these agencies are charged directly with the mandate to conduct R&D. The other three have mandates for policy research and capacity building in management of technology, intellectual property rights, and technology business incubation. National Agency for Science and Engineering infrastructure (NASENI) is charged with research in capital goods, production and reverse engineering; Federal Institute of Research (FIIRO) is mandated to accelerate industrialization in Nigeria; Sheda Science and Technology Complex (SHESTCO) has the mandate to develop research results for application in areas of agriculture, health, industry and environment; National Space Research & Development Agency (NARSDA) is vested with research in space and development. Promoting the development and utilization of Nigeria's industrial raw material is the responsibility of Raw Materials Research and Development Council (RMRDC); Nigerian Building and Road Research Institute (NIBBRI) is to ensure improvement in the quality of life of Nigerians in the areas of affordable housing; Nigerian Natural Medicine Development Agency (NNMDA) will do research, develop collate, document and promote the nation's natural medicine; Nigerian Leather and Science Technology (NILEST) is a Centre for development in the areas of Chemical and Leather technology. National Research Institute for Chemical

Technology (NARICT) develops the technologies required by the chemical industry and also undertakes R&D work in areas of agriculture, mineral and other raw material conversion to chemicals; Project Development Institute (PRODA) has part of his mandate to develop the technologies required by the power equipment industry; National Biotechnology Development Agency (NABDA) coordinates, promotes and regulates the development of biotechnology in Nigeria. Nigeria Institute for Trypanosomiasis Research (NITR) is to conduct R&D for the control and elimination of Trypanosomiasis and its vectors; The Nigerian Institute of Science Laboratory Technology (NISLT) conducts research in all the areas of science laboratory technology; Nigeria Atomic Energy Commission (NAEC) coordinates R&D activities for capacity building and infrastructure development in Nuclear technology. In charge of Intellectual property and research industry linkages are the National Office of Technology Acquisition and Promotion (NOTAP). National Board for Technology Incubation (NBTI) provides institutional infrastructure and mechanism for the development and commercialization of R&D outputs and inventions. National Centre for Technology Management (NACETEM) is mandated to provide knowledge support for the STI system in Nigeria through capacity building in management of technology, STI policy research and consultancy.

CONCLUSION

Research should be infused in teaching and academic freedom should reign as academic staff are empowered not to stick rigidly to daily work hours and be able to engage with the community and broader society. Universities require adequate and sustained budgets and they cannot hope to succeed if funding is scant.

Opined that appropriate approach to commercialization depend upon factors such as competition, market, funding and nature of the technology. Commercialization failures could result from weakness in R&D institutions to design R&D activities and marketing plan, lack of interaction among the elements of innovation system, poor/no incentives from government to drive industry to utilize local technology or research results (Syahrul, et al 2007). It is also critical to have a virile national innovation systems (NIS), individual re-orientation, and institutional rearrangement, functional government-university/research institutes-industry linkage through networking and effective Technology Transfer model (Siyanbola, et. al., 2015).

One of such interventions might be the creation of Professorial Chairs' Initiative which will fashioned to create interventions in the knowledge and human resources base. This will hopefully contribute towards helping universities achieve their research strategies. Thus Excellence in research is promoted vigorously and the following achieved;

1. Increased number of world-class researchers in Nigerian Universities'
2. Qualified research scientists to the higher education sector are attracted and retained in order to help reverse the decline in research outputs, focus and capacity.
3. Improve the capacity of institutions to generate and apply new knowledge;

4. Stimulate strategic research across the knowledge spectrum as well as create research career pathways for quality young and mid-career researchers that address historical racial, gender and age imbalances

5. Improve and accelerate the training of highly qualified personnel through research.

Hopefully such initiatives will assist in making the country more competitive in the global knowledge economy but our universities must further commit to the creation and dissemination of knowledge, in a wide range of disciplines and fields, and be supported by, for example, relevant laboratories and libraries etc. that facilitate and promote effective teaching and research which are catalysts for the innovation that is desperately needed to propel us into the future.

It should be a top priority to create enabling environments for seasoned and budding researchers in which knowledge production is nurtured and disseminated globally as part and parcel of a relevant and dynamic research culture. Increasing the number of PhD candidates and postdoctoral researchers is essential as is increasing researchers‟ local and global mobility. Focused education and training in the field of management of research and innovation is thus non-negotiable and universities must drive the implementation of greater research for National Development.

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Commercialization of Research Output For National Development: The Role of Technology Incubators in Nigeria

Abdulmalik Ndagi

Niger State Industrial Parks Development Agency, Minna

INTRODUCTION

Commercialization is a process that converts intellectual property into marketable products and services, this process requires different skills, funding, team and market analysis as compared to non-technology businesses. According to Infodev (2013). Technology commercialization is the process of taking a piece of technology, research and development results, invention or scientific intellectual property (IP) often, but not necessarily the result of university or similar research and turning it into a commercially viable product or service that is demanded by the market. Infodev therefore concluded, that Technology commercialization is often known as IP Commercialization or Technology Transfer.

The successful commercialization of research output to a large extend depend on the successful identification of market demand and capacity for effective and efficient science and technological research output that can stand test of time vis-a-viz benefit to mankind. Commercialization of research output for national development provides the basis for knowledge-based economic development, economic prosperity and scientific and technological growth. These are achievable through strategic systematic and integrated approach to valuing research and its findings as well as achieving an effective and efficient interaction of academia – industry and government which are the strategic components for national innovation system. One major cause of fast-growing and improved technology in industrialized countries according to Dehghani (2015) is a good deal of attention devoted to commercialization of research results conducted in such countries. He further posit that, inabilities to commercialize, apply research findings in new products and processes, and introduce them to market are major drawbacks of developing countries.

Commercialization is the process of introducing new products or services to the general market. It takes into account production distribution, marketing sales and customer support required to achieve the commercial success of the new product or services. it is assumed that to build a successful profitable business, creative ideas should be commercialized. As conceptualized in several literatures, commercialization is a complex non-linear cycle which necessitates that all relevant stakeholders effectively and efficiently perform their responsibilities.

Commercializing research output proposes directions for national development and integration accordingly, provides a catalyst to the knowledge-based economic development of countries. It also brings about economic prosperity and scientific growth which are critical starting points for countries to become a source of knowledge production and knowledge data bank. Such achievement is feasible

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through valuing research and its findings as well as achieving an effective interaction of components relevant to national innovation system. Commercialization process has some key features according to Reamer et.al (2003). Firstly, they assert that it is a cycle whereby a given input passes through a series of stages to reach a specific output and in every stage some value is added to it. However, the chain of stages gives the product more added value than the sum of added values at all stages. Secondly, this process, in an attempt to help investors reap benefits, exploits all possible potentials including labor force, organizational structure, rules and regulations, technology, and whatever which deserves to be considered potential. Thirdly, technology commercialization is a necessary part of innovation. If technological innovation is assumed to range from idea generation to initial market entry, there will be no innovation and, thus, no technology without commercialization.

Literature Review

Commercialization according to Dehghani (2015). It is a process through which knowledge and technology are transferred from universities and research centers to industries and new businesses. It is a complex process influenced by a variety of factors such as infrastructure, technological, social, political, and historical. These factors may either facilitate or impede the commercialization cycle. He further posit that it is one of the most controversial issues that is the reason number of companies and countries give first priority to this issue and allocate their attention among a number of activities. However, even within industrial countries there are some barriers to commercialization such as financial problems, inefficiency of organizational bureaucracy, paucity of research on the influence of organizational strategies and understanding necessary interaction between research teams, lack of mass production, evaluation of research findings, implementation of reforms, and product optimization which are being gradually eliminated. Lack of financial resources devoted to commercialization which supposes to be a facilitator then leads to poor access to commercialization guidelines and application of research findings.

Commercialization of research results according to Ogunwusi and Ibrahim (2014). has become the new catch-cry in most advanced economies as they embrace innovation as a key driver of economic policy. The transfer, exploitation and commercialization of public research results has become a critical area of science, technology and innovation. The knowledge and research generated by public research system is diffused through a variety of channels among which are the mobility of academic staff, scientific publications, conferences, contract research with industry and the licensing of university inventions. Effective commercialization of research results in any nation will depend on rapid technological innovation, effective strategic management of knowledge and a clear focus in value added goods, services and industries.

Technology commercialization according to Infodev (2013) further said, is not the same as technology adoption hence, Technology commercialization is the process of transforming innovative technologies developed by universities, companies and inventors into commercially viable products and services that are in market demand, whereas technology adoption is the normal cycle of acceptance of that technology by the market; by innovators, early adopters, early majority, late majority and laggards.

Technology commercialization remains a risky, difficult and expensive process that needs to be addressed cautiously by a business incubator manager to successfully commercialize it.

Incubation is a natural partner to Technology Transfer Offices which often exist within a university or research center to help the research institution benefit from the IP it has created. Some TTOs include an incubation function but many will partner with external incubators. There are many common mistakes that commercialization professionals try to avoid, keep expectations within the boundaries of what is likely; don't be over confidence.

The university is a key element of the triple helix of innovation system both as a human capital provider and a seed-bed of new firms (Etzkowitz *et al*., 2000; Laredo and Mustar, 2001). To realize the benefits of knowledge and to receive returns from these investments,(research and Development findings) the resulting innovations or inventions must be sold, or commercialized (Meyers, 2009). Knowledge-based economy is depended on high investment in education and training, research and development (R&D), the presence of high-quality scientific research institutions, extensive relationships between governments, academia, and industry and the protection of intellectual property (Lowe, 2005; World Economic Forum, 2010/2011).

knowledge, technology, innovation and human capital are generally understood as central and key drivers for generating sustainable economic growth and competitiveness and they represent key explanations for significant and persistent divergences in economic growth and development between countries and regions not natural resources or exports based on cheap labour. (Howells, 2005).

Commercialization is however, not a straightforward process; as many challenges must be overcome (Al Natsheh *et al*., 2015). It has been shown that new knowledge from universities must penetrate what is known as the knowledge filter in order to contribute to innovation, competitiveness and ultimately economic growth (Audretsch *et al*., 2006; Acs *et al*., 2010). The knowledge filter is defined as the barrier or gap between the investment in new knowledge and its commercialization (Al Natsheh *et al*., 2015); and has been associated with bureaucratic red tape and illogical government regulation (Audretsch, 2014). knowledge flows from universities (and research institutions) are much more diverse than they had been in the past, with publications and paper presentations at meetings being just two among a wide array of transfer mechanisms. Commercialization as a new form of technology transfer is becoming increasingly common which can be either directly, by nurturing academic entrepreneurship in incubation centres, or indirectly, by transferring knowledge and sharing expertise through consulting, joint research ventures, patenting, licensing of intellectual property, contract research or forming start-up companies (Cohen *et al*., 2002). Traditionally, teaching and research have been the university`s main roles. However, commercialization of research results or entrepreneurial science also referred to as, technology/knowledge transfer, third stream third mission or engagement, has emerged as an additional role for universities as stimulators and facilitators of knowledge transfer (Perkmann *et al*., 2012). The third stream is about the interactions between universities, industry and the rest of society, and can be said to be the stimulation and direct application and exploitation of knowledge for the benefit of the social, cultural and economic development of society - *i.e*., community outreach (Molas-Gallart *et al*., 2002), making technology available to end-users (Tahvanainen and Nikulainen, 2010). Studies into the third mission of academic institution highlight that universities have matured in their

approach to technology transfer, in what appears to be a more iterative and cyclical process of innovation diffusion, such that the double-helix character of DNA has been metaphorically adapted to describe the university-industry-government relationship, this time as a triple-helix to encourage development (Leydesdorff and Etzkowitz, 1996; Etzkowitz and Leydesdorff 2000). The intertwined (overlapping) relationship of the triple-helix suggests that the movement of knowledge is not necessarily one way but rather cycles in and out (iterative) of each triple-helix partner depending on the nature of the technology and the sources of intellectual capital best suited to its movement (Powers and Campbell, 2011). In this sense universities (and independent research institutions) are not only a source of knowledge, but are also active participants in the organization, development and commercialization of innovation. More recently, there has been the inclusion of the the market/society as a fourth strand to the helix, leading to a Quadruple helix model (Carayannis and Campbell 2010). This makes a perfect sense since the desired output of the triple helix activity is new and innovative products and services, which have to relate to the market and society in order to generate jobs and wealth and ultimately achieve greater competitiveness (Carayannis and Campbell 2010).

Bayh-Dole Act and Commercialization of University Research

In order to penetrate a formidable knowledge filter and facilitate university entrepreneurship and technology transfer from the university, the U.S. Congress attempted to remove potential obstacles to university technology transfer and commercialization by passing the University and Small Business Patent Procedures Act of 1980, more commonly known as the Bayh-Dole Act, 1980 (Link *et al*., 2007; Kenney and Patton 2009). This Act established the legal framework for commercializing the research that is developed within university settings by transferring the ownership of intellectual property (IP) from the publicly funded granting agencies to the universities. The logic was to give the universities incentives to support and build an infrastructure for the commercialization of research, with licensing preferences going to small businesses and industries within the United States (Link *et al*., 2007; Kenney and Patton 2009). This policy change stressed the expectations that the universities could contribute more directly to industrial development (Stevens, 2004). It played a critical role in rejuvenating the entire U.S. economic system, transforming it from a manufacturing base to an innovation base (Loise and Stevens, 2010). Prior to the Bayh-Dole Act, the United States government owned and managed intellectual property developed at academic institutions as the result of federal funds, hence, nobody could exploit the outputs of publicly funded research without tedious negotiations with a federal agency concerned (Kesselheim and Rajkumar, 2011). In view of this arrangement, patent protection and licensing of technology was rarely pursued (Kirschenbaum, 2002). Worse, companies found it nearly impossible to acquire exclusive rights to a government owned patent, and without that, few firms were willing to invest millions more of their own money to turn a basic research idea into a marketable product (Audretsch, 2014). Bayh-Dole Act 1980, led to a massive increase in funding to universities by venture capitalists (Valentine and Claasen, 2002), resulting in a rapid rise in commercial knowledge transfer from university to industry (Jensen and Thursby, 2001), through mechanisms such as, partnerships, licensing agreement and university start-ups, also known as spin-offs or Spin-outs (Banal-Estañol and Macho-Stadler, 2010).

A commercialization survey by the Association of University Technology Managers (AUTM – the technology transfer profession interest organization) among United States-based institutions showed that due to the Act, the number of patents granted to US universities increased from 589 in 1985 to more than 3200 in 2006 (AUTM, 2007). In addition, there were 16000 patent applications and 553 spin-off establishments in the same year. Start-ups are new firms created to exploit commercially some knowledge, technology, or research results developed within a university (Pirnay *et al*., 2003). Research has pointed out that there are two essential determinants explaining the process of knowledge transfer from universities to industry namely: (1) the linkages between researchers and research users, such as private firms and government agencies; and (2) the focus of the research projects on users' needs *i.e*., research that is-fit-for purpose (Landry *et al*., 2007). As a result, the United States has become very advanced in technology transfer and commercialization (TT&C) because of this Act, which has been in effect for more than 30 years (Loise and Stevens, 2010). The subsequent success of Bayh-Dole Act as a catalyst in the US for bringing new research findings to the marketplace inspired legislative changes in many OECD and beyond countries such as Germany, Denmark, Japan, Canada, India, the United Kingdom and Singapore to enact similar laws to this Act (Slaughter and Leslie, 1997; OECD, 2003; Mowery and Sampat, 2005).

Commercialization of research and development output in Nigeria according to NOTAP (2018). Depends heavily on its ability to acquire and apply technology indigenously to produce goods, processes, devices and provide services. To achieve this, a well-focused National Innovation System (NIS) is necessary in which technology acquisition is well matched to the needs of the market and industry. The Nigerian NIS is composed of ministries, departments and agencies, tertiary institutions, research institutions/Centres, financial institutions, the industries and civil society organization.

Traditionally, commercializing R&D outputs is meant to enhance competitiveness and capability of the NIS by promoting indigenous technologies resulting from R&D activities undertaken by tertiary institutions, research institutions/Centres, industry, individual researchers, inventors and traditional knowledge.

In the African context, Nigeria inclusive, university research capacity appears to be very limited, taking into account regional and country variations. Research capacity, defined by Volmink and Dare (2005), as comprising the institutional and regulatory frameworks, infrastructure, investment, and sufficiently skilled people to conduct and publish research, varies greatly across the continent. Indeed, a study by the RAND Corporation revealed that, with the exception of South Africa, Egypt, Mauritius, and Benin, African countries were part of a group of scientific laggards (RAND Corporation, 2001)**.** Furthermore, a 2007 report recognized that African higher education lacks capacity not only at the system and institutional levels, but also at the level of individual academics (Jones et. al. 2007). The research grant lying idle at Tetfund is Nigeria example according to (Bogoro, 2015) where he confirmed availability of research fund at tetfund totaling about four billion naira and only 20% have been accessed by university researchers over the years.

Research Institutions in Nigeria.

Research according to Ogunwusi and Ibrahim (2014) is a means of demonstrating one's ability and capability in solving an identified problem and it is an important pointer to the national technological

capability. One of the major roles of research is breeding industrialization which brings about jobs and wealth creation, arrests social menace and assists in curbing rural urban migration. The history of Research and Development in Nigeria can be traced to the establishment of a National Council for Scientific and Industrial Research (NCSIR) in 1964, following an international conference on the organization of Research and Training in Africa (FMST, 2010). According to Yusuf (2012), the Council's mandate was narrow and as such had structural weakness which made its function ineffective and inefficient. As a result, with assistance of UNESCO experts, four research Councils were established after the Civil war in 1970. These were:

1. Agricultural Research Council of Nigeria (ARCN)
2. Medical Research Council of Nigeria (MRCN)
3. Natural Sciences Research Council of Nigeria (NSRCN)
4. Industrial Research Council of Nigeria (IRCN)

In 1986, the first National Policy on Science and Technology (S&T) was launched (Yusuf, 2012). The policy identified that S&T-related activities in the country had been carried out without well-defined national direction. The public universities, research institutes and research outfits in private sector companies are expected to be drivers of research and development and home grown technologies. Also, R&D are expected to lead to home grown industries and power multinational companies within the country. However, since 1964 till now, despite the endowment of the nation with a large population and abundant natural resources, Nigeria is yet to advance economically. Up till now, the nation does not have any globally branded product, multinational company, technical and managerial expertise or worldwide range of Intellectual Property Rights exploited globally that emanated from its indigenous knowledge and industrial efforts (Bindir and Tandama, 2013).

Abubakar, (2019). That the newly approved universities were joining the largest university system in Africa in terms of number and enrolment, comprising of forty three (43) federal universities, forty eight (48) State universities and seventy nine private universities, translating into one hundred and seventy (170) universities in Nigeria. This shows that educational and knowledge infrastructure are abound in the country with also about 125 polytechnics, 98 colleges of education, over 300 institutions composed of research institutes, innovation agencies and policy implementation departments, multinational companies, large pool of skilled labour force including a sizeable number of diaspora, making up a total of approximately 693 institutions directly or indirectly involved in research yet nigeria economy is still technologically weak with a very high national poverty incidence that implies that over 100 million Nigerians are living below the poverty line.

Siyanbola et.al.(2012) There are a total of seventeen (17) agencies that constitute the Federal Ministry of Science and Technology; Fourteen (14) of these agencies are charged directly with the mandate to conduct R&D . The other three have mandates for policy research and capacity building in management of technology, intellectual property rights, and technology business incubation. National Agency for Science and Engineering infrastructure (NASENI) is charged with research in capital goods, production and reverse engineering; Federal Institute of Research (FIIRO) is mandated to accelerate industrialization in Nigeria; Sheda Science and Technology Complex (SHESTCO) has the mandate to develop research results for application in areas of agriculture, health, industry and environment; National Space

Research & Development Agency (NARSDA) is vested with research in space and development. Promoting the development and utilization of Nigeria's industrial raw material is the responsibility of Raw Materials Research and Development Council (RMRDC); Nigerian Building and Road Research Institute (NIBBRI) is to ensure improvement in the quality of life of Nigerians in the areas of affordable housing; Nigerian Natural Medicine Development Agency (NNMDA) will do research, develop collate, document and promote the nation's natural medicine; Nigerian Leather and Science Technology (NILEST) is a Centre for development in the areas of Chemical and Leather technology. National Research Institute for Chemical Technology (NARICT) develops the technologies required by the chemical industry

and also undertakes R&D work in areas of agriculture, mineral and other raw material conversion to chemicals; Project Development Institute (PRODA) has part of his mandate to develop the technologies required by the power equipment industry; National Biotechnology Development Agency (NABDA) coordinates, promotes and regulates the development of biotechnology in Nigeria. Nigeria Institute for Trypanosomiasis Research (NITR) is to conduct R&D for the control and elimination of Trypanosomiasis and its vectors; The Nigerian Institute of science laboratory technology (NISLT) conducts research in all the areas of Science Laboratory Technology; Nigeria Atomic Energy Commission (NAEC) coordinates R&D activities for capacity building and infrastructure development in Nuclear technology. In charge of Intellectual property and research industry linkages are the National Office of Technology Acquisition and Promotion (NOTAP). National Board for Technology Incubation (NBTI) provides institutional infrastructure and mechanism for the development and commercialization of R&D outputs and inventions. National Centre for Technology Management (NACETEM) is mandated to provide knowledge support for the STI system in Nigeria through capacity building in management of technology, STI policy research and consultancy.

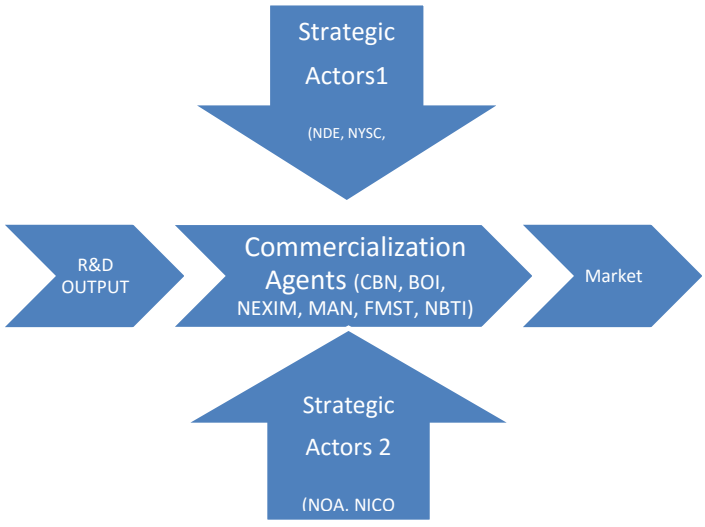
Another reason for the American success in commercialising public science is the substantial licensing income that universities such as Stamford, Colombia, MIT and the University of Florida have earned from patenting their inventions. The causes of failure by Nigerian scientists could be attributed to a wide range of factors including a lack of entrepreneurial spirit among scientists, barrier to the ability of public sector scientists to move to the private sector on a temporal basis to develop their discoveries and to poor Intellectual Property Right of university inventions. Currently in Nigeria, existing approaches for linking research with private enterprise take the form of research products fairs, experimental incubator models and incoherent outreach approaches (Binder and Tandama, 2013).

The most important implicit factors limiting the development of a virile Science, Technology and Innovation and consequently, technology development and transfer in Nigeria is funding of research and development activities. Technology transfer include a set of activities starting with investment in R&D, the R&D performance, decision on how to handle the intellectual property to demonstrate technology and commercialization which brings the products to the market. In this new economic order, developing nations can no longer compete based on their natural resource endowments and locational advantages. (Ogunwusi & Ibrahim 2014). In Nigeria, the Intellectual Property Right (IPRs) of innovators and industrialists are governed by Patent and Design Act cap 344 of 1990, Trademarks Act Cap 436 of 1990 and Copyright Act of 1998 (Ukpabi, 2009). Managing Intellectual Property Rights and Technology

transfer issues in Nigeria has been part of the core mandates of the National Office for Technology Acquisition and Promotion (NOTAP). To ensure a link between R&D activities carried out in the country and the market, and facilitate process of commercialization, NOTAP has established over 43 Intellectual Property and Technology Transfer Offices (IPTTO's) in tertiary institutions across Nigeria and assist innovators to prepare and file applications for property rights. Nevertheless the number of applications for rights protection filed by public RI's between 1999 and May 2012 was lower than those filled by private innovators, indicating that the public RI's are less interested in rights protection and consequently commercial exploitation of their results (Siyanbola et al, 2012). Presently, over 100 commercialisable R&D outcomes in the areas of Agriculture , Industry, Engineering, and Health have been successfully produced by agencies under the |Federal Ministry of Science and Technology in Nigeria. Less than 2% of R&D in Nigeria have been commercialised. In view of this, Siyanbola et al (2012) recommended a change in commercialization strategy in Nigeria through adoption of new strategic approach.

Commercialization Models

In Organization for Economic Cooperation and Development (OECD) countries, knowledge and research generated by public research system is diffused through a variety of channels. These include mobility of academic staff, scientific publications, conferences, contract research with industry and licensing of university inventions. Much of the policy forms in OECD countries has been centered on promoting knowledge transfer via a dual, but rather linear model of commercialization. This model is characterized by several push forces whereby universities and public research institutes transfer academic inventions via the sale, transfer or licensing of intellectual property, often on an exclusive basis to existing firms or new ventures. The converse of the model is a demand-pull model based on contract research or collaborative research and development whereby universities and public research institutes are solicited by industrial actors to find solution to production and innovation problems (OECD, 2012).

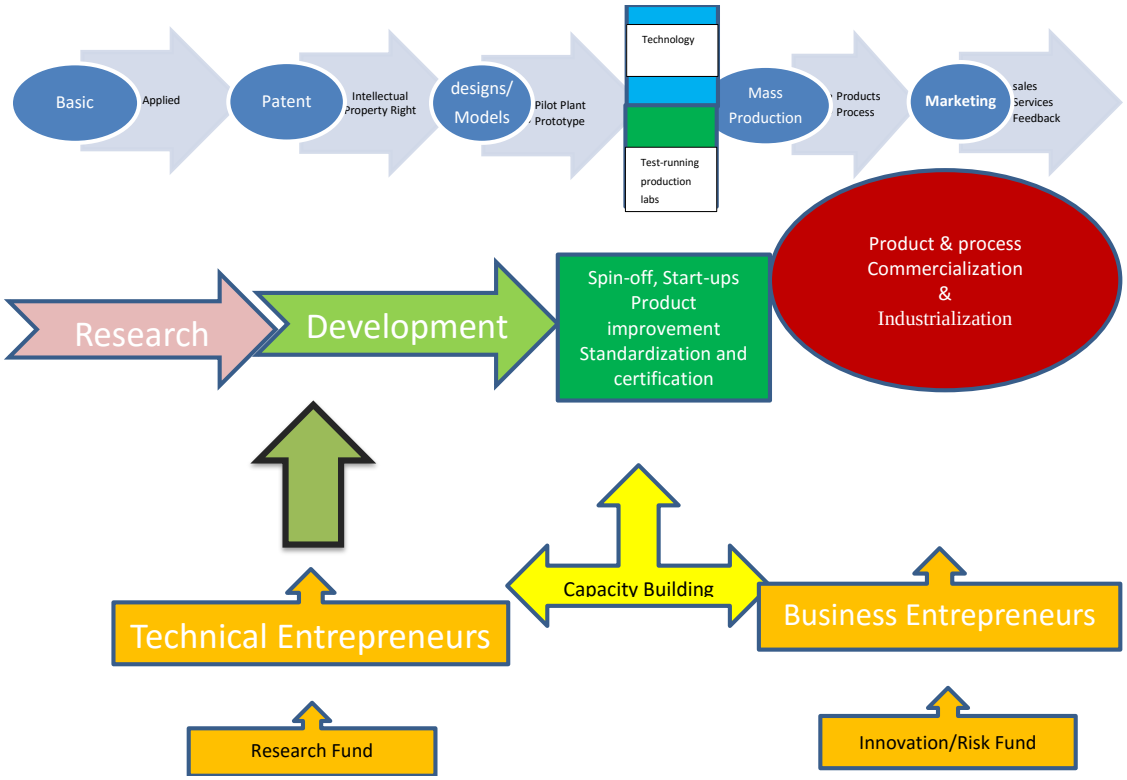


Commercialization Model One: Developed and Recommended by Siyanbola et.al (2013) for FMST.

1. R&D Output: Database of R&D from universities/HEIs, research institutes/centers, private company.
2. Commercialization Agents: These are group of experts who connects the R&D outputs with the market (CBN, BOI, NEXIM, MAN, FMST, NBTI etc.)
3. Strategic Actors1: These comprise of actors that make use of R&D outputs and process them into finished products for the market.
4. Strategic Actors2: These are actors responsible for sensitization and advocacy on patronizing goods produced in Nigeria. (NOA, NICO etc.)
5. Market: The whole essence of the activities starting from the laboratories through the other actors in the commercialization process.

Commercialization Model Two: Developed by Otto Lin of Taiwan University in 2001 and been used by Hong kong, china, Taiwan.

1. Basic Research: These are basic in nature as they failed to have specific focus mostly conducted by undergraduates.
2. Applied Research: These are market driven, need focused development drive research usually conducted by advance researchers and market demand.
3. Product and process Development: These involves the design and model phase for the product and process.
4. Pilot production, field trial and test running: This is to ascertain we are on cause to full manufacturing.
5. Manufacturing: Commence full scale commercial production according to specification and standards.
6. Sales & Services: Marketing expert to handle marketing for sales and after sales service to ensure appropriate feedback for correction.



Commercialization Model Three: Developed and Recommended by Ndagi (2017) for NBTI.

R&D output and Commercialization Challenges in Nigeria.

1. Funding of R&D activities in Nigeria according to Siyanbola et. al. (2013). has largely been by the federal government through the yearly budgetary allocation thus resulting in poorly funded institutions. The highest proportions of science and technology (S&T) activities in Nigeria are carried out by public institutions which invariably demand that it should be given more priority in the national budget. The limited funding of R&D activities in Nigeria, in practical terms, is a reflection of low appreciation of the benefits of R&D to national development. This stands in sharp contrast to government determination to leapfrog development through the application of science and technology. Although there are funding support for projects and R&D activities from international organizations, however, much investment and support is still needed in this sector

2. Lack of adequate publicity for Intellectual Property Right in Nigeria; Intellectual Property Rights (IPRs) of innovators and industrialists in Nigeria are generally governed by Patent and Design Act Cap 344 of 1990, Trademarks Act Cap 436 of 1990 and Copyright Act of 1998 [7]. Managing Intellectual Property Rights and technology transfer issues in Nigeria has been part of the core mandates of the National Office for Technology Acquisition and Promotion (NOTAP).This agency has been operating in compliance with the Trade Related Aspects of Intellectual Property Rights (TRIPS) of the World Trade Organization (WTO) which set the minimum acceptable standard for member countries. To ensure a link between R&D activities carried out in the country and the

market, and facilitate the process of commercialisation NOTAP has established over four-three (43) Intellectual Property and Technology Transfer Offices (IPTTOs) in tertiary institutions across Nigeria and assists innovators to prepare and file applications for property rights.

3. The Nigerian STI system overtly depends on government for nearly all its requirements. According to Bindir & Tandama (2013). As a result, a number of implicit and explicit factors influence the performance of Science and Technology Innovation system in Nigeria. Implicit factors such as state of art infrastructure to carry out meaningful research work on competitive basis is absent in most of the organisations as most of the universities and research institutes are not adequately equipped with modern facilities

4. The challenges of R&D output commercialisation according to Ogunwusi & Ibrahim (2014). is compounded by lack of steady power and water supply. The need for adequate information on global best practices, sources of grants and the information on current status of development in several disciplines are not available to most of the research scientists. In most universities and research institutes, latest relevant journals are scarce and most researchers are left with the information obtainable on the internet. Other important implicit factors such as training of personnel and funding are abysmally inadequate. In addition, the existing R&D reward systems are also clearly inadequate. The explicit factors germane to the successful performance of STI system which include commercialization of R&D results, linkages, quality assurance, Intellectual Property Rights (IPRs) system, entrepreneurship, investment, investors' confidence and marketing strategy are in most cases unorganized and inadequate in public R&D organisations and the universities. The most important implicit factors limiting the development of a virile Science, Technology and Innovation and consequently, technology development and transfer in Nigeria is funding of research and development activities. Technology transfer typically include a set of activities starting with investment in R&D, the actual R&D performance, decision on how to handle the intellectual property to demonstrate technology and commercialization which brings the products to the market.

5. Another significant challenge militating against successful technology transfer from laboratory to the market is the little or no linkage that exists between research institutes, universities and the industry. The opportunity for a country to initiate, maintain and sustain competitive advantage through innovation rests on its ability to create and advance synergy. Though, the number of universities and research institutes in Nigeria is high, the anticipated commercialization has failed mainly due to the lack of connectivity between industry and the academia. This is due to the fact that commercialization of R&D results has not been traditionally, a high priority of universities. The public universities in Nigeria are funded directly from the national budget. Private sector funding of R&D in Nigeria is lagging. (Ogunwusi & Ibrahim 2014).

6. There is weak and unorganized institutional framework to midwife and nurture linkages between university and industry which leads to inadequate and in some cases non-existing strategic partnership for commercialisation and innovation networking of the universities, business community and the government. Essentially, there is poor correspondence between

expectations/needs of the private sector and the research priorities in the universities and specialized research institutions.

7. The causes of failure by Nigerian scientists could be attributed to a wide range of factors including a lack of entrepreneurial spirit among scientists, barrier to the ability of public sector scientists to move to the private sector on a temporal basis to develop their discoveries and to poor Intellectual Property Right of university inventions. Currently in Nigeria, existing approaches for linking research with private enterprise take the form of research products fairs, experimental incubator models and incoherent outreach approaches. On the other hand, the organized private sector, including industrialists, business people and agriculturists, hardly shows confidence or stake in the existing linkage systems. Hence, the solutions to local industry problems are often sought without recourse to the skills, capabilities and opportunities within the university system (Bindir and Tandama, 2013).
8. The challenges to Commercialization of University research in Kenya according to Ayisi et al. (2016).are numerus among which are; Only few members of university staff are engaged in research and development this is due to too much teaching at the expense of research. Staff promotion policies demand prolific publications and dissemination of research results at conferences thus losing the patentability of inventions this requires need to move from publish or perish to innovate or perish
9. A recent report on the state of university-industry linkages in Africa revealed the following relevant findings that serve as a cautionary warning (AAU & AUCC 2012):
    a) University research output is limited by the low percentage of academic staff with PhD training and qualifications, and brain drain of qualified scientists;
    b) Many African universities have attempted to foster linkages with firms through the creation of offices and staff positions in charge of such affairs. However, such offices lack the material resources and expertise to handle industry partnerships and technology transfer effectively;
    c) There is a low number of science parks and technology incubators in academic institutions. Only a small percentage of universities surveyed reported being involved in managing science parks, technology incubators and engaging in technology transfer;
    d) The study suggests that support for establishing and managing technology incubators and science parks would respond to the needs and priorities of African universities.

CONCEPT OF TECHNOLOGY INCUBATION

There are several definitions and approaches to business and technology incubation. Conceptually, 'incubation' is a more diligent and planned process than clustering or `co-location' and therefore needs careful attention to the problems of prospective occupants, extending well beyond providing infrastructure and office services (Adelowo, Olaopap & Siyanbola 2012; Kiridena, 2001). According to the National Business Incubation Association (NBIA), Business Incubation catalyses the process of starting and growing companies, providing entrepreneurs with the expertise, networks and tools they need to

make their ventures successful. Incubation programmes diversify economies, commercialise technologies, create jobs and create wealth.

The term incubator, which is more widely known with the life-giving support to premature babies or phenomenon to enable them survive the critical early period of life, is what has been adapted to economic development and regeneration. Therefore, economically, definition of Incubation/Incubators varies with their services, their organizational structure and in the types of clients they serve. Technology Incubation has different goals which include job creation, new venture creation, wealth creation, value addition to clients' products, process and services and transferring technology from universities and major corporations to entrepreneurs/enterprises (Smilor& Gill, 1986). According to Lalkaka (2000), business incubation is a means by which visions of new businesses are turned into reality with reduced risks. Incubators aspire to have a positive impact on a community's economic health, by maximizing the success of emerging companies (Cassim, 2001). Business incubators have proved effective in many parts of the world. According to Rice and Matthews (1995), only 10 business incubators existed in the United States in 1980. There were nearly 500 by 1995, and a new incubator has been opening every week. The technology incubators generally focus on nurturing technology-intensive enterprises and knowledge-based ventures.

The technology incubation system (TIS) is variously represented by entities such as Techno-polis, Science Parks, Research Parks, Technology Parks, Technology and/or Business Incubators. These entities operate as separate organisations but are mostly integrated with other players in the innovation system. The terms Science Parks, Research Parks and Technology Parks as well as Technology Incubators (TIs), Technology Innovation Centres (TICs) and Technology Business Incubators (TBIs) are used interchangeably in many countries depending on the level and type of interaction between R&D community, venture funding and industry.

Relevant research thus comes from countries in Europe and North America. Several studies analyse the aims, structures and spatial impact of technology incubation centres and similar initiatives. In some countries, lengthy and comprehensive impact evaluations have already been conducted. With respect to technology incubation centres, Germany, United Kingdom, Sweden and the whole of the European Union (European Commission, 1996; Massey et al. 1992;) may still be the best researched countries. More or less comprehensive evaluations are found in other countries such as the USA (Luger and Goldstein, 1991).

In this paper, the term technology incubator is taken to mean a controlled environment-physical or virtual- that cares, and helps new ventures at an early stage until they are able to be self-sustained through traditional means while technology incubation apply generically to all the organizational forms for promoting technology-oriented SMEs respectively. The organizational format of technology incubations also varies and could generally be categorized as public or not-for-profit incubators, private incubators, academic-related incubators and public/private incubators, which are referred to as hybrid in most literatures. Also, technology incubations may thus have a wide range of goals and objectives giving rise to different forms of incubators specializing in accessing diverse resources

In the last two decades, African countries have embarked on establishment of technology parks and incubators to fast-track and sustain economic growth, creating jobs for fresh graduates and transition into the knowledge economy through commercialisation of research results.

The International Association of Science Parks (IASP) considers that the term "Science Park" could include Technology Park, Technopole and Research Park (Link and Scott, 2011), and defines it as an organization managed by specialized professionals, whose main aim is to increase the wealth of its community by promoting the culture of innovation and competitiveness of its associated businesses and knowledge-based institutions (International Association of Science Parks, 2014).

On the-other-hand IASP defines an incubator as an organization designed to accelerate the growth and success of entrepreneurial companies through an array of business support resources and services that could include physical space, capital, coaching, common services and networking connections (IASP, 2014). Many technology parks explicitly incorporate business incubators into their developments to provide facilities for the nurturing of firms at all stages of their business and technological life cycles, as they are a key mechanism for technology transfer. They are seen as a mechanism to support and establish new businesses/start-up and fledging companies to promote job creation, economic development, innovation and high growth, by providing a wide variety of services that are typical to most starting ventures: physical space and infrastructure (office space, secretarial and administrative services), business consulting and training, funding applications (government and private), IP protection, technology transfer, and networking (Jamil *et al*., 2015). Incubator program gives a chance to projects that are unable to attract commercial investors in the initial stages of development.

Incubators are available in various types rendering a range of long and short-term assistances and they help in the establishment of new enterprise in one way or the other. Many of these provide only guidance, technical assistance and consulting to entrepreneurs and offer business development services. ICT incubators are major examples of these Incubators where clients access to appropriate rental space, shared basic business services and equipment. Few incubators assist only in developing new ideas and arrange for venture capital funding. Incubators are sometimes known as Business Accelerator as it accelerates start-ups by providing quick knowledge, support services and resources (Lewis, 2001).

Highly adaptable incubators have differing goals, including diversifying rural economies, providing employment for and increasing wealth of depressed inner cities, and transferring technology from universities and major corporations (Smilor & Gill, 1986). Incubator clients are at the forefront of developing new and innovative technologies – creating products and services that improve the quality of our lives in communities around the world.

Essentially, the incubation programme is to assist and support the transformation of selected, early stage businesses with high potentials, into self-sufficient, growing and profitable enterprises (Lewis, 2001). By reducing the risks during the early period of business formation, the incubation sustains the new enterprises that might otherwise fail due to lack of adequate support. In doing so, the incubation programme contributes to the economic growth by creating jobs and offering other socio-economic benefits. According to Adelowo et.al (2012), technology incubation programme can therefore be seen as an economic development tool designed to accelerate the success of high technology entrepreneurial

enterprises through the provision of an array of technology business support resources and services in a controlled work environment.

Lewis (2001) sees technology incubation programme as an innovative system designed to assist entrepreneurs and inventors in the development of new technology -based firms. It seeks to link talents, technology, capital and know-how effectively, in order to accelerate the development of new businesses, and thus speeds the commercialization of technology. It is a facility that helps the early stage growth of technology-based enterprises by providing shared facilities such as space, office services, and business consulting services.

This concept, which constitutes a very potent economic development tool has generated great desire and has undergone extensive development in the USA and many other countries such as India, Japan, China, Korea, Israel, Germany, France etc. in the context of new global trend of engendering real sector development through small and medium scales businesses.

Technology incubation programme as a tool for economic development makes provision of job creation, employment opportunities targeting unemployed university graduates, retrenched public sector employees, retired research institution employees, retired private sector employees, and established industrialists desiring to expand or diversify their businesses (Lalkaka, 2000).

Promotion of small and medium scale development is yet another contribution of technology incubation programme on the economy, that is, it assists in incubating knowledge-based skilled and unskilled workers, start-ups into commercially viable products/services by providing specialists in various area of endeavors, skilled training, guidance, critical support services, such as invention and innovation, financing, laboratory, library, networking/ ICT, quality control workshop support services to all tenants or small and medium scale businesses at each centre, and a conducive environment (affordable, well-equipped workspace) to entrepreneurs.

Incubation programme was introduced to Africa in 1988 by United Nations Development Programme (UNDP) to test run the concept on pilot scheme in four (4) countries of Ivory coast, Nigeria, Equatorial Guinea and Zimbabwe. In 2012, the incubation programmes has spread across Africa with approximately about one hundred incubation centers. Nigeria has about forty (40) incubation centers, South Africa with about thirty-six (36) while the rest of other countries house the remaining twenty (20). Since 1988 with feasibility study for the establishment of incubator pilot centers at Lagos, Kano and Aba to ascertain the viability of Technology Incubation in these commercial cities. TIC Lagos was in 1993, TIC Kano was in 1994 and TIC Aba was in 1995. The success of these three pilot centers facilitated the establishment of TIC Minna, Nnewi and Calabar in 1998. Meanwhile, by 2005 there were seventeen (17) incubation centers in Nigeria but as at 2012 there are about forty (40) incubation centers in the country with about two hundred and eighty-seven (287) entrepreneurs and six thousand two hundred (6,200) jobs created. (NBTI. Annual report 2013)

The Policy Thrust of Technology Incubation Programme

The policy thrust of the Technology Incubation Programme according to Federal Ministry of Science and Technology [FMST] (2005) is to pursue the commercialization of technologies and technical innovations

using Technology Incubation as a tool in order to enhance the attainment of technological, industrial, social and economic competitiveness of the country and improve the quality of life of its citizens.

FMST (2005) explains that the vision of the programme is to make Nigeria a competitive nation through the commercialization of R&D results and other innovative efforts using technology incubation as a tool while the mission is to develop the necessary infrastructure to nurture technology starts-up to promote Nigeria's indigenous potential through value-added and technology-related activities and to create enabling environment for effective linkage amongst technology providers, entrepreneurs, and capital .

The larger objectives of our technology incubation programme according to FMST (2005) includes the following:

1. To impact practical and result-oriented training in several industrial trades. Special training programme will be introduced for rural artisans and vulnerable group of the society in collaboration with institutions of higher learning or research centers and other stakeholders located nearby.
2. To monitor closely the development of prototypes of machines, equipment and tools which could be passed on to the manufacturing units for commercial production.
3. To provide common facilities in such areas as testing, machining, castings, electroplating, quality control laboratories etc.
4. To encourage the production of machines and equipment, partly or fully as per market acceptability.
5. To develop testing and inspection of facilities for use by small scale units in collaboration with research centers.
6. To demonstrate R&D results in such critical areas as waste utilization, energy saving etc.
7. To help in ensuring that new ideas/products evolve into fruitful technical/manufacturing businesses using the abundantly available raw materials.
8. To help in solving specific problems for client entrepreneurs by continuously injecting innovation in materials processing.
9. To offer engineering services such as design testing, process/product performance monitoring and improvement, as well as training and general consultancy to client entrepreneurs.
10. To liaise with research centers and institutes in the design, development and production of improved tools for use by rural artisans for increasing their productivity and earning capacity.
11. To design tailor made/crash programme for our Centre Managers, entrepreneurs and industrialist to keep them abreast with the latest technology.
12. To provide assistance to the private sector in the creation and enhancement of institutional and technical infrastructure so that they can compete in the international market.

The scope of the Technology Incubation Programme

According to FMST (2005), the scope of the technology incubation programme is to nurture the development and commercialization of:

Low technologies;

Manufacturing of simple equipment and machineries; Up grading of traditional technologies; Handcrafts, etc;

Medium technologies;

Manufacturing of electrical and electronic components and equipment; chemical processes and manufacture of plastics items; manufacturing of scientific equipment, etc.

High technologies;

Biotechnology Processes and Products; software and hardware; space technology; artificial intelligence; robotics

Emerging technologies

Advance materials; nano technologies; and others.

Technology incubation schemes

Pre-incubation

Pre-incubation are the activities of the entrepreneurs prior to admission into the incubation programme.

Incubation

Resident Incubation: In this scheme, entrepreneurs are tenants of a Technology Incubation Centre (TIC). At the TIC, affordable share facilities such as working spaces, central workshop, equipment and laboratories, offices, hand-on management assistance, access to financing, networking and exposure to critical business support services are provided to enhance the success of the enterprise during the incubation period, which ranges from one (1) to three (3) years.

Non-Resident/Virtual Incubation: In this scheme incubation services such as access to resources (knowledge providers, finance, linkages/networking, etc) are extended to entrepreneurs outside the TIC.

Post Incubation

Some intervention measures such as monitoring services, linkages/networking to capital and knowledge providers (local and international), etc are extended to graduates of the programme to ensure their sustained competitive growth.

ROLES OF NATIONAL BOARD FOR TECHNOLOGY INCUBATION

The role of NBTI according to FMST (2005) includes the following:

i. Policy guidelines for the execution of Technology Incubation Programme;

ii. Undertaking feasibility studies for the establishment of Technology Incubation Centres;

iii. Providing technology focus for TIP;

iv. Management of the entire programme;

v. Provision of specialized and customized infrastructure;

vi. Provision of a central facility workshop, equipment, laboratories for the technical development needs of the entrepreneur;

vii. Granting of development fund (seed capital), a non-interest revolving loan, to entrepreneurs in partnership with relevant stakeholders for sustenance of the programme;

viii. Facilitating linkages with knowledge-based and external service providers; and

ix. Provision of marketing outlets through exposure to local and international trade fairs and exhibitions in collaboration with Nigeria Export Promotion Council,

In addition to the role of NBTI enumerated above, the host state, entrepreneurs that are involved in the programmes, private sectors as well as tertiary institutions have roles to play. These roles are as highlighted by FMST (2005) and are also listed below:

Roles of the State government.

i. Provision of technology parks for the relocation of the entrepreneurs after graduation;

ii. Provide technically feasible and commercially viable R&D results and inventions;

iii. Provide technical support to the TICs;

iv. Establish sustained Institutional linkages with the Centres;

v. Collaborate with relevant tiers of Government for the establishment of TICs; and

vi. Establish institusion-based Incubators to commercialize R&D results

Roles of entrepreneurs at the technology incubation centres

i. Provide Business Plan that translates commercially viable R&D results, inventions and/or indigenous knowledge into goods and services,

ii. Provide take-off capital for the business,

iii. Provide basic machinery for the take-off of the enterprise,

iv. Provide raw materials for the enterprise,

v. Provide adequate management for the business,

vi. Provide periodic report; and

vii. Abide by the rules and regulations of the TIP.

BENEFITS OF TECHNOLOGY INCUBATION PROGRAMME:

The benefits of Technology Incubation (when best practices are employed) to the different stakeholders include:

i. Entrepreneurs (Tenants)

It enhances the chances of success, raises credibility, helps improve skills, creates synergy among client-firms, and facilitates access to: mentors, information and seed capital.

ii. Governments

It would help overcome market failures, promotes regional development, generates jobs, incomes and taxes, and becomes a demonstration of the political commitment to small businesses.

iii. Research Institutes & Tertiary Institutions

It helps strengthen interactions and collaboration between the knowledge-base and the industries, promotes the commercialization of research results, and fosters enabling environment which encourages Faculties and students to maximize their potentials/capabilities

iv. Local Community

Enhances the creation of entrepreneurial culture, as well as local incomes with majority of graduating businesses settling within the area

Some Specific Research Commercialised at Technology Incubation Centres in Nigeria

S/N	TIC CENTRE	PRODUCT OF R&D	SOURCE OF R&D
1.	Lagos	1. Full Fat Soya 2. Beni Seed Oil Extract	RMRDC RMRDC, FIIRO
2.	Kano	1. Neem Oil for Neem Soap 2. Automatic Filling Machine	NARICT Private R&D
3.	Aba	1. Scourging Powder 2. Mr. Flush Chemical	Private R&D Private R&D
4.	Minna	1. Rice Husk in making ceramic glazing machine 2. Kunun Zaki preservation 3. Kunun Zaki processing machine 4. Chalk moulding machine 5. Cassava bread and cake 6. Bread fortification with protein 7. Poultry feed calcium enhancement 8. Shea butter free fact acid purification technique	PRODA Technology FIIRO SEDI – Enugu SEDI – Minna FIIRO FUT – Minna FUT – Minna FUT - Minna
5.	Nnewi	1. Amplifier 2. VHF/UHF Boaster 3. Stabilisers/Inverters	CAT - Awka CAT - Awka CAT - Awka
6.	Calabar	1. Bricks and Roofing Tiles	NBRRI
7	Enugu	1. Fire prevention/retardant solution	Private R&D

Source: Ndagi 2017

Conclusion

In accomplishing commercialization of R&D output, incubators use strategies such as increasing access to capital, the one stop shop approach, technical and business management training, contract procurement assistance, creating networking opportunities through clustering, export assistance and

technology transfer assistance. These services are provided through collaboration with other economic development and entrepreneurship development organization within the same region. The national policy on science, technology and innovation (NSTI) should provide a strategic framework for government-university/research-industry/entrepreneurship-society/market linkage to effectively and efficiently facilitate commercialisation of R&D output for national development.

Technology incubators play an important role in transferring research output from universities to industry. They are statutorily to support and nurture the development of technology value-added small and medium-sized enterprises (SMEs) and strengthen the country`s economic competitiveness for national development. Universities to commercialize their research outputs, technology incubators have been identified as key intermediaries to fill the gap between R&D and commercialization. As a result, many countries have supported these institutions as tools for commercialization and major contributors to knowledge-based economies. The most equipped commercialization team is one which is comprised of four characters, i.e., innovator or inventor, investor, technology expert, and entrepreneur (Shaverdi & Baghdadi, 2010). Inventor is a person who creates and presents a product or process which is either new or better to the existing ones and investor is an individual who invests in properties such as shares, products, and ideas with an aim of gaining profits. In addition, an entrepreneur is a possessor of an idea, an enterprise, or a high-risk investment company that voluntarily accepts the inherent risks associated with starting and growing a new business, product or service. Finally, technology expert is an individual who applies scientific knowledge to practical problems solving.

Recommendations.

- National research Fund, PSCII = National Risk fund, National Innovation fund
- Establishing and Strengthening of IPTTO

Universities require to establish fully fledged technology transfer units or consultancy bureaus, equipped to undertake patent searches, assess the novelty of innovations, pay the cost of processing patent applications and take care of the marketing of the invention and their commercialization, as well as the negotiation of the licenses and royalties.

- Monitoring and Evaluation

Universities should set targets on IP to be commercialized every given agreed period and commit budget for its implementation. In addition, there is need to develop an implementation strategy with guidelines for key performance indicators of all commercialization initiatives developed by universities to help monitor and measure their outcomes.

- Capacity-Building in Relevant Skills and Policy Development

Lack of entrepreneurial culture by staff (some faculty members have a purely academic orientation and don't have a lot of interest in dealing with private companies) need to be promoted. Support for training to students and early career researchers in commercialization to develop entrepreneurial skills and intellectual property management among academic staff and students is very key.

- University Science Parks and Technology Incubators

Supporting the establishment and management of university science parks and technology incubators for the purposes of technology transfer and management skills to run the facilities is strongly recommended

- Research Commercialization in humanities

Fields, like the humanities, may have limited possibilities for research commercialization. However, Innovation should also cover humanities areas such as governance, social, rural, urban, industrial corporate, education, health care, transportation, social safety nets and branding.

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Empowerment of Beekeepers on Modern Apicultural Management and commercialization of Beehive products in Lagos State, Nigeria

Joseph C. Anikwe

Applied Entomology and Pest Management, Department of Zoology, University of Lagos, Akoka, Lagos State.
Email: jachuks@yahoo.com; janikwe@unilag.edu.ng

Background

My research efforts have been centered on three areas of applied entomology and these are entomophagy which simply means the rearing of edible insects; insect pest management which involves the use of environmentally safe approaches to decimate obnoxious pest populations; and finally apiculture. For the purpose of this conference, my main focus will be on apiculture.

Apiculture (beekeeping) is the maintenance of honeybee colonies by humans for their highly desirable products and services. *Apis mellifera*, honeybees, in addition to providing products such as honey, beeswax, pollen, propolis, bee venom and royal jelly are responsible for about 80% of global agricultural pollination services. Although, the use of honeybees for pollination services in not a common phenomenon in developing countries such as Nigeria, it however, has been reported that bees are the most important pollinators. According to a Cornell study published in the May 22 issue of the journal Public Library of Science ONE, crops pollinated by honeybees and other insects contributed $29 billion to farm income in 2010 in the US alone.

The effectiveness of honeybees is due to their great number, their social life and their ability to pollinate a broad variety of different flowers. A colony can consist of 20-80 000 bees, and they will normally be visiting flowers over a distance of two kilometers when they are collecting pollen and nectar. If there is no flora in the neighbourhood, they can fly up to seven kilometers in search for food. An average *Apis mellifera* honeybee colony will make up to four million flights a year visiting about 100 flowers in each flight. The honeybee's pollination effectiveness also arises from the special constancy to flowers of one species. Scout bees communicate via the waggle dance to other bees in the colony which species to visit, and even give small tastes of nectar and scent from that flower.

As important as honeybees are to man and the ecosystem, they are usually confronted with a lot of biotic stressors which include pests and diseases. The impacts of bee pests on colony have been reported to cause about 15 % decline in honey bee colony establishment in some places in Nigeria. The behaviour of the bees such as regular absconding and aggressiveness has contributed to low colony establishment. It is usually difficult to work with the African bees because of their aggressive nature. It is therefore imperative to put an effective management strategy in place in order to fully exploit the benefits of honeybees. Bees make honey from the nectar that they collect from flowers, other plant

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saps and honeydews. With the vast floral resources and low capital requirement for beekeeping, it is an aberration to still import honey into Nigeria. African honeybees *Apis mellifera andersonii* compared to the western honeybees *Apis mellifera mellifera*, have a high foraging power but low productivity because they consume their honey hoards and more importantly, due to the crude hive type and management practices of beekeeping in Nigeria.

It was therefore imperative to train and empower beekeepers in modern apicultural techniques in order to address the issues of low productivity, improve honey quality and create employment opportunities for the youths.

Summary of our Research Efforts

In 2014, our proposal christened 'Participatory approach to modern apiculture management to empower farmers in selected rural communities in Lagos State' was selected among many others and funded by Lagos State Research and Development Council grant. The three major objectives of the project were to:

- train farmers on modern beekeeping techniques compatible with crop cultivation for augmented incomes.
- conduct melissopalynological analysis of honey samples from selected apiaries in Lagos State.
- implement Integrated Hive Management (IHM) strategy for increased honey productivity.

For the purpose of this write-up, I would not want to delve in details into the latter two objectives, although all three objectives were achieved at the end of the project. Communities and farmers were identified with the assistance of Extension Agents from the State's Ministry of Agriculture and Cooperatives. Twenty potential beekeepers each were recruited as beneficiaries of the project from Ikorodu, Epe and Badagry, giving a total of 60 beekeepers. The research team visited the selected communities on a fortnightly basis and held meetings with selected beekeepers at Odogunyan, Ikorodu; Agbowa-Ikosi, Epe; and Badagry. At the meetings, farmers were briefed on the importance of the project to their communities and how they would be empowered from the project to produce quality honey as well as the potentials of beekeeping in pollination of their crops for higher yields. Apiary sites were selected in each community in conjunction with the farmers to accommodate 20 hives that would be given to each location.

The farmers were invited to attend a training workshop on Modern Apicultural Management at the University of Lagos, Akoka. During the training, the farmers were encouraged to form cooperative societies at their respective locations. Experienced bee researchers presented papers on the advantages of modern hives over the traditional types, factors considered when siting apiaries and techniques for managing honeybees for increased honey production, as well as honey processing and packaging. At the end of the training workshop, a total of 60 modern hives (Kenya topbar and Langstroth) were distributed to benefiting farmers while three bee-suits were distributed to each community. Training materials were also made available to the farmers. Ten hives were retained at the University of Lagos for in-station apiary (Plates 1-4).

Plate 1: Cross section of participants at the training workshop

Plate 2: Cross-section of bee hives for distribution to farmers

Plate3: Presentation of bee-suits to a farmer from Agbowa Ikosi, Epe at the training Workshop

Plate 4: A cross section of farmers loading their beehives into trucks for onward delivery to their locations

Immediately after the training/sensitization workshop, the research team embarked on a fortnightly visits to the three communities to help the farmers (now beekeepers) with setting up of their hives, baiting of hives to attract bees, field training of farmers as well as monitoring and data collection. Bee smokers were distributed to each community on sites. These apiaries apart from commercial benefits they bring to their owners, also serve as extension and experimental sites for our some of our postgraduate students. We backstopped the project at the various locations until the beekeepers were able to stand on their own (Plate 5).

Plate 5: Demonstration on the use of honey comb foundation in Langstroth hive at Badagry

Selected farmers in Ikorodu, Badagry and Epe communities of Lagos state have been empowered with theoretical and practical knowledge of modern apiculture techniques compatible with crop cultivation for augmented incomes. The farmers have been equipped with basic beekeeping equipment and materials such as bee hives, beesuits, and smokers for honey production. Apiaries have been well established in selected locations and farmers are working as a team. Farmers now have knowledge of Integrated Hive Management (IHM). Apiaries have been well established in selected locations and active colonies of over 80% have been achieved across all locations including UNILAG. Quality honey production have since commenced and the farmers have been empowered (Plate 6). The project has produced four MSc students since inception and a PhD student in currently on research in modern apicultural management.

Plate 6: Harvested and ready to sell honey at UNILAG

Conclusion

With an average of 35kg honey yield per hive and a minimum retail price of N2,500/kg, there is no doubt these farmers have been greatly empowered. There is a ready market for honey especially when the source is trusted. Encountering adulterated honey in Lagos market is a common phenomenon, hence buyers are willing to pay premium price for genuine quality honey from our beekeepers. Honey is sold as raw honey in different container sizes but yet to be properly packaged and branded for international markets. Branding and packaging are the next phase which serve as a precursor to the process of full commercialization of their products. Full commercialization will require the process of registration with the National Agency for Food and Drug Administration and Control (NAFDAC), however, as a bee scientist, I would rather advocate for the establishment of National Honey Board, as practiced in other countries for standards in honey marketing.

Effect of Sodium Benzoate On The Quality Of 'Wara'– A Local Cheese Produced From Soybean

[*1]Abdulkadir F., [2]Igyor M.A., [1]Kolo S.I. & [1]Maude M.M.

[1]Department of Food Science and Technology, IBB University, Lapai, Niger State, Nigeria.
[2]Department of Food Science and Technology, University of Agriculture, Makurdi, Benue State, Nigeria.

*Correspondence: email address: timamakadir@gmail.com; Phone number: +23480631281844

ABSTRACT

The effect of sodium benzoate on the quality of "*Wara*" from soymilk was investigated. Soymilk samples (1 litre) with varying quantities of sodium benzoate (0 % 0.6 %, 0.8 %, 1.0 % and 1.2 %) were coagulated with Sodom apple (*Calotropis procera*) leave extract as enzyme source to produce "*Wara*" products which were subjected to standard analytical methods for residual sodium benzoate. The "*Wara*" samples were further subjected to ambient (28-35 ^{0}C) and refrigerated temperature (4±1 ^{0}C) storage conditions during which microbial load as well as sensory attributes were evaluated at 3 days' intervals. Results showed that residual sodium benzoate in the "*Wara*" samples ranged from 0.02-0.06 % in the treated samples which were within stipulated levels by regulatory bodies. Refrigerated Samples with 0.06 % sodium benzoate had lower values of 5.5×10^4 CFU/g for viable count and 3.3×10^3 CFU/g for fungal count at the end of the storage period. However, at both temperatures, higher concentrations of sodium benzoate had lower counts. Samples stored at ambient temperature were still acceptable at day 6 while refrigerated samples were acceptable at 15 days. Higher concentration of sodium benzoate produced "*Wara*" with longer shelf life.

Keywords: Soybean, Cheese, *Wara,* and sodium-benzoate.

Introduction

Cheese can be defined as a product made from the curd obtained from milk by coagulating the casein with the help of rennet or similar enzymes (Augustine *et al.,* 2014**).** It is a popular food product and its making started out as an accidental curdling of milk. In Nigeria, milk production is mainly produced by the Fulani nomadic people who are majorly pastoralists. Due to lack of refrigeration facilities, the Fulani women processed the surplus fresh milk into soft unripen cheese called '*Warankasi*' or '*Wara'* in short term (Adetunji and Babalobil, 2011).

However, due to scarcity of cow milk with attendant high cost, alternative soymilk has been used in production of *Wara*. In recent times, the consumption of soymilk and soy curd has increased, perhaps due to the perceived health problems associated with high consumption of animal products (Fasoyiro, 2011). Soy cheese production has become a great business for quite a number of women in developing countries (Chikpah *et al.,* 2015). The traditional processing method for making *soy Wara* does not take into consideration the quality control measures. Unhygienic conditions

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of processing of cheese make the risk of microbial contamination very high. These contribute not only to the short shelf life of the products but also more importantly its potential health hazards (Ashaye *et al.,* 2006). Traditional *Wara* is preserved in its whey which last barely a day or two. However, being boiled in water to make it tough could increase the keeping qualities which can last 3-4 days in chilled conditions (Zamal *et al.,* 2013). There are several different methods for soymilk processing. The traditional method involves soaking the beans overnight, followed by wet grinding (cold water grinding), filtering and cooking (Onuorah *et al.,* 2007). The traditional process results in a typical oriental soymilk with an intense characteristic of "beany" flavour, which is not generally accepted by some consumers. Such off-flavour characteristic results from the reaction catalysed by soybean enzyme lypoxigenase that acts almost immediately upon wet grinding (Amauri *et al.,* 2003). The nutritional composition of cheese depends on the type of milk used and the manufacturing and ripening procedures. Therefore, cheese contains relatively small amounts of the water soluble constituents (whey proteins, lactose, and water-soluble vitamins), which partition mainly into the whey (Nazim *et al.,* 2013). It is a valuable product that is meeting the nutritional requirement for improved intake of quality protein in both children and adult (Fasoyiro, 2011). Thus, chemical preservative has been employed to extend the shelf life of cheese in many ways. The shelf life of *Wagashie* was extended up to 15 days using different concentrations of NaCl (Appiah, 2000). Zamal *et al*. (2013) used different concentrations of potassium-meta-bisulphate (KMS), sodium benzoate, potassium sorbate and the mixture of KMS, sodium-benzoate and potassium sorbate to extend the shelf life of tofu for 2 days at room temperature and 14 days at refrigeration temperature. However, these preservatives were prepared as solution and added directly to the curd when it had been formed. This can result to uneven distribution of preservative in the curd. This research therefore, aimed to determine the residual sodium benzoate in the *Wara* products.

METHODOLOGY

Soybeans seed (*Glycine max*), was purchased from Kure market minna, Nigeria. The research was conducted at Federal University of Technology Minna and coagulant Sodom apple (*Calotropis procera*) was freshly harvested from Federal University of Technology Minna school premises. The preservative (sodium benzoate) used is of analytical grade.

Preparation of coagulant

The leaf extract of the sodom apple *(Calotropis procera)* was prepared by the method described by Olorunnisomo and Ikpinyang (2012). The extract was obtained by finely grinding 120g leaves of Sodom apple (*Calotropis procera)* using a laboratory mortar and pestle, then transferred into 500ml of the distilled water and was allowed to soak for 10 minutes after which the mixture was sieved to obtain the leaves extract.

Preparation of sodium benzoate solution

The sodium benzoate solution was prepared by separately weighing 0.6g, 0.8g, 1.0g and 1.2g of sodium benzoate with a weighing balance. This was then added into each 100ml of distilled water in a beaker. The solution was mixed thoroughly to have a homogenous solution.

Soy-milk preparation

The soy milk was prepared according to the method described by Ikuomola *et al.* (2013).

Raw soybeans (500 g) were handpicked to remove stones and dirt and then soaked in 2 litres of water for 24 hours at room temperature (32±2 °C). The soaked soybeans were drained and wet-milled with the aid of a blender (Kenwood BL440, 500 W) at high speed, sieved, using muslin cloth to separate chaff from the soymilk. The ratio of soybean to water used during wet-milling was one ratio eight (1:8). One litre each of the soy milk was then put in clean pot for further processing.

Preparation of *Wara* samples

The *Wara* sample was prepared according modification proposed Ikuomola *et al.* (2013). Soymilk (1 litre) each in the separate pots were stirred thoroughly and heated at 70 ^{0}C for 30 minutes. Different concentrations of sodium benzoate (0.6 %, 0.8 %, 1.0 % and 1.2 %) were separately added into each of the soymilk in the pot with the one without preservative as the control. The soymilk in each pot was stirred to have an even distribution of the preservative. Coagulant (100ml) each was then added with constant stirring and heating to prevent burning. Coagulation started within 12-30 minutes. The curd was heated for 20 minutes at same temperature (70 ^{0}C) to inactivate the enzyme and facilitates whey removal**.** The coagulated soymilk was sieved using a cheese cloth and the chaff separated from the milk pressed to drain out the whey. The coagulated curd was then cut into different shapes with a knife and fried in hot vegetable oil for about 5 minutes, cooled and separately packed into transparent polythene plastic (LDPE), sealed and then stored separately in a refrigerator (4±1 ^{0}C) and room temperature respectively prior to analysis.

Experimental design

Table 1: Experimental design for *wara* samples

Samples	Sodium benzoate(g/l)
A	0
B	0.6
C	0.8
D	1.0
E	1.2

Key
Sample A= control (no preservative)
Sample C= 0.8% sodium benzoate
Sample B = 0.6% sodium benzoate
Sample D = 1.0% sodium benzoate
Sample E = 1.2% sodium benzoate

Determination of Residual Sodium Benzoate in *Wara* Samples

This was carried out using the titrimeric method described by AOAC (2000). Ten (10g) of the sample was weighed and mashed in 20 ml of distilled water using motar and pistle then filtered. The filtrate was transferred into a conical flask and added 1ml of 10% NaOH solution and 12g NaCl. Distilled water was added to bring the volume up to 50 ml and allowed to stand for 30 minutes with frequent shaking. Two drops of phenolphthalein indicator were added to change the color and 3 drops of HCl added until the color change disappeared with the addition of excess 3ml of HCl. The mixture was transferred into a separatory funnel and 25ml of chloroform added and allowed to stand for 30 minutes with frequent shaking. Chloroform layer (12.5 ml) (low layer) was transferred into a conical flask and the chloroform was evaporated off on a water bath. Fifty (50ml) of 50 % ethanol solution was added and titrated with 0.05 M NaOH using phenolphthalein as indicator. Sodium benzoate retained in the samples was calculated using the formula below:

$$1ml\ of\ 0.05M\ NaOH = 0.0072\ sodium\ benzoate$$

$$Titre\ ml\ of\ NaOH = x$$

$$weight\ of\ sodium\ benzoate\ (x) = ml \times 0.0072$$

$$\%\ of\ sodium\ benzoate = \frac{weight\ of\ sodium\ benzoate}{weight\ of\ sample} \times 100$$

Total plate count of *Wara* samples

This was determined by pour plate method using serial dilution technique described by Oladipo and Jadesimi, (2012). Each of the cheese samples (25 g) was aseptically weighed using a weighing balance, crushed with 225 ml of diluents in a blender in order to have a homogeneous suspension. One milliliter (1 ml) was taken from each sample and introduced into sterile plates containing nutrient agar. The plates were incubated at 37 ^{0}C for 24 hours. The plate was examined and colonies present were counted and recorded to get the total colony count in CFU/g using the following formula. The total plate counts were compared with the international microbial legislation for soft cheese not exceeding 10^2-10^3 CFU/g and free from all pathogenic microorganisms (Salwa and Galal, 2002).

$$Total\ population = \frac{N}{V \times B}$$

Where N= Number of colonies
V= Volume of samples
B= Dilution factor

Similarly, for fungi, Saborauds dextrose agar (SDA) treated with tartaric acid was used to plate the dilutions and same procedure for serial dilution was used. The plates were incubated at room temperature in an inoculating hood for 72 hours. The mean of colony forming unit per gram were calculated and recorded.

The total viable bacterial and fungal counts were determined at room temperature (28-35 ^{0}C) for 9 days and refrigerated temperature (4±1 ^{0}C) for 27 days. This was determined using serial dilution technique and total viable count calculated using the formula above. This analysis was carried out at 3 days' interval for both samples.

Sensory Evaluation of *Wara* samples

Sensory evaluation of *Wara* samples were determined using the method described by Iwe, (2002). The organoleptic properties of *Wara* samples were examined by a panel of twenty (20) judges (final year undergraduate students). The panelists were asked to rate the samples for taste, appearance, texture, flavor and overall acceptability using a 9 point Hedonic scale where 1 to 9 represent dislike extremely (1) to like extremely (9) with 9-like extremely, 8-like very much, 7-like moderately, 6-like slightly, 5-neither like nor dislike, 4-dislike slightly, 3-dislike moderately, 2-dislike very much, 1-dislike extremely

Statistical Analysis

The data collected was subjected to Analysis of variance (ANOVA) and least significant difference at a level of ($p \leq 0.05$) was used to establish if significant differences exist among the sample using statistical package for social science (SPSS) version 20.0

RESULTS

Table 2: Residual Sodium Benzoate Retained in *Wara* Samples

Samples	Sodium benzoate(g/l)	Residual concentration (%)
A	0	ND
B	0.6	0.02 ± 0.00^{c}
C	0.8	0.04 ± 0.00^{b}
D	1.0	0.05 ± 0.01^{ab}
E	1.2	0.06 ± 0.01^{a}
LSD		0.01

Values are means ± standard deviation of duplicate determinants. Means followed by different superscript in the same column are significantly different (p≤0.05). ND = Not Detected

Key

Sample A= control (no preservative)

Sample B = 0.6% sodium benzoate

Sample C= 0.8% sodium benzoate

Sample D = 1.0% sodium benzoate

Sample E = 1.2% sodium benzoate

Table 3: Effect of Different Concentrations of Residual Sodium Benzoate on Total Viable Count of *Wara* Samples (CFU/g) During Storage at Ambient Temperature

Samples (%Residual benzoate)	Days of storage			
	0	3	6	9
A (0)	2.0×10^{3}	3.9×10^{4}	7.4×10^{4}	1.37×10^{5}
B (0.02)	NG	4.3×10^{4}	6.2×10^{4}	1.15×10^{5}
C (0.04)	NG	2.5×10^{4}	5.5×10^{4}	8.8×10^{4}
D (0.05)	NG	1.7×10^{4}	4.5×10^{4}	7.3×10^{4}
E (0.06)	NG	1.0×10^{4}	3.7×10^{4}	5.5×10^{4}

NG (No Growth)

Table 4: Effect of Different Concentrations of Residual Sodium Benzoate on Total Viable Count of *Wara* Samples (CFU/g) During Storage at Refrigeration Temperature

Samples (% Residual benzoate)	Storage Days									
	0	3	6	9	12	15	18	21	24	27
A (0)	NG	NG	NG	7.0×10^{3}	1.8×10^{4}	3.1×10^{4}	4.5×10^{4}	5.8×10^{4}	7.9×10^{4}	8.4×10^{4}
(002)	NG	NG	NG	1.0×10^{3}	1.2×10^{4}	2.8×10^{4}	3.7×10^{4}	5.3×10^{4}	6.3×10^{4}	7.8×10^{4}
C (0.04)	NG	NG	NG	NG	7.0×10^{3}	2.2×10^{4}	3.3×10^{4}	4.6×10^{4}	5.9×10^{4}	6.1×10^{4}
D (0.05)	NG	NG	NG	NG	4.0×10^{3}	1.3×10^{4}	2.6×10^{4}	3.9×10^{4}	4.7×10^{4}	5.6×10^{4}
E (0.06)	NG	NG	NG	NG	1.0×10^{3}	9.0×10^{3}	2.1×10^{4}	3.3×10^{4}	4.2×10^{4}	5.1×10^{4}

NG= No Growth

Table 5: Effect of Different Concentrations of Residual Sodium Benzoate on Total Fungal count of *Wara* Samples (CFU/g) During Storage at Ambient Temperature

Samples (% Residual benzoate)	Storage Days			
	0	3	6	9
A (0)	NG	9.0×10^{2}	2.7×10^{3}	5.7×10^{3}
B (0.02)	NG	7.0×10^{2}	1.9×10^{3}	4.4×10^{3}
C (0.04)	NG	1.0×10^{2}	1.7×10^{3}	3.8×10^{3}
D (0.05)	NG	NG	1.3×10^{3}	3.3×10^{3}
E (0.06)	NG	NG	1.0×10^{3}	3.0×10^{3}

NG= No Growth

Table 6: Effect of Different Concentrations of Residual Sodium Benzoate on Fungal Count of *Wara* Samples (CFU/g) During Storage at Refrigeration Temperature

Sample(% Residual benzoate)	Storage Days									
	0	3	6	9	12	15	18	21	24	27
A (0)	NG	NG	NG	5.0×10^{2}	2.8×10^{3}	3.5×10^{3}	4.8×10^{3}	5.1×10^{3}	6.3×10^{3}	7.5×10^{3}
B (0.02)	NG	NG	NG	1.0×10^{2}	1.2×10^{3}	2.5×10^{3}	3.3×10^{3}	$4.2.\times10^{3}$	5.4×10^{3}	6.9×10^{3}
C (0.04)	NG	NG	NG	NG	NG	1.3×10^{3}	2.3×10^{3}	3.2×10^{3}	4.6×10^{3}	5.1×10^{3}
D (0.05)	NG	NG	NG	NG	NG	6.0×10^{2}	1.9×10^{3}	2.6×10^{3}	3.9×10^{3}	4.0×10^{3}
E (0.06)	NG	NG	NG	NG	NG	2.0×10^{2}	1.3×10^{3}	2.2×10^{3}	3.0×10^{3}	3.3×10^{3}

NG= No Growth

Table 7: Effect of Different Concentrations of Residual Sodium Benzoate on Sensory Properties of *Wara* Samples during Storage at Ambient Temperature

Samples (% Residual benzoate)	Storage period (days)	Appearance	Texture	Aroma	Taste	Overall Acceptability
A (0)	0	4.80	6.40	6.20	5.60	6.70
	3	4.80	4.70	5.20	4.60	4.20
	6	1.20	1.40	1.20	1.00	1.20
B (0.02)	0	6.20	6.20	5.80	5.40	6.60
	3	5.70	5.50	5.60	4.00	4.30
	6	3.40	3.60	3.40	3.00	3.20
C (0.04)	0	7.30	6.80	7.30	6.30	7.80
	3	7.00	6.10	5.60	5.00	3.80
	6	5.80	5.30	3.60	3.40	3.90
D (0.05)	0	7.60	6.80	7.40	7.70	8.00
	3	6.70	6.30	5.90	5.60	4.60
	6	6.30	4.70	4.30	4.30	3.60
E (0.06)	0	8.30	7.70	7.80	8.20	8.60
	3	7.40	7.40	6.50	7.50	6.40
	6	5.90	5.80	4.50	4.80	4.50

Table 8: Effect Different Concentrations of Residual Sodium Benzoate on Sensory Properties of *Wara* Samples during Storage at Refrigerated Temperature

Samples (% Residual benzoate)	Storage period (days)	Appearance	Texture	Aroma	Taste	Overall acceptability
A (0)	3	5.70	5.80	5.80	6.00	6.60
	6	6.60	6.20	6.00	4.90	6.60
	9	6.50	5.80	6.00	5.40	6.00
	12	4.70	3.40	3.20	3.40	3.80
	15	4.00	2.70	2.00	4.90	6.70
	18	2.00	1.30	1.50	1.70	2.20
B (0.02)	3	6.30	7.30	6.80	7.10	7.30
	6	7.50	7.70	7.50	7.60	7.50
	9	7.50	7.40	7.30	7.90	7.70
	12	5.80	5.60	5.60	6.20	5.60
	15	5.70	4.70	5.70	4.70	4.30
	18	2.70	3.50	3.50	3.10	3.40
C (0.04)	3	6.60	7.50	7.00	7.60	7.50
	6	7.60	7.50	7.30	7.90	7.80
	9	7.60	7.80	7.90	7.80	8.00
	12	7.40	6.40	6.40	7.20	7.40
	15	5.50	6.10	6.00	5.70	7.00
	18	3.70	4.90	5.20	4.80	5.20
D (0.05)	3	6.60	7.30	7.20	7.60	8.00
	6	7.30	7.50	7.60	7.90	7.90
	9	7.50	7.70	7.60	7.90	7.90
	12	6.60	6.80	7.40	6.80	7.20
	15	6.70	6.70	5.60	6.30	6.70
	18	5.60	5.40	5.60	5.20	6.30
E (0.06)	3	6.70	7.60	7.40	7.90	8.00
	6	7.30	7.80	7.40	7.90	8.00
	9	7.60	7.80	7.70	7.90	8.00
	12	6.80	6.60	6.00	6.90	6.80
	15	5.70	5.10	4.70	6.70	6.70

DISCUSSION

Residual Sodium benzoate in *Wara* Samples

Table 2 shows the residual sodium benzoate in *Wara* samples. The samples had residual concentration of sodium benzoate lower than the stipulated levels by regulatory bodies. The concentrations of the residual sodium benzoate

differ significantly and increased with increased quantity of the sodium benzoate. The higher level (0.05 % and 0.06 %) retained in the samples D and E may be due to higher quantity of the sodium benzoate added. This may also be as a result of solubility of the preservative (sodium benzoate) in the whey as they are highly soluble in water. During salting process, moisture is expelled from the cheese and a percentage of salt is lost to the whey (Grummer *et al.*, 2012).

Effect of different concentrations of sodium benzoate on total viable count of *Wara* samples during storage at ambient and refrigeration temperature

The international microbial legislation for soft cheese should not exceed 10^2-10^3 CFU/g and should be free from all pathogenic microorganisms (Salwa and Galal, 2002). Samples had low levels of residual sodium benzoate has stipulated by international microbial legislation for soft cheese not exceeding 0.1 %. There was higher load of microbial counts in all samples at the end of storage period at both temperatures as shown in table 3 and 4. This may be due to the fact that *Wara* sample produced were high in protein. Rekha and Vijayalakshmi (2010) reported that *Tofu* is rich in protein making it susceptible to microbial growth. The low density polythene leather used for the packaging may have also been another source of microorganism as they were not further treated. There was no growth in all ambient stored treated samples on the first day of production and microbial count decrease as the concentrations of residual sodium benzoate increased in samples. This may be as a result of sodium benzoate concentrations retained by the samples. Sodium benzoate may have created hurdles (stress organism) which the organisms could not overcome their growth became noticeable when the environment became favorable (Nwafor and Ikenebomeh, 2009).

Effect of different concentrations of sodium benzoate on total fungal count of *Wara* samples during storage at ambient and refrigerated temperature

The fungi count during storage were relatively low when compare to bacteria. This showed that sodium benzoate inhibits fungi than bacteria. There was increase in fungi count in all samples. They were within safe limit stipulated by the regulatory bodies throughout the storage period at ambient temperature as shown in table 5. However, there was decrease in fungi counts as concentration of sodium benzoate increased in samples. The high number recorded in the control samples may be due to low pH and surface moisture as it is favorable for their growth (Badriah *et al.,* 2013). The lower counts observed in 0.06 % may be as a result of hurdles created by sodium benzoate their by inhibiting their growth.

Similar trend of increased in fungi counts at ambient temperature was observed during refrigeration storage as shown table 5. However, refrigerated samples had lower count compared to ambient samples as a result of low temperature of storage.

Effect of different concentrations of sodium benzoate on sensory properties of *Wara* samples during storage at ambient and refrigeration temperatures

The different concentrations of preservative (sodium benzoate) and storage temperatures (ambient and refrigeration) affected the quality changes of the products. There was a general decreased in sensory parameters during storage at both temperatures as shown in table 7 and 8.

Sensory parameters decreased as these storage indices increased at both storage temperatures. The *Wara* samples were accepted up to 6 days for ambient temperature and 15 days for refrigerated samples

The higher scores recorded for the 0.05 % and 0.06 % concentrations at the end of the storage period may be due to the higher concentrations of sodium benzoate added which may have inhibited microbial growth thereby slowing the rate of spoilage. Salt is added to cheese for several purposes such as controlling microbial growth and enzyme activities, modifying flavor, texture and other physical properties (Guinee, 2004; Johnson *et al.,* 2009).

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Radiological Implications of Artisanal Gold Mining Activities in Gababiyu, Minna Metropolis, Nigeria

[*1,2]Gomina M., [1]Kolo M.T. & [1]Awojoyogbe O.B.

[1]Department of Physics, Federal University of Technology Minna, Nigeria.
[2]Department of Physics, Ibrahim Badamasi Babangida University Lapai, Nigeria.

*Correspondence: Email address: gomina@ibbu.edu.ng; Tel: +234(0)8066801952

ABSTRACT

Radiological contamination of the atmosphere and human environment due to anthropogenic activities has been associated with significant human health challenges. 12 surface soil samples collected at random from Gababiyu artisanal gold mining site in Minna were assessed for their radiological contents using gamma spectrometric technique which employs NaI (Tl) detector. Specific activities of ^{226}Ra, ^{232}Th and ^{40}K ranged from 10.27±2.88 to 61.45±4.68 Bq kg^{-1}, 32.67±1.93 to 83.00±2.24 Bq kg^{-1} and 82.48±3.00 to 281.65±9.49 Bq kg^{-1} respectively, with mean values of 31.92±6.41, 63.57±2.67 and 198.91±1.38 Bq kg^{-1} in sequence. Computed average absorbed dose rate at 1 m above ground was 61.44 nGy h^{-1} with corresponding mean annual dose equivalent of 0.07 mSv y^{-1} and average excess lifetime cancer risk of 0.26 × 10^{-3}. These values were all below respective safety limits set by UNSCEAR. Results of this investigation indicated minimal radiological risk associated with mining in the artisanal goldmine in Minna Metropolis.

Keywords: Artisanal gold mining**,** NaI(Tl) detector**,** dose, Gababiyu**,** Nigeria.

INTRODUCTION

The world inhabited by man is naturally radioactive. Naturally occurring radionuclides are present everywhere and all living and non-living things are exposed to radiations (Odumo *et al.*, 2009). Naturally occurring radionuclides are nuclides that undergo spontaneous radioactive decay and emit mass-energy. The decay process occurs spontaneously and repeatedly until a stable nuclide is formed (Long *et al.*, 2012). Such radionuclides are generally termed "Naturally Occurring Radioactive Materials (NORM)". NORM refers to the primordial radionuclides of potassium (^{40}K), uranium (^{238}U and ^{235}U and their daughters), and thorium (^{232}Th and its daughters) synthesised during the creation of the solar system (IAEA, 2003). These primordial radionuclides are still in existence today due to their long half-lives in comparison with the age of the earth. They also emit gamma rays of sufficient intensity for gamma-ray mapping (IAEA, 2003).

Exposure to ionising radiation emitted by individual radionuclides of NORM pose radiological risk to humans and the environment (Alharbi, 2016). Radiation

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exposure can induce a range of effects in humans. The nature and probability of the effect depends on the radiation dose received by an individual. The effect may be somatic, occurring only in the exposed individual or genetic, occurring in the descendants of the exposed individual (NYSDH, 2007). Through proper regulations, such exposure can be controlled to ensure adequate protection to the environment and occupationally exposed people.

Elevated level of NORM in the environment may result from the activities and industrial processes related to the extraction and processing of ores (UNEP, 2016), such as uranium mining and milling, metal mining and smelting, phosphate production, coal mining and power generation from coal burning, oil and gas drilling, rare earth and titanium oxide industries, zirconium and ceramic industries and applications using isotopes of Radium and Thorium (UNEP, 2016).

Artisanal (small-scale) mining is an activity that encompasses small, medium, informal, legal and illegal miners who use rudimentary methods and processes to extract mineral resources (Sabo *et al.,* 2018). Environmental and health issues are risks associated with artisanal gold-mining, mining processes incite depletion of the environment such as land degradation, de-vegetation, air and water pollutions and loss of aquatic organisms (Ako *et al.*, 2014). Toxic matter discharged into the environment poses health risks to miners, their families and surrounding communities (Azubike, 2011; Ako *et al.*, 2014).

Associated with mining and mineral processing are potential adverse health risks that are more significant to occupationally exposed individuals. However, certain exposures and their associated risks may disperse via environmental pathways to the general population (Candeias *et al.*, 2018). According to the World Nuclear Association (WNA), radiation protection standards assumes that for any radiation dose, no matter how small, involves a possible risk to human health (WNA, 2014; Njinga *et al.*, 2015). Therefore, there is need to investigate the activity concentration of NORM in the artisanal goldmine in Gababiyu area of Minna Metropolis. This will yield radiological parameters to ascertain the radiological risk associated with small-scale mining in the region.

The study area (Gababiyu artisanal goldmine) is located in Minna Metropolis (Figure 1), off the City's Eastern bye pass, after the M.I Wushishi Estate. The area is also the site proposed for the El-Amin University. The area is part of Minna Sheet 164 and falls within the Basement Complex Terrain of Nigeria (Ahmed *et al.,* 2019).

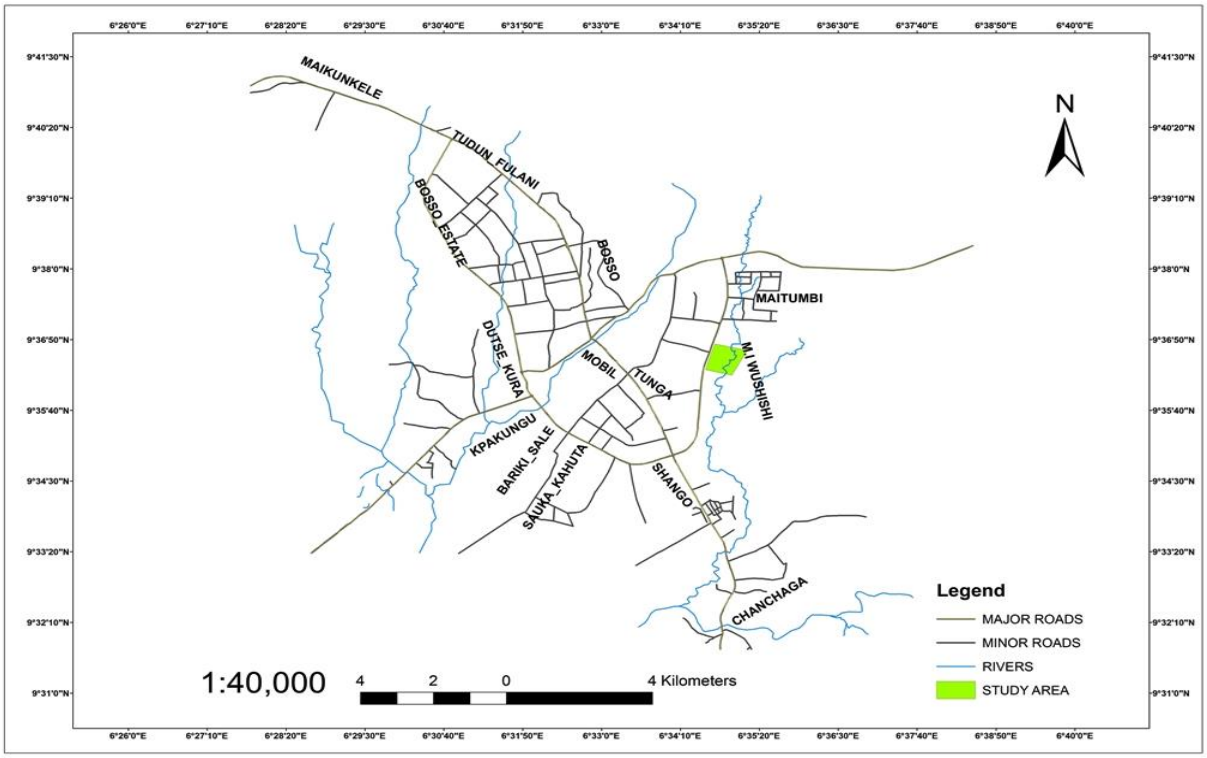

Fig. 1**:** Location map of the study area in Minna Metropolis (ArcGIS 10.1, 2012)

METHODOLOGY

Sample Collection and Preparation

Twelve (12) representative soil samples were randomly collected around the artisanal gold mine (Figure 1). All samples collected were cleared of any stones, pebbles and shrubs, and about 1 kg of each sample was packed neatly into well labelled polyethylene bags and conveyed to the laboratory for preparation.

Each of the soil samples were openly dried at room temperature in the laboratory and crushed using agate mortar and pestle. The crushed samples were sieved using a BSS 63 sieve to obtain homogeneous fine grain. Radon-impermeable cylindrical plastic containers selected based on the volume of the detector vessel (7.6 cm by 7.6 cm geometry) were used to package 300 g of the sieved sample. Ensuring the containment was air-tight to prevent radon-222 (^{222}Rn) from escaping, the packaging in each case was triple sealed. The sealing process was done by smearing of the inner rim of each container lid with Vaseline jelly, filling the lid assembly gap with candle wax to block the gaps between lid and container, and tight-sealing lid-container with masking adhesive tape.

Radon and its short-lived progenies were allowed to reach secular radioactive equilibrium by storing the samples for 30 days prior to gamma spectroscopy measurements (Veiga *et al.*, 2006; Ademola *et al.*, 2014).

Gamma Spectrometric Analysis

To determine the activity concentration of NORM in the soil samples, gamma spectrometric analysis was carried out using a 7.6 cm by 7.6 cm NaI (Tl) detector which has energy resolution of 72% at 661.16 keV peak of

^{137}Cs. The crystal is optically coupled to a photomultiplier tube (PMT). The assembly has a preamplifier incorporated into it and a 1 kV external source. The detector is enclosed in a 6 cm lead shield with cadmium and copper sheets to minimize the effects of background and scattered radiation. The data acquisition software is Maestro by Camberra Nuclear Products. Each of the samples was measured for a period of 29000 seconds. The activity concentrations were computed using the equation (Njinga *et al.*, 2015):

$$\mathrm{A\ (Bq.kg^{-1})} = \frac{\mathrm{C_n}}{\mathrm{I_{(\gamma)}\varepsilon MT}} \qquad (1)$$

where: A = activity concentration of a particular radionuclide in the sample, C_n = net count rate, $I_{(\gamma)}$ = emission probability of a specific energy photo peak, T = time for collecting the spectrum of the sample and M = mass of the sample.

Calibration and Efficiency Determinations

Calibration of the detector for energy and efficiency was done with two calibration point sources, ^{137}Cs and ^{60}Co before analysis. The standards used to check for the calibration are the IAEA gamma spectrometric reference materials RGU-1 for ^{226}Ra (^{214}Bi peak), RGTh-1 for ^{232}Th (^{208}Ti peak) and RGK-1 for ^{40}K. The energy peaks for the region of interest (ROI) used to compute the activity concentrations of ^{226}Ra ^{232}Th and ^{40}K are given in Table 1.

Table 1**:** Spectral energy windows used in the analysis

Isotope	Gamma Energy (Kev)	Energy Window (Kev)
^{226}Ra	1764.0	1620-1820
^{232}Th	2614.5	2480-2820
^{40}K	1460.0	1380-1550

Radiation hazard indices

The following hazard indices were computed from the measured activities of ^{226}Ra ^{232}Th and ^{40}K in the studied soil samples:

Radium Equivalent Activity (Ra_{eq})

The Radium Equivalent Activity (Ra_{eq}) was estimated using the equation (Ibrahiem, 1999; Munyaradzi *et al.*, 2018):

$$\mathrm{Ra_{eq}} = \left(\frac{\mathrm{Ra_A}}{370} + \frac{\mathrm{Th_A}}{259} + \frac{\mathrm{K_A}}{4810}\right) 370\ \mathrm{Bq/Kg} \qquad (2)$$

where: Ra_A, Th_A and K_A are respectively the specific activity concentrations of ^{226}Ra, ^{232}Th and ^{40}k in the soil samples.

United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) stipulates a threshold of 370 Bq/kg for (Ra_{eq}) (UNSCEAR, 2000; Suleiman *et al.*, 2018). This threshold activity corresponds to gamma radiation dose of 1.5 mGy/y.

Gamma Radiation Dose (D)

The gamma radiation dose (D) 1 m above ground was calculated by applying conversion factors of 0.462, 0.604 and 0.0417 for ^{226}Ra, ^{232}Th and ^{40}K respectively to convert their activity concentrations into dose rates using the equation (UNSCEAR, 2000; Munyaradzi *et al.*, 2018):

$$\mathrm{D} = 0.462\mathrm{Ra_A} + 0.604\ \mathrm{Th_A} + 0.0417\mathrm{K_A} \qquad (3)$$

where: D is the gamma radiation dose in nGy/h and Ra_A, Th_A and K_A are the activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K in sequence.

Annual Effective Dose Equivalent (AEDE)

Annual Effective Dose Equivalent (AEDE) in mSv/y is estimated as products of the gamma radiation dose, D (nGy/h), time in a year (8760 hours), dose conversion factor of 0.7 Sv/Gy and

occupancy factor of 20 % (0.2) for outdoor exposure (accounting for the duration the miners spend on the field in a year), using the equation (UNSCEAR, 2000; Taskin *et al.*, 2009):

$$\mathrm{AEDE} = \mathrm{D} \times 8760 \times 0.7 \times 0.2 \times 10^{-6} \quad (4)$$

External and Internal Hazard Indices

The external hazard index (H_{ex}) and internal hazard index (H_{in}) were estimated using the equations (Berekta and Mathew, 1985; Ademola *et al.*, 2014):

$$\mathrm{H_{ex}} = \frac{\mathrm{Ra_A}}{370} + \frac{\mathrm{Th_A}}{259} + \frac{\mathrm{K_A}}{4810} \leq 1 \quad (5)$$

$$\mathrm{H_{in}} = \frac{\mathrm{Ra_A}}{185} + \frac{\mathrm{Th_A}}{259} + \frac{\mathrm{K_A}}{4810} \leq 1 \quad (6)$$

For radiological safety, UNSCEAR stipulates a threshold of unity for both H_{ex} and $H_{in.}$

Excess lifetime Cancer Risk (ELCR)

Excess lifetime Cancer Risk (ELCR) was estimated using the equation (Taskin *et al.*, 2009; Munyaradzi, Anna and Makondelele, 2018):

$$\mathrm{ELCR} = \mathrm{AEDE} \times \mathrm{DL} \times \mathrm{RF} \quad (7)$$

where AEDE is the annual effective Dose Equivalent computed from Eq. (4), DL = the average duration of human life (estimated to be 70 years) and RF = the risk factor (Sv^{-1}). For stochastic effects, which produce low background radiation, the ICRP 60 stipulates a value of 0.05 Sv^{-1} for RF and a threshold of 0.29 × 10^{-3} for ELCR for the public exposure (Taskin *et al.*, 2009; Munyaradzi *et al.*, 2018).

RESULTS

The activity concentrations of naturally occurring radionuclides (^{226}Ra ^{232}Th and ^{40}K) in soil samples collected from Gababiyu artisanal goldmine are presented in Table 2. The computed radiation hazard indices are presented in columns 5 to 10 of Table 2. Distribution of ^{226}Ra ^{232}Th and ^{40}K across the soil samples are displayed on bar chart in Figure 2.

The specific activity concentration of ^{226}Ra varies from 10.27±2.88 to 61.45±4.68 Bq/kg, ^{232}Th specific activity ranges from 32.67±1.93 to 83.00±2.24 Bq/kg, while the activity concentration of ^{40}K varies from 82.48±3.00 to 281.65±9.49 Bq/kg. Radium equivalent activity (Ra_{eq}) ranges from 96.74 to 176.25 Bq/kg. Gamma absorbed dose 1 m from the ground (D) varies from 44.02 to 78.01 nGy/h. Annual Effective Dose Equivalent (AEDE) varies from 0.05 to 0.09 mSv/y with a mean of 0.07 mSv/y. The external hazard index (H_{ex}) varies from 0.26 to 0.48 while the internal hazard index (H_{in}) varies from 0.32 to 0.61. Excess Lifetime Cancer Risk (ELCR) estimated varies from 1.90×10^{-4} to 3.3×10^{-4} with a mean of 2.6×10^{-4}.

Table 2: Activity concentrations and radiation hazard indices of soil samples collected from Gababiyu artisanal goldmine

Sample	Activity concentrations (Bq/kg)				Radiological hazard parameters				
	^{226}Ra	^{232}Th	^{40}K	Ra_{eq}	D (nGy/h)	AEDE (mSv/y)	H_{ex} ≤ 1	H_{in} ≤ 1	ELCR ($\times 10^{-3}$)
GM01	10.27±2.88	60.12±0.31	183.84±8.56	110.39	48.72	0.06	0.30	0.33	0.21
GM02	19.18±2.80	48.21±0.94	144.85±5.47	99.27	44.02	0.05	0.27	0.32	0.19
GM03	25.05±0.92	63.62±0.20	233.34±8.85	133.99	59.73	0.07	0.36	0.43	0.25
GM04	28.33±1.08	32.67±1.93	281.65±9.49	96.74	44.57	0.05	0.26	0.34	0.19
GM05	34.52±2.00	68.10±0.98	168.77±6.76	144.90	64.12	0.08	0.39	0.48	0.27
GM06	17.50±2.24	78.76±1.53	277.74±7.94	151.51	67.24	0.08	0.41	0.46	0.28
GM07	22.22±1.68	68.34±1.42	135.46±6.06	130.37	57.19	0.07	0.35	0.41	0.24
GM08	33.52±2.60	60.43±2.01	82.48±3.00	126.29	55.43	0.07	0.34	0.43	0.23
GM09	38.48±3.00	66.33±1.34	247.06±6.86	152.36	68.14	0.08	0.41	0.52	0.29
GM10	51.06±3.80	73.41±1.61	232.26±5.68	173.92	77.62	0.09	0.47	0.61	0.33
GM11	61.45±4.68	59.88±2.08	189.57±2.63	161.68	72.47	0.09	0.44	0.60	0.31
GM12	41.40±4.44	83.00±2.24	209.90±5.63	176.25	78.01	0.09	0.48	0.59	0.33
Min.	10.27±2.88	32.67±1.93	82.48±3.00	96.74	44.02	0.05	0.26	0.32	0.19
Max.	61.45±4.68	83.00±2.24	281.65±9.49	176.25	78.01	0.09	0.48	0.61	0.33
Mean	31.92±6.41	63.57±2.67	198.91±1.38	138.14	61.44	0.07	0.37	0.46	0.26

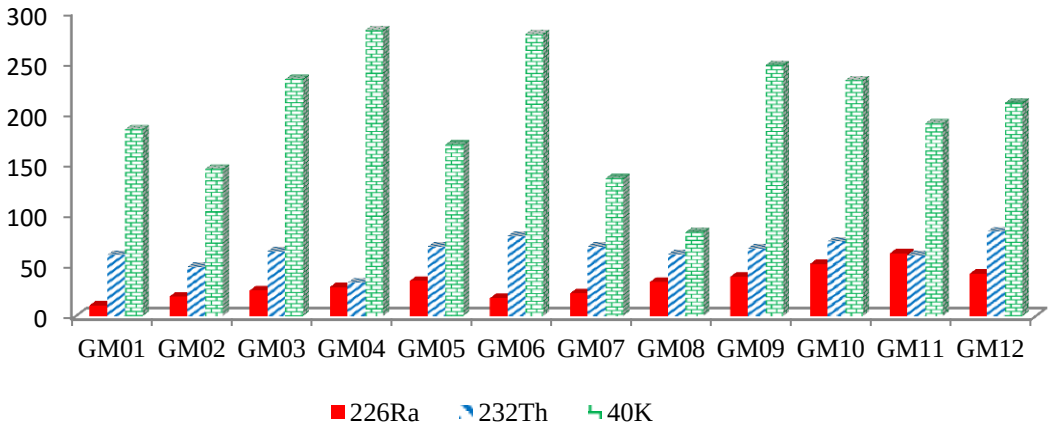

Fig. 2: Distribution of NORM across the soil samples

DISCUSSION

The specific activity concentration of ^{226}Ra, ^{232}Th and ^{40}K in the studied soil samples varied with mean values of 31.92±6.41, 63.57±2.67 and 198.91±1.38 Bq/kg in sequence. Global average values documented by UNSCEAR are 35 Bq/kg for ^{226}Ra, 30 Bq/kg for ^{232}Th and 400 Bq/kg for

^{40}K (Oluwaseyi *et al.,* 2017; UNSCEAR, 2000). Mean values for ^{226}Ra and ^{40}K are below UNSCEAR recorded average while that of ^{232}Th is twice above the recommended average. The distribution of ^{226}Ra, ^{232}Th and ^{40}K shown in figure 2 with unequal height of the bars depicting uneven concentration for the NORM (^{226}Ra, ^{232}Th and ^{40}K) across the region agrees with existing literature. Radium equivalent activity (Ra_{eq}) varies with mean of 138.14 Bq/kg that is below the 370.00 Bq/kg threshold recommended by UNSCEAR (2000).

Gamma absorbed dose 1 m from the ground (D) varies with 61.44 nGy/h mean yielding an Annual Effective Dose Equivalent (AEDE) with 0.07 mSv/y mean, that is far below the 1.00 mSv/y threshold value recommended for occupational exposure by UNSCEAR. Therefore, indicating minimal radiological risk associated with artisanal gold mining in the area.

The external and internal hazard indices (H_{ex} and H_{in}) indicated minimal radiological risk as they varied with mean values of 0.37 and 0.46 respectively. These values are below unity, implying an overall minimal risk to the artisanal gold miners and populace of surrounding communities.

Excess Lifetime Cancer Risk (ELCR) estimated varies with a mean of 2.6×10^{-4} that is slightly below the global recommended threshold of 2.9×10^{-4} (Taskin *et al.*, 2009; Munyaradzi *et al.*, 2018). Hence, indicating minimal cancer risk.

Conclusion

Human activities incite radiological contamination of the environment which results to significant human health challenges. Soil samples from artisanal gold mining environment in Gababiyu were assessed for their radiological contents. Average specific activity values for ^{226}Ra, ^{232}Th and ^{40}K were 31.92±6.41, 63.57±2.67 and 198.91±1.38 Bq/kg in sequence. These values except for ^{232}Th were below the safety limits stipulated by UNSCEAR. However, computed radiological hazard parameters for the studied soil samples were all within the safety limits for occupational exposure. Thus, the radiological impact of artisanal gold mining in the study area bears minimal significance.

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Molecular Study of Multi-Drug Resistance *Pseudomonas Aeruginosa* Among In-Patients In State Owned Hospital, Ilorin, Kwara State

***Bakare A.R. & Anibijuwon I.I**

Department of Microbiology, University of Ilorin, Ilorin, Kwara State, Nigeria.

*Correspondence: Email address: bakareabisoyerahmat@gmail.com Phone number: 07063094628

ABSTRACT

This research is built around the rising global spread of Multi Drug Resistance *Pseudomonas aeruginosa* coupled with the fact that there is lack of proper epidemiological statistics in Nigeria. This study isolated and identified Multi Drug Resistance *Pseudomonas aeruginosa* from patients admitted in Sobi Specialist Hospital (SSH), Cottage Hospital Adewole (CHA) and Civil Service Hospital (CSH) all located within Ilorin while taking into cognizance the incidence as well as corresponding risk factors associated with the occurrence of MDRPA, also tested the susceptibility of the Pseudomonas isolates to commonly used antibiotics as well as Risk factors associated with the increase and continuous use of antibiotics and growing numbers of invasive procedures, together with the development of intrinsic and acquired resistance mechanisms of *P. aeruginosa*. A total of 36 isolates were recovered collectively from the patients. Antibiogram profiling of isolates to standard gram negative antibiotics were determined using the disc diffusion method. 20 of the isolates were multi-drug resistant with notable resistant to nitrofurantin and cefixime. Molecular confirmation of isolates showed DNA size of 550 base pairs. Multi drug resistant *Pseudomonas* remains a major threat to the general public, antibiotic surveillance should be recommended to detect early occurrence of infections.

Keywords: Multi Drug Resistance, *Pseudomonas aeruginosa,* Antibiogram profiling.

INTRODUCTION

Pseudomonas aeruginosa is a leading cause of nosocomial infections ranked second among the gram-negative pathogens reported to the National Nosocomial Infection Surveillance System (Carmeli *et al* 1999, Porras-Gomez *et al* 2012). A species of considerable medical importance, *P. aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes. The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal (Balcht and Smith 1994). Among all gram-negative bacteria, *Pseudomonas aeruginosa* has been considered a chief opportunistic pathogen, which usually infects persons having some fundamental diseases and compromised immune status (Ali *et al* 2015).

Due to the alarming rate on the increase of antimicrobial resistance exhibited by

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Pseudomonas aeruginosa. It is of the most frequent cause of Nosocomial complications especially among surgical patients, this bacterium is popular for the alarming level of multi- drug resistance it exhibit to conventional antibiotics (Anibijuwon *et al* 2017). Multidrug Resistance *P. aeruginosa* is naturally resistant to a significant number of antimicrobials (Ampicillin, Amoxicillin, amoxicillin/clavulanate, first-generation cephalosporins, second-generation, cefotaxime, ceftriaxone, nalidixic acid.

This research was built around the rising global spread of Multi Drug Resistance *Pseudomonas aeruginosa* coupled with the fact that there is lack of proper epidemiological statistics in Nigeria. This research aims to determine the multidrug resistant strains of *Pseudomonas aeruginosa* in the clinical isolates.

METHODOLOGY

The study was conducted within Ilorin, it is located 8.50 Latitude and 4.54 Longitude and it is situated at elevation 320 meters above sea level. Kwara state in Nigeria. The state population is predominantly heterogeneous with people of varied religious and ethnic beliefs. This study was carried out in selected state hospitals within the state. These hospitals had facilities to attend to chronic health issues and they are accessible to the citizens and residents seeking medical consultation.

This study was carried out among all consenting age groups (children, adults and aged) who were hospitalized and also met the inclusion criteria in the selected hospitals that were surveyed. Also, a structured close-ended questionnaire was administered to the consenting subjects following proper clinical examination and diagnosis.

Ethical approval for the research was obtained foremost from the Ethics Review Board of the Faculty of Life Sciences, University of Ilorin. Then approval was also obtained from the Ethics Review Committee of the selected hospitals after which informed consent was obtained from the subjects and/or their caretakers.

Blood samples were obtained from subjects who meet the inclusion criteria by a laboratory scientist, placed in an ice chest and transported to the laboratory for analysis.

A total of 300 clinical samples were collected from the State Government Hospitals, in Kwara State, Nigeria. All of the specimens were enrinched in thiogycholate broth and incubated for 5 to 7 days before subculturing onto Cetrimide agar in order to isolate *P. aeruginosa* strains. The plates were then incubated at 37°C for 24 h.

Cetrimide Agar was used for the identification of *P. aeruginosa.* Sample to be tested was inoculated by spreading on the surface of the plates, Incubated for 24 hours at 37°C. The presence of growth was an indication of positive reaction. *P. aeruginosa*, isolated from blood sample, was identified by standard bacteriological methods which included: colony morphology, Gram staining, pyocyanin pigment production, catalase, oxidase and Triple Sugar Iron (TSI) fermentation tests.

Standardization of organisms: for the antibiogram profiling, the cells of the organisms were adjusted to 0.5 McFarland standard which is an estimated value of 1-2 × 10^8 CFU/ ml or 5 × 10^5 CFU/ ml. The standard was prepared by adding 0.1 ml of 1% Barium chloride ($BaCl_2$) to 19.9 ml of 1% sulfuric acid (H_2SO_4). (CLSI 2012).

The solution was mixed to form a turbid suspension. The resulting mixture was placed in

a foil-covered screw-cap tube. The isolates to be screened were subcultured overnight on Nutrient agar prior the test to get a 24 hour old culture. After incubation at 37 ˚C for 24 hours, inoculums were picked from each culture and inoculated in 9 ml peptone water by carefully adjusting the turbidity to the standard. The turbidity was observed and measured using a spectrophotometer until the turbidity reached ≥ 0.10D at 620 nm (CLSI 2012).

Antibiogram assay: Assay for the antibiogram of the isolates was carried out using the disc diffusion method as described by Bauer *et al.* (1985). The isolates were spread on solidified Mueller Hinton agar and antibiotic discs used for this study had been prepared into kit containing multiple discs, each with small discs impregnated with different types of antibiotics. The antibiotics used (Rapid labs, UK and Oxoid, UK) and their corresponding concentrations are as follows: Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Cefixime (5 µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Augmentin (30µg), Nitrofurantoin (300 µg).

Determination of susceptibility to antibiotics: Solidified Mueller Hinton agar plates were seeded with 100 µl of the standardized organisms and were spread evenly over the total surface area of the agar using a glass spreader. The plates were left on the laboratory bench for 30 minutes to ensure that the organisms are well absorbed on the agar surface. Multiple antibiotics discs containing eight of the aforementioned antibiotics were carefully and firmly placed on the surface of the agar using sterile forceps. The plates were then left for 1 hour to allow the antibiotics to diffuse. Afterwards, the plates were incubated at 37 ˚C for 18 – 20 hours. After incubation, the zones of inhibition generated by the antibiotics were measured on three axis using a ruler; the mean and standard error mean (SEM) of the values were calculated and recorded in millimeter (mm) (CLSI 2016). The values for each organism against the antibiotics were interpreted as sensitive, intermediate or resistant using the breakpoints interpretative criteria of Clinical and Laboratory Standard Institutes (CLSI 2016).

Determination of Multiple Antibiotic Resistance Index (MARI): the MARI was determined for each of the isolates by dividing the number of antibiotics to which the isolates were resistant by the total number of antibiotics used for the screening (Krumperman 1983, Paul *et al* 1997). The value of the index has a standard of ≤ 1.0, thus the higher the MARI, the higher the multiple antibiotic resistances exhibited by the isolate.

Descriptive statistics such as mean, frequency, standard deviation, level of significance, percentages and graphs were used to analyze the results. The data was analyzed using the statistical software SPSS version 22, Sigmaplot and Gradpad. The p-value used to constitute statistical significance was ≤ 0.05. The result of the research was also disseminated to the healthcare facilities for the purpose of clinical examination.

The confirmation of the isolates - *Pseudomonas aeruginosa* was done by polymerase chain reaction (PCR) using specific primers. Representative isolates of the specie was selected from the resistance isolate for the characterization.

RESULTS

Isolation of Pseudomonas aeruginosa

Three hundred samples were collected in total from in-patients of Sobi Specialist Hospital (SSH), Cottage Hospital Adewole (CHA) and Civil Service Hospital (CS). Of these, 12% (n=36)

samples yielded the growth of *Pseudomonas aeruginosa* species and 88% showed no bacterial growth.

Out of the 36 *Pseudomonas aeruginosa* isolates, twenty two (22) were isolated from blood samples of females while fourteen (14) were recovered from male samples. These isolates were recovered from all stipulated age groups with the highest frequency occurring within patients that are between 31- 40 years old (n=12). In Sobi Specialist Hospital (SSH), 22 isolates of *Pseudomonas aeruginosa* was isolated, of which 13 was from female samples and 9 from male counterparts. eight (8) isolates of *Pseudomonas aeruginosa* was isolated from Cottage Hospital Adewole (CHA) with 5 and 3 from female and male blood samples respectively while Six (6)

Antibiotic sensitivity pattern of Pseudomonas aeruginosa isolates

Pseudomonas aeruginosa isolates was found to be multi drug resistance due to its resistance to more than three (3) classes of antibiotics, which amount to 55.6% (n=20). The highest antibiotic resistance rate was observed in Nitrofuratin (NIT) with resistance of about 91% and highest sensitivity were seen in Gentamicin (GNT) 80.5%. None of the isolates showed resistance to all the used antibiotics, as depicted in Table 1

Table 1: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolates.

Antibiotics	Resistant		Intermediate		Sensitive	
	N	%	N	%	N	%
CAZ	31	86.1	2	5.6	3	8.3
CFX	16	44.4	9	25	11	30.5
GNT	6	16.7	1	2.8	29	80.5
CXM	32	88.9	1	2.8	3	8.3
OFL	12	33.3	-	-	24	66.7
AUG	26	72.2	6	16.7	4	11.1
NIT	33	91.7	1	2.8	2	5.6
CPR	13	36.1	1	2.8	22	61.1

CAZ: Ceftazidime 30µg, CRX: Cefuroxime 30µg, GEN: Gentamicin 10µg, CXM: Cefixime 5µg, OFL: Ofloxacin 5µg, AUG: Augmentin 30µg, NIT: Nitrofurantion 30µg, CPR: Ciprofloxacin 5µg.

Molecular identification of isolates

The result of PCR using specific primers confirmed the isolates that had been earlier identified using biochemical tests, with DNA size ranging from 550bp – 680bp. Lane 1 to 8 shows band for *Pseudomonas aeruginosa* isolates.

1 2 3 4 5 6 7 8 (Plate 1)

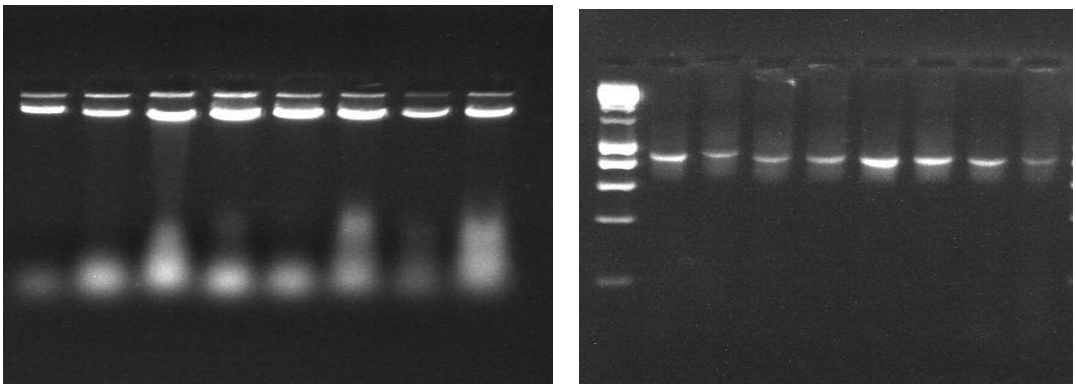

Figure 1: PCR products on Agarose gel electrophoresis

Pseudomonas aeruginosa is frequently implicated as a cause of health care acquired hospital with high mortality rates. One of the reasons for high pathogenicty of *Pseudomonas aeruginosa* is the intrinsic high resistance to numerous antibiotics, as well as the development of multidrug resistance in the hospital environment.

This study investigated symptomatic Nosocomial infection among in patients of all age ranges, in which 36 (12%) of the overall samples were positive for *Pseudomonas aeruginosa*. This indicates a low prevalence of cases of *Pseudomonas aeruginosa* Nosocomial infection in the sampled population. During the course of the study of this project, some consented to give their blood samples while others did not give theirs due to personal reasons.

Pseudomonas aeruginosa isolates were identified using Centrimide Agar (CA), which is a selective medium for the bacteria growth. All centrimide agar media with growth changed to green or greenish yellow which indicates that the positive isolates of *Pseudomonas aeruginosa*. Centrimide agar media contains Centrimide (cetyltrimethylammonium ammonium bromide) which is a quartner ammonium and can hinder the growth of other microorganisms by breaking (lysis) of the bacterial cell.

DISCUSSION

During the Five-month period of survey in the three government owned hospitals cited above, a total of 36 *Pseudomonas aeruginosa* isolates which is a predominant representative bacteria family in the cause of nosocomial infections and outbreaks (Cai *et al* 2017). Of which 20 (55.6%) exhibited that Multi Drug Resistant *Pseudomonas aeruginosa* in patients is common and contributes to mortality among all age ranges admitted in the Hospital ward at a Nigerian Tertiary Hospital.

Most of the isolates were recovered from female samples even though no cognizance was taken as regards the sex of a patient while the study was being conducted. Isolates were also more frequently isolated from patients of 31–40 years old. All the isolates were confirmed using specific primers by Polymerase Chain reaction.

The result of antibiotic activity testing showed that isolates were Multi Drug Resistant *Pseudomonas aeruginosa* (MRDPA), marked with the isolates that were resistant to three or more classes of antibiotics. This result is higher as compared to Akingbade research of 20% of isolates was MDRPA (Akingbade *et al* 2012). The antibiotics used in this study belonged to different classes of antibiotics including aminoglycosides, fluoroquinolones, Penicillin and cephalosporins. The isolates considered as most susceptible were resistant to at least 4 out of 8 antibiotics used. The isolates were most sensitive to Gentamicin and Ofloxacin.

Conclusion

Multidrug resistant saga has continued to pose critical health challenging issues especially in our environment with little or non-existence of functional health infrastructure to carter for increasing population of the citizens. Nonetheless, it may probably be believed that some of the reasons for the geometric increase of multi drug resistance cases might be linked to poor prescriptions expertise of antibiotic regimen and non- adherence to drug intake pattern recommendation by the subjects as suggested by the physician, even as it seems to be an evidence of lack of health awareness education on the subject matter among the subjects, in our remote communities.

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Diagnosis defect in developing countries: Insensitivity of Salmonella-shigella agar in Isolation of *Salmonella* spp. from Stool and Meat samples in Niger State and the role of Molecular Technique

***[1,2]Aremu K.H., [1]Musa A.D., & [3]Smith S.I**

[1]Department of Biochemistry, IBB University, Lapai, Niger State, Nigeria.
[2]Department of Biochemistry, Osun State University, Osogbo, Osun State, Nigeria
[3]Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research Yaba, Lagos, Nigeria

*Correspondence: Email address: harunaremu@gmail.com (+2348035592157)

ABSTRACT

Salmonella infections remain a major threat to public health in developing countries and to facilitate adequate treatment, effective and definitive diagnosis of *Salmonella* spp. is important. This study aimed to present emerging non-sensitivity of Salmonella-shigella agar as culturing media for *Salmonella* spp. and the use of molecular technique as an alternative choice. A total of 98 samples comprising 72 meat and 26 stool samples were analyzed for the presence of *Salmonella* spp. while molecular typing were carried out by the amplification of *fliC* gene in stool and *aroC* gene in both stool and meat samples. Thirty-one (31) isolates were positive for *Salmonella* spp. using Salmonella-shigella agar and conventional standard biochemical tests. The distributions of the isolates are meat (22) and stool (9). Result from molecular amplification of the respective genes shows that none of the isolates possess *fliC* and *aroC* genes. This study therefore affirms the insensitivity of salmonella-shigella agar as a culture media in *Salmonella* diagnosis and suggests the use of molecular technique or better sensitive culture media as preferred tool in diagnosis of *Salmonella*-related infections.

Keywords: *Salmonella* spp., Culture media, Molecular typing, Salmonella-shigella agar.

INTRODUCTION

Infectious diseases are a leading cause of death in developing countries (Yager et al. 2006) as vast majority of deaths occurs in children or young adults resulting in huge burden of disability and the loss of many years of viable health and productive life (Mabey et al. 2004). *Salmonella* infections remain endemic in Africa majorly due to scarcity of basic amenities like safe water and good sanitation practices. The infections have been associated with many food-borne diseases across the world (Jimenez et al. 2009). Various foods, such as chicken, beef, and pork, have been implicated in outbreaks caused by *Salmonella* spp. (Trevanich et al. 2010). The deficiencies and inadequacies of basic amenities have weigh on public health and, typhoid fever—a severe life-threatening illness caused by *Salmonella* Typhi is one of the many unfortunate consequences (Mogasale et al. 2014). From hospital-based studies in Africa,

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high proportion of children suffers from serious bacterial infections (Nadjm et al. 2010) with many of these deaths result from inadequate diagnosis to inappropriate treatment options as well as difficulty in accessing adequate health care infrastructure (Yager et al. 2008). Thus, effective methods for the isolation of *Salmonella* spp. are important to ensure food quality and safety. The choice of a suitable sampling procedure combined with a sensitive culture method is important for the successful detection of *Salmonella* spp. (Carrique-Mas et al. 2008). Identification of pathogenic *Salmonella* spp. and control of *Salmonella* infections are worldwide problems. Because of the ubiquitous occurrence of *Salmonella* spp. and the high incidence of salmonellosis (Rambach 1990), detection and identification of *Salmonella* spp. in food and water are important in both prevention and control of salmonellosis outbreaks (Rambach 1990).

Proper diagnosis is an important part of treatment of infectious diseases. In developing countries, due to limited resources been accrued to the development of diagnostic test and lack of market in richer countries to attract private-public investment in developing diagnostic tests, diagnosis have been poorly developed (Mabey et al. 2004). Owing to this, diagnosis in developing countries has been largely based on the use of culture media and widal test. The use of selective and differential plating media is a simple method for the isolation of *Salmonella* spp. A wide variety of selective and differential media has been developed for this purpose, including Salmonella-shigella (SS) agar, xylose lysine desoxycholate agar (XLD), Hektoen enteric (HE) agar, and bismuth sulfite (BS) agar (Cooke et al. 1999). Their differentiation abilities rely on characteristics of *Salmonella*, such as hydrogen sulfide production and the non-fermentation of lactose (Rambach 1990). Despite the effectiveness of these techniques, there are emerging limitations in their specificity and sensitivity. Several studies have reported bacterial colony share identical characteristics with other microorganisms, such as *Proteus* and *Citrobacter* (Eigner et al. 2001). Thus, numerous false-positive results are observed on these media which require further confirmation testing, a time-consuming and labor-intensive activity (Gaillot et al. 1999). These have reduced the specificity of these selective media. In addition, House et al. (2001) has reported reduced sensitivity of cultures when patients used antibiotic therapy prior to diagnosis. In the same vein, widal test which has been used extensively as a diagnostic tool in many developing countries in both acute and convalescent sera are often found to be unreliable in endemic areas (Khan et al. 1998). The processing of widal tests may take up to 18h, which disqualifies it as a rapid test (Rodrigues 2003). Also, Vollaard et al. (2005) has reported widal test to lack diagnostic value. Due to the increase in specie variation and their evolving inherent ability to grow on the available selective culture media; it is imperative to introduce techniques that are more rapid, reliable and sensitive.

Rapid and sensitive laboratory methods for diagnosis of typhoid fever and other salmonella-related infections are essential for prompt and effective therapy. As conventional method of detection of *Salmonella typhi* are unreliable, problematic, time consuming and cumbersome, molecular based PCR technique which is rapid, specific and more sensitive has been developed (Dorji et al. 2014). The use of amplification of DNA by PCR method is a revolutionary tool in diagnosis of pathogenic organisms (Dorji et al.

2014). Consequently, Haque et al. (2005) and other studies have reported the amplification of *fliC* (*stn* and *str*) (Song et al. 1993) and *aroC* genes (Kidgell et al. 2002) as target for molecular diagnosis of *Salmonella typhi* and *Salmonella* serovars respectively. Furthermore, WHO, (2011) has encouraged the use of molecular and sequence-based typing as a better technique for diagnosis of bacterial infectious diseases. In this study, using molecular technique, we aim to present the potential diagnosis defect in the use of Salmonella-shigella agar for the identification of *Salmonella* spp.

METHODOLOGY

This study was conducted in Niger State, Nigeria. The study areas of sample collection were: Zone A (General Hospitals in Lapai and Bida), Zone B (General Hospitals in Minna and Suleja) and Zone C (General Hospitals in Kontagora and Wushishi). A total of twenty-six (26) stool from suspected typhoid fever patients and seventy-eight (78) meat samples from meat sellers across zones of Niger State were collected and cultured on Salmonella-shigella agar for 24 hours and pure colonies were obtained and identified using biochemical tests as earlier described by Turki et al. (2012) and Farmer et al. (1999). This study was conducted with approval of ethics committee of Ministry of Health, Niger State.

Isolation and identification of *Salmonella*

Salmonella was isolated from samples using non- selective peptone water, nutrient broth enrichment and cultured on Salmonella–Shigella agar (Turki et al. 2012). Five grams (5g) of stool sample and 25g of meat sample were pre-enriched in 45ml and 225 ml sterile peptone water respectively and incubated at 37^{o}C for 24 h. One milliliter of the broth from both samples were transferred into 9ml nutrient broth for selective enrichment (Okafor et al. 2003) and incubated at 37^{o}C for 24hr. After the enrichment, *Salmonella* was detected by plating the broth on Salmonella–Shigella (SS) agar and incubation at 37^{o}C for 24hr. Typical *Salmonella* colonies which appeared colorless with black centers were picked and confirmed as Gram negative by Gram-staining. Pure cultures of isolates was made on slants of nutrient agar, incubated at 37^{o}C for 24hr and stored in refrigerator at 4^{o}C as stock cultures of presumptive *Salmonella* for further tests.

Biochemical characterization of isolates

Biochemical identification of isolates was as described by Farmer (1999). All isolates that gave reactions typical of *Salmonella* in the tests and substrates were considered to belong to the genus *Salmonella*. Typical *Salmonella* reactions are methyl red positive, citrate positive, nitrate positive, Voges Proskauer negative, indole negative, oxidase negative, motile in motility medium, produces H_2S, ferments glucose, maltose but fail to ferment lactose, sucrose.

DNA extraction

DNA extraction was carried out on samples using the Qiagen QIAmp mini DNA kit.

DNA Primers: The primers consist of *fliC* genes of *str* (495bp), *stn* (363bp) and *aroC* gene (639bp). The primers sequences and their corresponding genes are shown in Table 1.

PCR conditions for *Str*, *Stn* and the *aroC*: 2µl of the extracted DNA was mixed in a 1X PCR Master mix. The reaction mixture contains 1.5mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphates (dNTPs) (Solis Biodyne), 20 pMol of each primer (Jena Bioscience, Germany), 2 unit of FIREPol DNA polymerase (Solis Biodyne),

and sterile distilled water was used to make up 20µl reaction mixture. Amplification reactions were performed under the following conditions: initial denaturation of 95^0C for 5 minutes, followed by 35 cycles of denaturation at 95^oC for 30 seconds; annealing at 50^0C for 30 seconds (stn and str) and 57^0C (*aroC*) with extension at 72°C for 60 seconds, and a final extension for one cycle at 72°C for 5 minutes. The PCR product was run on a 1.5% agarose gel and electrophoresis was done at 100V for 1 hour 30 minutes for detection of amplified fragment.

Table 1 Primers for the Confirmation of *Salmonella typhi* and *Salmonella* sp.

Primers	Oligonucleotide sequence (5'-3')	Targeted gene	Predicted Amplicon size (bp)	Reference
STR (F) STR (R)	TATGCCGCTACATATGATGAG TTAACGCAGTAAAGAGAG	*fliC*	495	Song *et al*. (1993)
STN (F) STN (R)	ACTGCTAAAACCACTACT TGGAGACTTCGGTCGCGTAG	*fliC*	363	Song *et al.* (1993)
aroC (F) aroC (R)	GGCACCAGTATTGGCCTGCT CATATGCGCCACAATGTGTTG	*aroC*	639	Kidgell *et al.* (2002)

RESULTS

In this study, of the 26 stool and 72 meat samples from typhoid fever patients and meat-sellers respectively, only 9 (34.62%; stool) and 22 (30.56%; meat) showed reactions typical of *Salmonella* in the biochemical tests and substrates were considered to belong to the genus *Salmonella*.

Table 2: Distribution of sample from various sources

Sample	**No. of Sample**	**SS agar / Biochemical tests**
Stool	26	09
Meat	72	22
Total	98	31

Molecular analysis

(a) (b)

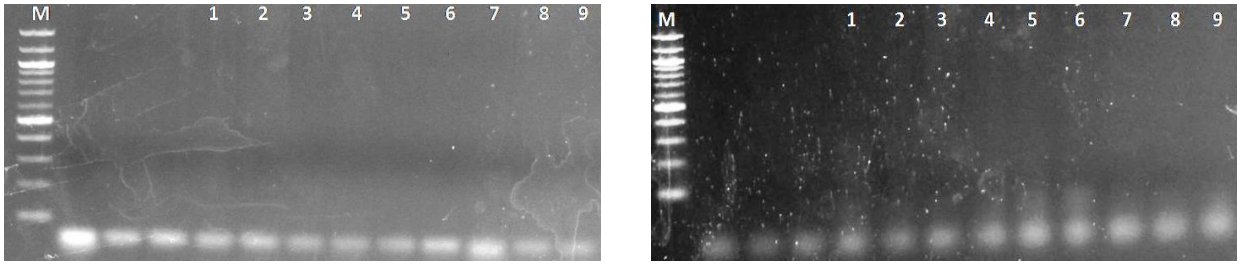

Plate 1a and 1b: PCR amplification of *fliC* genes using STN (a) and STR (b) primers. Lanes M: 100bp ladder; lanes 1-9 show no PCR products of *fliC* (495bp) and (363bp).

(a) (b)

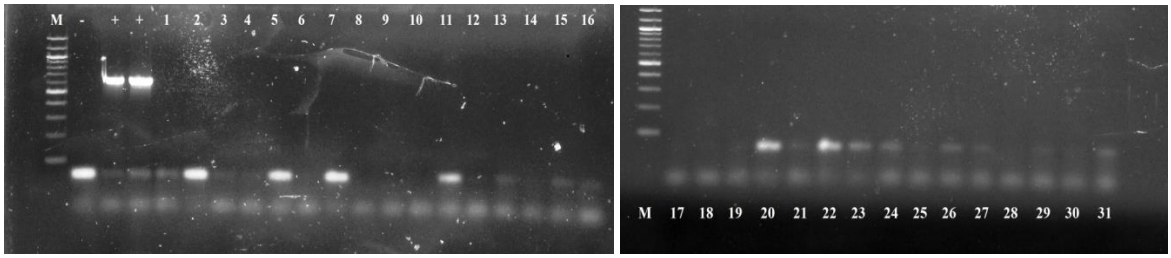

Plate 2a and 2b: PCR amplification of *aroC* (639bp) genes. Lanes M: 100bp ladder; Lane 1-9 show no *PCR product* of *aroC* gene from stool samples while lanes 10- 16 on Plate 2a and lane 17-31 on Plate 2b indicate no PCR product of *aroC* gene from meat samples.

DISCUSSION

Diagnosis of infectious diseases remains paramount towards effective treatment. The outcome of defective diagnosis is a leading threat to patients in developing countries causing treatment failures leading to deaths. In the industrialized world, infection control relies on results from individual patient-directed diagnostic microbiology laboratory tests. On the contrary, developing countries have yet to recognize basic clinical microbiology as a priority (Mabey et al. 2004). Due to the emerging defective sensitivity in the basic and conventional diagnosis methods especially with the use of culture media (Park et al. 2012), molecular diagnostic tools have been employed towards diagnosing infectious diseases which are relatively more specific, rapid and sensitive (Relman 1999). The result of culture confirmed technique and widal test have been a gold standard in diagnosis of *Salmonella* infections (Dutta et al. 2006). However, several studies have suggested the defective specificity and sensitivity of these diagnostic techniques (Park et al. 2012) hence, the use of molecular techniques.

In this study, 98 samples comprising 26 stool and 72 meat samples were analyzed, *Salmonella* spp. were confirmed in 9 stool (34.62%) and 22 meat (30.56%) samples. This confirmation was based on result of Salmonella-shigella (SS) agar and conventional biochemical

tests. The culture-confirmed *Salmonella* spp. from typhoid fever patients were subjected to molecular serotyping by the amplification of *fliC* genes however no band was observed as shown in Plate 1a and 1b while *aroC* genes amplification from both stool and meat samples show no band as illustrated in Plate 2a and 2b. These results present an anomaly in diagnosis of *Salmonella*-related infections using culture confirmation from Salmonella-shigella agar and biochemical tests.

The use of selective and differential plating media is a simple method for the isolation of *Salmonella* spp. Their characteristics differential abilities of *Salmonella* rely on hydrogen sulfide production and the non-fermentation of lactose. However, these characteristics are shared with other microorganisms, such as *Proteus* and *Citrobacter*, *Morganella* (Eigner et al. 2001). Thus, in this present study, the inability of *fliC* genes and *aroC* genes to be detected on the culture-confirmed *Salmonella* spp. from stool and meat samples respectively suggest that colonies with black centers observed on the Salmonella-shigella agar are not *Salmonella* strains rather they can be *Proteus* and *Citrobacter*, *Morganella* as suggested by Park et al. (2012). This is an indication of false-positive result observed on Salmonella-shigella media which require further confirmation testing, a time-consuming and labor-intensive activity (Gaillot et al. 1999). According to several studies, the specificity and sensitivity of Salmonella-shigella agar have been compromised (Eigner et al. 2001).

Conclusion

The outcome of this study affirms that treatment of patients for Salmonella infection based on diagnosis using SS agar is not reliable and could lead to false-positive diagnosis which in many ways increase the incessant occurrence of antibiotics resistance caused by inappropriate treatment and thus, death. Furthermore, the use of molecular techniques must be employed for better sensitivity and diagnosis of infections in developing countries.

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Molecular and Biochemical Characterization of Some Keratinophilic Fungi Isolated from Soil Samples of Murtala Amusement Park in Minna, Nigeria

[*1]Hamza U.I., [2]Emere M.C. & [3]Bulus T.

[1]Department of Biological Sciences, IBB University, Lapai, Niger State, Nigeria.
[2]Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria
[3]Department of Biochemistry, Kaduna State University, Kaduna, Nigeria

*Correspondence: Email address: uihamza@yahoo.com Phone number: 0806 333 5898

ABSTRACT

Keratinophilic fungi are not only known for their medical significance as the prime cause of various mycotic infections among children, but are as well adjudged rich sources of industrial enzymes. Murtala Amusement Park is amongst the preferred play grounds in Minna town. Isolation of the fungi was performed by hair-bait method of Vanbreuseghem. The isolates were identified using microscope and via molecular analysis using DNA sequence analysis. Internal Transcribed Spacer (ITS) region of rDNA was amplified and the PCR products were sequenced. A total of 542 isolates from eleven genera were identified; *Aspergillus, Candida, Fusarium, Paecilomyces, Mucor, Chrysosporium, Alternaria, Penicillium, Trichoderma, Microsporum,* and *Rhizopus.* Exactly 90.90 % of the isolates showed positive for amylase production, 63.60 % showed positive for lipase production, and 54.50 % showed positive for protease production. A very high incidence of keratinophilic fungi observed is a noteworthy finding for the development of indigenous biotechnological industries.

Keywords: Internal transcribed spacer, keratinophilic fungi, molecular analysis, biodiversity hot spot.

INTRODUCTION

Fungi belong to a very diverse group of eukaryotic organisms that populate various habitats and due to an extraordinary plasticity they can colonize different substrates such as keratin, collagen, elastin, lignin, cellulose, hemicellulose etc. using resources that are inaccessible or hardly accessible to other species (Chang and Miles, 2004). The estimated number of fungi in the world is considered to be between 1.5–3.5 million species, and many of them are still unidentified (Hawksworth, 2009). According to the 10th edition of Dictionary of Fungi, so far 97,330 species of fungi belonging to 75,337 genera are described (Hawksworth, 2009).

It has been demonstrated that pathogenic fungi secrete various lytic enzymes, such as proteases and lipases, which may play an important role in invading and parasitizing the host tissue. These enzymes enhance the parasites survival in tissues by chemically or physically altering the immediate environment or this act directly by digesting host proteins, lipids, thus providing a source of energy (Ogawa *et al.,* 992).

Enzyme production is a major field of contemporary biotechnology. The potentials of using microorganisms as biotechnological

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sources of industrially important enzymes have stimulated renewed interest in exploring the extracellular enzymatic activity in several microorganisms (Bakri et al, 2009). Selection of the right organism is very crucial since it plays an important role in high yield of enzymes of interest (Sathyaprabha *et al*., 2011). Fungi are microorganisms which are well known for their wide range of novelty of enzymes are used in the industrial process and this accounts for billions of dollars of revenue annually (Arunsasi *et al*., 2010). Filamentous fungi are particularly interesting due to their easy cultivation, and high production of extracellular enzymes of large industrial potential (Mishra and Dadhich, 2010).

Prevalence of Keratinophilic fungi from soil of playgrounds has been demonstrated in different locations which includes; Rizwana *et al*.. (2012) reported 86 % prevalence rate of keratinophilic fungi from the soils of public parks and playgrounds of Riyadh, Saudi Arabia, Gugnani *et al*.. (2012) reported a prevalence of 45 % and 69 % from St Kitts and Nevis, all in the West Indies, 43.75% prevalence was reported by Maruthi *et al*.. (2012) in India, Agu *et al*.. (2013) reported a prevalence rate of 45% from schools playing grounds in Sagamu, Ogun State of Nigeria.

Pakshir *et al*.. (2013) isolated keratinophilic fungi from public parks soil in Shiraz, Iran, Olajubu and Folorunso (2014) isolated keratinophilic fungi from AAU staff school playground in Nigeria. These playgrounds are often used by human beings and animals. These (human beings and animals) may leave some organic residues, which may contaminate the soil with keratinaceous debris and possibly spores of keratinophilic fungi.

Keratin substrates are generated in large amounts as waste products. Poultry processing plants produced millions of tons of feathers as waste products every year worldwide (Manczinger *et al*., 2003). These keratin substrates are degraded very slow in nature, hence, the European Union considered keratin substrates as hazardous waste materials (Balint, 2006). Decomposition methods such as incineration, chemical (Onifade *et al*., 1998) and landfilling are used, but these procedures are either expensive or environment-polluting (Balint, 2006). Biotechnology could offer environmentally friendly and economically cheap biodegrading methods (Balint, 2006). Exploration of potential keratin degraders will definitely find biotechnological use in various industrial processes involving keratin hydrolysis. There is need for isolation of microorganisms from various sources to meet the industrial and environmental demand. It is imperative to establish indigenous biotechnological industry in Nigeria. This would also solve the waste disposal problem of poultry waste and recycling of keratinacious waste would be beneficial financially and environmentally (Agrahari and Wadhwa, 2010).

Murtala Amusement Park is amongst one of the preferred outdoor recreation grounds in Minna (Abdrazack *et al*., 2013). It is patronized by young children mostly during festive periods. However, adults also patronize the park as venue for ceremonies such as marriage. There were no documented data about molecular identification of keratinophilic fungi from soil of Murtala Amusement Park and no documented report about screening of industrial important fungi from soil of Murtala Amusement Park.

As part of ongoing research aimed at isolating and identifying the fungi diversity in the park, this paper presents molecular and biochemical characterization of some fungal isolates found. The aim of this research was to isolate and identify keratinophilic fungi from soil samples of Murtala Amusement Park in Minna.

METHODOLOGY

The Study Area

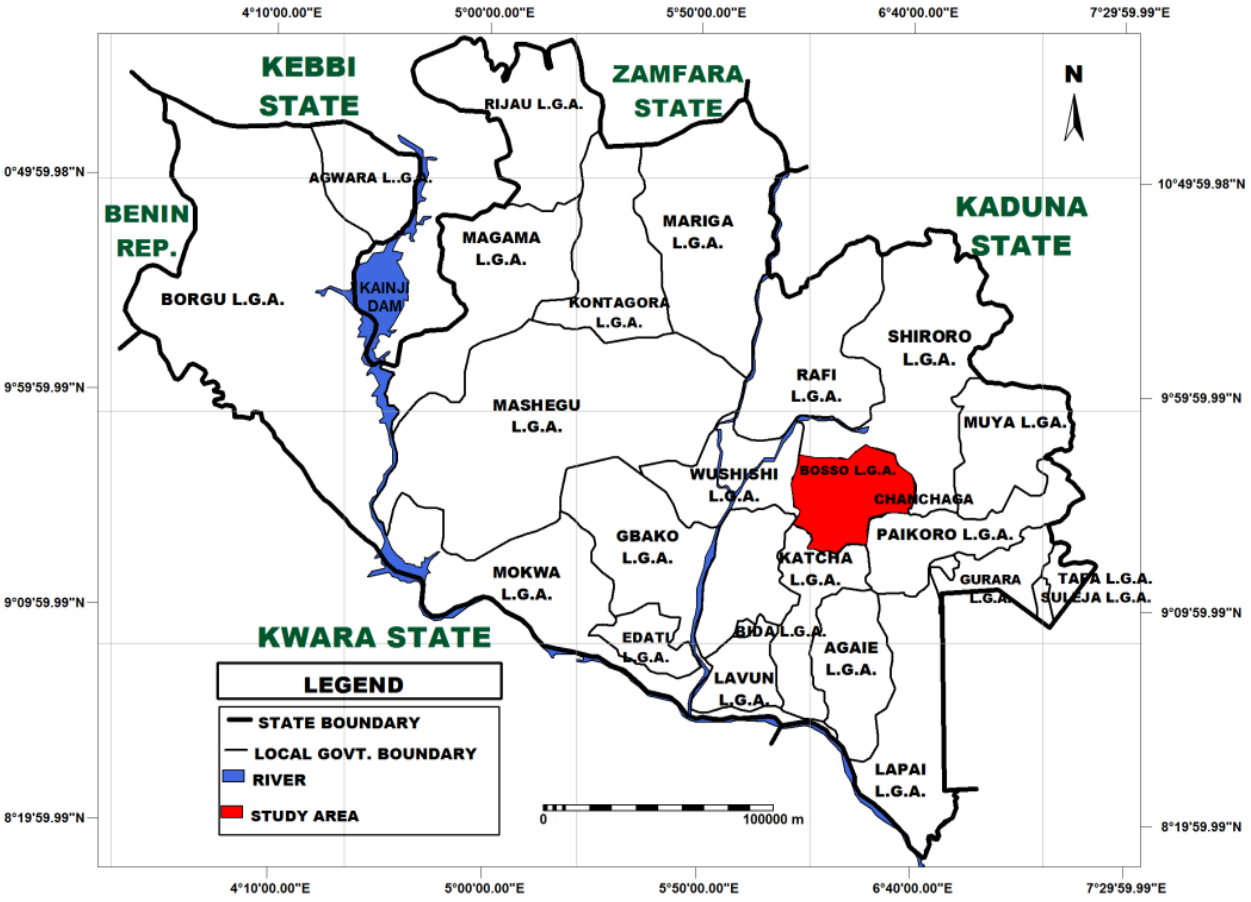

Figure 3.1 Map of Niger State

This study was carried out at Murtala Park which is located in Minna, Niger state capital. Minna lies on Latitude 903'N and Longitude 6033'E on geological base of undifferentiated basement complex of mainly gneiss and magmatite.

Collection of Soil Samples from the Study Area

Soil samples were collected from sites of six playing facilities in the park. These sites were mapped out into five study sites, North, South, West, East, and Central for sample collection. The soil samples were collected from topmost part of the soils (5 cm depth) with sterile hand trowel and transferred inside sterile polyethylene bags (well labeled). Finally, they were transported to the laboratory for further analysis.

Samples of human hair were obtained and washed several times in changes of distilled water and then defatted by soaking in diethyl ether for twenty-four hours. The hair was thoroughly washed again with distilled water and completely dried in hot air oven at 60 ^{0}C for 24 hours, and ground into powder with sterilized grinding stone.

Isolation and Identification of Fungi

The modified hair baiting method of Vanbreuseghem (1952) was employed for the isolation of keratinophilic fungi. Exactly 50 g of each soil sample was measured and transferred in to sterile Petri dishes and some quantity of pulverized defatted human hair were spread over the soil in the dishes. The hair-baited soils in the plates were moistened with sterile distilled water and incubated at room temperature. The soil samples were moistened at regular intervals with small quantities of sterile distilled water to prevent dryness. The soil samples were then examined routinely for fungal growths on the hair baits. Portions of fungal growth (usually observed after two weeks) were aseptically transferred to already prepared Petri dishes of Sabouraud Dextrose Agar (Titan Biotech ISO 9001: 2008 Certified) supplemented with 2 mg/ml gentamycin. The inoculated plates were incubated at room temperature and fungi that grow were sub-cultured severally in order to obtain pure cultures. The fungi were identified traditionally and with the aid of DNA based identification techniques.

DNA Extraction

Exactly 1000µL of Phosphate buffer solution (PBS) was added to cultures and wire loop was used to collect the mycelium in the cultures. Genomic DNA was extracted as described by Del Sal *et al*. (1989). Briefly, the solution (mycelium mixed with PBS) was transferred to an Eppendorf tube and 400 µL of lysis buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA; 1% sodium dodecyl sulfate [SDS]; 10 µL proteinase K) was added. Each sample was incubated for one hour at room temperature with vortexing at every ten minutes, and then 200 µL of 5 mol L^{-1} NaCl was added and incubated for 10 min at 65 ^{0}C. After that, 100 µL of 10% [w/v] cetyltrimethylammonium bromide (CTAB; Sigma) was added and incubation continued for a further 20 min at 65 ^{0}C. The solution was treated with RNase A (Roche) at a final concentration of 50 µg mL^{-1} for one hour at 37 ^{0}C, and then extracted with an equal volume of phenolchloroform- isoamyl alcohol (25:24:1[v/v]), and centrifuged at 12,000 g for 15 min at 4 ^{0}C. The DNA was precipitated with two volumes of ice-cold isopropanol at -20 ^{0}C for 20 min, washed twice in 500 µL of 70 % ethanol, air dried, and re-suspended in 100 µL of TE buffer (40 mM Tris-HCl, pH 8.0; 2 mM EDTA). DNA concentration was estimated by measuring the optical density at 260 nm.

DNA Amplification

The ITS1–5.8S–ITS2 rDNA region was amplified using ITS1 and ITS4 as forward and reverse primers as described by White et al. (1990). Amplification was performed in 50 μL reaction volumes containing 5 μL of 10× buffer, $MgCl_2$ (25 mm) 1.5 μL, dNTP (10 mM) 0.5 μL, 0.5 μL of each 0.2 Mm primer (ITS1: 3′-TCC-GTAGGT-GAA-CCT-GCG-G-5′ and ITS4: 3′-TCC-TCC-GCTTAT-TGA-TAT-GC-5′), Taq Polymerase (1.25 U) 0.5 μL, DNA sample 1 μL, and distilled water 40.5 μL. The PCR reaction was carried out using a Thermal Cycler with the following conditions: denaturation at 95 ^{0}C for 3 min, 35 cycles of (40 s at 94 ^{0}C, 45 s at 54 ^{0}C, and 1 min at 72 ^{0}C) extension at 72 ^{0}C for 10 min, and storage at 4 ^{0}C. Negative controls were also used in each set

of reactions. The final products were analyzed by electrophoresis on 1.2 % agarose gel and stained with 0.5 μgmL^{-1} ethidium bromide. A thermocycler with this brand name (qTOWER2.0) was used for amplification of segment of DNA. The PCR amplified products were then sequenced in both directions and sequences generated were processed by using the web-based blasting programme, Basic Local Alignment Search Tool (BLAST), at the National Center for Biotechnology Information (NCBI) site (http://www.ncbi.nlm.nih.gov/BLAST), and the data were compared with the NCBI/Genbank database (Altschul, 1990).

Enzymatic Screening of the Isolates

Screening for Amylase Production was done by inoculating the fungal isolates on starch agar (containing peptone, 1%; yeast extract, 1%; KH_2PO_4, 0.5%; agar 2% and supplemented with 1% (w/v) starch (HiMedia) as a carbon source and supplemented with antibacterial antibiotic Chloramphenicol) plate with fungal isolates. After incubation, the plates were flooded with Iodine solution (Iodine -0.2 g, Potassium Iodide- 0.4 g, Distilled water- 100 ml), and clear zone of hydrolysis surrounding the colony was taken as evidence of amylolytic activity (Aneja, 1996, kathiresan and Manivannan, 2006).

Screening for Lipase Production

The fungal isolates were inoculated on mineral media amended with Tween 80. The formation of opalescence (shimmering milky colour) surrounding the fungal colony was recorded as positive for lipase production (Rajan et al, 2011).

Screening for Protease Production

The fungal isolates were inoculated on Casein agar plates and incubated at 25 ^{0}C for 4 days. After incubation, the plates were observed for possible clear zone surrounding the colony (Aneja, 1996).

RESULTS

A total of five hundred and forty two fungal species were isolated and these comprised eleven genera namely; *Aspergillus, Candida, Fusarium, Paecilomyces, Mucor, Chrysosporium, Alternaria, Penicillium, Trichoderma, Microsporum,* and *Rhizopus*. *Aspergillus niger* had the highest frequency while *Aspergillus oryzae* had the least frequency. As shown in table 1 below.

All the fungi isolated were tested positive for amylase production except *Chrysosporium tropicum. Aspergillus flavus, Penicillium notatum, Fusarium solani, Rhizopus stolonifer, Chrysosporium tropicum,* and *Paecilomyces variottii* were tested positive for protease production. *Trichoderma spp, Mucor spp, Alternaria alternata*, and *Paecilomyces variottii* were tested negative for lipase production as shown in table 2.

The isolate 01 showed 97 % similarity with *Fusarium solani* with the following accessions LN828256.1, LN828156.1, and LN828110.1. Hence, this isolate is identified as *Fusarium solani*. While the isolate 02 was named as *Aspergillus flavus* because its similarity with *A. flavus*, LC106118.1 was 100 %. As shown in table 3.

The ITS region was amplified and final products were analyzed by electrophoresis on 1.2 % agarose gel and stained with 0.5 μgmL^{-1} ethidium bromide. The agarose gel electrophoregraph of amplified PCR products are shown in figure 1.

Table 1: Number of Fungal species isolated from soil samples of Murtala Amusement Park, Minna, Niger State.

Fungal species	Number of fungi
Aspergillus oryzae	3
A.niger	107
A.flavus	58
A.fumigatus	41
Penicillium notatum	33
Candida albicans	55
Fusarium solani	30
F. oxysporium	43
Trichoderma species	29
Rhizopus stolonifer	23
Mucor species	21
Microsporum gypseum	22
Chrysosporium tropicum	33
Alternaria alternata	27
Paecilomyces variottii	17
Total	54

Table 2: Enzyme production potentials of some fungal species isolated from soil samples of Murtala Amusement Park, Minna, Niger State

Fungal species	Amylase	Protease	Lipase
Aspergillus niger	+	-	+
A.flavus	+	+	+
A.fumigatus	+	-	+
Penicillium notatum	+	+	+
Fusarium solani	+	+	+
Trichoderma spp.	+	-	-
R. stolonifer	+	+	+
Mucor species	+	-	-
C. tropicum	-	+	+
A.alternata	+	-	-
P. variottii	+	+	-

+ Present - Absent

Table 3: Identification of fungal isolates based on genetic analysis of the ITS region.

Isolates code	**Morphological identification**	**Genebank strains (BLAST)**	**E-value**	**Identity**	**Query cover**	**Accession**
Isolate 01	*Fusarium species*	*Fusarium solani*, isolate CIB 35484	0	97%	100%	LN828156.1
		Fusarium solani, isolate CIB 3	0	97%	100%	LN828156.1
		Fusarium solani, isolate LEMM 110148	0	97%	100%	LN828110.1
		Fusarium solani, isolate CIB 20	0	97%	99%	LN828149.1
Isolate 02	*Aspergillus species*	*Aspergillus flavus*, strains FIB PP 2.2	0	100%	100%	LC106118.1

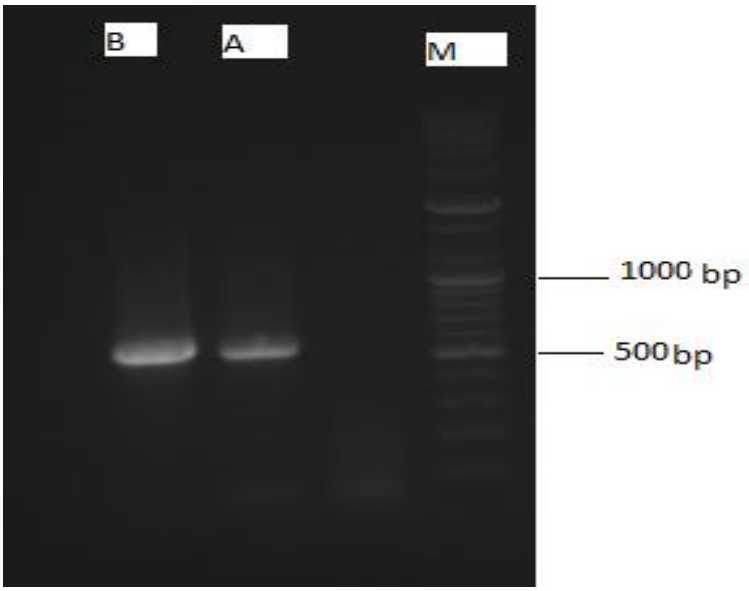

Figure 1 Agarose gel electrophoregraph of PCR products of the amplification of ITS region of *Fusarium solani* and *Aspergillus flavus* A= *Fusarium solani* B= *Aspergillus flavus* M= molecular ladder

DISCUSSION

In this study, *Aspergillus niger* was the fungus with the highest frequency. This result is in line with Mini et al (2012), Olajubu and Folurunso (2014), Ashok et a1 (2015) but disagrees with the findings of Agu et al (2013) who in their study isolated *A.flavu*s as the most predominant fungus. The dominance of the *Aspergillus niger* in the soil may be due to its greater rate of spore production, dispersal, extreme resistance to environmental conditions, and its suitability to grow in different soil pH concentration. Furthermore, the genus *Aspergillus* is known to produce some toxins such as aflotoxins, achrotoxins. These toxins, if secreted may inhibit the growth of other fungi.

Many researchers have demonstrated that pathogenic fungi secrete various lytic enzymes, such as proteases and lipases, and these enzymes enhance survival in tissues by digesting host proteins, lipids, thus providing a source of energy for the fungi (Ogawa et al, 1992).

Banakar et al (2012) worked on the isolation and screening of forest soil of Bhadra Wild Life Sanctury, for potent amylolytic fungi. The isolated fungi were mainly belonged to *Penicillium, chrysogenum*, *Aspergillus candidus*, *Aspergillus fumigatus.* It was found that *Penicillium sp* showed more amylase activity for both 3rd day and 7th day incubation. *Penicillium chrysogenum* was found to produce more soluble crude protein. Sathyaprabha et al (2011) reported the isolation of fungi namely, *Aspergillus fumigatus, Aspergillus versicolor, Aspergillus nidulans* and *Aspergillus niger* from soil samples obtained from crude petroleum oil contaminated soil. They screened the isolates for amylase and cellulose production. Kathiresan and Manivannan (2006) isolated Strains of *Penicillium* sp. from the coastal soil of a mangrove habitat and later identified as *P. fellutanum* and screened for amylase production. Tiwari (2007) isolated a fungus from soil identified as *P. rugulosum* and screened it for amylase.

In our present study, exactly 90.90 % of the isolates were positive for amylase production, 63.60 % of the isolates were positive for lipase production and 54.50 % of the isolates were positive for protease production. All the isolates degraded the keratin substrate (human hair), but not all produced clear zone surrounding the colony on casein agar. This could be due to the fact that the substrate used is a pure form of keratin. Thus, it is perfectly logical to say that Murtala Amusement Park could be a source of fungi of industrial importance. Many researchers have isolated fungi of industrial importance. Such researchers include Banakar et al (2012). In this study, *Aspergillus flavus* produced maximum zone of hydrolysis for amylase production. This result is in consonance with the findings of Arunsasi et al (2010) who screened *A. flavus* for amylase production and found it to produce maximum zone of hydrolysis. In addition, in the present study, *Fusarium solani* produced appreciable zone of hydrolysis for amylase production and positive for lipase. Kannahi and Ancy (2012) had screened *F. solani* and *A. flavus* and found both promising in the production of protease and lipase. However, *A. flavus* produced high levels of protease and lipase enzymes compared to *F. solani*. This disagrees with the work of Mukunda et al. (2012) who found *A. flavus* negative for protease production and *F. solani* negative for protease and lipase production. The variations in these results could be due to differences in the pathogenic effect of the strains of the organisms isolated and screened for the enzyme production potential.

Basic Local Alignment Search Tool (BLAST) programme was developed by Stephen Altschul of NCBI in 1990 and has since become one of the most popular programmes for sequence analysis. The BLAST uses heuristics to align a query sequence with all sequences in a database. The objective is to find high-scoring ungapped segments among related sequences. The existence of such segments above a given threshold indicates pairwise similarity beyond random chance, which helps to discriminate related sequences from unrelated sequences in a database (Xiong, 2006).

In order to verify the results of the morphological examinations and identify the strains at the species level, they were subjected to ITS1-5.8S-ITS2 gene sequence analysis. The results obtained from this sequence analysis corresponded with those from the morphological identification. The sequencing results were blasted against Genbank. Sequence similarity, which is the percentage of aligned residues that are similar in physiochemical properties such as size, charge, and hydrophobicity was found to be 97 and 100 % for *Fusarium solani* and *Aspergillus flavus* respectively. The rule says you can label proteins as “homologous” if 25 percent of the amino acids are identical, for DNA you will require at least 70 percent identity to draw the same conclusion (Claverie and Notredame, 2007). Sequence similarty and sequence identity are synonymous (Xiong, 2006). Thus, it was concluded that the nucleotide sequence of *Fusarium solani* and the nucleotide sequences in the NCBI database with these accessions LN828256.1, LN828156.1, and LN828110.1 have homologous relationship, and also the nucleotide sequence of *Aspergillus flavus* and nucleotide sequence in the NCBI database with this accession LC106118.1 have homologous relationship.

Expectation value (E-value), which tells you how likely it is that the similarity between your sequence and a database sequence is due to chance. It was found to be zero for the two

isolates. The lower the *E*-value, the less likely the database match is a result of random chance and therefore the more significant the match is (Xiong, 2006). Thus, the match in this research was highly significant.

Conclusion

This research shows that the soil of Murtala Amusement Park, Minna, Nigeria was contaminated with keratinophilic fungi: *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus oryzae, Candida albicans,Penicillium notatum, Fusarium solani, Fusarium oxysporum, Trichoderma species, Rhizopus stolonifer, Mucor species, Chrysosporium tropicum, Alternaria alternata, Microsporum gypseum,* and *Paecilomyces variottii* and some of these fungi (*Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Penicillium notatum, Fusarium solani, Trichoderma species, Rhizopus stolonifer, Mucor species, Chrysosporium tropicum, Alternaria alternata,* and *Paecilomyces variottii*) are potential sources of enzymes such as amylase, lipase, and protease. The DNA technology used has provided an easier means of confirming the isolates that were initially identified culturally. A very high incidence of keratinophilic fungi demonstrated in the soil of Murtala Amusement Park is a noteworthy finding for the development of indigenous biotechnological industries.

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Potentials of Residual Fungal Contaminants of Imported Cigarette Tobacco Sold in Niger State to Produce Ochratoxin A

[*1]Gomina M., [1]Musa A.D. & [2]Shehu D.

[1]Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria.
[2]Department of Science Laboratory Technology, Federal Polytechnic, Bida, Nigeria

*Correspondence: Email address: gmuftanatu@gmail.com; Phone number: +2348187623231

ABSTRACT

Ochratoxin is one of the most abundant mycotoxins produced by several species of *Aspergillus* and *Penicillium* species. This study was conducted to investigate the incidence of fungal contamination of some imported cigarette tobacco sold in Niger state and determine if they produce Ochratoxin A. Fifteen different brands of cigarette were obtained from supermarkets in Lapai and Minna. The cigarette tobacco was cultured on potato dextrose agar and observed for fungal growth for 72 hours. Isolates of the fungi were subcultured on fresh media until pure cultures were obtained. The pure isolates were identified morphologically. Each identified pure isolate was cultured on rice medium for elicitation of mycotoxins. Liquid-liquid extraction method was used to determine the presence of Ochratoxin A in the medium. The concentration of Ochratoxin A from each isolate was determined using thin layer chromatography. The results showed that *Aspergillus fumigatus* had the highest incidence of 100% in the fifteen cigarette analysed, while *A. nidulans* had the lowest incidence of 6.7%. Other fungi identified were *A. niger, Aspergillus flavus* and *Chrysonilia sitophilia* with an incidence of 66.7%, 33.3 % and `20% respectively. The concentration of Ochratoxin A elicited by *Aspergillus nidulans*, *Aspergillus flavus* and *Chrysonilia sitophilia* were <25µg/kg each, while *A. niger* and *A. fumigatus* produced 25µg/kg of Ochratoxin A in the medium. The level of Ochratoxin A elicited by the contaminating fungi all exceeds the tolerance range of 5-20µg/kg as given by the European commission and Codex Committee of food additives and contaminants (CCFAC). The results of the study shows that these cigarette samples sold in Niger State contain residual fungal contaminants and these fungi are capable of eliciting Ochratoxin A. Further studies to determine the presence of Ochratoxin A in cigarette tobacco is therefore suggested.

Keywords: Ochratoxin A, Cigarette tobacco, Fungi, *Aspergillus*, Contamination.

INTRODUCTION

Cigarette tobacco has attracted attention over the years as a result of the health risks it causes, which are prevalent than with other tobacco products. Previous researches have always implied that the smoke from the cigarettes are responsible for these health risks but new researches are proposing that these health risks are caused not only by the cigarette smoke but by microorganisms that contaminate tobacco during its manufacture, processing and storage (Wigand, 2006).

The microbial composition of some tobacco

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products have been analyzed for a long time now. Some studies have reported the identification of toxic microbes in tobacco products such as bacterial and fungal spores (Wood, 1968; Forgacs and Carll, 1996; Varma, 1991; Warke, Kamat and Kamat 1999; Rubenstein, 1992; Verweij *et al.,* 2000). A review article by Paul and Paszkiewicz (2011) addressed the microbes that have been identified and quantified in cigarettes, cigarette smoke and smokeless tobacco products. The prominent fungi that have been isolated from tobacco products are of the Genus *Aspergillus* (Papavassiliou, Piperakis and Marcelou-Kinti, 1971; Welty and Nelson, 1971; Varma, 1991). *Aspergillus* species are capable of producing toxic secondary metabolites (known as mycotoxins} such as Aflatoxins and Ochratoxins (Sweeney and Dobson 1998).

Therefore, this study is aimed at determining to potentials of residual fungal contaminants of imported cigarette tobacco sold in Niger State to elicit Ochratoxin A.

METHODOLOGY

Collection of Samples

Fifteen different brands of imported cigarettes were collected from markets in Lapai and Minna in April 2016.

The names of these cigarettes, their production, expiry dates and origin are given on table 1.

Table 1: Cigarette brands, origin, production and expiry dates

S/N	Cigarette Name	Production Date	Expiry Date	Origin
1	Sample A	February 2015	August 2016	Korea
2	Sample B	November 2014	May 2016	Senegal
3	Sample C	-	-	UAE
4	Sample D	-	-	UAE
5	Sample E	August 2015	February 2017	UAE
6	Sample F	January 2015	July 2016	Germany
7	Sample G	September 2015	March 2017	UAE
8	Sample H	-	-	Foreign
9	SampleI	September 2015	March 2017	Korea
10	Sample J	June 2015	December 2016	Korea
11	Sample K	December 2014	June 2016	Korea
12	Sample L	October 2015	April 2017	Korea
13	Sample M	August 2015	August 2016	Senegal
14	Sample N	August 2015	August 2016	Senegal
15	Sample O	-	-	-

Isolation of Fungi from Samples

The tobacco from the cigarette samples were cultured on Potato Dextrose Agar (PDA). The PDA was prepared by heating 500g of thinly sliced peeled white potatoes with 1000ml distilled water at about 1 hour and then filtered using muslin cloth.20g of glucose, 15g of agar agar and 0.5g of chloramphenicol was added to the potato broth while stirring the mixture which was then made up to 1000ml with distilled water. The mixture was shaken to homogeneity and then autoclaved. Cooled PDA broth (20 ml) was pipette into sterile Petri dishes using sterile pipette in a previously

fumigated inoculating hood. It was then allowed to cool and gel .

For inoculation of the samples, three cigarette sticks of each sample were emptied into a sterile porcelain dish in a previously fumigated inoculation hood. A forcep was used to mix the tobacco leaves together and then inoculated to an appropriately labeled PDA plates. Triplicates were inoculated for each sample and later transferred to another previously fumigated inoculation hood and left at room temperature. Three plates of uninoculated PDA plates were kept as control. The plates were then observed after 48 hours for fungal growth. Plates that had growth with multiple fungal colonies were further separated into different plates to obtain pure cultures of the fungi. The pure cultures obtained were transferred into PDA slants for storage and further investigation.

Identification of fungal Isolates

The pure cultures obtained were identified using morphological identification. This was done by examining each isolate under the microscope for Macroscopic and macroscopic characteristics to identify the fungi with references to Barnett and Hunter (1998) and Kenaga *et al.* (1971).

Culturing of Isolates on Rice Medium

Distilled water (100ml) was added to 250g of rice in 1000ml conical flask and then allowed to stand for 24 hours at room temperature for moisture equilibration. It was then autoclaved to sterility using a prestige medical clinical autoclave. The pure isolates were inoculated onto appropriately labeled sterile rice medium in a previously fumigated inoculation hood. The inoculated rice medium was then maintained in a Grieve laboratory oven at about 35^0C for 21 days. After 21 days of maintaining the fungal culture on rice medium at 35^oC, 750ml of methylene chloride was added to the conical flask containing the rice culture and allowed to stand for 1 hour. The culture was then pulverized using a commercial blender.

Extraction of Ochratoxin A

A multimycotoxin assay method developed and used in U.S. Department of Agriculture, Southern Regional Research Center, and New Orleans was used (Ehrlich and Lee, 1984). 50g of the pulverized samples were weighed into 500ml conical flask and 25ml of 1M-phosphoric and 250ml of dichloromethane was used. The flask was shaken for 30 minutes using a shaker and the content filtered was collected from this. 50ml aliquot was placed in separate 100ml conical flask with glass stoppers for ochratoxin assay.

Ochratoxin Assay

50ml of filtrate was measured into separating funnel; 70ml of $NaHCO_3$ solution (4gm $NaHCO_3$ /100ml distilled water) was added and shaken for one minute. After phase separation (about 15mins) the upper layer ($NaHCO_3$) was transferred to 400ml beaker with which the lower layer methylene chloride (CH_2Cl_2) was transferred into second separating funnel , this was then rewashed twice with 35ml of $NaHCO_3$ and $NaHCO_3$ layers were combined into the beaker in which lower layer methylene chloride was discarded. The $NaHCO_3$ solution was acidified with H_2SO_4 (about 25ml) to pH 2 using pH strip as an indicator. Then the acidified solution was transferred into a clean 200ml separating funnel with which 50ml of $CH_2Cl_2$was added and shaken carefully and CO_2 was evolved. The lower layer CH_2Cl_2 was drained through sodium sulphate ($NaSO_4$) salt into 250ml round bottom beaker. Another 50ml CH_2Cl_2 was added to the aqueous solution in the separating funnel and shaken carefully CO_2 was

also evolved and the lower layer CH_2Cl_2 was also drained through sodium sulphate and collected in the same round bottom beaker, finally sodium sulphate was washed with 5ml of CH_2Cl_2. The extract was evaporated to near dryness on a steam bath of rotary evaporator. The extract was quantitatively transferred with CH_2Cl_2 into a 4ml glass vial fitted with Teflon line screw cap, the solvent was dried using hot plate, this was then stored in deep freezer refrigerator.

For the thin layer chromatographic analysis, 100µg of benzene: acetonitrile (98:2) was added to the extract in glass vial. 2, 5, 7 and 10µl of the OTA standard were spotted along with 20µl of each of the samples on the TLC plate. The plate was allowed to dry and developed in a mobile phase of Benzene: Hexane (3:1) after which it was removed and dried. The plate was developed again in a mobile phase of Benzene: Acetic acid (95:5). The TLC plate was also sprayed with Aluminium chloride ($AlCl_3$) and viewed under the UV at a wavelength of 365nm. The color intensity of each sample spotted was then compared with that of the standard under the UV.

Calculation of Ochratoxin estimate

The formula used to estimate the concentration of OTA in the sample is given below:=

$$\frac{\frac{\#\text{ of standard}}{\text{with same color density of sample}}}{\mu\text{l of sample spotted equivalent to standard}} \times \frac{\text{concentration of standard } (\mu\frac{g}{ml})}{\text{weight of sample actually used in gram}} \times \frac{\text{dilution of evaporaed sample extract}}{1}$$

The unit is given in µg/kg or ppb

RESULTS

A total of five different fungal species were obtained from the morphological identification, four of these species belong to the Genus of *Aspergillus* while the other belong to the *Monilinia* species.

Table 2: Number and Types of Isolates Obtained from the 15 Cigarette Samples

Samples	Total Number of Isolates	Number of Types of Isolates
Sample A	4	2
Sample B	3	2
Sample C	4	2
Sample D	5	2
Sample E	7	3
Sample F	6	3
Sample G	5	2
Sample H	2	1
SampleI	3	2
Sample J	2	1
Sample K	2	2
Sample L	3	3
Sample M	5	4
Sample N	6	3
Sample O	2	2

Table 3: Incidencesof Fungi Isolatedfrom 15 Samples of Cigarette

Fungi	Incidence
Aspergillus niger	21
Aspergillus fumigatus	25
Aspergillus flavus	9
Chrysonilia sitophilia	3
Aspergillus nidulans	1

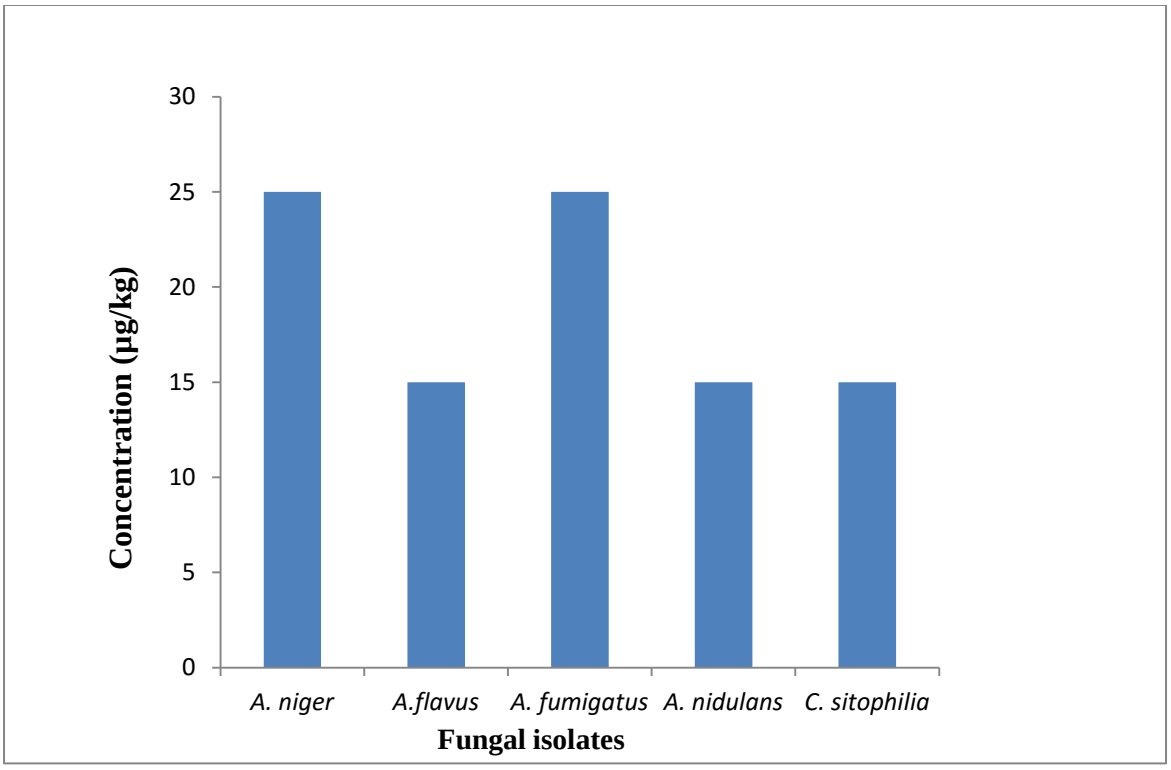

Figure 1: Ochratoxin produced by the isolated fungi species

DISCUSSION

From the results of the morphological identification, it was shown that all the fifteen cigarette samples have fungal contamination as shown in Table 2. This may suggest that there is residual fungal contamination of cigarette sample from the curing process, which involves the use of microorganism. Five different fungal species were isolated from the cigarette tobacco analyzed; *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans and Chrysonilia sitophilia.* Of these, *Aspergillus fumigatus* has the highest incidence of occurrence, and the leaset incidence of occurrence was *A. nidulans*. This suggests that Aspergillus fumigatus is the most common contaminant of cigarette tobacco. This study agrees with some previous studies that reported the isolation of fungi in Tobacco products. In 1971, Greek scientist Papavassiliou and coworkers studied cigarettes produced in different countries and isolated hundred strains of fungi. The most prominent isolated fungi were *Aspergillus.* A study by Varma in 1991 reported the isolation of nine species of *Aspergillus i*n stored leaves of chewing tobacco. Wigand (2006) reported that species such as *Bacillus subtilis, Aspergillis niger* and other microbiological organisms (molds, bacteria, fungi, protozoa, arthropods and nematodes) have been found in tobacco final product.Welty and colleagues of the United States Department of Agriculture (USDA) examined 'fungi isolated from flue-cured tobacco at Time of sale and after storage' in 1969, and subsequent research has indicated that many of these species regularly found on tobacco are capable of producing aflatoxins or other dangerous mycotoxins. All the isolated fungi from this study produced Ochratoxin in varying concentration. *A. niger* and *A .fumigatus* had

Ochratoxin concentration of 25µg/kg while *A. nidulans*, *A. flavus* and *C. sitophilia* had Ochratoxin concentration of <25µ/kg. The only fungal species that have been reported to produce Ochratoxin are the *Aspergillus* and *penicillium* species (Yan Wang *et al.,* 2016). Ochratoxin A has been known to possess immunotoxic, nephrotoxic, carcinogenic, tetratogenic properties (Krogh, 1987). The tolerance range of Ochratoxin contamination by the European commission and Codex committee of food additives and contaminants (CCFAC) is 5-20µg/kg. Ochratoxin produced by *A. niger* and *A. fumigatus* exceeds this tolerance range.

Various factors are known to influence fungal development and mycotoxins production which can occur during harvest, after harvest or storage (Beardall, 1994). Among these factors are moisture, temperature, aeration and substrate. The results of the study therefore indicates that the Ochratoxin A contamination cigarettes could occur as our tropical weather could encourage the production of this mycotoxin by the fungi already present in the cigarette tobacco.

Conclusion

From the results of this study, all the cigarette tobaccos were contaminated with fungi. All the fungi species isolated elicited Ochratoxin A in different concentrations. *Aspergillus niger* and *Aspergillus fumigatus* produced 25µ/kg Ochratoxin, a concentration above the tolerance range given by European Commission and Codex Committee of food additives and contaminants (CCFAC). Therefore, there are Ochratoxin A associated health risks with smoking of these cigarettes.

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Microbiological Assessment of Mobile Phones of Food Vendors in Zungeru Town Niger State, Nigeria

[*1]Mohammed A.S., [1]Muhammad A. & [2]Musa B.M.

[1]Department of Biological Sciences, Niger State Polytechnic, Zungeru, Nigeria.
[2]Department of Anatomy and Physiology, Niger State School of Nursing Bida, Nigeria.

*Correspondence: Email address: Yamann15@gmail.com; Phone number: 08030951435

ABSTRACT

The microbial assesment of mobile phones belonging to food vendors in different canteens in Zungeru town was investigated. One hundred and two (102) swab samples were randomly collected from the food vendors. Sterile swabs were firmly pressed on the handset both front and back and then inoculated into nutrient, MacConkey and Sabroud dextrose agar for bacteria and fungi growth. Microbial growth was observed in 80 samples (78.4%) and the remaining 22 (21.6%) were sterile. The predominant bacteria isolated were *Staphylococcus aureus* (47%), *Staphylococcus epidermidis* (33%), *Bacillus cereus* (15.6%), *Escherichia coli* (23.5%), *Pseudomonas aeruginosa* (7.8%), while the fungi isolated were *Penicillium notatum* (16.6%), *Aspergillus fumigatus* (8.8%), *Aspergillus niger* (20.5%), *Mucor sp.* (6.8%) and *Aspergillus flavus* (2.9 %). It was concluded that mobile phones of food vendors pose a potential health threat to consumers. Personal hygienic and sanitation measures such as hand washing, cleaning of the environment and washing of hand before and after handling of food and phone decontamination should be adopted by food vendors to prevent food borne disease.

Key words: Mobile Phone, Food Vendors, Bacteria, Fungi.

INTRODUCTION

The global system for mobile telecommunication (GSM) was established in 1982 in Europe with a view to providing and improving communications networks (Naubauer *et al*., 2005). Today, mobile phones have become one of the most indispensable accessories of professional and social life. Although they are usually stored in bags or pockets, mobile phones are handled frequently and held to the face (Yusha'u *et al*., 2008; 2010). The constant handling of the mobile phones by users makes it a breeding place for transmission of microorganisms as well as hospital-associated infections (Glodblatt *et al*., 2007; Yusha'u *et al*., 2010). Growing evidences have indicated that contaminated fomites or surfaces play a key role in the spread of bacterial infections (Kawo and Rogo, 2008; Kawo *et al*., 2009; 2012). The first study of bacterial contamination of mobile phones was conducted in a teaching hospital in Turkey with bed capacity of 200 and one intensive care unit (Karabay *et al*., 2007). Another study on mobile phones in New York has shown that t Mobile phones have become widely spread accessories in today's

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life. In 2013, more than 1.6 billion smart phones were in use worldwide, and it is estimated that this number will approximately double within the next 4 years (Strategy Analytics, 2013). In addition to the standard voice function of a telephone, mobile phones can support many additional services such as SMS for text messaging, email, pocket switching for access to the Internet, and MMS for sending and receiving photos and video. With all the achievements and benefits of the mobile phone, it is easy to overlook the health hazard it might pose to its many users (Tagoe *et al*., 2011). In addition, mobile phones might act as fomites as they are carried with their owner to places such as toilets, hospitals and kitchens, which are loaded with microorganisms (Bhoonderowa *et al*., 2014)**.**

Unlike fixed phones, mobile phones serve as a perfect habitat for the microbes to breed– providing higher temperature and humid conditions (Srikanth *et al*., 2009). Mobile phone usage has increased dramatically. In such environments where the percentage presence of bacteria is likely high, such as in hospitals, abattoirs, market places and toilets, this could enhance pathogen transmission and intensify the difficulty of containing disease spread (Butcher and Ulaeto, 2005). Sources of infection may be exogenous such as air, medical equipment, hands of surgeons and other staff, or endogenous such as the skin flora in the operative site, or rarely from blood (Ducel *et al*., 2002). The human skin is constantly in contact with microorganisms and becomes readily colonized by certain microbial species. The adult human is covered with approximately 2m2 of skin, with surface area supporting about 1012 bacterial cells/person (Mackowiak, 1982) he examined phones were found to harbor pathogenic microorganisms (Goldblatt *et al*.,2007). The usage of cell phone in Nigeria commenced in 2000.Since then, the number of subscribes has increased greatly to more than forty million in more than eight service providers. Thus, increases the number of base stations in villages and cities all over the countries (Nwadike, 2007). Research has also shown that mobile phone could constitute a major health hazard. In 2000, World Health Organisation (WHO), described electromagnetic radiation has been reported to alter the electric activity of the brain causing sleeplessness, headache, malaises, memory retentiveness and low sperm quality. It damages the DNA of manufacturing sperm cell.

Mobile phone has also been reported to be a reservoir for microorganisms. It has been reported that mobile phone can harbor more microorganisms than a man's lavatory seat, the sole of a shoe or a door handle. The combination of constant handling and the heat generated by phones creates a prime breeding ground for all sorts of microorganisms that are normally found in our skin. Soto *et al*. (2006) showed that mobile phones could be contaminated via source such as human skin or hand, bag, phone pouch, bags, pockets, environment and food particles, these sources are links through which microorganisms colonized the phone, thus causing diseases that range from mild to chronic. Although, microorganisms isolated so far by health researchers are mostly normal flora of the source of contamination, they can cause opportunistic infections. The aim of this present study is aim at investigating the microbial contamination of mobile phone belonging to food vendors in Zungeru town.

METHODOLOGY

Study Area

Samples were collected from the mobile phones for a period of 6 weeks duration during 1 July 2017 to 6 August 2017 in Zungeru town situated at 9.81^0 N Latitude, and 6.16^0 E Longitude, 147 meters elevation above the sea level. Zungeru is a small city in Niger State, Nigeria having about 24,447 inhabitants.

Sampling/Sampling Size

Sample size consisted of 102 mobile phones from food vendors were randomly collected from food canteens in Zungeru town.

Microbiological Analysis

Sampling was done using sterile cotton swab sticks. The swab sticks were rubbed all over the surface of mobile phones and immediately streaked on three plates of Nutrient agar and Potato Dextrose agar. The plates were incubated at 37°C for 24 hours for bacteria and 25 ^{0} C for 48 hours for fungi. Pure cultures of isolates were characterized based on Morphological description of colonies, gram stain reaction and biochemical tests were used for bacterial identification according to the conventional method described by (Cheesbrough, 2002).

Statistical Analysis

Data from this study was analysed descriptively using mintab version 21 software. Perrson Chi-Square test at 0.05 level of significance was used to determine the significant difference.

RESULTS

Out of 102 mobile samples of food vendors, growth was observed in 80 samples (78.4%) and the remaining 22 (21.6%) were sterile. Five bacterial isolates were detected including, 34(33%) *Staphylococcus epidermidis,* 48(47%) *Staphylococcus aureus*, 24(23.5%) *Escherichia coli*, 16(15%) *Bacillus cereus* and 8(7.8%) *Pseudomonas aeruginosa* as shown in (Table 1). There were fungal isolates as follows: 17 (16.6%) *Penicillium notatum*, 9(8.8%) *Aspergillus fumigatus*, 7 (6.8%) *Mucor spp*, and 3 (2.9%) *Aspergillus flavus* based on their mycelia, color and spores (Table 2). Post experimental analysis using Perrson Chi-Squre test at 0.05 level of significance Table (3) indicated the microbial growth isolated from mobile devices according to gender group , Single growth for males was 29(28.4%) and for females 18(17.6%), while multiple growth for males was 10 (9.8) and for females was 23 (22.5%).

Table, 1: Freuency of bacteria isolated from mobile phone

Bacteria	Mobile phones (n=102)	Prevalence rate (%)
Staphylococcus aureus	48	47
Staphylococcus epidermidis	34	33
Bacillus cereus	16	15.6
Escherichia coli	24	23.5
Pseudomonas aeruginosa	8	7.8

Table 2: Frequency of fungi isolated from mobile phone

Fungi	No of Occurrence	Prevalence rate (%)
Penicillium notatum	17	16.6
Aspergillus fumigatus	9	8.8
Aspergillus niger	21	20.5
Mucor sp.	7	6.8
Aspergillus flavus	3	2.9

Table 3: Microbial growth isolated from mobile devices, depending on the gender

	Growth No. (percentage%)			
Gender	Single microbes	Multiple microbes	Sterile	Total
Male	29 (28.4)	10 (9.8)	4 (.9)	43 (42.2)
Female	18 (17.6)	23 (22.5)	18 (17.6)	59 (57.8)
Total	47 (46.1)	33 (32.4)	22 (21.6)	102 (100)
P value		0.05*		

DISCUSSION

Microbiological standards in hygiene are necessary for a healthy life, most especially in food selling booths or canteen. However, practices that shift from normal standards of hygiene have been observed in both the developing and developed world. This investigation confirms such a deviation, as a variety of microbes were found on mobile phones. This present study concurs with that of Akinyemi *et al*. (2009) in Lagos, Nigeria in which food vendors and marketers' mobile phones had the highest rate of contamination when compared with the other groups of people whose mobile phones were sampled. The high prevalence of bacterial agents isolated from their mobile phones was attributed to the poor hygienic and sanitary practices associated with the low level of education among marketers and food vendors, especially those involved in handling raw meats and vegetables There was also a correlation between the microorganisms isolated in the work done by Walther and Ewald (2004) and the organisms found on the mobile phones of the food vendors of Ago-Iwoye town in that *S. aureus* had the highest occurrence in the mobile phone (50%) of the food vendors. Ekrakene and Igeleke (2007) also reported that *S.aureus* was isolated from mobile phones of health care staff and showed that infection may occur through this organism. The main reservoir of *S. aureus* is the hand, from where it is introduced into food during and after preparation (Hui *et al*., 2001). *S. aureus* is a well-known micro biota of the human skin which could be transferred into food via hand to hand or contact. It causes illnesses ranging from pimples and boils to pneumonia and meningitis which are not unlikely to be found in vendors and their consumers as corroborated by the high population of colonies. *Streptococcus faecium* apart from been a normal commensal of the skin, can also be found in the vaginal and intestinal tract, can cause diseases such as urinary tract infection, biliary tract, ulcers, abdominal wound, endocarditis and so on. *Bacillus cereus* is a normal flora of the water,vegetables, cereals and cooked food. It can cause food poisoning and opportunistic infections in immunocompromised persons. This undoubtly contributes a great deal to food prepared or eating with infected hands (Jay, 2000). *Escherichia coli* and *Micrococcus luteus* are both normal flora of the skin, soil and water. *E. coli* can also be found in the intestinal tracts. They are member of the coliforms, which presence on the mobile phones indicated the presence of faecal contamination. *E. coli* produces endotoxins which could aid their pathogenecity in man (Al-Abdalall, 2010). Therefore, the presence of *E. coli*, *S. faecium* and *Micrococcus luteus* in food sold by food vendors has shown that there is presence of faecal and soil contamination in the food during preparation or after preparation. This is in agreement with the work done by Roth and Jenner (1998). The presence of the isolated fungi showed that the food vendors' phones and environments had been contaminated by fungal spores. Ekrakene and Igeleke (2007) also

isolated some of these fungi in their research. Tiny food particles, soil particle and decayed dirt in the pouch or pocket over a long period of time will favour the growth of saprophytic fungus. These isolates can significantly cause food spoilage and food infection through the production of toxins. Most of the isolated fungi are also natural inhabitants of the soil and air. Their colonization of phones can cause infections like Aspergillosis, food intoxication, food spoilage and allergic reactions if these spores are introduced into foods, drinking water or inhaled (Flavia *et al*., 2001). Mobile phones could pose a great threat at food selling spots, hospitals, among children, etc. (Al-Abdalall, 2010). These pathogens may cause food borne infections, lowering of semen, brain disorder, cancer, headache , nosocomial infections, cell damages, etc.(Neely and Sittig, 2002).

The result of this study showed that microbial contamination of food vendors' mobile phones in different canteens in Zungeru town is high and these mobile phones may assume the vehicle of pathogenic agents of food borne disease outbreak and transmission. Food vendors are encouraged to adhere to strict personal hygiene and environmental sanitation in order to prevent disease outbreaks and transmission. Apart from those who sell foods, the phone user can personally contaminate his or her food when eating. For instance, using the phone while in the toilet or bathroom and thereafter going to eat food could easily lead to the contamination of the food, despite washing hands after using the toilet hence mobiles phones should not be taken to toilets, bathrooms or put on dirty surfaces. Control measures such as disinfecting mobile phone surfaces such as tables and other platforms where food is placed, regular hand washing and the wearing of gloves by the food vendor should be practiced.

Conclusion

Users of mobile phones are found everywhere: in the market, the home, hospitals, and schools. They could therefore, be the cause of the spread of the infection in the community. Our results indicate that isolates were associated with various strata of society. Today, mobile phones are important equipment for physicians and other health workers. Since restrictions on the use of mobile phones by HP is not a practical solution, many researchers suggest that adherence to such infection control precautions as hand hygiene should be strict. In addition, people should be informed that these devices may be a source for transmission of hospital-acquired infections to and from the community. Further studies for the possible means of decontamination of mobile phones, such as the use of alcohol and/or disinfection tissues, should be found and employed everywhere. Simple cleaning of computers and telephones with 70% isopropyl alcohol may decrease the bacterial load. Control measures are quite simple and can include engineering modifications, such as the use of hands-free mobile phones, surfaces that are easy to clean and disinfect, hand washing, and the wearing of gloves by the appropriate personnel.

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Investigating the Potentials of *Citrullus lanatus* seed as Phytobiotic to Improve Feed Efficiency, Growth Performance and Blood Biochemical Parameters in Broiler Chickens

***Kure H.M & Aliyu-Paiko M.**

Department of Biochemistry, IBB University, Lapai, Niger State, Nigeria.

*Correspondence: Email address: kurehajara@gmail.com; +2347065528343

ABSTRACT

Phytobiotics are compounds of plant origin used in animal nutrition to enhance productivity by improving nutritional and other properties. This study investigated the use of *Citrullus lanatus* whole seed powder as a phytobiotic to enhance growth performance, feed efficiency and some blood biochemical parameters on broilers. Sixty day-old chickens were grouped into three; A, B and C. Group A, the control received commercial diet only. Group B and C were fed commercial diet supplemented with 1% and 2% of *C.lanatus* powder, respectively. Both feed and water were offered *ad libitum.* Water consumed was measured daily, birds were weighed on a weekly basis and feed intake was measured weekly. At the end of the experiment, Results for haematological parameters showed a significant difference in white blood cell number and urea level. Growth performance of group C were higher compared to groups A and B, although this difference was statistically insignificant ($p>0.05$). Feed consumption and feed conversion ratios were similar for all groups. Haemoglobin, haematocrit and red blood cell number showed insignificant difference at ($p>0.05$). From the results of this study, it may be concluded that *C. lanatus* could improve the immune status of broilers and can be supplemented in feeds for broilers at 1% and 2% without interfering with the overall health and performance of the chickens.

Keywords: *Citrullus lanatus*, antibiotics, antioxidant enzymes, broilers.

INTRODUCTION

Antibiotics are common type of growth enhancers used in poultry industries. They are naturally occurring, semi-synthetic, or synthetic compounds with antimicrobial activity (Brooks *et al*., 2004; Kirbis, 2007). However, one of the drawbacks of excessive use of antimicrobial drugs is that they get accumulated in the tissues and organs of treated animals as residues and eventually become part of the food pyramid (Goetting *et al,* 2011).

Application of antibiotics in food producing animals can serve as a reservoir of antibiotic-resistant bacteria strains which may be transferred to humans (Marshall and Levy, 2011). Subsequently, the effectiveness of antibiotics in humans decreases, resulting in treatment failures (Aarestrup *et al*., 2008; Mellata, 2013). Due to the fear of creating cross resistance between animals and humans, the European Union has placed a ban on the application of antibiotics as growth promoters since 1st January 2006, which was followed by

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other parts of the world including North America (Yegani and Korver, 2008).

Restriction of antibiotic growth promoters has however, resulted in an increased incidence of enteric disorders such as necrotic enteritis and dysbacteriosis in poultry (Huyghebaert *et al*., 2011). Due to the restriction of antibiotic growth promoters, poultry industries have now focused on the use of biological products, including enzymes, probiotics, prebiotics, synbiotics, organic acids and plant extracts (phytobiotics), as alternatives to antibiotic feed additives in diets for monogastric animals (Bedford 2000; Wenk 2003, Rasschaert *et al*., 2016). Phytobiotics are plant-derived natural bioactive compounds used in animal nutrition as alternatives to antibiotic growth promoters that are added to feed to enhance performance in animals (Windisch *et al*., 2008; Jacela *et al*., 2010).

Researchers have documented the beneficial effects of phytobiotics on the performance of poultry (Denli *et al.,* 2004; Alçiçek *et al*., 2003; Jamroz and Kamel, 2002; Tucker, 2002). Tucker (2002) demonstrated a significant improvement of performance and survival rate in broilers fed diet with different types of plant extracts. Tollba *et al,* (2007) reported that at two, four and six week of age, the broiler receiving varying levels of black pepper showed better body weight gain. Many phytobiotics show promising results for applications in organic and conventional poultry production.

Watermelon (*Citrullus lanatus*) is a creeping plant belonging to the family cucurbitaceae. The center of diversity and possible center of origin of *Citrullus* is southern Africa (Erickson, 2005). It is mainly propagated by seeds. It is a tropical plant and requires a lot of sunshine and high temperature of over 25°C for optimum growth (Okonmah, 2011).

Watermelon fruit is known to be a very good source of Lycopene, a red pigment of the carotenoid class found in only a few fruits and vegetables, as it is a powerful oxygen radical scavenger and highly effective antioxidant (Gerster, 1997, Fraser and Bramley, 2004). It helps quench the free radicals that contribute to conditions like asthma, atherosclerosis, diabetes, colon cancer and arthritis. It is also high in fibre and citrulline; an amino acid the body uses to make arginine (Oyeleke, 2012). Watermelon seeds are known to be highly nutritional; they are rich source of protein, vitamin B, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others, as well as some phytochemicals (Braide, 2012).

METHODOLOGY

Overview of research: This research was carried out to know the effect of watermelon seed powder when added to poultry feed. The research answered some questions relating to growth performance and feed efficiency, effect of the seed powder on bird's blood, immunity and antioxidant enzymes. The research lasted for a period of 6 weeks which is a bench mark for starter period. This paper gives a vivid explanation of how the research was carried out and results obtained.

Experimental Location: This research was conducted at the animal research facility of Ibrahim Badamasi Babangida University, Lapai, Niger State. The experiment lasted for a period of six weeks.

Additive preparation: Watermelon seeds were retrieved from watermelon fruits, washed and

dried under room temperature. Dried seeds were grinded to the size of chikun super starter feed (commercial feed from Chikun Olam Feed Limited).

Experimental Animals: Sixty Day old broilers of CHI product were purchased from minna and transported to the animal research facility of Ibrahim Badamasi Babangida University, Lapai. Birds were kept in cages and allowed to acclimatize for 3 days before commencement of the research. Broilers were fed commercial feed from Chikun Olam Feed Limited and water was given *ad libitum*. Lighting system was provided from 7pm to 7am.

Experimental design and diet: After acclimatizing the birds for a period of 3 days, broilers were grouped into three, A, B and C, with each having a replicate group. Each group had 10 birds making a total of 60 broilers.

Group A and its replicate were control groups and consumed feed without the addition of watermelon seed powder (additive).

Group B and its replicate consumed feeds with the inclusion of 1% watermelon seed powder.

Group C and its replicate group consumed feed with the inclusion of 2% watermelon seed powder.

No further additive was included in their drinking water. Water given to them was fetched from taps within the school premises. Good hygiene was maintained throughout the 6 weeks of the experiment. Cages were cleaned on a weekly basis.

Broiler performance responses: Broiler chicken growth performance responses, such as body weight gain (BWG), feed intake (FI), mortality, feed conversion ratio (FCR) and specific growth rate (SGR) were determined on a weekly basis during the 6 experimental weeks. Growth performance was calculated by making use of the formulas below;

Weight gain (WG) = (W_f - W_i)

Where W_f = final weight

W_i = initial weight.

Feed intake was calculated by subtracting feed leftovers from total feed offered.

Feed intake (g) = $\frac{total\ feed\ intake}{number\ of\ birds}$

Feed conversion ratio was calculated by dividing the weight gain by feed intake expressed in the formula below:

Feed conversion ratio (FCR) = weight gain (g)/feed intake (g)

Mortalities (M) were recorded throughout the experimental period. Percentage mortality was calculated as:

Survival rate (%) = $\frac{number\ of\ birds\ that\ survived}{initial\ number\ of\ birds}$ × 100

Specific growth rate was calculated during the feeding trial, using the formula below:

Specific growth rate (SGR %) = [(ln W_f - ln W_i)/T] x 100

Analysis of blood samples: After 6 weeks of feeding trial, blood samples were collected from the right brachial vein into tubes without anticoagulant (3 birds from each group and its replicate combined to make a total of three samples per treatment). Serum was collected from the clotted blood and stored in a refrigerator. Activities of enzymes (alkaline phosphatase, superoxide dismutase, Alanine aminotransferase and aspartate aminotransferase) and concentrations of

metabolites in serum were measured photometrically using commercial kits (DIALAB diagnostics, Switzerland GmbH and AGAPPE diagnostics, Switzerland GmbH). Another sample of whole blood was collected with EDTA containing anticoagulant and this was used to analyze for haemoglobin (Hb), white blood cell (WBC), lymphocytes (LYM), mean cell haemoglobin (MCH), red blood cells (RBC), haematocrit (HCT) and mean cell volume (MCV) using the haematology analyzer, Abacus 380.

Statistical analysis: The data were analyzed by One-way ANOVA using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and the means were compared using Turkeys test. Results were expressed as treatment means with their pooled standard error of the mean (SEM). A probability value of $P < 0.05$ was described to be statistically significant.

RESULTS

None of the chickens died during the experiment, survival rate was 100 % throughout the experimental groups. Results for proximate composition are presented below.

Table 1: Result for proximate composition of experimental feeds and *C. lanatus* seed

Treatments	MC%	CF%	ASH%	FAT%	CP%	CHO%
0% WSP	8.44	4.90	4.30	14.70	23.62	44.04
1% WSP	7.56	7.70	6.40	6.49	13.10	28.87
2% WSP	8.14	8.80	7.30	13.17	30.62	31.97
WSP	5.40	33.80	3.35	30.25	23.62	3.60

Where MC=Moisture content, CF= Crude fibre, CP=Crude protein and CHO= Carbohydrate (Nitrogen free extract).
Control: Chikun commercial feed.
Treatment 1: Chikun commercial feed+ 1% watermelon seed powder.
Treatment 2: Chikun commercial feed + 2% watermelon seed powder.

Table 2: feed efficiency and growth performance of broiler chickens fed graded level of *C. lanatus* seed powder for 6 weeks starter period.

Treatments	0% WSP	1% WSP	2% WSP
Average initial weight [g]	70.25 ± 1.80^a	70.80 ± 1.84^a	71.70 ± 2.74^a
Average final weight [g]	2084.15 ± 52.37^a	1961.20 ± 117.11^a	2171.05 ± 42.98^a
Weight gain[g]	40,278	37,808	41,987
Feed conversion ratio	0.5560	0.5229	0.5756
Specific growth rate%	8.07	8.03	8.12
Feed intake [g]	72445	72307	72945

Data are expressed as mean ± SEM (n = 20).
Mean ± SEM followed by different letter within a row are significantly different (P <0.05).

Both initial and final average weight of broiler chickens fed different percentages of *Citrullus lanatus* seed powder for a period of 6 weeks showed an insignificant difference. Although the group fed the

higher percentage of *C.lanatus* recorded a higher average weight compared to the control group and 1% WSP group. Feed intake of 2% WSP was higher followed by 0% WSP and the least being 1% WSP.

Table 3: Water intake of broilers fed different percentages of *C. lanatus* seed powder for started period of 6 weeks

Treatments	Week1	week2	week3	week4	week5	week6	TOTAL
0% WSP	11,330	21,050	28,260	41,450	48,390	47,830	198,310
1% WSP	11,210	21,540	29,020	47,410	60,160	54,380	223,720
2% WSP	10,930	22,250	29,071	48,550	55,420	57,950	224,171

Table 3 shows the weekly water intake of birds. The group with the highest additive consumed more water followed by the group receiving 1% additive, with the least being the control group.

Table 4; Result for full blood count of broilers fed different percentages of *C. Lanatus* seed powder for started period of 6 weeks

GROUP	WBC	LYM	MID	GRA	RBC	HGB	HCT	MCV	MCH
CONTR	85.31±1.07^{a}	66.42±3.82^{a}	12.28±3.20^{a}	6.61±0.99^{a}	2.25±0.04^{a}	14.30±0.21^{a}	26.73±0.34^{a}	119.33±1.20^{a}	64.87±0.64^{a}
1%	54.37±1.93^{b}	51.33±5.72^{a}	8.82±0.87^{a}	4.54±0.97^{a}	2.41±0.09^{a}	15.03±0.34^{a}	28.17±0.73^{a}	117.60±1.73^{a}	62.53±1.21^{a}
2%	53.06±2.76^{b}	53.61±5.72^{a}	11.41±1.92^{a}	7.68±3.04^{a}	2.29±0.64^{a}	15.03±0.55^{a}	27.55±0.78^{a}	120.33±1.33^{a}	64.27±1.58^{a}

Table 5: Serum metabolic parameters of broiler chickens fed different percentages of *C. lanatus* seed powder for starter period of 6 weeks.

Treatments	0% WSP	1% WSP	2% WSP
Total protein (g/dl)	2.07±0.29^{a}	2.07±0.13^{a}	1.53±0.09^{a}
Creatinine (mg/dl)	1.77±0.14^{a}	1.57±0.12^{a}	1.60±0.15^{a}
Urea (mg/dl)	46.00±0.23^{a}	48.70±0.15^{b}	49.03±0.14^{b}
Potassium (mmol/l)	2.23 ± 0.22^{a}	3.73 ± 00.14^{a}	3.93 ± 0.37^{a}
Chloride (meq/l)	61.33±1.01^{a}	57.93±3.62^{a}	56.47±2.13^{a}
Sodium (meq/l)	117.03 ± 6.43^{a}	102.40 ± 23.36^{a}	139.07 ± 24.77^{a}

Data are expressed as mean ± SEM (n = 3).
Mean ± SEM followed by different letter within a column are significantly different (P <0.05).

Table 6: Hepatic/Renal function biomarker of broiler chickens fed graded level of *C. lanatus* seed powder for 6 weeks starter period.

Treatments	AST (µl)	ALT (µl)	ALP (µl)	BIL-TOTAL (mg/dl)	BIL-DIRECT (mg/dl)
0% WSP	13.90 ± 0.67 [a]	17.77 ± 0.33 [ab]	28.50 ± 0.45 [a]	1.90 ± 0.23 [a]	1.13 ± 0.03 [a]
1% WSP	15.07 ± 0.77 [a]	20.03 ± 0.47 [a]	27.87 ± 1.67 [a]	2.00 ± 0.29 [a]	1.57 ± 0.14 [a]
2% WSP	14.37 ± 1.22 [a]	17.10 ± 1.13 [b]	29.87 ± 2.33 [a]	2.23 ±0.78 [a]	3.63 ± 2.04 [a]

Values were expressed as mean ± SEM of 3 determinations. Columns with same superscripts are significantly the same while those with different superscripts were significantly different ($P < 0.05$).

DISCUSSION

The result for proximate composition of watermelon seed is presented on Table 1. The moisture content was found to be 5.40, fat 30.25, fiber 33.80, protein 23.60 and carbohydrate 3.60. This result varies from results for proximate analysis of *Citrullus lanatus* obtained by Oyeleke *et al.* (2012), the possible reason for such a difference could be attributed to varietal and regional differences. The addition of watermelon seed powder to chikun feed increased crude fiber, and crude protein. Therefore, birds consuming feed with watermelon seed powder at the different percentages received more fiber and protein than the control group fed only chikun commercial feed. The survival of all chickens from all treatment is an indication that *C.lanatus* may be free from any toxic substances. No vaccines or antibiotic growth promoters were used in this present study.

Table 2 shows the result for feed efficiency and growth performance of broiler chickens fed graded level of *C. lanatus* seed powder for 6 weeks starter period. There was no significant difference observed in both initial and final average weight of broiler chickens fed different percentages of *Citrullus lanatus* seed powder. Although the group fed the highest percentage of *C.lanatus* recorded a higher average weight gain compared to the control group and 1% WSP group. However, this result is not in agreement with the findings of Guo *et al.* (2000), Jamroz and Kamel (2002) who reported that herbs and herbal products had a significant positive effect on broiler body weight gain.

Generally, it has been shown that inclusion of phytogenic compounds in broiler chicken diets improves feed conversion ratio (Fallah *et al*., 2013; Mountzouris *et al.,*2011). Although other findings contradicts this statement, a study on phytobiotics suggests that inclusion of 0.2% peppermint or thyme (w/w) characterized by menthol and thymol (70 mg/kg diet) in broiler diets does not affect feed intake and feed conversion ratio (Ocak *et al*., 2008), which is also in line with this present study. Feed intake of 2% WSP was higher followed by 0% WSP and the least being 1% WSP

Table 3 shows the total water consumed during the 6 weeks of the experiment. Water, in addition to being a vital nutrient, is involved in many aspects of poultry metabolism including body temperature control, digestion and absorption of food, transport of nutrients, and the elimination of water products, via urine,

from the body (Jafari *et al.,* 2006). The group receiving 2% of the additive had the highest water consumption of 224,171 litres compared to control group (198,310 litres) and group B (223,720 litres). This could be due to the added fibre in experimental diets as factors like dietary fibre content and its characteristics primarily affect water intake (Dirk *et al* 2013). Also, High levels of dietary crude protein stimulate water intake, as demonstrated in young broilers by Marks and Pesti (1984)

Table 4 shows the result for full blood count. Blood parameters are major indices of physiological, pathological and nutritional status of an organism; changes in the constituent compounds of blood when compared to normal or reference values could be used to interpret the metabolic stage of an animal as well as quality of feed (Wheater *et al*., 1987). All three experimental groups were within the normal range of HCT (26.1 to 29.5%) as reported by Ikhimioya *et al*. (2000), birds did not differ significantly in HCT, HB and RBC values. Values for HCT range from 26.73 to 28.17, HB from 14.30 to 15.03 and that of RBC ranged from 2.25 to 2.41 with control group having the least HCT, HB and RBC numbers. Inadequate intake of energy and protein decreases HCT and haemoglobin (Hb) concentration which indicates anaemia (Rastogi, 2007; Muhammad and Oloyede, 2009). This revealed that birds in groups B and C consumed more energy and protein and were not prone to anaemia. The MCV value in all the treatment groups in this study fall within the normal range of 90 to 140 fl, this finding is in line with what has been earlier reported by Tuleun *et al.* (2007) in broiler chickens. The results for WBC count showed significant ($p > 0.05$) difference between control group and the treatment groups, this is in line with the work of Azine *et al* 2018 who supplemented broilers diet with 1 mg and 2 mg of Monosodium glutamate.

Table 5 shows the result for Serum metabolic parameters of broiler chickens fed different percentages of *C. lanatus* seed powder for starter period of 6 weeks.

Blood biochemical analysis is widely used to assist the diagnosis and characterization of diseases in most animal species. However, it is an important tool, as some metabolic disorders are difficult to detect only by clinical signs (Andreasen *et al*., 1996). It can also assist the monitoring of poultry health, the diagnosis and treatment of diseases, and to assess their health status (Schmidt *et al*., 2007). The amount of creatinine in the serum of experimental birds was statistically the same. This suggests the safety of *C. lanatus* on renal function. According to Aslam *et al*. (2010), an increase in serum creatinine is indicative of poor renal function. The serum total protein levels of the treatment groups were unaffected by the addition of *C. Lanatus* to diets in this study. This result is consistent with that of Erdog˘ an *et al* 2010 who also reported that serum total protein level was unaffected by the addition of synbiotics and phytobiotics to poultry diets

The results for serum urea showed a significant difference between the control group and the groups receiving *C.lanatus* additive. This is due to the increase in protein content brought about by the addition of *C.lanatus* seed powder. Abdourhamane *et al* 2016 revealed that cake from cucurbits seeds are a good source of proteins and could be used in poultry feed. Iyayi and Tewe (1998) showed that serum urea and total protein depend mainly on the quality and quantity of protein in the diet.

Hepatic/renal function biomarkers of broiler chickens fed graded level of *C. lanatus* seed powder for 6 weeks starter period showed that the Amounts of ALT, AST and ALP in supplemented birds with different levels of *C. lanatus* were not significantly different compared to control group (table 6). Alanine Amino Trasferase (ALT) and AST considered as liver enzymes indicating liver damage, thus no increase in serum concentration of ALT and AST may provide evidence of liver protection against hepatocellular degeneration (Al-Jaff., 2011). This result is in line with that obtained by Tollba *et al* 2010 who reported that adding the aromatic herbal extract to broiler diet did not alter the ALT and AST when compared to control. Similarly, Moomivand *et al*. 2015, also reported that Amounts of ALT, AST and ALP in birds supplemented with different levels of drinking thyme essence were not significantly different from the control group.

Based on the results of this study, it can be concluded that *C. lanatus* supplementation improves the immune status of broilers, thereby making them more resistant to infection and can be used in feeds for broilers at 1% and 2% without interfering with the overall health and performance of the chickens.

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Performance of Broiler Chickens Fed Varrying Levels of Decorticatted Baobab (*Adansonia digitata)* Seed Meal Diet At Starter Phase

***Ango H.S., Malik A.A. & Adama J.**

Department of Animal Production, Federal University of Technology Minna, Nigeria.

*Correspondence: Email address: hauwaango@gmail.com; **Telephone: 08036552572**

ABSTRACT

A research study was conducted to assess the growth performance and nutrient digestibility of broiler chicken fed varying levels of decorticated *Adansonia digitata* (baobab) seed meal diets. A total of 117 day-old chicks were randomly allocated to three treatments in a completely randomized design experiment. Each treatment was replicated three times with thirteen birds per replicate. The treatments were control (0 % inclusion level), 9 % inclusion level and 18 % inclusion level tagged as T1, T2, and T3 respectively. Feed and water were supplied *ad libitum*. The results show there were no significant ($p>0.05$) differences in initial weight, final weight and total weight gain among the birds fed the three dietary treatments but the mortality differed significantly ($p<0.05$). The digestibility of the crude fibre content, ether extract and nitrogen free extract as well as the total digestible nutrient differed significantly ($p<0.05$) among the treatments. It can be concluded that the inclusion of decorticated Baobab seed meal at 9 % and 18 % in broiler starter diets as a protein source is detrimental to the growth performance and nutrient digestibility of the birds. Better processing methods should be employed to reduce the anti-nutritional factors present in the seed meal.

Keywords: Broiler chicks, decorticated Baobab seed, growth and nutrient digestibility.

INTRODUCTION

Feed is one of the most important inputs in all livestock production systems (Gatenby, 2002). Recently, there has been an increased competition for feed ingredients that are used in manufacturing animal feed since some of these ingredients are also used as human food (Gadzirayi *et al.,* 2012). The population of the world is expected to increase by about 29 % from the current 7 billion to 9 billion in 2050 (Meissner *et al.,* 2013). Hence, there is great need to look for alternatives that can compensate the high demands for such ingredients.

Some multipurpose trees have nutritional properties that can be beneficial if incorporated in livestock feed. Utilizing some of these to their full potential can result to sustainable livestock production (Melesse *et al*., 2011). One such example is the baobab tree it has been used for many traditional purposes but little has been said about its potential as an animal feed resource (Osman, 2004). It is a tree that can grow for years and capable of adapting to harsh conditions.

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Feed constitutes about 70 % of the entire production in poultry and of the total cost; up to 95 % is required to meet the protein and energy requirements (Gadzirayi *et al*., 2012; Mohanta, 2012). Voluntary intake, feed digestibility and animal performance can be improved by using alternative low quality multipurpose trees (Melesse *et al*., 2011). Seed and leaf meals function as protein sources and aid in providing some essential vitamins, minerals, oxycaretenoids as well as bioactive compounds that function at cellular level (Melesse *et al*., 2013). African baobab seeds have been shown to be a superb source of protein, with most of the essential and non-essential amino acids (De Caluwé *et al*., 2010). From previous reports, the baobab seed cake is a potential low-cost and locally available protein source for livestock feeding (Chimvuramahwe *et al.,* 2011).

In most developing countries, the major sources of protein in commercial poultry production are fishmeal (FM) and oil seed cakes. However, these are usually scarce, expensive and used extensively by other livestock and humans. Nutrition accounts for 60-70 % of the total production cost in modern poultry production systems (Smith, 1990). Furthermore, feeding has a great effect in poultry growth, egg production and meat quality. This situation has created a need to look for cheap, locally available and less competitive substitutes to some ingredients of poultry feeds and in particular, sources of protein. There is continued scarcity and consequent high prices of conventional protein (soya beans) and energy sources for livestock in the tropics (D'Mello *et al.,* 1987) and this hinders poultry production. As a result of this, the use of Multipurpose Tree Species (MPTs) in poultry feeding by livestock farmers in northern Nigeria has become a normal practice. However, the feeding values of some of these trees is not known and one of them is *Adansonia digitata.*

The use of leguminous Multipurpose Trees (MPTs) and shrubs has been suggested to be a viable alternative source of proteins, vitamins and minerals for poultry feeding (Church, 1991). A review of available literature shows that *Leucaena leucocephala, Gliricidia sepium, Sesbania sesban, Manihot esculenta* have been widely used in feeding non-ruminants and especially poultry resulting in improvement of their productivity (Lopez, 1986; D`Mello et al., 1987). However, the use of MPTs are limited by their high fibre contents and in some cases, presence of toxic factors or metabolic inhibitors.

This research was therefore aimed at determinining the performance of broiler chicken fed different levels of decorticated baobab (*Adansonia digitata*) seed meal diet at starter phase.

METHODOLOGY

Experimental Site

This research study was carried out at the Animal Production Teaching and Research Farm of the Federal University of Technology, Minna, Niger State. Minna, which lies between latitude 9^{o} 28' N and 9^{o} 37' N and longitude 6^{o} 23' E and 6^{o} 33' E, and has a temperature range of 38^{o} to 42^{o} C, with lowest temperature in August and highest in March. It has a mean annual rainfall range of between 1000 mm – 1500 mm. Minna is within Southern Guinea Savannah Ecological Zone (FUTMIN, 2012).

Experimental Feed Preparation and Formulation

Baobab *(Adansonia digitata)* fruits (kuka) was be obtained from Gwari market in Minna Bosso Local Government of Niger State and crushed, to obtain the seeds. Maize, maize offal and groundnut cake were equally purchased from Gwari market. Processing of the baobab was carried out in accordance with the procedure of Emmanuel (1995).

Baobab *(Adansonia digitata)* seeds was soaked in water to dissolve the pulp, and was gently scrubbed in order to obtain the soft kernel. The clean seeds were then boiled at 100^{o} for 1 hour after which the seeds were soaked for 12 hours and dehulled by rubbing between the palms to remove the hard seed coat. They were then rinsed with water and sun dried, after which they were milled using an attrition mill and stored in air tight containers.

The Baobab *(Adansonia digitata)* seeds were used to formulate a complete diet with 0, 9, and 18 % inclusion levels which will be designated as treatments A, B, and C respectively. The gross compositions of the experimental diets are shown in Table 2.

Table 1**:** below shows the Proximate composition of decorticated *Adansonia digitata* seed meal

Parameters	Decorticated *Adansonia digitata* seed (%)
Dry mater	91.68
Crude protein	36.75
Crude fibre	15.60
Ether extract	18.30
Ash	12.32
Nitrogen free extract (NFE)	8.71
Calculated ME kcal/kg	3146.86

Table 2: Gross compositions of the experimental diets containing varying levels of decorticated *Adansonia digitata* seed meal

Ingredients (%)	Treatment (%)		
Gross Composition	A	B	C
AS seed meal	0.00	9.00	18.00
Maize	44.5	53.00	53.00
Maize offal	11.00	4.00	2.00
Groundnut cake	25.00	17.0	10.00
Full fat soya	10.00	7.50	8.30
Fish meal	3.00	3.00	3.00
Lime stone	1.00	1.00	1.00
Bone meal	2.00	2.00	2.00
Palm oil	2.00	2.00	3.00
Lysine	0.25	0.25	0.25
Methionine	0.25	0.25	0.25
Salt	0.50	0.50	0.50
Premix	0.50	0.50	0.50
Total	100.00	100.00	100.00
Calculate Analysis			
Crude protein	22.14%	22.00%	22.00%
Metabolizable energy (kcal/kg)	2989.35	2937.66	2768.33
Crude fiber	3.83	3.09	2.84
Ether extract	6.20	5.84	5.64

AS = *Adansonia digitata*

Experimental birds and Their Management

A total of one hundred and seventeen (117) day old broiler chickens were bought from CHI farms along Lagos-Ibadan express way, Ibadan, Oyo State. Each replicate contained 13 birds. The birds were fed with the experimental diets; fresh and clean water was provided *ad-libitum*. The housing system was intensive (deep litter) with concrete floor, covered with wood shavings, equipped with adequate feeders and drinkers. The pen was cleaned and washed with water and detergents. The pen was disinfected with formaldehyde solution to get rid of

harmful organisms a week prior to the arrival of the chickens. The birds on arrival were randomly apportioned into brooding pen; three treatments with three replicate each.

Appropriate drugs and vaccines were administered when due. Artificial lighting was provided for 24 hours throughout the brooding period with the use of rechargeable lamps. Heat was provided with the aid of charcoal and kerosene stoves. Regulation of temperature was done by observing the behaviour of the chicks.

Experimental Design

One hundred and seventeen (117) day old chicks were randomly allocated to 3 treatment diets with thirteen (13) birds in each replicate with three replicates per treatment based on a Completely randomized design (CRD) as outlined by Steel and Torrie (1980). The diets contained 0, 9 and 18 % dietary inclusion levels of *Adansonia digitata* seed meal forming diets A, B and C respectively.

Data Collection

Average feed intake of broiler chickens

The feeding was designed to last for a period of 8 weeks during which daily records of feed intake and live weight changes were taken. The birds were weighed before the experiment began. Daily record of feed intake was obtained by subtracting the left over feed from the amount offered every day throughout the period of the experiment. Record of body weight gain was obtained from subtracting the initial weight from the new weight of the animal after every week. This continued throughout the experimental period. The records of daily day and weekly feed intake was obtained for each of the replicate and mean weekly feed intake per/birds was calculated.

$$\text{Avearage feed intake (g)} = \frac{Quantity\ of\ feed\ given\ (g) - Quantity\ of\ leftover\ (g)}{Number\ of\ birds}$$

Average body weight gain of boiler chickens

The initial-weight of the birds was recorded at the beginning of the experiment, and the birds were weighed weekly thereafter. Body weight measured at the end of the previous week was deducted from that of the current week to obtain the weight gained / bird / week.

Feed conversion ratio (FCR)

To determine the FCR, average feed intake was divided by the average weight gain. It is expressed mathematically as:

$$FCR = \frac{Mean\ feed\ intake\ (g)}{Mean\ weight\ gain\ (g)}$$

(Esonu *et al.,* 2010)

Digestibility trial

The last seven days of the research work was used for digestibility trial. Two birds were randomly picked from each replicate to determine the nutrient digestibility. Total collection method was used. The birds were managed using specially designed metabolism cages. The first three days were adjustable period. A known measure of feed was fed to the animals per day; the rejected feed was also measured. Feacal sample were collected for four days. These were oven-dried for 24 hours at temperature of 50^{o}C. The oven-dried samples were probed per replicate at the end of the week and representative sample were taken for proximate analysis. Digestibility coefficient of the nutrients was calculated as follows;

$$Digestibility\ Coefficient = \frac{Nutrient\ intake\ in\ feed - Nutrient\ voided\ in\ droppings}{Nutrient\ intake\ in\ feed} \times 100$$

(Aduku and Olukosi, 1990)

Proximate Analysis

Representative samples of the experimental diets and the collected feacal samples during the digestibility trial were analyzed for their proximate components as outlined by AOAC (1990) and Vansoest *et al*., (1991), respectively.

Statistical Analysis

The data generated was subjected to Analysis of Variance (ANOVA) using completely randomized design (CRD) as outlined by Steel and Torrie (1980). Duncan's multiple range test (DMRT) was used to separate the means (Duncan, 1955).

RESULTS

Proximate composition of experimental diet fed to broiler chicken

The result of the proximate composition of the experimental diets fed to broiler chicken is shown in Table 4.1. The result shows that the dry matter of the diets ranged from 93.08 - 95.20 %, the crude protein values ranged from 19.25 - 20.12%, crude fibre 6.1 – 7.4%, ash ranged from 5.37 – 9.4%, ether extract 8.61 – 8.94% and nitrogen free extract from 49.58 - 54.62% respectively.

Dry matter content in treatment T_1 T_2 and T_3 were similar values. Crude protein content for treatments T_1 and T_3 were alike while that of T_2 was slightly higher. Values obtained for treatments T_1, T_2R_1 and T_2R_2 had similar crude fibre contents that of T_2R_3 and T_3 were also similar. Treatment T_3 had the highest content of ash followed by treatments T_2 and then T_1. Ether extract content for the treatments T_1, T_2 and T_3 were similar. Nitrogen free extract (NFE) content for treatment T_1 was the highest while treatments T_2 and T_3 had similar values.

Table 4.1 Proximate composition of the experimental diet

TREATMENT	%CP	%ASH	%MC	%FAT	%CF	%NFE	DM%
T1R1	19.25	5.37	5.1	8.86	7.2	54.22	94.9
T1R2	19.25	5.38	4.95	8.9	7.3	54.42	95.05
T1R3	19.25	5.39	4.8	8.94	7.4	54.62	95.2
T2R1	20.12	8.65	5.32	8.89	7.1	49.72	94.68
T2R2	20.12	8.65	5.11	8.75	6.9	50.47	94.89
T2R3	20.12	8.65	4.9	8.61	6.7	51.22	95.1
T3R1	19.25	9.4	4.98	8.91	6.7	50.76	95.02
T3R2	19.68	8.9	5.95	8.9	6.4	50.17	94.05
T3R3	20.11	8.4	6.92	8.89	6.1	49.58	93.08

Diet (T_1) = 0% inclusion level of decorticated baobab
Diet (T_2) = 9% inclusion level of decorticated baobab
Diet (T_3) = 18% inclusion level of decorticated baobab
NFE = Nitrogen free extract

Growth Performance of Broiler Chickens Fed Varrying Levels of Decorticated Baobab (*Adansonia digitata*) Seed Meeal Diets at Starter phase

The result of the effect of feeding diets containing varying levels of decorticated baobab seed (DBS) meal diets on the performance of broiler chickens fed for 28 days is presented in Table 4.2. The result shows that decorticated baobab seed meal diet had no significant effect (P<0.05) on initial weight, final body weight, body weight gain, total feed intake and feed conversion ratio. Mortality was significantly higher (P < 0.05) in birds fed diets containing decorticated baobab seed meal diets than those fed control diet.

Table 4.2 Growth performance starter phase

Parameters	T1	T2	T3	P-value	SEM	LOS
Initial weight	57.92	57.36	57.84	0.36	0.16	NS
Final Weight	202.44	199.02	184.52	0.74	8.80	NS
Total weight gain	144.52	141.66	126.68	0.73	8.83	NS
Total feed intake	630.92[a]	474.36[ab]	524.89[b]	0.97	31.38	NS
Feed conversion ratio	4.43	3.60	4.15	0.58	0.30	NS
Mortality	0.00	23.07	82.05	0.00	12.70	*

[abcd] = means in the same row with different superscripts are significantly different (P<0.05)
SEM = standard error of means
P-Value = probability value
Diet (T_1) = 0% inclusion level of decorticated baobab seed meal
Diet (T_2) = 9% inclusion level of decorticated baobab seed meal
Diet (T_3) = 18% inclusion level of decorticated baobab seed meal

Nutrient digestibility of Broiler Chickens Fed Varying Levels of Decorticated Baobab (*Adansonia digitata*) Seed Meeal Diets at Starter phase

Table 4.3 Nutrient Digestibility Starter phase

Parameters	T1	T2	T3	P-value	SEM	LOS
Dry matter (%)	92.16	84.55	88.09	0.16	1.63	NS
Crude protein	90.01[a]	83.27[ab]	8O.42	0.08	1.88	NS
Crude fibre	81.45 [a]	68.36 [ab]	59.24 [b]	0.04	4.01	*
Ether extract	96.02 [a]	90.75 [b]	90.12 [b]	0.04	1.15	*
Ash (%)	73.77	68.08	67.00	0.60	2.65	NS
NFE (%)	95.60	89.78	87.92	0.03	1.38	*
TDN (%)	94.54	84.66	81.70	0.01	2.15	*

[abc] = means in the same row with different superscript were significantly different (P<0.05)
SEM = standard error of means
P-Value = probability value
Diet (T_1) = 0% inclusion level of decorticated baobab seed meal
Diet (T_2) = 9% inclusion level of decorticated baobab seed meal
Diet (T_3) = 18% inclusion level of decorticated baobab seed meal
NFE = nitrogen free extract
TDN = Total digestible nutrient

Table 4.3 shows nutrient digestibility of broiler chickens fed varying levels of decorticated baobab seed meal diet at starter phase. The result showed that decorticated baobab seed meal diet had significant effect ($P < 0.05$) on crude fibre, ether extract, nitrogen free extract digestibility and on total digestible nutrient (TDN). Dry matter, crude protein and ash digestibility showed no significant difference ($P < 0.05$) among the treatment groups. The result of crude fibre indicated that T_2 had the best significant ($P < 0.05$) performance over the treatment groups followed by T_2 then T_3. However, in TDN, treatments one (T_1) the control group recorded significantly highest values over all other treatment groups.

Dry mater, crude protein and ash were not significant across treatments. However, crude protein digestibility was lower in T_3 than in the other treatment. Ether extract decreases slightly with increase inclusion of decorticated baobab seed meal diet up to T_3.

DISCUSSION

ANOVA showed that there was better performance ($P < 0.05$) from broiler chicks fed the control diets compared to those fed decorticated baobab seed (DBS) based diets, this is in agreement with the findings of (J Chimvuramahwe,*et.,al* 2011) who reported that ANOVA showed better performance ($P < 0.05$) from broiler chicks fed the control diet as compared to those fed baobab seed cake based (BSC) diets. (J.Chimvuramahwe, *et.,al* 2011). The highest feed intake was recorded in broilers fed 0 % (DBS) based diet across the experimental period (four weeks).This is in line with the report of (J.Chimvuramahwe, *et.,al* 2011). Feed intakes of broilers fed BSC0 based diet were significantly different ($P < 0.05$) from those fed BSC5, BSC10, and BSC15 based diets in weeks three to eight.

The results of this experiment were in line with the findings of Mwale et al. (2008) Chimvuramahwe et al., 2011; Sola-Ojo et al., 2013; Saulawa et al., 2014) who stated that Inclusion levels that are beyond 10% in a monogastric animal diet can result in a decline in intake and conversion rate of feed (Mwale et al., 2008; Chimvuramahwe et al., 2011; Sola-Ojo et al., 2013; Saulawa et al., 2014). It is because the seed has an unappreciable amount of fibre and some anti-nutritional factors that are discouraged for poultry diets. Therefore inclusion rates higher than 10% cause a cumulative increase in fibre and anti-nutritional factor levels that cause detrimental effects on poultry performance and even increase mortality rates (Osman, 2004; Nkafamiya et al., 2007; Mwale et al., 2008; Ezeagu, 2009; Gadzirayi *et al.,* 2012).

Baobab seeds contain some anti-nutritional factors, such as phytate (2%), oxalate (10%), tannins and saponins (3-7%) which reduces digestive efficiency and utilization of dietary nutrients in poultry. (Price Chisoro *et al., 2017).*According to Mwale et al. (2008) and Chimvuramahwe et al. (2011) the effects of these nutritional factors of the baobab seed meal on growth, nutrient digestibility and meat quality of broilers is scarce and unidentified. Even though the best performance is noted when birds are fed diets at 5-10% inclusion levels, Chimvuramahwe et al. (2011) and *Prince Chisoro et al. (2017)*

Similar to the findings of this research, Baobab seed inclusion beyond 10 % in the diet brought about a decline in feed consumption and conversion rate (Mwale et al., 2008). Adewusi and Matthew (1994) reported that increase in

dietary fiber content in rat diets resulted in a corresponding decrease in feed consumption, conversion rate and true digestibility. Their analysis however, showed that the diets had more crude fiber, which is discouraged in poultry diets (Mapiye et al., 2008), and this could be one of the reasons why the highest mortality was recorded from 18 % Decorticated Baobab Seed (DBS) based diet. Mortality was highest in birds fed on the highest Baobab seed inclusion level. This can be attributed to the cumulative effect of anti-nutritional factors to toxic levels since baobab seed cake contains some anti-nutritional factors such as oxalate, phytate, saponins and tannins (Nkafamiya et al., 2007).

The high mortality rate in this study can be attributed to the processing method adopted for Baobab seeds.. Similarly, the findings of Adeyemo and Oyejola (2004) reported about 50% mortality of birds from day old to eight weeks.

Broilers fed 9 % and 18% DBS diets had significantly lower feed intake compared to those on control diet. This can be attributed to high fat content of DBS. Baobab seed contains high energy (3000 – 4500 kCal/kg (Murray et al., 2001; Nkafamiya et al., 2007). These findings agree with Lesson (2000), Veldkamp et al., (2005) and Nahashon et al., (2005) who found out that birds consume feed to primarily meet their energy requirement. This is also consistent with Plavnik et al., (1997) and Nahashon et al., (2006) who suggested that as dietary energy increases; birds satisfy their energy needs by decreasing feed intake. Decrease of feed intake with high energy in diets was supported by Veldkamp et al. (2005) who showed that feed intake decreases linearly as dietary energy increases. However, BSC diets were acceptable to the broiler chicks even at the highest inclusion level (18% BSC). This may be attributed to the good aroma of this cake. The baobab seed cake has a good aroma that improves feed intake (Booth and Wickens, 1988).

The highest feed intake obtained in treatment fed on control diet could have been due to the diet's palatability and nutrient composition (Acamovic, 2001). The low feed intake of the diets in early growth stages (week 1 to 3) could be attributed to the underdeveloped gastro-intestinal tract and adaptation of chicks to the feed. Research has shown that digestibility of feeds in poultry increases with age (Corless and Sell, 1999). Both protein and energy requirements for growth and development increase with age (Pal and Singh, 1997), thus broiler chicks increase their feed intake to meet this requirement. Feed intake is a major factor that influences both the body weight gain and feed conversion rate in meat-type poultry (Nahashon et al., 2006; Nkafamiya et al., 2007). The Body weight gain of broilers was high in those fed on control diet which significantly increased from week1 to 4. This may be attributed to high feed intake value reported for the control diet.

Contrary to the findings of this research, Saulawa *et al*. (2014) reported that even higher inclusion levels above 10% chicks can still tolerate the diet due to the good aroma of the baobab seed meal which improves feed intake (Mwale *et al.,* 2008; Belawu and Ibikunle, 2009; Chimvuramahwe et al., 2011; Madzimure *et al.,* 2011; Saulawa *et al*., 2014). The other contributing factor to having an inclusion level of 5-10% is due to the fat content of the baobab seed oilcake. Increased inclusion levels result in lowered feed intake since birds are known to consume feed principally to meet their energy requirements. High energy diets in birds,

frequently as a result of the relative fat content, on average have, reduced feed intakes owing to the decreased ease of passage rate of digesta though the gut (Nahashon et al., 2006). Therefore, there is an inverse relationship between the energy content of the diet and bird feed intake. Nahashon et al. (2006) and Nkafamiya et al. (2007) noted that feed intake in meat-type birds is an important factor that has influence on both body weight gains and feed conversion rates.

Conclusion

It can be concluded that the inclusion of decorticated Baobab seed Meal Diets at 9% and 18% in broiler Starter diets as a protein source is detrimental to the growth performance and nutrient digestibility of the birds. Better processing methods should be employed to reduce the anti-nutritional factors present in the seed meal.

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Prince Chisoroa, Tinashe Kagurua, Never Assanb,* *aDepartment of Livestock and Pasture Science, University of Fort Hare, Private Bag X1314, Alice, Eastern Cape, South Africa. bZimbabwe Open University, Department of Agriculture Management, Faculty of Agriculture, Bulawayo Regional Campus, Box 3550, Bulawayo, Zimbabwe.*

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Evaluating the Potentials of *Carica papaya* seed as Phytobiotic to Improve Feed Efficiency, Growth Performance and Serum Biochemical Prameters in Broiler Chickens

***Muazu U. & Aliyu-Paiko M.**

Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria.

*Correspondence: Email address: umarmuazu1321@gmail.com; Telephone: +2347063529882

ABSTRACT

Phytobiotics are plant derived products added to feed to improve the performance of farmed animals. They exhibit variety of properties, including antioxidant, growth promoting, appetite enhancing properties and stimulation of enzyme activities. This study investigated the potentials of *carica papaya* seed powder (PSP) to improve feed efficiency, growth performance and serum biochemical activities of broiler chickens. Sixty day old chickens were randomly distributed into three treatments and fed diets (commercial starter feed) mixed with 0, 0.5 and 1% PSP for the six weeks starter period. Feed and Water were offered to the birds in all the different treatments *ad-libitum*. Feed consumption, growth performance and water intake were monitored weekly when cages were also cleaned. Lipid peroxidation was also assayed in the serum of the chicks from all treatments. At the end of the experiment, results showed that feed efficiency and growth performance of broilers were not significantly ($p>0.05$) influenced by dietary treatments. There were also no significant ($p>0.05$) influence of PSP on serum biochemical parameters such as SGPT, SGOT, ALP, bilirubin, albumin, creatinine, proteins, potassium, chloride, sodium and urea. Furthermore, lipid peroxidation decreased significantly ($p<0.05$) in treatments with PSP when compared to the control. From the results of this experimentation, feeding broiler chickens with diets mixed with PSP significantly and positively reduced serum lipid peroxidation profile without negatively affecting feed efficiency, growth performance and serum biochemical parameters. It may be concluded that supplementation of PSP has potentials as antioxidant when supplemented in the diets of broiler chickens.

Keywords: Phytobiotic, Pawpaw seed powder, broiler, antioxidant.

INTRODUCTION

Poultry farming refers to the process of rearing domestic birds such as chickens, ducks turkeys, geese, etc for the purpose of farming meat or eggs for food (Beutler, 2007). Over 50 billion chickens are raised globally per annum as source of food for both meat and eggs (Atteh, 2004). Poultry raised for meat are often called broilers while those raised for eggs are usually called layers. In Africa, agriculture and agro-industries account for over 30% of national incomes on average, as well as for the bulk of export revenues (Heise *et al*, 2015). Nearly three-quarters of the African population depend on agriculture to secure their livelihoods (Connolly, 2014; Oram, 2012).

As a result of high population growth in Africa and the growing income, the demand for eggs

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and poultry meat has significantly increased in recent years across large parts of the African continent (WHO, 2010). Based on estimates by the United States Agency for International Development, this trend is likely to continue over the next few years (Heise *et al,* 2015). Therefore, the consumption of poultry and eggs is projected to increase by about 200% between 2010 and 2020 in some countries in sub-Saharan Africa, including Nigeria (USDA 2013; Obi, 2003).

To increase production, use of feed additives in animal feeding began in the 1940s, to improve the organoleptic characteristics of raw materials, fodders, and/or animal products, and to prevent diseases (US Food Administration 2009; Sapkota *et al,* 2007). Additives are also used to improve production efficiency by decreasing the mortality rate and stimulating weight gain (Upadhayay and Vishwa, 2014; Castanon, 2007; Dibner and Richards, 2005). Additives used in animal feeds are diverse and heterogeneous. Different categories are common depending on their properties and functions (Marroquin-Cardona *et al*, 2010). The European Union (EU) classified food additives as antibiotics; antioxidants; aromatics and flavorings; coccidiostats and other medicinal substances; emulsifiers, stabilizers, thickeners and gelling agents; colorants including pigments; preservatives; vitamins, provitamins, and other chemically well-defined substances with similar effects; trace elements (oligo elements); binders, anti-caking agents, and coagulants; acidity regulators; enzymes; microorganisms; radionuclide binders (Barton, 2000).

Unfortunately, the risk posed by antibiotic growth promoters (AGPs) to create cross-resistance to antibiotics used in human medicine and their presence in animal products, has led to their use to drop significantly, this is as they are banned in some cases in the formulation of fodders and for use in general animal husbandry (Gaucher *et al*, 2015). Some researchers however, suggested that the ban on these substances has caused increase in the incidences of bacterial infections (i.e., diarrhea, coccidiosis and intestinal necrosis) (Allen *et al*, 2013; Castanon, 2007). The prohibitions on AGPs resulted in economic impact on the livestock industry because it led to increased production costs. The American livestock industry demonstrated that the use of AGP in poultry production was associated with losses for producers (Dibner and Richards, 2005; Graham *et al*, 2007).

The restrictions on AGPs has stimulated interest in medicinal plants, which was revived in recent times because of their efficacy in providing cost effective therapy to several diseases because of secondary metabolites abundant in plants. These compounds known as phytochemicals, have been found to be responsible for the antioxidant properties of plants (Boots *et al,* 2008). Of paramount importance among them are polyphenols which where reported to exhibit anticancer, antimicrobial, antiplasmodic, anti-inflammatory, antiulcer, antidiabetic and antihypertensive properties (Balch and Balchi, 2000; Jisika *et al*, 1992). Interestingly, *Carica papaya* is rich in polyphenols which makes them versatile tools for the treatment of ailments in folklore medicine (Wang *et al,* 2008).

Papaya (*Carica papaya*) is a common human fruit; available throughout the year in the tropics. It is referred to as the "medicine tree" or "melon of health", and papaya is also rich in nutrients (Jackwheeler, 2003). It contains medicinal properties and the major active ingredients recorded include; carpine, chymopapain and papain, a bactericidal

aglycone of glucotropaeolin, benzyl isothiocyanate, a glycoside sinigrin, the enzyme myrosin, and carpasemine (Jackwheeler, 2003). The mature fruits have a round or star-shaped seed cavity (Nakasone and Paull, 2003) in which several hundreds to thousands of seeds are formed (Tsuchihashi, 2003). Papaya seed can be preserved for long period of time at relative humidity of 9 to 12% (Ellis *et al,* 1991), and they can survive for three years at low temperature and in a dry place (Malo and Campbell, 1994).

Phytogenic effects have been shown with papaya extracts in poultry for feed palatability and quality (sensory aspects), gut function and nutrient digestibility (improved growth), (Rumokoy, 2016), gut parasites and production parameters (improved weight gain and feed conversion ratio, reduced mortality), (Nideou, 2017), gut microflora (less diseases of the GIT, improved growth, reduced mortality) (Feroza1, 2017; Bauri *et al,* 2015), immune function (improved health), and carcass meat safety and quality (reduced microbial load, improved sensory quality) (Mountzouris *et al*, 2009; Bolu, *et al,* 2009).

This present study was designed to investigate the effects of dried *Carica papaya* seed powder on growth performance, feed efficiency, antioxidant enzyme activity, and serum biochemical parameters in broiler chickens.

METHODOLOGY

Location and duration of the study

The feeding trial phase of this research study was carried out at the aquatic animal research facility of biochemistry department Ibrahim Badamasi Babangida University, Lapai, Niger State. The experiment lasted for a period of 6 weeks of 7 days each.

Husbandry Conditions and feeding trial

Sixty day-old broiler starter birds were purchased from a commercial supplier in Minna, Niger state, and transported to the research facility in ventilated paper cages. Day old broiler chicks were exposed to cross-ventilation during the period of the study. Chicks were reared in cages of dimension 1.5 m x 1.5 m x 2.0 m; length x breadth x height, that were previously cleaned and disinfected. Chicks were acclimated for 5 days. During the acclimation, the chicks were fed commercial starter feed (23.62% Crude Protein, 14.7% Crude fat) and water was supplied *ad libitum* under strict biosecurity control according to previously published protocol (Molla *et al*, 2012).

Sixty day old chickens were randomly distributed into three treatments and fed commercial starter feed (Chikun, Olam poultry feed mill, Kaduna, Nigeria) mixed with 0, 0.5 and 1% PSP for the six weeks starter period. Pre-weighed feed were provided to all treatments while drinking water were measured every morning to monitor feed and water intake. Feed and water were offered to the birds in all the different treatments *ad-libitum*. All birds were reared without vaccination. Chicks were reared under approximately natural photoperiod of 12/12 hours of light/dark cycles for period of six weeks and cages were cleaned at the end of every week.

The live weight of individual birds were taken at the beginning and end of the experiment, but batch weighed once weekly, for 6 weeks to monitor growth performance and feed consumption. The weights were taken using a top loading weighing balance (Scout-pro, Spinebrook, USA).

Proximate composition analysis of experimental feeds and papaya seeds powder

Moisture content, crude fat, crude fiber, and crude protein (Microkjeldahl N x 6.25) were all determined following standard Methods of the Association of Analytical Chemists (AOAC, 2002).

Calculations for feed efficiency and growth performance parameters

Various parameters were calculated by applying the appropriate formulae where necessary, using the following:

Chicks survival (%) = (total chicks survival/ total chicks stock) x 100

Feed intake (FI) = total feed intake/number of live chicks

Feed conversion ratio (FCR) = wet weight gain (g)/feed intake (g)

Weight gain %(WG %) = $[(W_f - W_i)/W_i] \times 100$

Specific growth rate (SGR %) = $[(\ln W_f - \ln W_i)/T] \times 100$

Where W_f refers to the mean final weight, W_i is the mean initial weight and T is the feeding trial period in days.

Determination of Serum biochemical analysis

Chicks serum biochemical analysis such as SGPT, SGOT, ALP, bilirubin, albumin, creatinine, proteins, potassium, chloride, sodium and urea were determine spectrophotometrically following the manufacturer's instructions of AGAPPE diagnostics, Cham, Switzerland while Lipid peroxidation was assayed according to the method described by Health and Parker (1968).

Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) and the means were compared using Turkeys test. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using SPSS software Version 20.0.

RESULTS

No chickens died during the experiment, as 100 % of the chickens from all treatments survived. Results for proximate composition analysis of PSP and all dietary treatments are shown in Table 1. From the Table, it may be observed that PSP is a very good source of fat, protein and fibre. However, adding PSP up to 1 % did not significantly ($P > 0.05$) modify the nutrient composition of all dietary treatments.

Table 1: Proximate composition of feeds and *Carica papaya* seed powder

Treatments	MC%	CF%	ASH%	FAT%	CP%	CHO%
PSP	6.81	30.80	2.09	28.19	26.25	5.86
0% PP	8.44[a]	4.90[a]	4.30[a]	14.70[a]	23.62[a]	44.04[a]
0.5% PP	7.56[a]	5.90[a]	5.95[a]	12.0[a]	27.12[a]	34.97[a]
1% PP	7.66[a]	6.50[a]	7.10[a]	13.67[a]	32.37[a]	39.20[a]

MC=Moisture content CF= Crude fibre CP=Crude protein CHO= Carbohydrate (Nitrogen free extract)

Control: normal commercial feed (Chukun feed)

Treatment 1: normal commercial feed+ 0.5% carica papaya seed powder

Treatment 2: normal commercial feed + 1% carica papaya seed powder

Results for feed efficiency and growth performance of broiler chickens fed graded level of *C. papaya* seed powder for 6 weeks starter period are shown in Table 2. From the table, it may be observed that weight gain increased with increasing level of PSP in the diet from 0.5% to 1.0 %. Generally, better feed conversion ratio values were obtained in all treatments, but getting poor with increase of dietary PSP up to 1 % PP. Chicks fed diet containing 0.5 % PP had better feed conversion ratio. Feed intake was significantly ($P < 0.05$) the highest in 1% PP.

Results for hepatic/renal function biomarkers of broiler chickens fed graded level of *C. papaya* seed powder for 6 weeks starter period are shown in Table 3. From the Table, it may be observed that there was no significant difference ($P > 0.05$) across all the treatments, of the biomarkers monitored.

Results for serum metabolic parameters of broiler chickens fed graded level of *C. papaya* seed powder for 6 weeks starter period are shown in Table 4. From the Table, it may be observed that serum metabolic parameters such as total protein, creatinine, urea, potassium, chloride and sodium did not significantly ($P > 0.05$) differ across all the treatments. However, lipid peroxidation profile decreased significantly ($P < 0.05$) in treatment with PSP when compared to the control, and peroxidation decreased with more PSP in the diet.

Table 2: feed efficiency and growth performance of broiler chickens fed graded level of *C. papaya* seed powder for 6 weeks starter period

Treatments	0% PP	0.5% PP	1% PP
Mean Initial weight (g)	69.20^{a}	66.55^{a}	68.25^{a}
Mean Final weight (g)	2001.70^{a}	2079.75^{a}	2175.67^{b}
Weight gain (g)	1932.50^{a}	2013.25^{a}	2107.42^{a}
Av. Daily weight gain (g)	46.00^{a}	47.93^{a}	49.64^{a}
Feed conversion ratio FCR	1.81	1.72	1.96
Specific growth rate SGR(%)	8.00	8.19	7.88
Feed intake FI (g)	3498.75^{a}	3462.75^{a}	4084.28^{b}

MC=Moisture content CF= Crude fibre CP=Crude protein CHO= Carbohydrate (Nitrogen free extract)
Control: normal commercial feed (Chukun feed)
Treatment 1: normal commercial feed+ 0.5% carica papaya seed powder
Treatment 2: normal commercial feed + 1% carica papaya seed powder

Table 3: Hepatic/Renal function biomarker of broiler chickens fed graded level of *C. papaya* seed powder for 6 weeks starter period.

Treatments	AST (µl)	ALT (µl)	ALP (µl)	BIL-TOTAL (mg/dl)	BIL-DIRECT (mg/dl)	ALBUMIN (µl)
0% PP	16.80±1.25^{a}	18.90±0.76^{a}	25.97±1.86^{a}	1.93±0.14^{a}	1.43±0.07^{a}	1.63±0.07^{a}
0.5% PP	16.20±0.46^{a}	17.93±2.72^{a}	27.23±1.88^{a}	1.87±0.23^{a}	1.67±0.23^{a}	1.43±0.07^{a}
1% PP	15.17±1.89^{a}	17.97±0.59^{a}	28.87±0.82^{a}	1.47±0.18^{a}	1.43±0.03^{a}	1.63±0.03^{a}

Data are expressed as mean ± SEM (n = 3).

Mean ± SEM followed by different letter within a column are significantly different ($P < 0.05$).

Table 4: Serum metabolic parameters of broiler chickens fed graded level of *C. papaya* seed powder for 6 weeks starter period.

Treatments	0% PP	0.5% PP	1% PP
Total protein (g/dl)	1.70±0.06[a]	1.67±0.09[a]	2.00±0.15[a]
Creatinine (mg/dl)	1.40±0.11[a]	1.30±0.06[a]	1.27±0.03[a]
Urea (mg/dl)	46.50±0.26[a]	46.57±0.26[a]	47.63±0.43[a]
Potassium (mmol/l)	3.43±0.22[a]	3.90±0.21[a]	3.47±0.13[a]
Chloride (meq/l)	53.23±1.57[a]	61.33±3.56[a]	95.43±17.09[a]
Sodium (meq/l)	114.17±1.82[a]	148.57±4.37[a]	127.07±5.47[a]
LPO (nmol/ml)	5.98±1.33[a]	2.86±0.04[b]	1.77±0.21[b]

Data are expressed as mean ± SEM (n = 3).
Mean ± SEM followed by different letter within a column are significantly different (P <0.05). LPO=Lipid peroxidation assay**.**

DISCUSSION

The survival of all chickens from all treatment is an indication that PSP may not contain any toxic substances. No vaccines or AGPs were used in the study. The crude protein content for PSP is high indicating that it is a very good source of protein. Protein is an essential component of diet needed for the survival of both animal and human of which basic function is to supply adequate amount required (Pugalenthi, 2004). Protein represent key nutrient for muscle and bone health, and thereby function in the prevention of osteoporosis in chickens (Bonjour, 2011). The protein content of PSP noted in the present study was similar to that reported by Makanjuola, (2018) and Maisarah, *et al.* (2014).

PSP is also noticed to be a good source of fiber. According to Eromosele, (1993), fibre helps in the maintenance of animal health and has been known to reduce cholesterol level in the body. A high fibre food expands the internal wall of the colon, causing the passage of waste, thus making it an effective anti-constipation. Fibre also reduces the risk of various cancers, bowel diseases and improves general health and well-being of animals. The crude fibre content of PSP noted in the present study was close to that reported by Adebisi, and Olagunju, (2011).

Fat contents of PSP were recorded to be high indicating that PSP is a good source of oil which can serve as energy source. According to Wood, *et al,* (2003), dietary intake of fat (unsaturated fatty acids) reduces the risk of cardiovascular disease and possibly the incidence of some cancers, asthma and diabetes in chickens. The fat content of PSP noted in the present study was similar to that reported by Makanjuola, (2018).

However, inclusion of PSP up to 1 % did not significantly alter nutrient composition of all dietary treatments. This result obtained from this study is likely due to the quantity added. According to Windisch *et al.* (2009) supplementation levels of products to poultry diets vary between 0.1 and 40 g/kg for dried products and plant extracts. In addition, phytobiotics (PSP) are substance added to feed to improve the safety, flavor, taste, and other qualities of the feed (FDA, 2018; Erich, 2002) and do not add significantly to the main nutrients of the feed.

Weight gain increased with increasing level of PSP in the diet from 0.5% to 1.0 % (Table 2).

This increased weight gain by broiler chicks fed PSP containing diet could be attributed to the fact that herbal plants provide compounds that enhanced digestion and absorption of nutrients in diets leading to improved growth of chicks (Safa, 2014). The result of this study is in accordance with the findings of Mohamed *et al.*, (2012), and Arshad *et al*. (2012) who reported significant ($p<0.05$) increase in live body weight of broiler chicks fed ginger-supplemented diet.

Generally, better feed conversion ratio values were obtained in all treatments, but getting poor with increase of dietary PSP up to 1 % PP. Chicks fed diet containing 0.5 % PP had better feed conversion ratio. Better feed conversion ratio of the broiler chicks fed diet containing PSP additive could be attributed to the antibacterial properties of these additive, which resulted in better absorption of the nutrients present in the gut and finely leading to improvement in feed conversion ratio of the treatments group (Khatun *et al.*, 2013).

The broiler chicks fed PSP diets showed better feed intake and utilization compared to the control (Table 2). Feed intake was significantly ($P < 0.05$) the highest in 1% PP. better feed intake and utilization of broiler chicks fed PSP diets could be attributed to enhanced secretion of digestive enzymes resulting in an improved gut functioning (Windisch *et al*., 2009). Arshad, (2012) and Herawati, (2011) reported similar results in feed intake when broiler chicks were fed diet containing ginger powder as phytobiotics additives.

Hepatic/renal function biomarkers of broiler chickens fed graded level of *C. papaya* seed powder for 6 weeks starter period showed AST, ALT and ALP values not significantly varied ($P > 0.05$) across all the treatments. Notably, the value of AST, ALT and ALP obtained in broiler chicks fed PSP containing diet were within the normal range and likely influenced the ability of birds to withstand the effect of anti-nutritional factors (if any) which could cause liver damage (Harper *et al*., 1979). This was in line with the findings of Ekpenyong and Biobaku, (1986) who stated that the values of AST and ALT are normally low in blood but becomes high when there is occurrence of liver damage by toxic substances. Similar values of AST and ALT that was not significantly varied ($p>0.05$) in this study for the control (0% PP) relative to treatments are indicative of normal liver and kidney functions (Bolu *et al*., 2006; Reitman and Frankel, 1957) that was not made worse by addition of PSP.

The result of the serum metabolic parameters; serum albumin, creatinine, urea and total protein of treatments (0.5% PP and 1% PP) (table 3) were similar with that for the control (0% PP). High urea and creatinine values are a measure of amino acid degradation (Shukla and Parahaurii, 1995) and early pointer to depressed liver and kidney functions (Wards *et al*., 1985).

Elevated creatinine value in chickens suggests depletion of tissue creatinine phosphate and this may adversely affect the muscle mass (Eggum *et al*., 1982; Alleyne *et al*., 1970). This implies that there was slightly better protein metabolism and utilization in the treatment group than the control. Therefore, PSP can be used with confidence in broiler diet to provide adequate nutrition.

The oxidative-antioxidative system imbalance leads to the pathology called oxidative stress. Oxidative stress occurs when there is an excessive free radical production and/or low antioxidant defense and this results in chemical

alterations of biomolecules, which cause structural and functional modifications like lipid peroxidation, damage to proteins and DNA (Ramanathan, *et al.*, 1999). The levels of reactive oxygen species is controlled by enzymes like Superoxide Dismutase, Catalase, Glutathione Peroxidase (GPx) and nonenzymatic scavengers like Vitamin C and Vitamin E (Kinalski, *et al.*, 2000)**.**

In the present study, lipid peroxidation profile decreased significantly ($p<0.05$) in treatments with PSP when compared to the control (Table 4). Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals steals electrons from the lipid in cell membranes, resulting in cell damage (Ostrea, 1985). In this study, lipid perodixidation values tended to decrease as the level of additive has increased from 0.5% PP to 1% PP. This may be attributed to the antioxidant effects of PSP (Kinalski, *et al.*, 2000).

Antioxidant functions by suppressing the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), affecting enzyme activities (Maisarah 2014). The decreases value of lipid perodixidation recorded for broiler chicks fed PSP diet could be explained by the effect of water-soluble antioxidant (result not shown) present in PSP which is capable of neutralizing ROP before lipid peroxidation is initiated.

From the results of this experimentation, feeding broiler chickens with diets mixed with PSP significantly and positively reduced serum lipid peroxidation profile without negatively affecting feed efficiency, growth performance and serum biochemical parameters. It may be concluded that supplementation of PSP has potentials as antioxidant when supplemented in the diets of broiler chickens.

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Inclusion of Cashew Nut Meal as Phytobiotics in Feeds to Improve Feed Efficiency, GrowthPerformance and Blood Metabolic Profile of Broiler Chickens

***Yusuf H.U. & Aliyu-Paiko M.**

Department of Animal Production, Federal University of Technology Minna, Nigeria.

*Correspondence: Email address: yusufhulelatu@gmail.com; Telephone: +2347033937666

ABSTRACT

Phytobiotics are plant derived products added to feeds inorder to improve performance of agricultural livestock. Dietary inclusion of phytobiotics increases general performance offarm animals. This study evaluated the effects of including cashew nut meal (CNM)in feedon growth performanceand other parameters inbroiler chickensduring feeding trial. Sixty birds were grouped into three treatments,where T_1(control) was fed commercial diet only. T_2 and T_3 were experimental groupsmaintained on diets containing 2% and 4% CNM, respectively.Feed and water were supplied *ad-libitum* during the experimental period of six weeks. Growth performance, feed consumption and water intake were monitored weekly, when also cages were cleaned.Results revealedthat T_2differed significantly ($P< 0.05$) in growth compared to$T_1$and T_3. However, no significant ($p>0.05$) difference was noted in theconcentrations of other monitored blood biochemical parameters. Similarly, the values for hematological parameters such ashaematocrit (PCV), Hb, RBC, WBC, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), and mean cell volume (MCV)were also not significantly affected ($P>0.05$) by the dietary treatments. Based on results in this study,it may be concluded that CNM supplementation in dietscould improve immune parameters of broiler chickens without adverse effect on growth performance and feed efficiency.

Keywords: Phystobiotics, Cashew nut meal, antioxidant enzymes, haematological parameters.

INTRODUCTION

Global chicken production makes a substantial contribution to food security (WHO, 2014). Supply of chicken through poultry production remains the most important source of animal protein in the developed and developing countries like Nigeria. Poultry production is estimated to contributing about 25% of the agricultural domestic products of Nigeria's economy. This massive production has rated Nigeria as the fourth in Africa for broiler production (USDA, 2013). This report indicated the need for improvement on the production of broiler birds in Nigeria.

The challenges of food security and sustainability in developing countries of Africa like Nigeria pose a serious threat to the region. Furthermore, sufficient supply of animal protein is very critical and has resulted in the growing demand in the production of foods of animal origin especially from poultry (FAO 2010). Population growth, urbanization, and income improvements are the main drivers of increased demand for foods of animal origin in Nigeria

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(Abdullah *et al*., 2011).

As a result of high population growth in Africa and the growing income, the demand for eggs and poultry meat has significantly increased in recent years across large parts of the African continent (WHO, 2010). Based on estimates by the United States Agency for International Development, this trend is likely to continue over the next few years (Heise *et al,* 2015). Therefore, the consumption of poultry and eggs is projected to increase by about 200% between 2010 and 2020 in some countries in sub-Saharan Africa, including Nigeria (USDA 2013; Obi, 2003).

Unfortunately,commercial farming systems for broiler chickens to meet increasing population and the demand for cheap protein/meat suffer from numerous problems. These problems include low capital base, inefficient management, technical and economic inefficiencies, infectiousdiseases, high costs of feeds, poor quality of day-old chicks, and inadequate extension and training facilities (Heise*et al*., 2015; Alabi and Isah 2002). However, the most prominent among these challenges include outbreak of diseases, high cost of quality feeds and poor quality seeds (Heise*et al*., 2015).

Overcoming these prominent challenges and attaining higher body weight with better feed conversion ratio in a short period of time is the main objective of efficient broiler production (Toghyani*et al*., 2011).Sub-therapeutic antibioticshave been widely used as growth promoters (AGPs) inlivestock to maintain health and enhance productivity(WHO, 2014).However, heavy reliance on the use of antibiotics to promote growth and animal performance, and combat bird diseases has adversely resulted to induction of resistant strains of pathogenic microorganisms, inhibition or killing of beneficial microbiota in the gastrointestinal (GI) ecosystem and the bio-accumulation of antibiotic residues in poultry products that are harmful for human consumption (Saleha*et al*., 2009). Due to the ban on usage of antibiotics as growth promoters in the EuropeanUnion (**EU**) and other countries since 2006, intensive research has focused on the development of efficient alternatives to AGP (Huyghebaert*et al*., 2011; Brown *et al*., 2017).

Natural growth promoters (NGPs), such as probiotics, prebiotics, and phytobiotics, have been exploited, as alternatives to antibiotics in livestock production.As alternative to antibiotics, phytobiotics are plant derived productsadded to feed in order to improve performance of agricultural livestock (Windisch and Kroismayr, 2006). Phytobiotics are relatively new feed additives, Knowledge about their modes of action andapplication rates are still rather limited (Windisch et al., 2009).

Several studies reported presence of essential secondary metabolites with phytogenic properties in cashew kernel. Despite these documented medicinal benefits, no substantive studies have been conducted about the use of cashew kernel as phytobiotic additive in feed for poultry production. However, these vital bioactive compounds could elicit many health benefits in poultry.

METHODOLOGY

Experimental Location

The research work was conducted at the Poultry Unit of the Department of Biochemistry, Ibrahim BadamasiBabangida University Lapai,

Niger State Nigeria from April 2018 to June 2018.

Experimental Feeds

ChikunOlam commercial starter feed (broilers chick feed) was purchased from Minna central market and transported to Animal house of IBB University, Lapai, Niger state.

Cashew nut meal

Fresh cashew nuts were purchased from Lapai central market. The collected seeds were cleaned and dried for two weeks. The kernels were obtained by cracking the shell and subsequently milled. The kernel meal was then stored at ambient temperature until used.

Experimental animal

A total of sixty-five (65) day-old mixed sex broiler chicks were purchased from a commercial hatchery in Minna, Niger State Nigeria and transported to the Animal house of IBB University, Lapai, Nigeria. The chicks were acclimated to the experimental facility conditions and fed with control feed for three days.

Experimental design

Sixty (60) birds (mean initial weight 75.5g) were randomly distributedinto three treatments. Each treatment has two replicates with ten (10) chicks per replicate. Treatment A was fed with the control diet (commercial feed with 0% cashew nut meal). Treatment B and C were administered with experimental diets containing 2 % and 4 % cashew nut meal respectively as additives to the commercial feed. Birds were fed the assigned experimental diets *ad-libitum* with constant access to water for 6 weeks.

Birds were weighed individually at the beginning and once weekly, to monitor growth performance and feed consumption. At the end of the six week experiment, surviving chicken were randomly pooled per treatment and used to determine feed efficiency, growth performance, haematological parameters, and liver biomarkers

Proximate Analysis

Proximate composition of the cashew nut meal and commercial feed were analyzed using standard method of AOAC (2010).

Feed Efficiency and Growth Parameters Adopted and Calculations

Various parameters were calculated by applying the appropriate formulae where necessary, using the following calculations:

Feed Efficiency Parameters

Feed intake (FI) =$\frac{totalfeedintake}{numberofbird}$

Protein intake (PI) =

$feedintake(g) \times percentproteinindiet$

Feed conversion ratio (FCR) =$\frac{wetweightgain\ (g)}{feedintake\ (g)}$

Protein efficiency ratio (PER) =$\frac{wetweightgain\ (g)}{totalproteinintake}$

Growth Performance Parameters

Bird survival (%) =$\frac{finalnumberofsurvivingbird}{initialnumberofbird} \times 100$

Weight gain (WG) = Wf-Wi

Weight gain (WG %) =$\left[\frac{(W_{f-W_i})}{W_i}\right] \times 100$

Specific growth rate (SGR %) =$\left[\frac{\ln W_{f-\ln W_i}}{T}\right] \times 100$

WhereW_f refers to the mean final weight,W_i is the mean initial weight and$T$ is the feeding trial period in days.

Determination of Hematological Parameters

The haematological components including haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and white blood cells (WBC).were determined using the automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by Dacie and Lewis, (1991).

Assay of Liver Enzymes activitiess

Alanine transminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) were spectrophotometrically assayed according to the methods of Reitman and Frankel (1957), Thefeld, *et al.,* (1974) and Wright *et al.,* (1972) respectively.

RESULTS

This section describes the results obtained for each of the analysis carried out after the 6 weeks of feeding trial.

Table 1: Proximate composition of feeds and cashew nut meal

Treatments	Moisture	crude fibre	Ash	Fat	protein	Carbohydrate
CFD	8.44±0.34^{b}	4.90±0.45^{b}	4.30±0.35^{b}	14.70±0.99^{a}	23.62±0.87^{a}	44.04±1.57^{c}
CNM	4.05±0.48^{a}	2.91±0.49^{a}	3.10±0.07^{ab}	38.90±0.98^{b}	28.00±0.05^{b}	23.05±0.45^{a}
2% CNM	8.34±0.37^{b}	7.50±0.34^{c}	7.20±0.56^{c}	14.20±0.68^{a}	27.12±0.34^{b}	35.64±0.43^{b}
4% CNM	8.12±0.03^{b}	10.60±0.19^{d}	2.09±0.34^{a}	14.20±0.43^{a}	29.75±0.95^{b}	35.24±0.57^{b}

Values are expressed as Mean ±SEM. Each mean is an average of three replicate (n=3).Values with different superscript are significantly different ($p<0.05$).

- CFD: commercial starter feed (Chikun feed)
- CNM: cashew nut meal
- 2% CNM: commercial starter feed + 2% cashew nut meal
- 4% CNM: commercial starter feed + 4% cashew nut meal

Table 2: Feed efficiency of broiler chickens fed experimental diets for 6 weeks starter period

Treatments	Control	2% CNM	4% CNM
Feed intake (g)	3614.12±25.67^{a}	3805.81±21.56^{b}	3707.29±28.65^{ab}
Feed conversion ratio	1.86±0.05^{a}	1.85±0.03^{a}	1.97±0.45^{a}
Protein efficiency ratio	82.38±9.43^{b}	75.80±4.56^{b}	63.14±3.45^{a}

Values are expressed as Mean ±SEM. Each mean is an average of three replicate (n=3).Values with different superscript are significantly different ($p<0.05$).

Table 3: Growth performance of broiler chickens fed experimental diets for 6 weeks starter period

Treatments	Control	2%CNM	4% CNM
Initial weight (g/bird)	77.54±4.56^{a}	75.53±4.56^{a}	73.51±5.43^{a}
Final weight (g/bird)	2023.52±21.56^{b}	2126.53±19.44^{b}	1944.52±18.45^{a}
Weight gain (g/bird)	1945.85±12.34^{b}	2055.93±11.56^{c}	1878.40±11.84^{a}
Weight gain (%)	2510.77±27.66^{a}	2721.28±24.66^{b}	2550.44±22.34^{a}
Specific growth rate (%)	7.77±0.95^{a}	7.95±0.55^{a}	7.80±0.50^{a}

Values are expressed as Mean ±SEM. Each mean is an average of three replicate (n=3).Values with different superscript are significantly different ($p<0.05$)..

Table 4: Haematological parameters of broiler chickens fed experimental diets for 6 weeks starter period

Treatments	Control	2% CNM	4% CNM
WBC (× $10^{3}mm^{-3}$)	86.66±0.96^{a}	84.66±0.33^{a}	84.33±1.76^{a}
PCV (%)	27.00±0.45^{b}	24.94±0.26^{a}	27.01±0.79^{b}
RBC (× $10^{6}mm^{-3}$)	2.13±0.02^{a}	2.04±0.29^{a}	2.22±0.65^{a}
Hb	14.06±0.18^{a}	13.63±0.08^{a}	14.33±0.38^{a}
MCV	126.33±2.33^{a}	122.00±1.52^{a}	121.67±1.20^{a}
MCH	65.76±0.14^{a}	66.96±0.57^{a}	45.50±19.51^{a}
MCHC	52.06±0.93^{a}	54.73±0.62^{a}	53.10±0.20^{a}

Values are expressed as Mean ± SEM. Each mean is an average of three replicate (n=3).Values with different superscript are significantly different ($p<0.05$).CNM= Cashew nut meal

Table 5: Liver Biomarker Enzymes of broiler chickens fed experimental diets for 6 weeks starter period

Treatments	Control	2% CNM	4% CNM
ALT	17.530.14^{a}	15.00±0.40^{a}	26.70±1.60^{a}
AST	19.13±0.98^{a}	14.83±0.32^{a}	27.83±2.01^{a}
ALP	16.60±0.79^{a}	15.43±1.61^{a}	29.36±0.33^{a}

Values are expressed as Mean ± SEM. Each mean is an average of three replicate (n=3).Values with different superscript are significantly different ($p<0.05$)

DISCUSSION

Animal health and wellness depends on the quality of the feed given to them. In the present study the proximate composition of commercial feeds and cashew nut meal are shown in Table 1. The determined nutrient content of the cashew nut meal obtained in this trial was slightly at variance with the findings of Fetuga *et al*. (1975). Various factors ranging from the processing method, length of storage and storage facility, the type of soil on which the crop was grown and specie differences could be responsible for such variations. The percentage composition of the experimental diets is shown in Table 1, the crude protein and Metabolizable

energy of the experimental diets are adequate for broiler production in the tropics (Aduku, 2004).

The numerically higher mean weight gain observed for birds fed 2% CNM in Table 3 could be due to adequate proportion of cashewnut meal in the diet. This could have provided a positive balance of amino acid for the birds (Odunsi, 2002; Faniyi, 2002). The significantly ($p<0.05$) higher feed intake (Table 2) and weight gain (Table 3) obtained in broilers fed the diet supplemented with 2.0% CNM compared to those broilers fed 4.0 % CNM and the control diet could be due to improved palatability while the 4.0 % CNM could have slightly depressed appetite on the broilers. Since according to Hill and Dansky (1950) and Mark and March (1985), energy rather than protein concentration seems to be the major determinant of feed intake. The values obtained for growth parameters and nutrient utilization in broilers fed 2% CNM is evidence that inclusion of cashewnut meal as phytobiotic seems profitable for productive performance.

Blood represents a means of assessing clinical and nutritional health status of animals in feeding trial and the haematological parameters most commonly used in nutritional studies include PCV, RBC, HBC, MCHC, MCV and clotting time (Aletor and Egberongbe, 1992; Olorede and Longe, 2000; Adeyemi *et al*., 2000). Table 4 shows the results of haematological parameters. The results showed the packed cell volume (24.94±0.26 - 27.01±0.79) are statistically similar, this indicate the better utilization of all the energy sources, the values reported in this study are in agreement with (22.23-26.83%) reported by Afolayan *et al*. (2014), it is however fall below the normal range of (31-33.5%) reported by Mitruka and Rowley (1977).

The values of haemoglobin (Hb) obtained were within the normal range for chickens (7-14g/l) as stated by Mmereole (1996). Haemoglobin values of 6.3-7.8g/l (Mmereole, 1996) and 8.7-9.3g/l (Ikhimioya*et al*., 2000) had been reported in indigenous chickens and 8.6-10.7g/l (Madubuike, and Ekenyen, 2006) and 11.26-13.1 g/l (Afolabi *et al*., 2010) in broilers. The significant value of MCH obtained was within the normal range since the Hb and RBC obtained were within normal range for chicken. This result indicated that the nutrients were adequately utilized by the broilers and posed no problem to the birds. It explains why the birds were healthy, not anaemic and were capable of withstanding stress.

The values of PCV, RBC, MCV and MCHC obtained in this study which were not significantly different across the treatments and within the normal range for chickens supported the findings of (Ikhimioya and Arijeniwa, 2000). This showed that the bone marrows of the birds were functioning normally. It revealed the absence of macrocytic and hypochronicanaemia. The normal values of PCV obtained in the study showed that the CNM increased the availability of protein, energy and the degradation of anti-nutritional factors. This according to (Cary *et al*., 2002) improves broiler performance. This confirmed that haematological traits, especially PCV and Hb were correlated with the nutritional status of the animal (Cary *et al*., 2002) and agreed with (Oyawoye and M. Ogunkunle, 1998) who stated that PCV is an index of toxicity in the blood and high levels usually suggest the presence of toxic factors which has adverse effect on blood formation. The values of MCV, MCH and MCHC are not statistically different among the treatment groups indicating the quality of CNM

in maintaining the integrity of blood indices in broiler birds (Afolabi *et al.,* 2010).

Alanineaminotransferase (ALT) and Aspartateaminotransferase(AST) are biomarkers of hepatic integrity and to a certain level can be used to assess the extent of hepatocellular damage (Shittu*et al*., 2016). Alkaline phosphatases (ALP) are often used to assess the integrity of plasma membrane and endoplasmic reticulum (Ekanem and Yusuf, 2008). The serum AST, ALT and ALP activities reported in Table 5 are within the range reported by other previews works (Adamu *et al*., 2015; Ogueji and Auta, 2007; Gabriel *et al.,* 2010). Therefore, the activity reported in this study followed the reported pattern signifying that the broilers are not responding to any environmental challenges. This was in line with the finding of (Ekpenyong, andBiobaku, 1986) who stated that the values of AST, ALP and ALT are normally low in blood but becomes high when there is occurrence of liver damage by toxic substances.

Based on results in this study, it may be concluded that CNM supplementation in diets could improve immune parameters of broiler chickens without adverse effect on growth performance and feed efficiency.

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Comparative Hazard Analysis and Critical Control Point (HACCP) Concept In Peanut Cake (Kulikuli) Production

***[1]Mohammed S.S.D. & [2]Garba R.S**

[1]Department of Microbiology and Biotechnology, Nile University of Nigeria, FCT, Abuja.
[2]Department of Microbiology, Kaduna State University, Kaduna, Nigeria.

*Correspondence: Email address: mosada78@gmail.com; Tel: +234(0)8035861774

ABSTRACT

Peanut cake (Kulikuli) is a snack delicacy indigenous to the West African coast. Kulikuli is usually produced from groundnut during groundnut oil extraction and then fried to obtain the final product. This study "Comparative Hazard Analysis and Critical Control Point (HACCP) Concept in Peanut cake (Kulikuli) Production" investigates bacterial and fungal contaminations (hazards) at 4 (four) different points during 'kulikuli' production in Kaduna, Nigeria. Eight (8) Samples were collected from Badarawa and Rigasa during the 4 (four) points of production. Paste after oil extraction and molded paste were the critical control points (CCPs), while the remaining stages were the control points (CPs). The laboratory kulukuli was produced using standard procedures with regards to hazard analysis and critical control points. Proximate composition on both the laboratory produced kulikuli adhering to HACCP and the locally produced kulikuli were carried out using standard techniques. Microbiological evaluation of the kulukuli samples were investigated through isolation, enumeration and identification using pour plate techniques , morphological, biochemical tests and mycological evaluation using lactophenol cotton blue under 40X magnification and atlas for identification. The bacteria and fungi isolates were tested against selected antibiotics and antifungal agents. All the proximate composition parameters were closely related but sample one showed a notable difference in crude fat content. Bacteria associated with the kulikuli samples from Badarawa and Rigasa were in varying numbers in CFU/g and were identified as *Escherichia coli*, *Proteus* sp., *Klebsiella* sp., *Salmonella* sp. while fungi species include *Aspergillus* sp, *Penicillium* sp and a *Mucor* sp. But no bacteria and fungi species were detected in the laboratory produced kulikuli samples. Bacterial isolates showed sensitivity to all the antibiotics used except *E.coli* while the fungi isolates were also sensitive to all the antifugal agents employed. *Salmonella* was resistant to all antibiotics used in this study but *E coli, Klebsiella* and *Proteus* were sensitive to antibiotics employed at different concentrations. The antibiogram of selected antifungal against the fungal isolates showed that Fesovin had a sensitive concentration of ≥17 and resistance of ≤13; Itraconazole is had concentration of >16 and resistance of <9; Fluconazole had concentration of ≥19 and resistance of ≤14; Griseofulvin had concentration of ≥19 and resistance of ≤14; Ennotab had concentration of >16 and resistance of <9. Therefore, the point of oil extraction is very critical and can render the overall product unsafe for consumption as it the highest of microbial contamination. Hazard analysis and critical control point approach in quality management can be used in the preparation of local snacks. This will increase consumer confidence and in turn more patronage of the product (s).y.

Keywords: HACCP, bacteria, kulikuli, proximate, antibiotics.

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INTRODUCTION

All food business operators are responsible for making sure that the food produced by their business is safe to eat (Regulation (EC) No 178/2002 Article 14.2 of the European Parliament). This means that it is neither injurious to health nor unfit for human consumption. To do this, Regulation (EC) 852/2004 Article 5 of the European Parliament requires the operator to put in place, implement and maintain permanent procedures based on HACCP principles. The Hazard analysis and Critical Control Point (HACCP) concept is a systematic approach based on hazard identification, assessment and control but places premium on both process and raw material control than the testing of final products (Savage 2009). To produce safe food for consumers, all the important safety hazards that are associated with the production of food need to be prevented, eliminated or reduced to an acceptable level. These food safety hazards may be biological, physical or chemical. The seven hazard analysis and critical control point (HACCP) principles provide a systematic way of identifying food safety hazards, making sure that they are being managed responsibly and showing that this is being done continuously (Nahemiah *et al* 2014). The HACCP is based on seven key principle : the potential hazards in foods are identified and the risks assessed; Critical Control Points (CCP), the point in the process where control could be exercised to minimize or eliminates the potential hazards, are to be identified; specifications are established for each CCP; corrective actions to be taken when deviation occurs at the CCP identified; a record is kept of the process flow chart; and verification procedures are put in place to ensure that the HACCP identifies hazards and preventive measures for their control. Its effect is to focus control at the CCPs and to provide a bases on which the performance of food production system and the expected safety of food processed by it can be assessed immediately by the processes (Nahemiah *et al* 2014). The HACCP is a proactive approach to building food safety into one's food production or preparation process that depends on the common sense application of both scientific and technical methods in the plant (FAO 2008 &Nathai-Balkissoon & Arumugadasan 2014). An efficient and accurate record keeping within HACCP is essential. This provides the manufacturer with confidence that their product is safe and allows auditors to do their job. Documentation includes details of the component raw materials, the processing and the requirements of final products. Additionally, details of the HACCP plan, staff training, audit and verification details are needed. Nevertheless, people's resistance to change is the main obstacle to the HACCP implementation. Other barriers include inadequate support and facilities such as, the layout, space limitations, and poor design facility (Pun & Bhairo-Beekhoo 2008). The HACCP concept is a systematic approach based on hazard identification, assessment and control but places premium on both process and raw material control than the testing of final products. But it is often necessary to carry out individual analysis of the critical control point (CCP) required for a given production process rather than applying generalized procedure for all types of foods. Kulikuli is a northern snack that is made from dry roasted peanut/groundnut, ground into a semi-paste, mixed with selected spice and deep fried until it hardens. Kulikuli is one crunchy snack that brings back lovely childhood memories. The

essential ingredient for the sustenance of life is food with either plant and animal origin and therefore, its demand is necessary, people depend mostly on indigenous technology for food preparation in Nigeria, more especially food of plant origin (Adjou 2012). Peanut cake (Kulikuli) is a snack delicacy indigenous to the West African coast. Kulikuli is usually produced from groundnut during groundnut oil extraction and then fried to obtain the final product (Adjou *et al* 2012). Apart from being a part of the diet of most age ranges, peanut cake is most commonly consumed by the middle aged and younger persons, particularly students. Peanuts and its derivatives are often classified as street food which satisfies essential need of the urban population by being affordable and available (Boli *et al* 2014). Kulikuli is rich in protein and crude fat similar to its parent material, groundnut (Aletor & Ojelabi 2007 & Kolapo *et al* 2012 & Ejoh & Ketiku 2013). Moreover, groundnut is often referred to as a poor man's protein due to its availability and affordable prices. Due to its high nutritive content, peanut cake in Nigeria is prone to contamination by a wide variety of microorganisms including many bacterial species ranging from the simple commensals to the pathogenic types and fungal organisms (Ezekiel *et al* 2014).Contamination by these microorganisms occurs during handling, storage and transportation as a result of improper processing during production and storage conditions, thereby exposing groundnut and its products to the risk of contamination with aflatoxin (Polixeni & Panagiota 2008 & Mutegi *et al* 2012). Mycotoxins are secondary metabolites of fungal origin which produce toxic responses when ingested by animals or humans. Mycotoxicosis is a term used to denote the diseases that result from the ingestion of mycotoxin by animals and humans (Frisvard *et al* 2017). Plant products such as spices (ginger and chilli) have been used not only to provide flavor and aroma in foods but also for their antimicrobial properties in many food production including kulikuli. Groundnut (*Aranchis hypogea*) and its popular derivative snack product, kulikuli are particularly prone to contamination during the production process because it's mostly a locally produced and attention is not paid to HACCP principles to ascertain where contamination is likely to occur during the production, making it unsafe for consumption. The study was aimed at the comparative hazard analysis and critical control point concept in kulikuli production.

METHODOLOGY

Collection of Samples

Samples of kulikuli for analysis were collected from Badarawa (Coordinates:10.4707^{0}N and 7.4435^{0}E) and Rigasa (Coordinates: 10.5286^{0}N and 7.3866^{0}E) in Kaduna. The method adopted involved monitoring of the Kulikuli preparation, identification of possible sources or routes of contamination. Samples were collected during production and tested for microbiological hazards, categorizing risks and documenting a flow chart, indicating relevant critical control points. That was used to evaluate the quality of Kulikuli prepared in the laboratory.

Hazard and Critical Control Points (HACCP)

The hazard analysis and critical control point in Kulikuli production were carried . The methods used were identification of the control points (CPs) and critical control points (CCPs) using the method described by Nahemiah *et al* (2014).

Laboratory Preparation of Kulikuli Stock

A measured quantity of shelled peanut was sorted, roasted and milled. Oil was extracted

from the ground peanut seeds in a previously heat sterilized mortar and pestle by adding hot sterile distilled water. After the oil removal process was repeated severally, the final paste was then used in preparing the snacks.

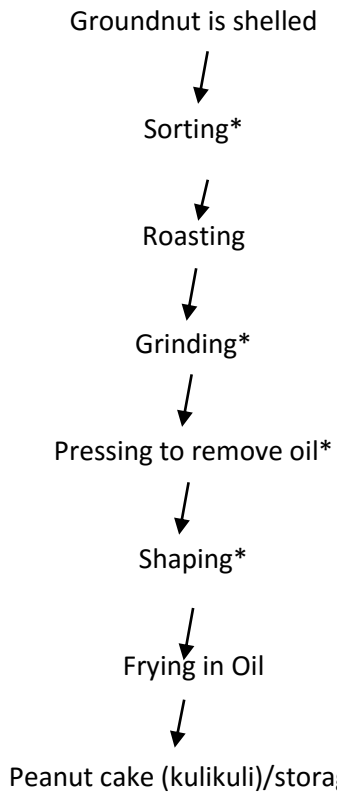

Fig.1: Flow chart of kulikuli production indicating CCPs (Akano & Atanda, 2009).

Proximate Analysis of Kulikuli

The proximate analysis of the kuli kuli samples were carried out to determine the percentage (%) mosture content, ash, protein, fat, fibre and carbohydrate using standard procedure described by Association of Official Analytical Chemists (AOAC) (2009).

Media Preparation

Potato dextrose agar, Mueller-hinton agar, Mac Conkey agar and plate count agar were prepared according to manufacturer's instruction. All media were sterilized in an autoclave at 121^0C for 15 minutes.

Proximate Analysis

Proximate composition of the cashew nut meal and commercial feed were analyzed using standard method of AOAC (2010).

Isolation Bacteria from Samples of Kulikuli

Enumeration of aerobic mesophilic bacteria: plate count agar (PCA) was used for bacterial counts. Approximately 10 ± 1.0 g samples was mixed with sterile peptone water (1:10) to prepare the initial homogenate. This was homogenized for about 3 minutes using hand agitator followed by making serial dilutions with 1ml of the initial homogenate being added to 9ml of sterile peptone water (Oxoid). Twenty (20) µl of the initial dilution (10^{-1}) and through each of the dilutions up to 10^{-5} was added, respectively, to PCA using the drop plate technique. The PCA agar plates were incubated at 35^0C for 24 hours after which plates were read. All colonies that grew on PCA were expressed in CFU/g and propagules/g, respectively (Oyeleke & Manga 2008). The total viable bacterial count (TVBC) was carried out according to the standard methods described by Oyeleke & Manga (2008).

Isolation of Fungi from Kulukuli Samples

Serial dilution was done for each of the kulikuli samples using a 5-fold dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) with each test tube containing 9ml each of sterile distilled water. It was vigorously shaken, after which a selected dilution was taken. .Exactly 0.1ml of the suspension was poured and plated on PDA in the vicinity of a flame, and the plates were swirled and allow to solidify at room temperature. The solidified plates of the fungi were inverted and incubated at 25^0C for 72

hours so as to have a mixed culture of fungal growth, thus total fungal count was determined by counting the colonies on the agar plates. The mixed culture of fungal were then sub-cultured to obtain a pure culture on separate plate containing PDA. Pure cultures of fungi species isolated from kulikuli samples were preserved on slant of PDA contained in sterile Bijou bottle and were kept in the refrigerator at 4^0C as reference culture (Jonathan *et al.* 2013).

Characterization and Identification of the Bacteria Isolates

Bacteria isolates were sub-cultured from their respective stocks cultures to an appropriate sterile medium and also incubated at 37^0C for 24 hours . Characterization and identification of the isolates were determined based on Grams staining and biochemical tests: catalase, indole, urease, motility, methyl red-voges proskauer (MR-VP) as described by Oyeleke & Manga (2008).

Identification of Fungi Isolates from Kulikuli Samples

The cultural characteristics of the fungi isolates/growth on plate were observed using mycological atlas. The mycelia of the fungi were carefully picked using sterile needle and was placed gently on drop of lactophenol cotton blue on a clean glass free slide. And was viewed under 4X and 40X magnification of the microscope and results were recorded.

Antibiotic Sensitivity Test

Inoculum was made with a broth culture diluted to match 0.5 Mcfaland standards, which is roughly equivalent to 150 million cells per ml. The media used for Kirby-Beuer testing was Mueller Hinton agar. Using an aseptic technique, a sterile swab was dipped into the broth culture of a specific organism and then and the excess water was gently removed by gently pressing it or rotating the swab against the inside of the tube. Using the swab, the Mueller Hinton agar plate was streaked to form a bacterial lawn. The plate was allowed to dry for approximately 5 minutes an antibiotic disc containing specific antibiotics was then dispensed on the place using a flame sterilized forceps and was incubated at temperature 37^0C (Bonev *et al* 2008).

Antibogram of Selected Antifungal against the Fungal Isolates

Susceptibility testing was conducted using disk diffusion technique on Mueller-hinton agar (MHA) plates as described by Ali *et al* (2013). On MHA, methylene blue glucose solution (assisted yeast growth and enhanced visualization of inhibition zone) was added to the surface of the agar and allowed to air dry prior to the addition of yeast inocula. Inoculation was carried out by dipping a sterile swab into the inoculums suspension adjusted to the turbidity of 0.5 McFarland standards (10^{-8} cells/ml) and the agar surface was streaked a four direction. The plates were dried at ambient temperature for 15 minutes in a laminar flow cabinet and antifungal drugs (Itraconazole, Fluconazole, Fesovin, Vab, Enotab and Griseofulvin) commercially prepared in different concentrations were applied. The diameter zones of inhibition were measured in millimeter after 24 hours of incubation.

RESULTS

Table 1 showed the assessment and identification of HACCPs in kuli kuli production. The result revealed that bacteriological, mycological and chemical contamination (s) of the food product occurred at different processing point. The result further proffer the

CCPs and critical limits to which the kuli kuli can be safe for consumption. Table 2 showed the proximate composition of selected samples of kulikuli analyzed. The percentage moisture content for sample1 was 12.36% and sample2 had 12.22%; Ash content for sample1 was 3.91% and sample2 had 3.95%; Protein content for sample1 was 12.39% while sample2 had 12.60%; Crude fat for sample1 was 15.56% and sample2 had 15.40%; Crude fiber for sample1 was 0.22% and sample2 had 0.26% while carbohydrate content for sample1 was 55.69% which was highest and sample2 had 55.55%. Table 3 showed the mean total bacteria viable count of laboratory produced kulikuli. It indicated that there was no growth in all production stages of the mean total bacteria viable count (TCV) in the laboratory prepared kulikuli. Table 4 showed the mean total bacterial viable count (TVC) of kulikuli from two locations at four production stages. The result are presented in colony forming unit per milliliter (CFU/mL). The production stage of A at both locations, it was observed that there were no growth. Kuli kuli samples from Badarawa showed that the colony forming unit per milliliter (CFU/mL) of production stage of B, C and D were 1.2 x 10^4 CFU/mL**,** 2.1 x 10^4 CFU/mL and 4.5 x 10^4 CFU/mL; while samples from Rigasa showed that sample B had bacteria load of 1.6 x 10^4 CFU/mL, C and D were 1.6 x 10^4 CFU/mL and 2.3 x 10^4 CFU/mL respectively. Table 5 showed the characterization and identification of bacteria isolates from the sampled kuli kuli with their respective morphology. Majority of the bacteria isolates were coliforms. The species of bacteria were identified as *Escherichia coli, Proteus* sp, *Klebsiella* sp and *Salmonella* sp while the produced kulikuli were free of bacterial contaminations. Table 6 showed the frequency of occurrence of bacteria isolated from sampled kulikuli. *Escherichia coli* had the highest occurrence of 42.25% while *Proteus* sp. had the lowest occurrence of 16.75%. Table 7 showed the colony characteristics of fungi isolates from sampled kulikuli . The fungi were identified as *Aspergillus* sp, *Penicillum* sp and *Mucor* sp, Table 8 showed the mean fungal count CFU/mL x 10^3 of sample A1, A2, A3, A4 and B1, B2, B3 and B4. Sample A1 and B1 had no growth while sample A2, A3, A4 had bacteria load of 1.24 x 10^3 CFU/mL, 1.81 x 10^3 CFU/mL, 1.32 x 10^3 CFU/mL and B1, B2, B3 had 1.20 x 10^3 CFU/mL, 2.00 x 10^3 CFU/mL, 2.40 x 10^3 CFU/mL respectively. Table 9 showed the morphological characteristics of moulds isolated from kilkuli. The probable species was *Aspergillus* sp which was identified having black surface and grey-green coloured mycelium colony while *Penicillum* sp and *Mucor* sp had white with tint blackish colonies and white colored coyyon respectively. Table 10 showed the antibiogram of bacteria isolates against selected antibiotics. *Salmonella* was resistant to all antibiotics used in this study but *E coli, Klebsiella* and *Proteus* were sensitive to the different antibiotics at varying concentrations; Sparfloxacin (10µg), Chloramphenicol (30µg), Pefluxacein (30µg), Tarivid (30µg), Septrin (30µg), Gentamicin (10µg) and Ciprofloxacin (30µg).Table 11 showed the antibiogram of selected antifungal against the fungal isolates. Fesovin is had a sensitive concentration of ≥17 and resistance of ≤13; Itraconazole is having concentration of >16 and resistance of <9; Fluconazole has concentration of ≥19 and resistance of ≤14; Griseofulvin has concentration of ≥19 and resistance of ≤14; Ennotab has concentration of >16 and resistance of <9.

Table1. Assessment and Identification of Hazard Analysis and Critical Control Points (HACCPs) in Kuli kuli Production

Steps	Hazard	CCps	Critical limit (s)
Purchase of peanut	B/M	Yes	Healthy peanut with low contaminations
Sorting	B/M	Yes	Sort into clean containers
Roasting	Chemical	No	Heat treatment
Grinding	B/M	Yes	Wash machine/ grind into clean container
Mixing/pressing	B/M	Yes	Mix with hot water in a clean container
Shaping	B/M	Yes	Avoid using bare hands and dirty containers
Frying	Chemical	No	Reduces fat and moisture/protein contents
Ready to eat Kulikuli	B/M	Yes	Keep in clean container/use of foil paper.

CCPs: Critical Control Points, B: Bacteriological, M: Mycological.

Table 2: Average Mean Proximate Composition of Selected Kulikuli

Parameters (%)	Sample	
	1	2
Moisture content	12.36	12.22
Ash content	3.91	3.95
Protein content	12.39	12.60
Crude fat content	15.56	15.40
Crude fiber content	0.22	0.26
Carbohydrate content	55.69	55.55

Table 3: Mean Total Bacterial Viable Count (TVC) of Laboratory Produced Kulikuli

Production Stage	x 10^{-4}CFU/mL
A	NG
B	NG
C	NG
D	NG

A = Grounded paste prior to oil extraction, B = Paste after oil removal, C = Moulded paste
D = KuliKuli , NG = No growth

Table 4: Mean Total Bacteria Viable Count (TVC) of Sampled Kulikuli

Production Stage	**Location**	**x 10^{-4} CFU/mL**
	Badarawa	
A		NG
B		1.2
C		2.1
D		3.5
	Rigasa	
A		NG
B		1.6
C		2.3
D		4.5

A = Grounded paste prior to oil extraction, B = Oil extraction , C = Moulded paste , D = Kulikuli
NG = No growth

Table 5: Characterization and Identification of Bacteria Isolates from Kulikuli

Gram Reaction	Shape	Cat.	M	U	I	MR	VP	Probable Organisms
-	Rod	+	+	-	+	+	-	*Escherichia coli*
-	Rod	+	+	+	+	+	-	*Proteus* sp
-	Rod	+	-	+	-	+	-	*Klebsiella* sp
-	Rod					+	-	*Salmonella* sp

Cat. = Catalase test, ,M = Motility test, , U = Urease test, , I = Indole test, MR = Methyl Red test,
VP = Voges Proskauer test.

Table 6: Frequency of Occurrence of Bacteria Isolates from Kulikuli

Microorganisms	Total Occurrences	Percentage (%)
Proteus species	6	16.75
Escherichia coli	10	42.25
Klebsiella species	8	20.75
Salmonella spp	8	20.62
Total	32	100

Table 7: Colony Characteristics of Fungi Isolate from Kulikuli

Size	Color	Fungal
Large	Black, grey-green	*Aspergillus* sp
Large	White	*Penicillum* sp
Large	White	*Mucor* sp.

Table 8: Average Mean Fungal Count from Produced Kulikuli

Sample code	**Mean Fungal Count CFU/mL x 10^3**
A1	NG
A2	1.24
A3	1.81
A4	1.32
B1	NG
B2	1.20
B3	2.00
B4	2.40

A& B = Sample code,1- 4 = Production stage

Table 9: Morphological Characteristics of Moulds Isolated from Kulikuli

Colony morphology	Microscopic features	Probable species
Black surface	Hyphae septate, conidiosphores borne on hyphae	*Aspergillus* sp
Grey-green colored mycelium	Hyphae septate, coindiospores borne on hyphae	*Aspergillus* sp
White with tint blackish colonies	Hyphae septate, conidiophoresm, conidia, phalide	*Penicillum* sp
White colored coyyon	Hyphae non-septate with large	*Mucor* sp

Table 10: Antibiogram of Bacteria Isolates against Selected Antibiotics

Bacterial Isolates	Antibiotics (mm)							
	SP (10µg)	AU (10µg)	CH (30µg)	PEF (30µg)	OFX (30µg)	SXT (30µg)	CN (30µg)	CPX (30µg)
E coli	-	-	-	18	14.5	12.5	19	16.5
Salmonella	-	-	-	-	-	-	-	-
Klebsiella	18.5	-	14	-	-	17.5	-	-
Proteus	-	-	15	-	-	-	-	17.5

- = No zone of inhibition, + = Zone of inhibition, SP = Sparfloxacin, AU = Augmentin, CH = Chloramphenicol, PEF = Pefluxacein, OFX = Tarivid, SXT = Septrin, CN = Gentamicin,CPX = Ciprofloxacin

Table 11: Antibiogram of Selected Antifungal against the Fungal Isolates from Kulikuli

Antifungal drug	Drug	Concentration sensitive	Zone of inhibition dose dependent	(mm) Resistance
Fesovin	0.1	≥17	14-16	≤13
Itraconazole	0.1	>16	10-15	<9
Fluconazole	0.15	≥19	15-17	≤14
Griseofulvin	0.5	≥19	10-14	≤14
Ennotab	0.2	>16	10-18	<9

DISCUSSION

In the kulikuli production processing line, the oil extraction and molding stages were the most critical control points (CCPs) and a point of major contaminations. This could be due to the fact that the water used for the extraction was not potable or treated resulting in a heavy contamination with the indicator organisms. However, the equipments used for the oil extraction were not sterile and which could serve as a vehicle of transmission and can easily increase the rate of contaminations. The molding stage was characterized with a heavy fungal contamination, which could be as a result of the unhygienic handling of the molding platforms that were left unkept and not thoroughly clean before use, cross contamination could have also occurred from handlers touching or engaging in other activities while working on kulikuli. The result of this study is in accordance with the result of Ezekiel *et al* (2014) on hazard analysis in kulikuli. The present result revealed that roasting the groundnut and grounding it into a paste is a control point and a point of minor contamination in kulikuli production as the sample collected at this stage of production showed no bacteria contamination in the laboratory produced kulikuli .*Aspergillus* sp however was significant in sample 2. This implies that unless a heavy contaminated container is used to collect the paste during grinding or there is a cross contamination from the grinding machine, contamination in this stage is minimal. The proximate composition of the samples of kulikuli analyzed showed varying similarities and differences in the parameters. The difference could be associated to the type of heat applied during the production of the different kulikuli. This is in accordance with the report of Ezekiel *et al* (2014). Contaminations by microorganisms as seen in this study could occur during handling, storage and transportation as a result of improper processing and storage conditions, thereby exposing groundnut and its products to the risk of contamination with moulds, they eventually produce aflatoxin (Polixeni & Panagiota, 2008 & Mutegi *et al* 2012). In this study, the kulikuli were highly contaminated with bacteria including pathogenic enterobacteria. Fungi were also recovered ,although in lower counts as compared to bacteria load in the samples. The finding in study is in accordance with the reports of Akano and Atanda (2009) & Adebesin *et al* (2011) who evaluated the microbial load of kulikuli from Bauchi, a northern Nigeria city and found the bacterial count to be higher than the fungal loads. They also reported kulikuli to have higher microbial count than other groundnut cereal-based products. Oladimeji & Kolapo (2008) also reported that bacterial loads tend to be significantly higher in food samples than fungal counts. This may be due to the fact that

the generation time of bacteria is lesser than that of fungi especially mould and also because bacteria being unicellular, may reproduce by binary fission unlike moulds which mostly after mycelia extension or spore development. The occurrence of *enterobacteriaceae* especially indicator bacteria such as *E.coli* and *Salmonella* in the kulikuli samples obtained in the sample sites is of a public health concern since this two culprits reflects the poor quality of product offered to the consumers. The indicator bacteria alongside other isolated *enterobacteriaceae* such as *Klebsiella* and *Proteus* have been implicated in several human infections. *Salmonella* and *E.coli* are specially known for their potential to incite food poisoning and intoxication. These bacteria are usually conveyed in food, drink or water by vectors or fecally-contaminated handlers who maintain low level of hygiene as reported by Adegoke (2014). Therefore, the occurrence of these *enterobacteriaceae* in kulikuli samples available for human consumption is alarming and poses great health hazard since their counts exceed the acceptable limits set by the International Commission of Microbiological Specification of Food (ICMSF). The presence of *Aspergillus* species such *as A. flavus and A. niger* and *A fumigatus* and *Penicillim* sp. in kulikuli sample pose a toxicological threat to the consumers since majority of the strain of these fungal species are toxigenic (Jimoh & Kolapo, 2008). Akano & Atanda (2009) reported the presence of these fungi and aflatoxin in kulikuli from Ibadan, Oyo state Nigeria after incidence of death resulting from aflatoxin-contaminated food in Nigeria. The presence of *A. niger, A. flavus, mucor* sp. on these products might be due to improper handling during processing and hawking. Some of these fungi especially *Aspergillus* sp are able to survive in situations where free water is not available. Also the production of spores by these organisms on dried food products makes it possible for them to survive, since their spores are to some extent more resistant to dry conditions than the vegetative mycelia. The presence of these molds on roasted groundnut, Dankwa, Yaji and Kulikuli, may result in production of toxic substances which could lead to health hazard for the consumers. The fungus *A. flavus* attacks groundnut seeds producing the important metabolite, aflatoxin, which has been shown to be highly toxic to man, all domestic and laboratory animals. The bacterial isolates from this study showed sensitivity to the antibiotics as shown in the result except *E. coli* which showed resistance to the entire antibiotic at difference concentrations.

Conclusion

Based on the findings of the study conducted, The HACCP approach has been shown; to identify areas of concern where failure has not yet been experienced, making it particularly useful for new operation. Hazard analysis and critical control point approach in quality management can be used in the preparation of local snacks. The hazards identified in this study are of great health concern. The proximate composition showed little to no significant difference in all the samples analyzed.

Recommendations

1. Enforcement of the Hazard Analysis and Critical Control Points (HACCPs) concepts in food production.
2. Regulatory agency like national agency for food and drugs administration and control (NAFDAC) and public health agency should be mandated to regulate the sales and distribution of these snack so as to prevent food borne diseases outbreaks

3. Water intended for preparation of this snacks should be potable, chemically treated to destroy all potential pathogenic bacteria in proper hygiene and sanitary measures should be practice during processing and handling of these snacks to limit or avoid introduction of bacteria.
4. The hawkers of these products should also handle them in such a way that they would be prevented from insects, especially houseflies, which are known to transmit microbes.
5. These groundnut products could also be packed in sealed transparent polyethylene (nylon) bags after production, before being displayed on the market stands as against exposing them in bowls or glass boxes.
6. Quality control measures (although may be difficult should be adopted) to ensure that the snacks are save and free from any pathogen before human consumption.
7. Care should be taken from the harvesting stage of groundnuts through the processing stage to prevent contamination and infection with microbes.

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Study of Heavy Metal Analysis and Pyhtotoxic Activities of Crude Mathanolic Extract Of *Annona senegalnsis* Stem Bark

***Yakubu N., Amuzat A.O., Abubakar H. Mayaki F.G. & Usman H.B**

Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Abuja.

*Correspondence: Email address: mosada78@gmail.com; Tel: +234(0)8035861774

ABSTRACT

This study was aimed to determine heavy metals concentrations and phytotoxic activities of crude methanol extracts of *Annona senegalensis* stem bark. The fresh stem bark of *A. senegalensis* was collected, air-dried at ambient temperature, ground into the powder and extracted by serial extraction. The concentrations of ten (10) heavy metals in *A. senegalensis* stem bark extracts were determined using atomic absorption spectroscopy (AAS) while the phytotoxic activity was studied using radish seeds in various concentrations of *A. senegalensis* stem bark (1000, 500, 250 and 125mg/ml). The result of the heavy metals analysis showed that the concentrations of Pb, Mg, Mn, Fe, Zn, Cu, Ca, Ni, Hg and Cd were 3.03±0.01, 289.86±2.51, 44.03±0.99, 12.89±0.36, 30.48±0.03, 5.29±0.07, 88.78±2.01, 0.63±0.05, 0.02±0.01 and 0.07±0.03 respectively which were all below maximum permissible levels set by different standard organizations like WHO, FAO and FDA. For the phytotoxic activities, the result shows that the root and shoot lengths of the crude methanol extracts were generally higher than the control. However, there was higher % seed inhibition in the different concentrations of the crude methanol extracts (66.67%, 53.33%, 46.67%, and 80.00%), unlike the control which had only 40.00% seed inhibition. Therefore, *A. senegalensis* could be a promising medicinal plant and plant product based natural herbicides.

Keywords: *Annona senegalensis*, heavy metals, phytotoxic, crude methanolic extract, AAS, radish seeds.

INTRODUCTION

Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine. Over the past century, the phytochemicals in plants have been a pivotal pipeline for pharmaceutical discovery. The importance of the active ingredients of plants in agriculture and medicine has stimulated significant scientific interest in the biological activities of these substances (Moghadamtousi *et al.*, 2015). One of such plant with abundance of phytochemicals is *Annona senegalensis*.

Recently, researchers have shown increasing interest in phytotoxic medicinal plants (Wang et al., 2013; Yang et al., 2012). These could be due to the following (i) the easier screening process of phytotoxic plants from medicinal plants (Chevallier, 1996) or (ii) the ability to contain more phytochemicals in medicinal plants than other plants. Some of these medicinal plants could be used in several ways to control weeds, e.g. (i) transplanting/sowing the plants as cover crops to main crops, (ii) as bioherbicides by direct application of their crude extracts, or (iii) using their active substances as a tool for new natural and biodegradable herbicides

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development upon isolation and characterization (Islam and Hisashi, 2014).

Annona senegalensis, commonly known as Wild Custard Apple and Wild Soursop is a shrub or small tree 2-6 m tall but may reach 11 m under favourable conditions. The species occur along river banks, fallow land, swamp, forests and at the coast. It commonly grows as a single plant in the understory of savannah woodlands (Orwa, *et al.*, 2009). They are found growing throughout Nigeria. It is very common in Northern Nigeria, primarily in Nasarawa, Kaduna, Kano, Plateau, Niger States and in the Federal Capital Territory, Abuja and usually known as Numgbere (Nupe), Gwándàn dààjìì (Hausa) or dukuu-hi (Fulani) (Mustapha, 2013). All parts of *A. senegalensis* plant have been found useful for traditional medicine applications. The leaves have been used in treating yellow fever, tuberculosis, and smallpox (Aiyeloja & Bello, 2006; Mustapha & Uthman, 2013). The stem bark has been used in snakebite and hernia treatment (Dambatta & Aliyu, 2011). The root is used in conditions such as difficulty in swallowing, gastritis, snake bites, male sexual impotence, erectile dysfunction, tuberculosis, and as antidote for necrotizing toxins; the root bark is effective in infectious diseases (Ofukwu & Ayoola, 2008; Noumi & Afiatou, 2015; Jiofack *et al.*, 2009). Juice from the tree is used in the treatment of chickenpox (Faleyimu & Akinyemi, 2010; Ahombo *et al.*, 2012).

Several works have been reported on the phytochemical and biological activities of medicinal plants, although there are few reports in regard to the heavy metal concentrations in the medicinal plants and herbal drugs used (Karayil & Vivek, 2014). The medicinal herbs can cause health risks due to the presence of toxic metals such as Nickel, Lead, Cadmium, Manganese, and Mercury, which are hazardous to humans. Pharmacological evaluation of the medicinal plants was recommended for the purity and quality of the drugs coming from the botanicals (Peter & Smet, 2002). Heavy metals are ubiquitous in trace concentrations in soils and the plants grown in these soils face the heavy metal stress and causes changes in the production of secondary metabolites. High levels of heavy metal contamination in medicinal or other plants may suppress secondary metabolite production. It is essential to maintain safety quality and efficacy of the plants and their products to avoid serious health problems (Karayil & Vivek, 2014).

Thus, according to these backgrounds of *A. senegalensis* multimedicinal uses, the present study was conducted to evaluate specifically stem bark methanol extracts for heavy metal analysis and phytotoxic properties.

METHODOLOGY

Chemicals

Paraquat which was used as a positive control in the phytotoxic assay was purchased from Sigma-Aldrich EC 231-791-2. Methanol used as a solvent in extraction and reagents used for heavy metals analysis were of pure analytical grade.

Sample Collection

The fresh identified stem bark of *A. senegalensis* was carefully harvested and collected from Lapai town, Lapai LGA of Niger state, Nigeria in May 2019. The identification and authentication were performed by a Botanist in the Department of Biological Science, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria.

Sample Preparation

The collected fresh stem bark of *A. senegalensis* was washed with tap water to remove the particulate sand. The collected plant part was dried in an oven equipped with forced air circulation at 40 °C for 72 h. Then, the dried samples were pulverized in an electric mill, weighed, vacuum packaged in plastic bags and stored at ± 4 °C.

Extraction Method

From the dried sample (powder) prepared, treatment extract was produced. Briefly, one hundred

(100) grams of ground plant material was soaked and shaken independently in 1L of 70% (v/v) aqueous methanol for 2 days on an orbital shaker at room temperature. Extraction with methanol was repeated three times, added to the original extract and filtered through one layer of filter paper (number 2; Advantec Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The filtrate was concentrated to dryness under lessened weight at 40°C utilizing a rotary evaporator, Heidolph Laborota 4000, Schwabach, Germany (Islam and Hasashi, 2014). All concentrates were re-suspended in methanol to yield 50 mg/ml crude methanol extract stock fractions. The resulting extract stock fraction was diluted to have working concentrations of 1000, 500, 250, 125 and 0mg/l (control) for the evaluation of phytotoxic effect.

Heavy Metals Analysis

For heavy metal analysis, chemicals of analytical grade were used to prepare a sample of the crude methanolic extract. From the stock fraction, a sample weighing 2 g was taken in a crucible and was ignited in a muffle furnace at 550°C for 6 hrs. The ash produced was digested in 5 ml of concentrated hydrochloric acid followed by evaporation on a hot plate. Then, the small amount of distilled water was added to the digested residue, filtered and volume was made to 30 ml using distilled water. The formed solutions were evaluated using atomic absorption spectrophotometer (model 1100; Perkin Elmer, Waltham, Massachusetts, USA) for the heavy metals, such as, iron (Fe), lead (Pb), zinc (Zn), manganese (Mn), magnesium (Mg), copper (Cu), calcium (Ca), nickel (Ni), mercury (Hg) and cadmium (Cd) (Akram et al., 2015, Abera et al 2015).

Table 1: Working Parameters of Atomic Absorption Spectrophotometer

S/N	Heavy Metal	Wavelength (nm)	Slit Width (cm)	Reference(s)
1	Iron (Fe)	248.3	0.7	Akram *et al.* (2015).
2	Zinc (Zn)	213.9	0.7	Akram *et al.* (2015).
3	Magnesium (Mg)	285.2	0.7	Akram *et al.* (2015).
4	Lead (Pb)	283.3	0.7	Abera *et al*. (2015)
5	Manganese (Mn)	279.4	0.7	Akram, *et al.* (2015).
6	Copper (Cu)	324.7	0.7	Abera *et al.* (2015)
7	Calcium (Ca)	422.7	0.7	Akram *et al.* (2015)
8	Nickel (Ni)	232	0.7	Abera *et al.* (2015)
9	Mercury (Hg)	253.6	0.7	Abera *et al.* (2015)
10	Cadmium (Cd)	228.8	0.7	Abera *et al*. (2015)

Determination of Phytotoxic Activity

Phytotoxic effects were evaluated based on bioassay diaspore germination and early growth of seedlings. The radish seed (Raphanus sativus L. was used as the target species.

Germination bioassay

This was performed in 90-mm (diameter) Petri dishes containing two sheets of filter paper moistened with 5 mL of methanol (negative control) and the same volume of various working concentrations (1000, 500, 250, 125 mg/ml) of the extracts obtained from the powder of *A. senegalensis* stem bark with 25 radish seeds per petri dish were placed at sufficient distance using sterilized forceps.. Each treatment plate was replicated four times, and the dishes were capped and sealed with plastic film. Completely randomized was used as the experimental design. Germination was measured at every 0.5-day interval up to 4 days (the time when no further seeds germinated) and was considered when the radical emerges by rupturing the seed coat as described by Islam and Hisachi (2014). Other germination criterions involved are the measurement of the average root and shoot lengths and number of seeds inhibition (Zeb *et al.*, 2014). All plates were incubated at 25°C in dim light. After 3–5 days of incubation, number of seeds germinated and percent inhibition of root and shoot lengths were calculated using formula: $\%\ inhibition\ of\ the\ root/shoot\ length = \frac{Root/shoot\ length\ in\ test\ sample}{Root/shoot\ length\ in\ control}\ x\ 100$

Statistical Analysis

The data recorded were subjected to Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to separate the means at a 95% confidence interval ($p<0.05$). IBM SPSS, Statistics 20 package, version 9.01 (SAS Institute Inc., Cary, NC, USA) was used.

RESULTS

The concentrations of heavy metals found in the crude powder of *A. senegalensis* stem bark are presented in table 3.1. The results show that the concentrations of Pb, Mg, Mn, Fe, Zn, Cu, Ca, Ni, Hg, and Cd were 3.03±0.01, 289.86±2.51, 44.03±0.99, 12.89±0.36, 30.48±0.03, 5.29±0.07, 88.78±2.01, 0.63±0.05, 0.02±0.01 and 0.07±0.03 respectively. All the values were below maximum permissible levels of the respective heavy metals in medicinal herbs/products as set by different standard organizations like World Health Organization (WHO), Food and Agricultural Organization (FAO) and Federal Drug Administration (FDA).

The result of the phytotoxic effect of the crude extract of *A. senegalensis* stem bark is presented in Table 3. The result shows that the root and shoot lengths of the crude methanol extracts were generally higher than the control. However, there was higher % seed inhibition in the different concentrations of the crude methanol extracts (66.67%, 53.33%, 46.67%, and 80.00%), unlike the control which had only 40.00% seed inhibition. More so, there was statistically significant difference ($p<0.05$) in the results for shoot lengths, unlike the root length that was not.

Table 2: Heavy metals concentrations in the crude powder of *A. senegalensis* stem bark

S/N	Metal	Conc. (mg/mL)	PL (mg/mL)	Reference
1	Pb	3.03±0.01	10	Hussain (2006)
2	Mg	289.86±2.51	2000	Khan *et al.* (2013)
3	Mn	44.03±0.99	200	Khan *et al.* (2013)
4	Fe	12.89±0.36	20	Khan *et al.* (2013)
5	Zn	30.48±0.03	50	Khan *et al.* (2013)
6	Cu	5.29±0.07	10	Maobe *et al.* (201)
7	Ca	88.78±2.01	614	Khan *et al.* (2013)
8	Ni	0.63±0.05	1.5	Khan *et al.* (2013)
9	Hg	0.02±0.01	0.1	Maobe *et al.* (2012)
10	Cd	0.07±0.03	0.3	Khan *et al.* (2013)

Values are presented in means ± standard deviations of triplicate analyses. PL: Permissible limit.

Table 3: Phytotoxic effect of crude methanol extract of *A. senegalensis* stems bark

S/N	Conc. (mg/ml)	ARL (cm)	% RLI	ASL (cm)	% SLI	% SI
1	Control	5.60±0.90[a]	-	5.17±0.87[a]	-	40.00[a]
2	1000	4.06±0.99[a]	72.05	5.00±0.91[a]	96.71	66.67[b]
3	500	5.01±0.83[a]	91.07	5.07±1.14[a]	98.07	53.33[c]
4	250	4.09±0.71[a]	73.04	4.98±1.20[a]	96.32	56.67[c]
5	125	4.93±1.20[a]	88.04	5.10±1.45[a]	98.65	50.60[c]

[a, b] Means with different superscripts on the same column are significantly different ($p<0.05$). All values in the table are means of total no of seeds that germinated in each treatment ± standard error of the mean (SEM). ARL: average root length, RLI: root length inhibition, ASL: average shoot length, SLI: shoot length inhibition and SI: seed inhibition.

DISCUSSION

The availability of heavy metals and their relationships with essential trace elements can generate serious health problems. World Health Organization (WHO) recommends that plant materials, which are used to make finished products for use, may be checked for the occurrence of heavy metals contamination. Pb and Cd are non-essential trace elements that have no useful function in the body. The consequences of chronic Cr intake include skin rash, nasal itch, kidney and liver damage, bleeds, stomach upsets and lungs cancer (McGrath & Smith, 1990) According to WHO, FAO and FDA, the specific limit of heavy metals under study are presented in Table 2. It was observed that crude powder of *A. senegalensis* stem bark recorded lower concentrations of heavy metals under study than the limit of permissibility (Table 2). This finding is similar to the reports of Karayil & Vivek (2014) and Akram *et al.* (2015). Some of these heavy metals, although very toxic at high levels, play some crucial physiological and metabolic roles. For instances, Copper (Cu) deficiency can cause anemia and congenital inabilities (Maobe *et al.*, 2012) while Nickel (Ni) is required by the body in very small quantity for insulin production in

the pancreas. Zinc is co-factor of over 200 enzymes and is also involved in metabolic pathways (Khan *et al.*, 2012).

Germination bioassay is the widely used method to determine the phytotoxic activity (Rice, 1984, Putnam & Tang, 1986) and the early seedling growth is the most sensitive stage to evaluate the phytotoxicity (William &Hoagland, 1982; Wardle et al., 1991; Gong et al., 2001). A number of indices have been introduced by previous researchers to study the phytotoxic activities of phytotoxic substances on germination process (WHO, 2005; Maobe et al., 2012). To investigate the phytotoxic potential of *A. senegalensis* stem bark extracts on the germination process, we determined some important germination indices: % seed germination inhibition (%SI), root length (RL), % root length inhibition (%RLI), shoot length (SL), and % shoot length inhibition (%SLI). High significant % seed germination inhibitions were recorded in all extract concentrations used compared to control. However, no significant differences were observed in root and shoot length of germinated seeds in all tested concentrations compared to control while the % RLI and SLI were significantly greater than that of control (Table 3). All these signify the phytotoxic potential of *A. senegalensis* stem bark extracts on targeted seeds. Anjum and Bajwa (Anjum & Bajwa, 2005) and Hussain et al. (2008) demonstrated that phytotoxic plant extracts or substances caused inhibition or delay of the germination process. All these are in line with the findings of Ayaz, *et al.* (2014). This signifies that crude methanolic extract of *A. senegalensis* stem bark could be a good source of natural herbicides for weeds control in a sustainable manner to increase per acre yield. Different weeds are amongst the most key factors responsible for the significant reduction in crops yield (Ayaz, *et al.*, 2014). To counteract these unwanted weeds, different chemicals are used. However, such chemicals are frequently associated with environmental pollution, residual toxicity, carcinogenesis, high cost and hence their use is restricted (Khuda, *et al.*, 2012; Zeb, *et al.*, 2014). Consequently, the search for alternative herbicides which are safe and cost-effective is needed. Natural herbicides may be among these alternatives (Ayaz, *et al.*, 2014).

The sensitivity of early growth of seedling (% RLI and SLI) to *A. senegalensis* stem bark extracts as compare to % seed germination inhibition as indicated in Table 3, probably could be due to (1) the presence of seed coat that serves as a barrier between the seed embryo and its environment (Araujo & Monteiro, 2005), (2) the seed coats selective permeability that may prevent the inhibitory activity of phytotoxic extract/substances to penetrate the seed coats (Wierzbicka & Obidzinska, 1998), (3) the protrusion of the root through the seed coat (parameter used to measure germination) which does not necessarily mean growth by cell division (Sawatore et al., 2008).

Conclusion

From the findings of this study, it can be concluded that crude methanolic extract of *A. senegalensis* could be relatively not toxic and safe for use in the preparation of medicinal products. The results also indicated that *A. senegalensis* stem bark extracts have phytotoxic properties and could contain phytotoxic substances. Hence, *A. senegalensis* could be a promising medicinal plant and plant product based natural herbicides.

Recommendations

Further studies should be carried out on the isolation and identification of allelopathic substances, which may promote the

development of new natural herbicides to replace the chemical herbicides currently available which have become sources of serious environmental pollution.

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Proximate Composition and Some Selected Metals Contents of *Neocarya macrophylla* (Gingerbread Plum)

***Elele U.U. & Okonkwo T.P.**

Department of Chemistry, Ibrahim Badamasi Babangida University, Lapai, Abuja.

*Correspondence: Email address: uuelele@yahoo.ca; *Telephone: +2348033803880*

ABSTRACT

The leaves, barks, stems, and unripe fruits of *Neocarya macrophylla* (Gingerbread Plum), cultivated in Lapai, Niger State, Nigeria and its environs were collected randomly from four locations: IBBUL, Lapai town, Agaie, and Jangada. Soil samples were also collected at a depth of 0-20 cm from the vicinity of the *Neocarya macrophylla* plants in order to assess the possibility of using *Neocarya macrophylla* in phytoremediation. The plant samples were analyzed using standard methods for their proximate composition and some metals content (Cd, Fe, Mg, Mn and Zn) using AA500 Atomic Absorption Spectrophotometer while the soil samples were analyzed for their metal contents only. Blank determinations were also carried out. Two standard reference materials: SRM 1573a and SRM 2709 were analyzed by the same digestion and analytical methods. The SRMs results showed coefficient of variation of less than 7 % between the certified values of the metals under study and those obtained in this study. The nutrient values varied amongst the *Neocarya macrophylla* parts and from location to location. Cadmium (Cd) was not detected in any of the plant and soil samples while Fe content was highest in all the samples. Also, the concentrations of Fe, Mg, Mn and Zn also differed in the samples from one location to another possibly due to differences in the rock parent materials, soil properties, age of the plants and anthropogenic activities. The concentrations of the metals in the samples determined did not exceed phytotoxic levels reported in literature. *Neocarya macrophylla* is not a hyperaccumulator and cannot be used in phytoremediation of polluted soils. However, it is advisable that heavy metal concentrations in edible plants be monitored regularly so as to avoid toxic effects on livestock and human beings.

Keywords: *Neocarya macrophylla*, proximate, heavy metal, AAS, soil, Lapai.

INTRODUCTION

Neocarya macrophylla Sabine (formerly *Parinari macrophylla* Sabine) is commonly known as Gingerbread plum (English), Gawasa or *Furar rura* (Hausa), Pútú'bá (Nupe) or Neou oil tree. It belongs to the *chrysobalanaceae* family (Muhammed et al, 2015). It is a small, bushy evergreen tree growing up to 6-10 meters or sometimes often less making it possible to harvest its fruits from the ground (Amza et al, 2011). *N. macrophylla* is native to western and central Africa from Senegal to Nigeria. It is grown in arid and semi-arid regions mainly in the western part of Africa (Ajayi, Ifedi & Adebola, 2019). The plant is grown in northern part of Nigeria (Amza et al, 2011). It bears large, edible, starchy fruits, which are about 5 cm long and can be used for different purposes. Many of the fruits are eaten fresh or boiled with cereals and fragrant syrups are prepared using the fruits, which has proved to be much better than

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some fruit juices (National Research Council, Development, Security, Cooperation Policy and Global Affairs, 2008, Amza et al, 2011). The fruit's flesh is soft and yellowish in colour when fresh with a flavour similar to that of the avocado fruits and the endocarp is covered with soft mealy pulp that is edible, the wood is light brown in colour and fairly hard. The living tree provides villagers with shelter, dye, glue, fodder, firewood, soap, structural materials, and even termite repellents (Ifedi, Ajayi & Anibuko, 2017). The kernels inside the seeds are usually roasted like cashew or almond nuts.

N. macrophylla is a plant that is available throughout the year with multipurpose utility. It is used traditionally for the treatment of cancer, ear and eye infections, asthma, skin infections, toothache, etc. The bark and leaves are used as mouth wash, internal troubles and for inflamed eye (Frederick, 1961). The leaves can be chewed to relieve pain, playing the role of an analgesic (Amza et al, 2010). The stem bark of *N. macrophylla* was observed to be effective against *Eschericia coli, Bacillus subtilis, C. albicans, Streptococcus, pyogenes* and *Bacillus cereus*, which are the causative agents for some sicknesses namely, diarrhea, dysentery, skin and mouth infections (Yusuf et al, 2015). The leaves have antihelminthic activities (Barnabas et al, 2011). It is also used to impart pleasant scent to ointments (Warra et al, 2013). Its oil extracts and the ash obtained from its burnt nut/seeds is used for soap making. Recently, the toxicological evaluation of its seed oil had been studied (Ajayi et al, 2019).

Multipurpose utility of *Neocarya macrophylla* necessitated this study aimed at determining the proximate composition of leaves, barks, stems, and unripe fruits of *Neocarya macrophylla* cultivated in Lapai, Niger State, Nigeria and its environs as well as some metals (Cd, Fe, Mg, Mn and Zn) contents so as to ascertain the possibility of its use in phytoremediation.

METHODOLOGY

Materials/Apparatus

The following materials/apparatus were used in this study: stainless steel knife, soil auger, beakers (50 cm^3, 250 cm^3 and 1000 cm^3), 1 and 2 mm mesh sieve, MP5002 weighing balance, spatula, watch glass, porcelain crucibles, Jenway 1000 hot plate with stirrer, deluxe power blender, mortar and pestle, DHG-9202 series thermal electric thermostatic drying oven, Soxhlet extractor, AA500 Atomic absorption spectrophotometer, Model SXL-1008 Muffle furnace, plastic trays and sample bottles, funnel, volumetric flask (50 cm^3 and 100 cm^3), Whatman No. 41 ashless filter paper, and masking tape.

Reagents

The reagents were HNO_3 and HCl (Loba Chemie, India); NaOH, H_2SO_4, $HClO_4$ and petroleum ether (40-60 °C) (Sigma Aldrich, USA), 30 % H_2O_2 (Alpha Chemika, India) and were of analytical grade. The reagents were used in the preparation of 1:1 HCl, 10 % HNO_3, 0.25 M H_2SO_4 and 0.31 M NaOH solutions.

Sample collection and handling

The samples were randomly collected from the premises of Ibrahim Badamasi Babangida University, Lapai (IBBUL); Lapai town, Jangada and Agaie. The plant samples (leaves, bark, stem and fruits) of *N. macrophylla* were collected using stainless steel knife and placed in labeled envelopes while the soil samples were collected from the vicinity of the plants at a depth of 0-20 cm using a soil auger. Care was

taken to prevent contamination of the plant and soil samples. Some part of the plant samples were immediately subjected to moisture content determination at 105 ^{O}C. The remaining plant samples were dried in an oven at 70 ^{O}C for 5 hours. The dried plant samples were homogenized and sieved with the 1 mm sieve. The soil samples were air-dried at room temperature on plastic trays for 7 days, after which they were pounded and sieved using the 2 mm mesh sieve. The sieved plant and soil samples were packed into clean dry appropriately labeled polythene leather, and stored room temperature ready for analysis.

Proximate analysis of the plant samples

Standard procedures of the Association of Official Analytical Chemists (AOAC) (2006) were used for proximate analysis. The moisture content was determined by weighing 5.00 g of the fresh samples into well labeled crucibles and oven-drying at 105 °C to constant weights. The dried samples were then cooled in a desiccator for 30 minutes and weighed. Ash content was determined by weighing 2.00 g of the dried samples into porcelain crucibles; they were firstly ignited and then transferred into the Model SXL-1008 Muffle furnace at 550 ˚C for 8 hours. Then, the crucibles were cooled in a desiccator and weighed. For the crude lipid, 2.00 g of the dried sample was extracted with petroleum ether (40-60 °C) for 8 hours in a Soxhlet extractor. The solvent was recovered and the oil dried in the oven at 70 °C for 1 hour. The crude fiber was determined by treating 2.00 g of the sample with 0.25 M H_2SO_4 under reflux for 30 minutes and 0.31 M NaOH; followed by refluxing for 30 minutes and filtration under suction. Crude protein analysis was determined by the micro-Kjeldahl's method and calculated as % N_2 (Nitrogen) × 6.25. Blank determinations were carried out and all determinations were in triplicate. The total carbohydrate was obtained by difference.

Wet digestion of plant and soil samples for metal analysis

Method 975.03 of AOAC (2006) was used in the wet digestion of the plant samples and the digests were finally analyzed with the AA500 Atomic absorption spectrophotometer while the soil samples were wet digested using the United States Environmental Protection Authority (USEPA) (1996) Method 3050B.

Statistical Analysis

All statistical analyses were performed using Microsoft Office Excel 2007 on Windows 7 system and data obtained were presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Table 1 shows the results of the heavy metal contents of NIST Standard Reference Material - SRM 1573a (Tomato leaves).

The precision of the metals were 3.2, 2.4, 6.8, 3.9, and 1.2 % for Cd, Fe, Mg, Mn, and Zn respectively indicating that the AOAC (2006) method used in preparing the plant samples for atomic absorption spectrophotometry was good with small degree of variation.

Table 1. Heavy metals contents of SRM 1573a (Tomato Leaves)

Metal	Certified Value, mg kg^{-1}	Measured Value*, mg kg^{-1}	Coefficient of Variance, %
Cd	1.52 ± 0.03	1.54 ± 0.05	3.2
Fe	367.5 ± 4.3	362.0 ± 8.80	2.4
Mg	12000 (estimate)	11740 ± 795	6.8
Mn	246.3 ± 7.1	244.04 ± 9.40	3.9
Zn	30.94 ± 0.55	31.52 ± 0.38	1.2

*Values are mean of triplicate analysis ± standard deviation.

Table 2. Heavy metals contents of SRM 2709 (San Joachin Soil)

Metal	Certified Value, $mgkg^{-1}$	Measured Value*, $mgkg^{-1}$	Coefficient of Variance, %
Cd	0.371 ± 0.002	0.35 ± 0.02	5.7
Fe	33600 ± 700	33730 ± 480	1.4
Mg	14600 ± 200	14550 ± 230	1.6
Mn	529 ± 18	533.4 ± 1.8	3.4
Zn	103 ± 4	101.6 ± 0.9	0.9

Values are mean of triplicate analysis ± standard deviation.

Table 2 shows the results of the heavy metal contents of NIST - SRM 2709 (San Joachin Soil).

The precision of the metals in Table 2 were 5.7, 1.4, 1.6, 3.4, and 0.9 % for Cd, Fe, Mg, Mn, and Zn respectively indicating that the USEPA (1996) Method 3050B used in the preparation of the soil samples was quite good. For both SRMs, the coefficient of variation was less than 7 %.

Figure 1 shows that the moisture content (fresh weight basis) varied amongst the *N. macrophylla* parts and from location to location. The stem of *N. macrophylla* collected from Agaie had the highest percentage of moisture (67.65 ± 1.22 %) while the leaves of Lapai *N. macrophylla* had the lowest percentage moisture (20.01 ± 0.02 %). The differences observed could be due to the age of the plants used in this study as well as the frequency of rainfall in the areas where the plants grew. The moisture content of the unripe fruits from the different locations was almost the same. The moisture contents of the plant parts used in this study from the different locations were less than the 80.05 ± 0.17 % reported by Muhammad and Umar (2015) for the fruit peels. According to Hassan and Umar (2004), the higher the moisture content of a plant material, the higher the tendency of microbial activities that affects the stability and quality of the material.

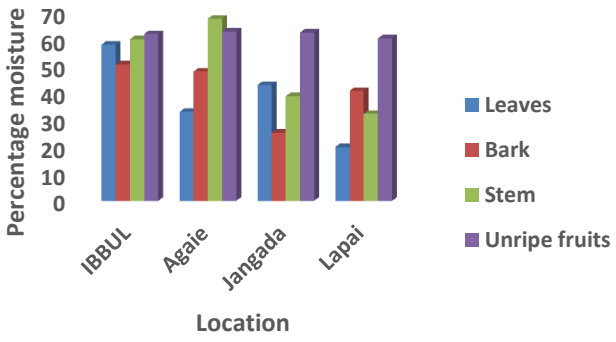

Figure 1: Percentage moisture in various plant parts at different locations.

The ash content of the bark of *N. macrophylla* ranged from 2.02 ± 0.00 % in the stem collected from Agaie to 8.33 ± 0.03 % in the leaves collected from Lapai. The ash content of a material is indicative of its mineral contents: the higher the percentage ash, the greater the mineral contents and vice versa. The ash content of 4.4 ± 0.8 % reported by Amza et al (2010) for *N. macrophylla* kernel and 5.20 ± 0.19 % reported by Muhammad and Umar (2015) for *N. macrophylla* fruit peels fall within the range obtained in this study.

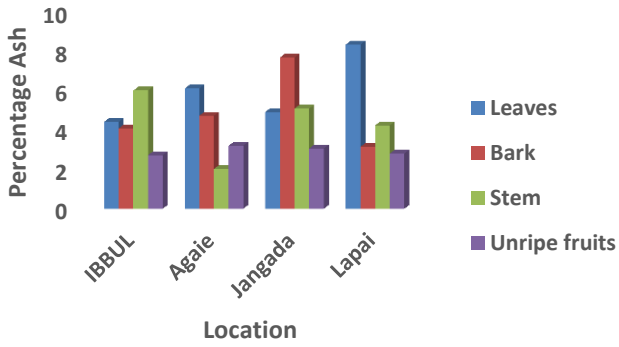

Figure 2: Percentage ash in various plant parts at different locations.

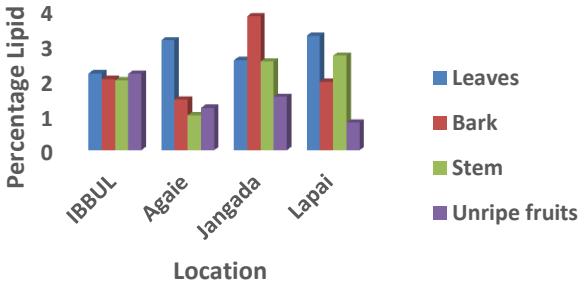

Figure 3: Percentage crude lipid in various plant parts at different locations.

The unripe fruits of *N. macrophylla* collected from Lapai had the lowest crude lipid (0.79 ± 0.10 %) while the bark collected from Jangada had the highest value (3.84 ± 0.04 %). Muhammad and Umar (2015) reported percentage crude lipid for *N. macrophylla* fruit peels of 2.30 ± 0.70 % in their study, which falls within the range of values obtained in this study.

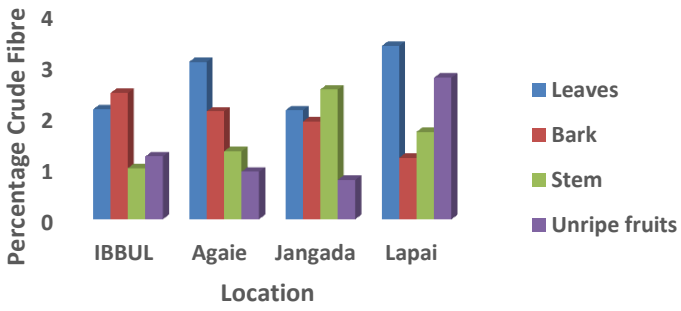

Figure 4: Percentage crude fibre in various plants parts at different locations.

Figure 4 shows that the leaves of Lapai *N. macrophylla* had the highest value for crude fiber (3.39 ± 0.06 %) while the Jangada unripe fruits contained the least crude fibre (0.77 ± 0.03 %). However, a higher crude fibre of 5.83 ± 0.18 % was reported for *N. macrophylla* fruit peels (Muhammad & Umar (2015). Dietary fibre is important in the body system as helps to control blood sugar levels, maintain healthy bowel, lowers cholesterol and helps to achieve healthy weight (Wasagu et al, 2013).

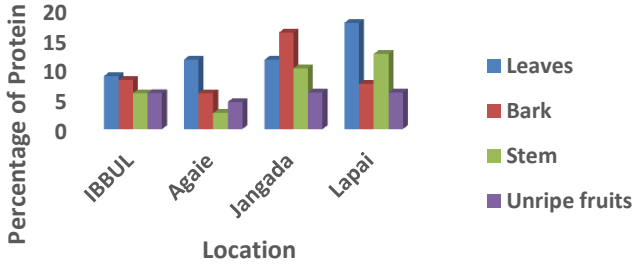

Figure 5: Percentage of crude protein in various plants parts at different locations.

The crude protein content ranged from 2.68 ± 0.03 to 17.74 ± 0.10 % with the lowest and highest contents determined in the Agaie *N. macrophylla* stem and Lapai *N. macrophylla* leaves respectively. In general, the *N. macrophylla* leaves contained the highest amount of protein and could be used as ingredients in compounding animal feeds since plant foods with about 12 % protein are regarded as good sources of protein (Watt & Merrill, 1963). The crude protein contents for the leaves, bark, stem and unripe fruits of *N. macrophylla* were generally higher than the 2.50 ± 1.11 % reported for the fruit peels by Muhammad and Umar (2015).

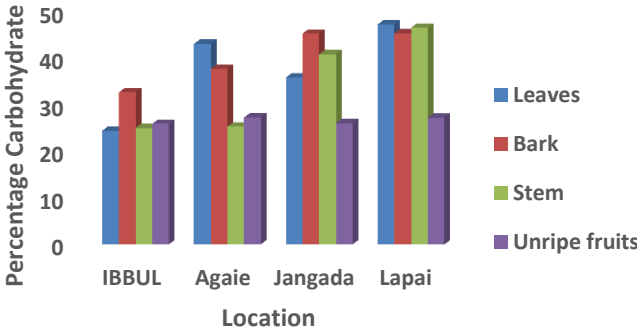

Figure 6: Percentage carbohydrate (CHO) in various plants parts at different locations.

The carbohydrate content was calculated by subtracting the sum of the percentages of moisture, ash, crude lipid, crude fibre and crude protein from 100 %. The various parts of *N. macrophylla* showed high percentage of carbohydrate that ranged from 24.40 ± 0.02 % in the leaves of *N. macrophylla* collected from IBBUL to 47.25 ± 0.10 % also in the leaves of *N. macrophylla* collected from Lapai.

Figure 7: Concentration of some selected metals in the soils at different locations.

All the metals studied are heavy metals with densities greater than 5.0 gcm^{-3} except Mg, which is an alkaline-earth metal with a density of 1.74 gcm^{-3}. Cadmium (Cd) was not detected in all the soil and *N. macrophylla* samples used in this study. This is possibly due to the fact that the study areas are non-industrialized areas with low intensity of chemical usage (Akinola, Njoku & Ekeifo, 2008). The World Health Organization (WHO) (1998) permissible limit for Cd in dried medicinal plants is 0.3 $mgkg^{-1}$ while that of soil is 3 $mgkg^{-1}$.

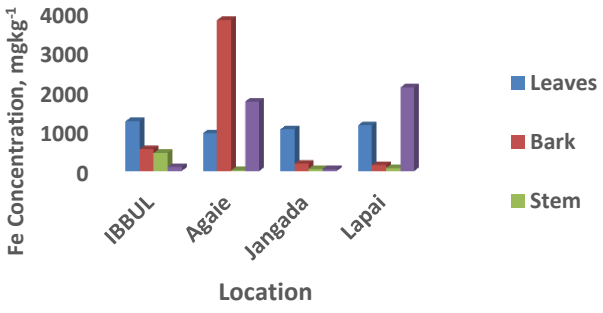

Figure 8: Concentration of Fe in various plant parts at different locations.

From Figure 8, the concentration of Fe in the soil samples in the locations used in this study ranged from 1730.0 ± 0.2 to 6480.0 ± 0.3 mgkg^{-1} and was the highest amongst the concentrations of the metals determined. Iron is an essential metal in plants and animals. The WHO (2001) permissible limit for iron in edible plants is 425 mgkg^{-1}. The concentration of Fe in the leaves of *N. macrophylla* from all the locations were higher than the WHO permissible limit for edible plants while the barks of *N. macrophylla* collected from Jangada and Lapai; the stems collected from all locations except IBBUL; and the unripe fruits collected from Agaie and Lapai were lower than the WHO permissible limit. The permissible limit for Fe in soil is 35,000 mgkg^{-1} (Kabata-Pendias, 2011). The concentrations of Fe in soils from the four locations were IBBUL (6,480.0 ± 0.3 mgkg^{-1}), Agaie (6,480.0 ± 0.2 mgkg^{-1}), Jangada (2,430.0 ± 0.1 mgkg^{-1}) and Lapai (1,730.0 ± 0.2 mgkg^{-1}). These Fe concentrations are lower than the WHO permissible limit. IBBUL and Agaie have higher concentration of iron in their soils than Jangada and Lapai. The level of iron at these locations does not show potential hazard to the users. Iron is an essential heavy metal important in plants for the development of chlorophyll and a range of enzymes. In the human body, iron is very important for the production of hemoglobin and transport of oxygen; its deficiency causes anaemia (Thomas, 2002).

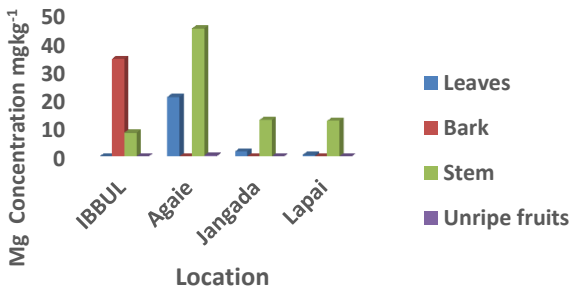

Figure 9: Concentration of Mg in various plant parts at different locations.

In the soil samples, the concentration of Mg from the four locations ranged from 0.00 ± 0.01 to 75.5 ± 0.3 mgkg^{-1} (see Figure 7). Magnesium (Mg) contents in the various parts of *N. macrophylla* at all the locations were generally low and ranged from 0.0 ± 0.0 to 45.0 ± 0.4 mgkg^{-1} with the stem containing moderate amounts of Mg (see Figure 9). The stem of NM at Agaie had the highest Mg content *N. macrophylla* bark at IBBUL (34.3 ± 0.2 mgkg^{-1}). Amongst the leaves, *N. macrophylla* leaves at Agaie had the highest concentration of 20.9 ± 0.6 mgkg^{-1}. From Figure 9, the concentration of Mg in all the samples was lower than its WHO permissible limit of 32,500 mgkg^{-1}. Magnesium was absent in soils collected from IBBUL and Agaie while Jangada soil contained the highest concentration of Mg (75.5 ± 0.3 mgkg^{-1}) which is lower than WHO permissible limit.

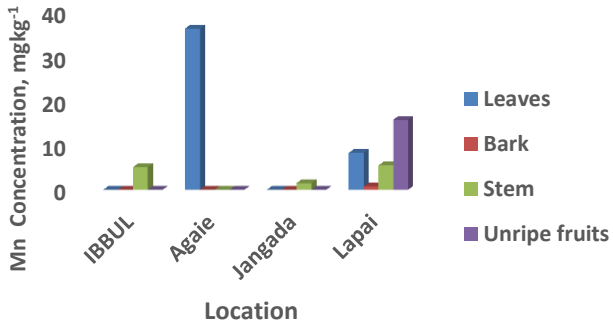

Figure 10: Concentration of Mn in various plant parts at different location.

The concentration of Mn in the soil samples from the four locations ranged from 74.5 ± 0.1 to 262.6 ± 0.2 mgkg^{-1} (see Figure 7). Manganese was not detected in most parts of *N. macrophylla* and its contents were generally lower than the Mg contents (see Figure 10). The range of Mn in the *N. macrophylla* parts was from 0.0 ± 0.0 to 36.4 ± 0.3 mgkg^{-1}. The highest concentration of was detected in the leaves of *N. macrophylla* collected from Agaie and was seconded by the unripe fruits collected from Lapai (15.08 ± 0.4 mgkg^{-1}). Other plant parts at various locations showed Mn content that was less than 10.0 mgkg^{-1}. The WHO permissible limits of Mn in soils and plants are 2,000 and 500 mgkg^{-1} respectively.

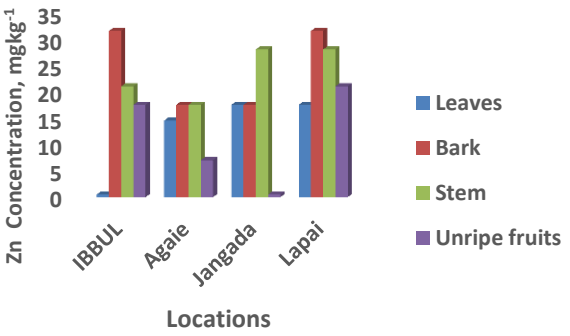

Figure 11: Concentration of Zn in various plant parts at different location.

From Figure 11, the concentration of Zn was highest in the bark of *N. macrophylla* collected from IBBUL and Lapai with the values 31.5 ± 0.2 and 31.5 ± 0.3 mgkg^{-1} respectively. Zinc contents of most of the other plant parts at the various locations were less than 14.0 mgkg^{-1} with the exception of the leaves of *N. macrophylla* collected at IBBUL and the *N. macrophylla* unripe fruits collected at Jangada and Agaie. The WHO permissible limit for Zn in soils is 300 mgkg^{-1} while in plants, it is 50 mgkg^{-1}, which is higher than the values obtained in *N. macrophylla* parts used in this study. The concentration of zinc in the soil samples from the four locations ranged from 65.0 ± 0.2 to 73.0 ± 0.0 mgkg^{-1}, which is lower than the WHO permissible limit for soil. This shows that the plant parts and soils analyzed do not have potential of zinc poisoning. The trace heavy metals like Fe, Zn, and Mn become harmful and toxic when their concentration exceeds the recommended standards (Shah et al, 2013). According to Kabata-Pendias (2011), Hyperaccumulators are plants and/or genotypes that accumulate metals above certain concentrations in leaves and should contain trace metals in leaves above the following levels (mgkg^{-1}): >100 for Cd; >1000 for Cu, Pb and >10,000 for Mn, Zn. None of the concentrations of the metals studied exceeded these stipulated values.

Conclusion

The findings of this study have shown that *Neocarya macrophylla* is a good source of carbohydrates and protein with a moderate value of lipid and fibre. The concentrations of metals in the plants varied from location to location possibly due to the differences in soil

properties, age of the plants, extent of fertilization, and climatic conditions. *Neocarya macrophylla* is not a hyperaccumulator and cannot be used in phytoremediation of polluted soils. It is safe to consume *Neocarya macrophylla* cultivated in Lapai, Niger State, Nigeria and its environs.

Acknowledgement

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Effect of Shea Nut Conditioning on The Quality of Shea Kernel: Optimisation of Process Variables Using RSM

***[1]Saba A.M., [2]Okafor J.O., [2]Adeneyi O.D., [3]Egwim E.C., [2]AZEEZ O.S. & [4]Muhammad A.**

[1]Department of Chemical Engineering, The Federal Polytechnic, Bida, Nigeria.
[2]Department of Chemical Engineering, Federal University of Technology, Minna, Nigeria.
[3]Department of Biochemistry, Federal University of Technology, Minna, Nigeria
[4]Science Department, of Chemistry, Ibrahim Badamasi Babangida University, Lapai, Abuja.

Correspondence: Email address: sabaam2006@gmail.com; Telephone: +2348039662075*

ABSTRACT

Shea kernel is a product of Shea tree from where a highly valued vegetable oil with wide range of applications in industries and home care products are obtained. Thus, the need to improve Shea kernel quality in Nigeria becomes necessary. This study investigated the effects of optimizing processing factors, responses, development and analysis of predictive response models of Shea kernel leading to optimization of Shea kernels. The methodology adopted in this research involves collection of fresh and riped Shea fruits that have fallen to the ground, removal of Shea pulp to expose the Shea nut, conducting preliminary experiment on the Shea nut to establish the effects of Shea nut conditioning period (SNCP), Shea nut boiling duration (SNBD) and Shea nut drying temperature (SNDT) on the Free fatty acid of Shea kernel. The boundary conditions obtained from the preliminary experiments were used for the design of experiment (DOE) by Box-Bekehn method of response surface methodology. Fresh Shea kernels were then processed according to the experimental design and the responses of free fatty acid, peroxide value and percentage oil content were determined. The upper, middle and lower limits obtained for SNCP, SNBD and SNDT are 1, 6.5, 12day; 0, 60, 120min and 30, 70, 110^{o}C respectively. The optimum conditions of SNCP, SNBD and SNDT obtained after the optimization of Shea kernel are 4.0day, 120min and 86^{o}C and the corresponding predicted responses of PV, percentage oil content, FFA and desirability are 2.868 meq/kg, 0.628%, 53.85% and 0.878 respectively.

Keywords: Shea-kernel, SNCP, SNBD, SNDT and Optimization

INTRODUCTION

Researchers and scientist all over the world are continuously looking for ways and means of improving the yield and quality of vegetable oils to meet both domestic and industrial applications, (Aculey, 2012).Shea butter is one of these numerous vegetable oils and it's a fatty extract obtained from the kernels of Shea fruit. It is also a mixture of fatty acids usually Oleic, Stearic, Palmitic, Linoleic and Arachidic acids with Oleic and Stearic acids predominating and together constituting about 85% of the fatty acid content of Shea butter (Coulibaly *et al*., 2009 and Julius *et al*. 2013). The presence of these fatty acids in Shea butter varies in proportion depending on the source of the Shea nuts. The nut is obtained from Shea tree which is a native of Africa and it is either called *Vitellaria paradoxa or Butyrospernumparkii* in West Africa or *"nilotica"* in East Africa. Nigeria is well endowed with this tree accounting for

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about 50 to 60% of West African Shea tree population. In Nigeria, these trees are concentrated in North Central states of Niger, Kwara, Nassarawa, Zamfara, Adamawa, Edo, Yobe Plateau, Kaduna and some parts of Kebbi, Bauchi, Kogi, FCT and Oyo (Naspan, 2018 and Koloche *et al.,* 2016)**.** Statistically, it has been shown that 40–50% of these Shea trees are found in Niger state, even though the statement is not updated, (Koloche *et al,* 2016). The tree starts flowering and fruiting in January and harvest begins between May to June through to August. After the harvest, the pulp is removed, the nuts are dried, the shell is removed and the Shea kernels processed for Shea butter.Lack of standard methods in Shea nut processing andproper documentation to show the variation in Shea kernel quality with processing factors have call for this research work. The aim of this research work is to evaluate and optimize the process variables for Shea kernel processing with a view to obtain optimum quality parameters of peroxide value (PV), percentage oil content and free fatty acid (FFA) for grade 'A' Shea butter production.This research shall contribute and improvedthe quality of traditional processing method of Shea kernel through Shea kernel optimization on the percentage oil content and quality processing method.

METHODOLOGY

Collection of Shea Fruit

The Shea fruits were collected from 4.5 hectares ofexperimental field housing 67 Shea trees along Sonmajigi village (N09^{o} 11$^{'}$ 56$^{''}$; E05^{o} 35$^{'}$ 45$^{''}$), in Lavun Local Government Area of Niger State, Nigeria. The Shea fruits that had matured, fallen to the ground werepicked, taken to theresearch site and kept in the open for the required duration of time. The picking was done between the hours of 9am to 11am daily to ensure consistency in timing of the Shea fruits picking throughout the period of collection (July to August). On eachpicking day the Shea pulp were removed manually by applying hand pressure to expose the Shea fruit.

Preliminary Experiment to Establish the Boundary Conditions of Variables for Optimization of Shea kernel

Effect of SNCP on the Free Fatty of Shea Kernel

A batch of 5kg of fresh fruits was collected as described and conveyed to the Federal Polytechnic Bida, the research site in Nigeria. The jute bags were used to reduce the heat generated within the bags to soak the surface moisture on the fruits. At the site where they are kept under a tree in the open space to mimic natural habitat conditions in the experimental site four pieces of Shea fruits considered adequate for the analysis were taken each day for 16 day. The pulp of the Shea fruit which is soft and tender was removed simply by applying hand pressure. Thereafter, the brown hard-shell enclosing the kernel was cracked using wooden pestle and mortar to expose the kernel. The kernel obtained was ground using 1.5kW Atlas grinding machine and screened using laboratory sieve shaker to a particle size of between 0.05 mm to 0.1 mm and then analyzed for FFA.The experiment was repeated for SNCP of between 2 day to 16 day and their corresponding FFA calculated and the resultsare as shown in figure 3.1.

Effect of Boiling Duration on the FFA of Shea Kernel

Similarly, 5kg was collected, measured using diamond weighing balance and the pulp removed. The fresh Shea nut obtained was washed severally with water to remove all the

left over pulp and then carefully poured into boiling water in an aluminium pot heated on a laboratory hot plate. As the boiling was continued, 4 pieces of Shea nut considered adequate for the FFA analysis were collected randomly from the lot at intervals of 20 min for up to 120 min. The nuts collected were then cooled in a medium size desiccator. The hard shell of the cooled Shea nut was then cracked to expose the kernel; the kernel was ground, screened to between 0.05 mm to 0.1 mm size and then analyzed for FFA as described in subsection 2.3.1. The experiment was repeated for SNBD of 40 min, 60 min, 80 min, 100 min and 120 min and their corresponding FFA was calculated and the resultsare as shown in figure 3.2.

Effect of Drying Temperature on FFA of Shea Kernel

Another 5kg of fresh Shea fruit collected from the experimental field was measured using Diamond weighing balance. The fruit was de-pulped and washed. The nuts were fed into the cylindrical chamber of the designed and fabricated rotary dryerwith the aid of a removable feed-in hopper, then temperature was first set at 30^{o}C, and the power button was switched on and as the cylindrical chamber rotates at a speed of 13.3 rpm, the blower attached to the burner was switched on and hot air from the burner is convected to the chamber. Inside the chamber, heat is transferred from the wall and to the pipes through conduction to the nuts. The heat produced in the burner was generated by the Shea shell briquette. At intervals of one hour, the power button was switched off; the Shea nut was rolled out through the discharge slot by jacking up the cylindrical chamber of the dryer with the aid of a manually operated hand crank. The weight of the Shea nutwas measured using diamond weighing balance to check for moisture loss. The drying was continued until two successive constant weights were obtained. The shell of the Shea nut was then cracked using wooden pestle and mortar to expose the dried kernels. The dried kernels were ground using a 0.75 Solitaire laboratory blender and screened to between 0.05 mm - 0.1mm using laboratory sieve shaker**.** 2 g of the screened ground kernels was measured using Mettler Toledo balance and was analyzed for FFA as described in Subsection 2.3.1. The experiment was repeated for Shea nut drying temperatures set at 50^{o}C, 70^{o}C, 90^{o}C, and 110^{o}C and their respective FFAs were calculated and the result is as shown in figure 3.3.

Methods of Analysis

Determination of FFA of Shea Kernel

This was done according to AOCS (1994) and the volume of NaOH consumed was recorded and used in equation 2.1 to calculate the free fatty content of the kernel.

$$\mathrm{FFA}\,(\%) = \frac{(\mathrm{V}-\mathrm{B})\times \mathrm{N_f} \times 28.2}{\mathrm{W}} \qquad 2.1$$

Where V = Volume of NaOH ethanolic solution used for titration (ml), B =Volume of NaOH consume during FFA determination blank titration (ml), N_f =Normality of NaOH factor, W = Weight of Oil sample (g).

Percentage Oil Content Determination and Extraction Efficiency

The percentage oil yield was determined by using the relationship below:

$$Oil\,Yield, Y = \frac{W_u - W_e}{W_u} \times 100 \qquad 2.2$$

Where,W_u = Weight of unexpressed sample (g), W_e = Weight of expressed sample (g)

The Extraction Efficiency, E_E is defined as follows

$$E_E = \frac{S_y}{T_{sy}} \times 100 \qquad (2.3)$$

Where,S_y = Sample oil yield in percentage (%) and T_{Sy} = The total seed oil content in percentage (%)

Determination of PV of Shea Kernel

This was done according to ISO 3960: 2005and the PV determined for each sample using the expression below;

$$Peroxide\ Value\ (\text{mEq/100g}) = \frac{(T \times M \times 1000)}{\text{Weight of Sample } (W)} \qquad 2.4$$

Where, T = Titre value Na_2SO_3 (cm^3), M = Molarity of Na_2SO_3.(mol), W =Weight of Oil sample (g)

Optimization Procedure and Validation of Responses from Shea Kernel Processing

Box–Behnken Design (BBD) response surface methodology was used for the design of experiment (DOE) for the Shea kernel optimization and model development for good quality Shea kernel processing. Validation of the optimum responses obtained from the optimized Shea kernel is to be carried out by collecting fresh Shea nut and processed according to the optimum conditions obtained from Design Expert 11.2.1.0.

RESULTS AND DISCUSSION

The Result of Preliminary Experiment

Prior to the optimisation of Shea kernel, preliminary experiments were conducted to show the effects of SNCP, SNBD and SNDT on the percentage FFA of Shea kernel. The results of these experiments are respectively shown in figures 4.1 to 4.3.

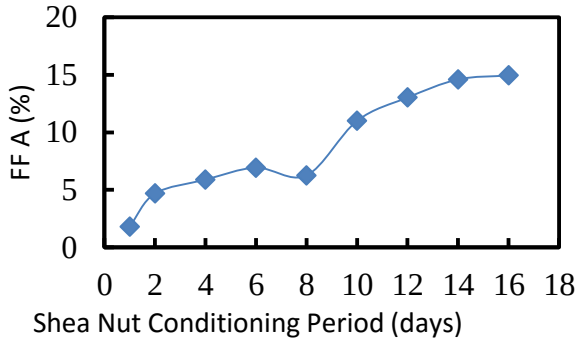

Figure 3.1: Effect of SNCP on FFA

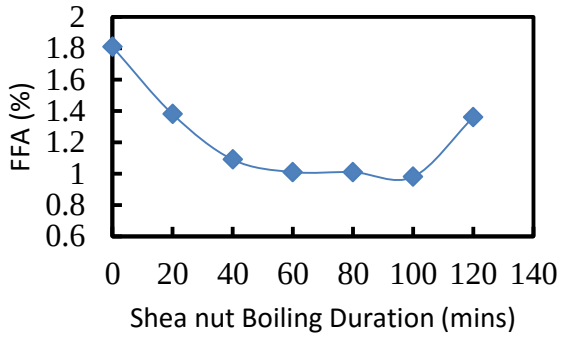

Figure 3.2: Effect of SNBD on FFA

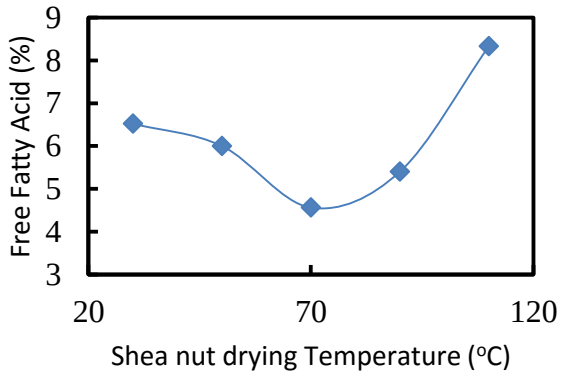

Figure 3.3: Effect of SNDT on FFA

Figure 3.1 shows the changes in FFA of Shea kernel as the SNCP increases from the initial period of day 1 to 16 day,within this period the average FFA increases from 1.810% to 14.946%. The high value of FFA at the 16th day may result from slow drying rate which enhanced activity of enzyme lipase present in the kernel. This observation is consistent with the findings of (Aculey *et al.,* 2012). The sharp drop observed on the 8th day, may also result from partialactivation of phenolic compounds during kernel degradation, (Nahm, 2011). This sharp drop as observed may also suggest the upper range of SNCP within which the Shea nut should be processed. Farabove 8^{th}day, the FFA builds up at a higher rate. Thiseffect of SNCP onFFA is modelled by quadratic equation 3.1with correlation coefficient, $R^2 = 0.941$.

$$Y_{FFA} = 0.002x^2 + 0.817x + 1.908 \qquad 3.1$$

The effect of SNBD on the FFA of Shea kernel is depicted in Figure 3.2 and the curve shows that as the SNBD increases from 0 min to 100 min, the FFA decreases from 1.810% to 0.980%, indicating leaching out of FFA as boiling is done. This trend favours the processing of higher quality Shea kernel since low FFA is desired in high quality Shea kernel. In other word increases the chances of getting high quality Shea butter. Further increase in SNBD to 120 min shows significant increase in the FFA of Shea kernel from 0.980% to 1.360%. This trend suggests that higher boiling duration do not favour lower FFA. It can be deduced that SNBD can both decrease and increase FFA of Shea kernel and hence should be controlled within 100 min. This observation is consistent with the findings of (Lovett, 2012) which suggest moderate SNBD for good quality Shea kernel. The curve is adequately represented with quadratic equation 3.2 with correlation coefficient of $R^2 = 0.972$.

$$Y_{FFA} = 0.000x^2 - 0.023x + 1.801 \qquad 3.2$$

Figure 3.3 shows how SNDT affects the FFA of Shea kernels. As SNDT increases from 30 ^oC to 70 ^oC, the FFA of Shea kernel decreases from 6.420% to 4.560% and from 70 ^oC to 110 ^oC the FFA increases from 4.560 to 8.330. The lower the SNDT, the longer it takes the Shea kernel to dry and hence more FFA is produced within the kernel as a result of increase in lipase activity and moisture content in the kernel. Also, with high SNDT above 70 ^oC thermal degradation of fatty acids begins to occur thereby producing more FFA. This variation suggests that moderate SNDT of about 70 ^oC favours lower FFA in Shea kernel. The graph is modelled by quadratic equation 3.3 with correlation coefficient of $R^2 = 0.866$.

$$Y_{FFA} = 0.001x^2 - 0.214x + 11.82 \qquad 3.3$$

Experimental Results of Shea kernel Processing

Table 3.1: Responses obtained from experimental design of Shea Kernel Processing using Box-Behnken Design Method

Std	Run	Factor 1 A:SNCP (day)	Factor 2 B:SNBD (hrs)	Factor 3 C:SNDT (^{o}C)	Response 1 PV (meq/kg)	Response 2 Oil Content (%)	Response 3 FFA (%)
10	1	1	60	30	1.40±0.01	52.00±1.23	7.98±0.19
14	2	12	60	30	2.70±0.03	51.67±1.22	5.57±0.13
7	3	6.5	120	30	1.80±0.02	53.67±1.27	5.49±0.13
8	4	6.5	60	70	3.20±0.03	48.67±1.15	2.26±0.05
11	5	6.5	60	70	3.40±0.03	52.67±1.25	1.68±0.04
12	6	6.5	120	110	3.80±0.04	53.33±1.26	1.04±0.02
9	7	6.5	0	30	1.45±0.01	54.67±1.29	5.98±0.14
6	8	6.5	60	70	3.50±0.04	48.33±1.14	1.48±0.03
5	9	6.5	0	110	3.10±0.03	50.00±1.18	1.12±0.03
4	10	12	0	70	3.31±0.03	56.00±1.32	3.37±0.08
15	11	1	120	70	1.45±0.01	53.67±1.27	2.24±0.05
2	12	1	0	70	1.29±0.01	47.67±1.13	2.00±0.05
17	13	6.5	60	70	3.50±0.04	52.33±1.24	1.63±0.04
1	14	1	60	110	1.90±0.02	45.33±1.07	0.60±0,01
13	15	12	120	70	4.50±0.05	52.00±1.23	2.52±0.06
3	16	6.5	60	70	3.80±0.04	52.00±1.23	1.53±0.04
16	17	12	60	110	4.90±0.05	49.67±1.17	4.85±0.11

* Response values are means ± SD (*n* = 3).The FFA ≤ 3%; percentage Oil content≥50% andPV ≤ 5meq/kg, Megan G, (2013).

'A' threshold of Shea kernel obtained but the percentage oil content of 47.67 % is lower than the realizable % oil content.Run 10 in Table 1 gave the highest percentage oil content of 56%, which is above the realizable standardand the PV of 3.31meq/kg is within and the FFA of 3.37% is outside the grade 'A' threshold. Run 14 also gave the lowest FFA of 0.60% and its PV of1.90meq/kg fell inside the grade 'A' threshold of Shea kernel and the percentage oil content is lowest 45.33 %. To improve the percentage oil content and the quality parameters of FFA and PV of Shea kernel the process variables can be optimized.

Analysis of Variance (ANOVA) for Shea kernel Processing

Table 3.2, 3.3 and 3.3 shows the ANOVA for response surface quadratic model of PV, percentage oil content and FFA of Shea kernel and statistical parameters obtained from the analysis.

Table 3.2: Analysis of Variance for Response Surface Quadratic Model of PV for Shea kernel

Response	Source	Sum of Squares	df	Mean Square	F-value	p-value	
PV	Model	20.35	9	2.26	40.99	<0.0001	Significant
	A-SNCP	10.97	1	10.97	198.93	0.0001	
	B-SNBD	0.7200	1	0.7200	13.05	0.0086	
	C-SNDT	5.04	1	5.04	91.36	0.0001	
	AC	0.7225	1	0.7225	13.10	0.0085	
	A^2	0.4516	1	0.4516	8.19	0.0243	
	B^2	1.12	1	1.12	20.24	0.0028	
	C^2	0.7695	1	0.7695	13.95	0.0073	
	Residual	0.3862	7	0.0552			
	Lack of Fit	0.1982	3	0.0661	1.41	0.3639	Not significant
	Pure Error	0.1880	4	0.0470			
	Cor Total	20.74	16				
Fit Statistics							
Std. Dev.		0.2349		R^2		0.9814	
Mean		2.88		Adjusted R^2		0.9574	
C.V. %		8.15		Predicted R^2		0.8330	
PRESS		3.46		Adeq Precision		21.8159	

Table 3.3: Analysis of Variance for Response Surface Quadratic Model of % oil content for Shea kernel

Response	Source	Sum of Squares	df	Mean Square	F-value	p-value	
%Oil Content	Model	101.62	9	11.29	3.86	0.0444	Significant
	C-SNDT	23.39	1	23.39	8.00	0.0255	
	AB	25.00	1	25.00	8.54	0.0222	
	B^2	24.10	1	24.10	8.24	0.0240	
	Residual	20.48	7	2.93			
	Lack of Fit	2.57	3	0.8553	0.1910	0.8975	Notsignificant
	Pure Error	17.92	4	4.48			
	Cor Total	122.10	16				
Fit Statistics							
Std. Dev.		1.71		R^2		0.8323	
Mean		51.39		Adjusted R^2		0.6166	
C.V. %		3.33		Predicted R^2		0.4345	
PRESS		69.05		Adeq Precision		7.7530	

Table 3.4: Analysis of Variance for Response Surface Quadratic Model of FFA for Shea kernel

Response	Source	Sum of Squares	df	Mean Square	F-value	p-value	
FFA	Model	73.04	9	8.12	97.50	< 0.0001	Significant
	A-SNCP	1.52	1	1.52	18.29	0.0037	
	C-SNDT	37.89	1	37.89	455.20	< 0.0001	
	AC	11.09	1	11.09	133.22	< 0.0001	
	A^2	4.91	1	4.91	58.95	0.0001	
	C^2	16.08	1	16.08	193.24	< 0.0001	
	Residual	0.5826	7	0.0832			
	Lack of Fit	0.1877	3	0.0626	0.6338	0.6312	Not significant
	Pure Error	0.3949	4	0.0987			
	Cor Total	73.62	16				

Fit Statistics			
Std. Dev.	0.2885	R^2	0.9921
Mean	3.02	Adjusted R^2	0.9819
C.V. %	9.55	Predicted R^2	0.9508
PRESS	3.62	Adeq Precision	34.7195

Analysis of Variance (ANOVA) for PV, percentage oil content and FFA of Shea Kernel

From Table 3.2, 3.3 and 3.4 the modelsfor PV, Percentage oil content and FFA had F-value of 40.99,3.86 and 97.50 respectively, which implied that there were statistically significant and had only 0.5901%, 0.9614% and 0.0250% chance that there magnitudes could occur due to noise (induced variation under normal operating conditions by uncontrollable factors). The "Lack of Fit F-value" of 1.41,0.1910 and 0.6338 for PV, percentage oil content and FFA implies the Lack of Fit are not significant relative to the pure errors of 0.047, 4.48 and 0.0987. There is 36.39%, 89.75% and 63.12% chance that the "Lack of Fit F-value" of these large could occur due to noise. The models have p-value < 0.0001, 0.0444 and 0.0001respectively for PV, percentage oil content and FFA. These were able to explain 99.99%, 99.96% and 99.99% variation in PV, percentage oil content and FFA. Thus, suggesting that the models show a good fit. The significant model terms are as shown in Table 4.2, 4.3 and 4.4.

Fit Statistic for PV, percentage oil content and FFA of Shea kernel Processing

From table 3.2, 3.3 and 3.4 the quadratic regression model showed satisfactory value of determination coefficient; R^2=0.9814, 0.8323 and 0.9921 for PV, percentage oil content and FFA respectively.No significant lack of fit at p > 0.05, which means that the calculated models were able to explain 98.14%,83.23% and 99.21% of the results. The results indicated that the models used to fit response variable were significant (p<0.0001, 0.0444 and 0.0001) and adequate to represent the relationship between the response and the independent variables. R^2 adj (adjusted determination coefficient) is the

correlationmeasure for testing the goodness-of-fit of the regression equation, (Li-Chun *et al,* 2012); Hossain *et al.,* (2012) and Kim *et al.,* (2012). The R^2 adj value of these modelsare0.9574, 0.6166 and 0.9819 which indicatesthat only 4.26%,38.34% and 1.81% of the total variations could not be explained by the models. The "Pred R-Squared" and the "Adj R-Squared" are in reasonable aggrement there differences less than 0.2. Meanwhile, relative lower value of CV<10% showed a better precision and reliability of the experiments carried out with 8.15, 3.33 and 9.55 for each of the responses.This means that the deviation between predicted and experimental values were not much as reported by Maran and Manikanda (2012). "Adeq Precision" of 21.816,7.7530 and 34.7195 all indicates an adequate signal ratio greater than 4 which are desirable and it shows that this model can be used to navigate the design space.

Analysis of PV for Shea Kernel Quality

$$Y_{Peroxide}=3.48+1.17125*SNCP+0.3*SNBD+0.79375*SNDT+0.425*(SNCP*SNDT)-0.3275*(SNCP)^2-0.5158*(SNBD)^2-0.4275*(SNDT)^2 \quad 3.6$$

The desired objective is to minimize the PV of the Shea kernelwhich indicates the oxidation of fat/oil and possible formation of off flavour. Equation 3.6 suggests that decreasing any of the three quadratic terms influenced the other by causing decrease in PV. Table 3.5 also showed the interaction between SNCP and SNDT (Shown infigure 3.4 and 3.5) on the PV of Shea kernel at optimum 60 min SNBD. The PV with the desire objective is to obtained good quality Shea Kernel that can produce grade 'A' Shea Butter around $10 \leq PV \geq 0$.

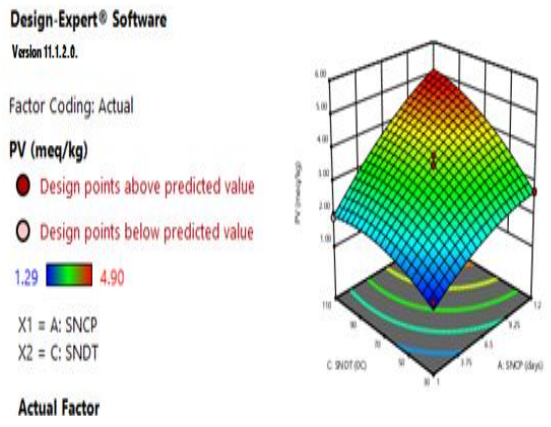

Figure 3.4: 3D RSP showing double effects of SNCP and SNDT on Peroxide value

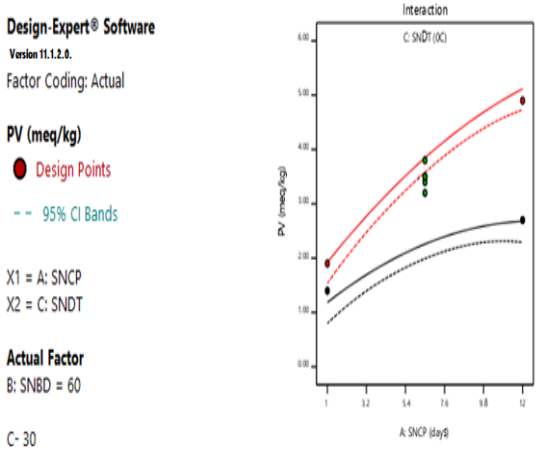

Figure 3.5: Interactive Plot showing effects of SNCP and SNDT on Peroxide value

Table 3.5: Design point of interaction between SNCP and SNDT on PV at confidence interval bands of 95 % and optimum 60 minof SNBD

Lower limit		PV	Upper limit		PV
SNCP	SNDT		SNCP	SNDT	
LL-1	LL-30	1.40	UL-12	LL-30	2.70
Lower limit		PV	Upper limit		PV
LL-1	UL-110	1.90	UL-12	UL-110	4.90

Note: Equilibrium point of zero SNCP per SNDT

As presented in figure 3.5, the Shea kernel processed at 1 day SNCP, 30 ^{o}C SNDT of and 60min SNBD has the lowest PV of 1.40meq/kg. This might result from the kernel not been exposed to other active variables such as O_2, moisture contentsand UV rays from sunlight since it is collected on the first day, with minimum FFA and hence low PV.Also the Shea kernel processed at SNCP of 12 day, constant 110 ^{o}C SNDT and 60min SNBD gave the highest PV of 4.9meq/kg; this figure is 3times the PV obtained at constant 1 day SNCP, 110 ^{o}C SNDT and 60min SNBD. And almost twice the PV obtained at 12day SNCP, 30 ^{o}C SNDT and 60min SNBD. These differences in PV may be due to combining effect of prolong SNCP of 12day and constant 110 ^{o}C SNDT as described in preliminary experiment shown in figure 1 and 3. This indicatesthat an increase in SNDT up to 70 °C resulted in reduction of FFA and by implication the PV. This observation is similar to the findings of Afaf, (2003) which states that the decomposition of hydroperoxides and other side reactions are minimized around 70°C. This might give the moderate temperature value of the graph, where lowest PV was measured and no generation of hydroperoxides above 70 ^{o}C. Thus, suggesting that SNCP of 1.00 day or less and SNDT of 30 ^{o}C must be maintained in order to obtain the lowest PV.

Analysis of percentage Oil Contentof Shea Kernel

$$Y_{Oilcontent}=50.80-1.71*(SNDT) \quad 2.50*(SNCP*SNBD)+2.39*(SNBD)^2 \qquad 3.7$$

The percentage oil content was significant (P<0.0444) and positive to quadratic effect of SNBD as shown in equation 3.7, this leads to maximum percentage oil content of 56.00% at 12 day, 0 min and 70 ^{o}C SNDT. Table 3.6shows the interaction between SNCP and SNBD (extracted from figure 3.6 and 3.7) on the percentage Oil content of Shea kernel at optimum 70 ^{o}C SNDT. The objective is to obtain Shea Kernel with the maximum possible oil content ≥50%.

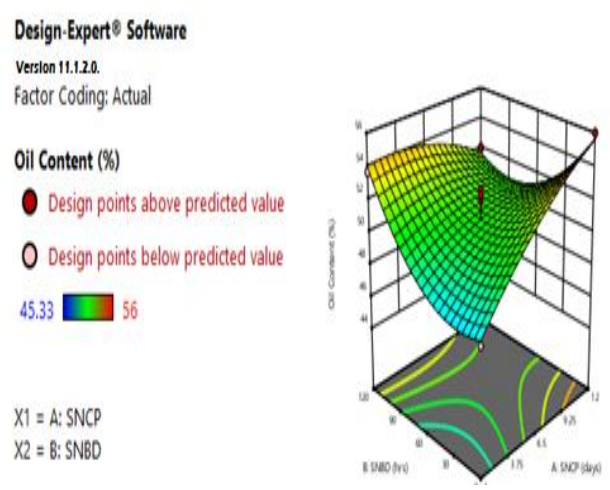

Figure 3.6: 3D RSP showing double effects of SNCP and SNBD on percentage oil content

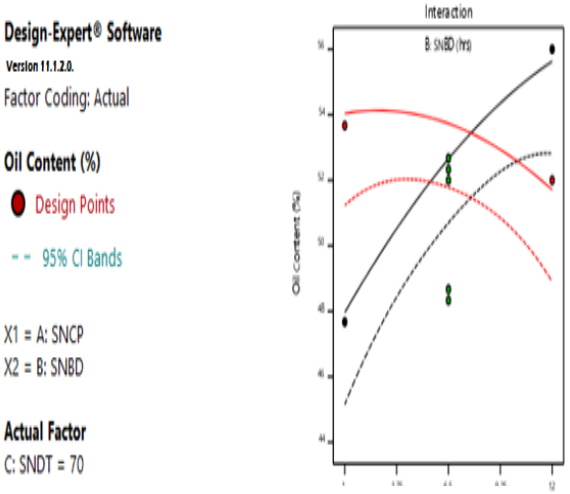

Figure 3.7: Interactive Plot showing effects of SNCP and SNBD on percentage oil content

Table 3.6: Design point of interaction between SNCP and SNBD on percentage oil content at confidence interval bands of 95 % and optimum 70 ^{o}Cof SNDT

Lower limit		% Oil content	Upper limit		% Oil content
SNCP	SNBD		SNCP	SNBD	
1	0	47.67	12	0	56.00
Lower limit		% Oil content	Upper limit		% Oil content
1	120	53.67	12	120	52.00

Note: Equilibrium point of 7.875 SNCP per SNBD

As presented in figure 3.7,the Shea kernel processed at SNCP of 1 day, 0 min SNBD and optimum 70 ^{o}C of SNDT produced the lowest percentage oil content of 47.67%. This may due to high retention of the proteins binding the fibres; holding the moisture and lipids at 1 day SNCP thereby making the fresh nut difficult to dry causing unavailability of the total fats content for the extraction of oil content, (Lovett, 2005; Harris, 1998 and Hyman, 1991).Similarly, the Shea kernel processed at 12 day SNCP, 0 min SNBD and SNDT of 70 ^{o}C had the highest percentage oil contents of 56.00%. This high percentage oil content of 56.00% obtained might be due to prolonged SNCP of 12 day which might have allowed polymerization of more oil the Shea kernel at constant SNDT of 70 ^{o}C. Also as a result prolonged SNCP moisture content contained in the kernel must have reduced, hence increases the percentage oil contents. According to Yonas *et al.,* (2016) the highest percentage oil content obtained from the Shea kernel may result fromconstant SNDT of 70 ^{o}C at prolong SNCP of 12 day which might also be attributed to thermal polymerization and decarboxylation of the oil. The high percentage oil content obtained may also result from denaturing of the proteins binding the fibres; and holding the moisture and lipids, thus making the fresh nut easier to dry and increasing the availability of the total fats content for extraction, (Lovett, 2005; Harris, 1998 and Hyman, 1991).

3.4.3. Analysis of FFAfor Shea Kernel Quality

$$Y_{FFA}=1.72+0.4363*(SNCP)-2.18*(SNDT)+1.67*(SNCP*SNDT)+1.08*(SNCP)^2+1.95*(SNDT)^2 \quad 3.8$$

The negative linear coefficient term of SNDT was the only significant term in single effect in relation with FFA; thusleading to minimum value of FFA 0.60% around SNCP.Table 3.7shows the interaction between SNCP and SNDT (extracted from figure 3.8 and 3.9) on FFA of Shea kernel at optimum 60 minof SNBD. The objective is to obtain a grade 'A' Shea kernel with 3≤ FFA ≥ 0.

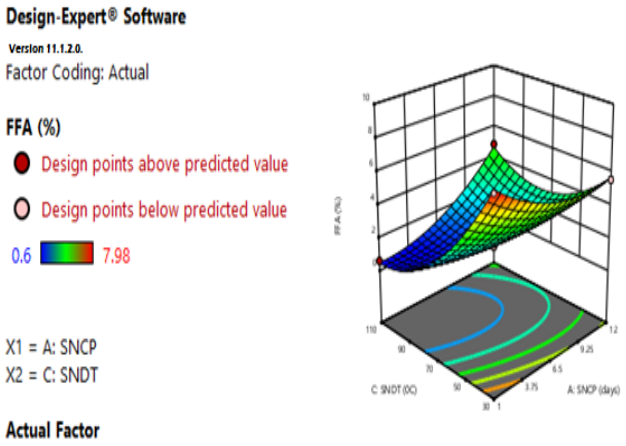

Figure 3.8: 3D RSP showing effects of SNCP and SNDT on Free Fatty Acid

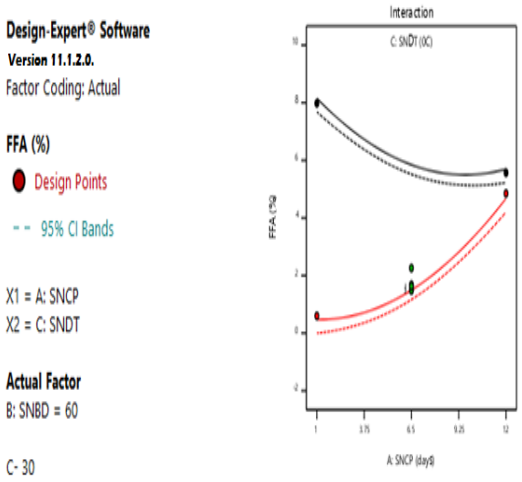

Figure 3.9: Interactiveplot showing effect of SNCP and SNDT on Free Fatty Acid

Table 3.7: Design point of interaction between SNCP and SNDT for FFA at confidence interval bands of 95 % and constant optimum conditions of 60 ^{o}C SNBD

Lower limit		FFA	Upper limit		FFA
SNCP	SNDT		SNCP	SNDT	
1	30	7.98	12	30	5.57
Lower limit		FFA	Upper limit		FFA
1	110	0.60	12	110	4.85

As presented in figure 3.9, the kernels obtained at 1 day SNCP and 30^{o}C SNDT has the highest FFA value of 7.98%. At this low SNDT of 30 ^{o}C the enzymes and microorganisms that would catalyze the formation of free fatty acids are active and can lead to the formation of more FFA, Badoussi *et al.*, (2015).Similarly, the kernel obtained at 12day SNCP and 30 ^{o}C SNDT gave the FFA value of 5.57%. This slight decrease in FFA might be as a result of the Shea fruits been exposed to a lower temperature (<30 ^{o}C) for 12 day before the commencement of drying at 30 ^{o}C. On the other hand, the kernel obtained at 1 day SNCP and 110 ^{o}C SNDT gave the lowest FFA value of 0.60%. This might be as result of inactivation of enzymes and faster rate of moisture removal from the kernel leading to lower FFA value.

Optimization of Shea kernel using Response Surface Methodology

When all the factors investigated were combined, it was observed that the highest required percentage oil content and quality responses of grade 'A' Shea kernel were not met. Thus, the factors were optimised to guarantee high percentage oil content, low PV and low FFA, a multi objective optimization was carried out by Design expert (11.1.2.0) to estimate the individual desirability using BBD of RSM

Optimization Result

Numerical Optimization Result

The goal is to determine the optimum conditions that will maximize percentage oil content from 45.33% to 56%, minimizes the PV from 4.9meq/kg to 1.29meq/kg and FFA from 7.98% to 0.60%.The optimum conditions of the factors were found to be 4.333 day of SNCP, 120 min of SNBD, 86.056 ^{o}C of SNDT while the responses are 2.869 of PV, 53.58% of percentage oil content and 0.628 of FFA (%) with high desirability of 0.878 (87.8%) were selected.

Validation of PV, % oil content and FFA of Shea kernel quality

The optimized data were validated by carrying out three parallel experiments under the same optimal conditions and the average experimental values of the responses obtained from Shea kernel processed as shown in table 3.8. Comparing these values of standard deviations≤1.02 with that of Mohammed *et al.,* (2017), it implies that the optimization in this study is reliable as shown in Table 3.8.

Table 3.8: Validation of Shea kernel Optimization

Shea kernel	SNCP (day)	SNBD (min)	SNDT (^{o}C)	PV	% Oil Content	FFA
Optimum conditions (predicted)	4.3333	120.00	86.00	2.87	53.579	0.627754
Experimental conditions (Actual)	4.30	120.00	86.00	2.887	52.14	0.663913
Standard deviation				0.012021	1.017527	0.025568

Conclusion

The optimization of these processing factors using Box-Behnken method of response surface methodology was carried out, which led to Grade 'A' Shea kernel obtained at a processing condition of 4.0 day of SNCP, 120 min SNBD and 86 ^{o}C of SNDT giving Shea kernel with 2.868 meq/kg of PV, percentage oil content of 53.82%, 0.628% of FFAand desirability of 0.878.

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Electric Discharge Development in Insulation Systems

Umar S.

Department of Physics, Ibrahim Badamasi Babangida University Lapai, Nigeria

*Correspondence: Email address: sumar@ibbu.edu.ng; Telphone: +2347030976078

ABSTRACT

A natural ester dielectric fluid was synthesized from neem oil seed. The oil is renewable, biodegradable (eco-friendly), less flammable and has high dielectric constant compared with the conventional mineral oil (transformer oil) used as coolant and dielectric fluid in high voltage facilities. Pre-breakdown characteristic of the oil was investigated adapting a rod-to-plane electrode with kraft paper geometric configuration in a computer simulation using COMSOL Multiphysics software. Combined permittivity of the oil-impregnated kraft paper was calculated. Voltages in the range of 1.0 kV to 10.0 kV were applied to the system and the electric field distribution plotted. Results from the simulation show that for the same voltages applied, insulation system with mineral oil produced greater field intensities compared with that with natural ester fluid, an indication that natural ester has better electric breakdown properties since electric discharge initiation is associated with electric charge emission at electrically stressed regions in insulation systems. Natural ester dielectric fluid from neem oil seed is suggested as a potential alternative insulating fluid for power applications.

Keywords: Natural ester, Dielectric constant, Insulation fluid, COMSOL, Finite Element Method (FEM).

INTRODUCTION

Natural ester oil (vegetable oil) is obtained from agricultural products which, unlike the conventional mineral base transformer oil, is bio-degradable, renewable and relatively less flammable. Insulation liquid forms a fundamental and essential component of high voltage power transformers and circuit breakers. They serve both as coolants and insulation to the power facility.

One of the important parameters to the performance of a material as a dielectric is its dielectric strength. It is the maximum electric field applicable to the dielectric material. And, when this field is exceeded, partial and localized discharges are initiated in the material under high and sustained application of the field over time resulting eventually in a complete breakdown of the material i.e. dielectric breakdown.

Natural ester dielectric fluids have been shown to possess superior breakdown properties compared with the conventional mineral base transformer oil (Martin *et al.*, 2007; Oommen, 2002). Partial discharge initiation in insulation material is dependent upon multiple factors such as; nature of the dielectric i.e. its molecular structure, presences of impurities such as conducting micro-particles (iron filings), hydrostatic pressure, water or moisture which originate from evaporation in the insulating liquid around high field strength region which may be due to local temperature rise in these regions as a result of either increased dielectric losses or joules heating effect, it may also originate from bond breakage due to mechanical stress (Chen *et al.*, 2012).

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Oxidation of liquid insulation when it comes into contact with air may result in the production of moisture or more acidic regions (i.e. higher conducting regions) which gives rise to partial discharge initiation and eventually result in complete breakdown. However, an oxidized insulation fluid is stable to re-oxidation i.e. Epoxidized insulation liquid possesses some level of thermal oxidation stability at elevated temperatures (Oommen, 2002). Epoxidized natural ester fluid from neem seed oil is considered in this work and its performance against that of the conventional mineral based transformer oil is compared. Partial discharge initiation and development in this oil is studied numerically using COMSOL multi-physics as numerical model. The model allows for accurate prediction of electric field distribution in electrical systems. In Comsol, the finite element method is used for solving defined physics governing each of a fractional domain (i.e primitive cells in a mesh of the entire material domain) of the electrical system under study to generate field distribution in it.

The design of the geometry of the conducting parts of high voltage facilities is also influential in determining breakdown characteristics in liquid insulation which it is in contact with. This is because points of high surface curvature are prone to high field enhancement due to high accumulation of space charges on them. A number of electrode geometries which are present in real-life power facilities have been considered in pre-breakdown studies of insulation liquid by many researchers; point to plan electrode configuration which is applicable mostly in simulating and studying the presence of conducting particles in insulating liquids, other geometric models include; rod to plane, elevated rod to plane, rod to plane with oil-impregnated paper insulation, wedge with VDE electrode, oil-filled void, inter-turn electrode configurations (Berg & Lundgaard, 1999; Martin *et al.*, 2007; Chen *et al.*, 2012). Rod to plane electrode geometry with kraft paper insulation is considered in this work. This is because, in addition to liquid insulation, most high voltage facilities currently in use employ the use of paper insulation due to mechanical support it offers in addition to its insulation property.

METHODOLOGY

A natural ester fluid was synthesized through trans-esterification of epoxidized neem seed oil as presented in (Umar, Abdelmalik & Sadiq, 2018).

The performance of the natural ester fluid was investigated against that of conventional mineral oil (i.e. Transformer Oil) in a computer simulation which was carried out using COMSOL Multiphysics (software) which employs the finite element method (FEM) as solver to obtain electric field distribution in the oil-solid insulation system using natural ester fluid and also using mineral oil as the insulating fluids in the system.

Simulation of the model

The following steps were observed in creating the model;

A 2D model environment was selected. Then geometric objects were constructed (see Figure 1) and materials specified. Electrostatics was chosen as the appropriate physics module and the appropriate boundary conditions specified. Extremely fine mesh was selected as the suitable mesh type and the field patterns were plotted.

In meshing, COMSOL splits a domain into smaller sub-domain also known as primitive

cells such that it creates a web of different points within the geometry and then uses those points to calculate the physical results from the module (electrostatics) defined i.e the governing partial differential equation of the physics are then solved using the finite element method (FEM). The FEM gives an approximate solution, hence the finer the mesh the higher the accuracy of the approximate solution.

Electrostatics is the chosen module for the simulation: it allows electric potential, charges, and grounds to be applied to the different components of the constructed geometry. And, the resulting electric field and electric potential field can be graphed. Charge conservation, Gauss' law, and Classical electrostatics govern the physics of the module. In theory, the electric field intensity, E can be defined as a function of a scalar potential, V;

$$E = -\nabla V \qquad 1$$

and $$\nabla \cdot D = \rho \qquad 2$$

Where ρ is space charge density and D electric displacement.

The intensity of field distribution over the domain is a direct consequence of the magnitude of space charges present in it i.e. in a non-zero charge density domain;

$\nabla^2 V = -{}^{\rho}/_{\varepsilon}$ i.e. (Poisson's equation) 3

However, in a source free domain, the FEM solves the governing differential equations assuming Laplacian condition i.e.;

$$\nabla^2 V = 0 \qquad 4$$

The combined permittivity of the oil-paper was determined from the equation (Martin *et al.*, 2007);

$$\varepsilon_{oil-kraft\ paper} = \left[1 - K^2\left(1 - \frac{1}{1+K\left(\frac{\varepsilon_{kraft\ paper}}{\varepsilon_{oil}}-1\right)}\right)\right] \qquad 5$$

Where K is evaluated from the equation;

$$K = \sqrt[3]{{}^{V_{oil}}/_{V_{kraft\ paper}}} \qquad 6$$

For high density pressboard (about 1.2 g/cm^3), K is approximated to be 0.45 (Martin *et al.*, 2007) and using measured permittivity of the ester fluid which is 4.6 and the literature value of 2.1 for mineral oil and also setting the dielectric constant of kraft paper (cellulose) as 5.0, the combined permittivity was determined to be 4.4 for ester oil-impregnated kraft paper and 4.0 for mineral oil impregnated kraft paper.

Construction of the Geometry

The electrode configuration considered in this work is shown (see figure 1). The grounded electrode is 110 mm in length and 10 mm thick. The high voltage electrode is 23 mm long and 10 mm thick as well. The kraft paper insulation is placed in contact and in-between the two electrodes.

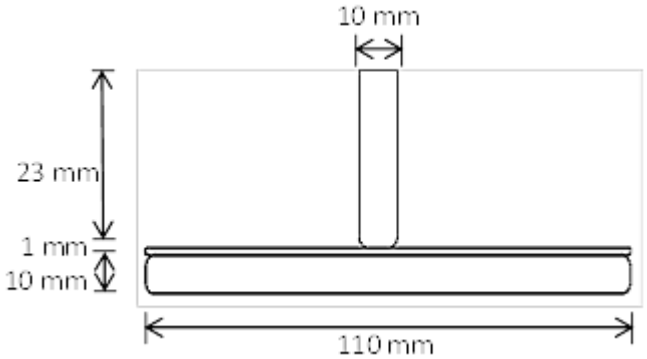


Figure 1: Rod to plan geometry with kraft paper.

RESULTS AND DISCUSSION

The rod to plane geometry with kraft paper configuration is symmetric about the vertical axis and hence, only half of the physical model simulated is displayed (see figure 2).

The field in the mineral oil-filled electric system indicates higher field strength at the oil wedge region than in the natural oil-filled system. Most of the field generated in the natural oil-filled system is absorbed by the paper insulation than it is in the mineral oil-filled insulation system. Figure 3 shows the correlation between the applied voltage and the maximum field intensity recorded. Mineral oil produced greater maximum field intensities than natural ester oil at the same applied voltages.

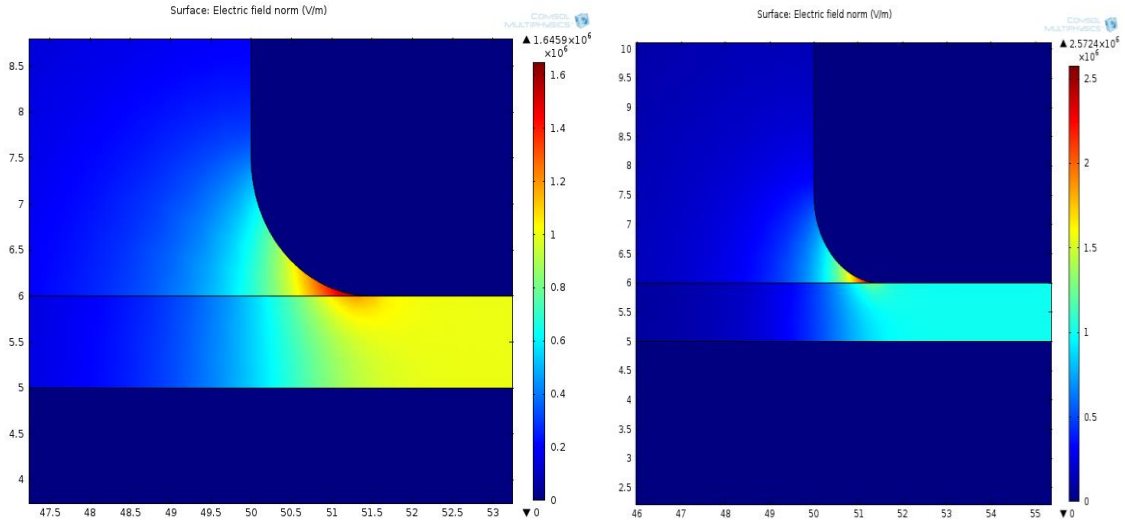


Figure 2: Electric field distribution at the triple junction at applied voltage of 1kV (a) using natural ester fluid as insulation fluid and (b) using transformer oil as insulation fluid

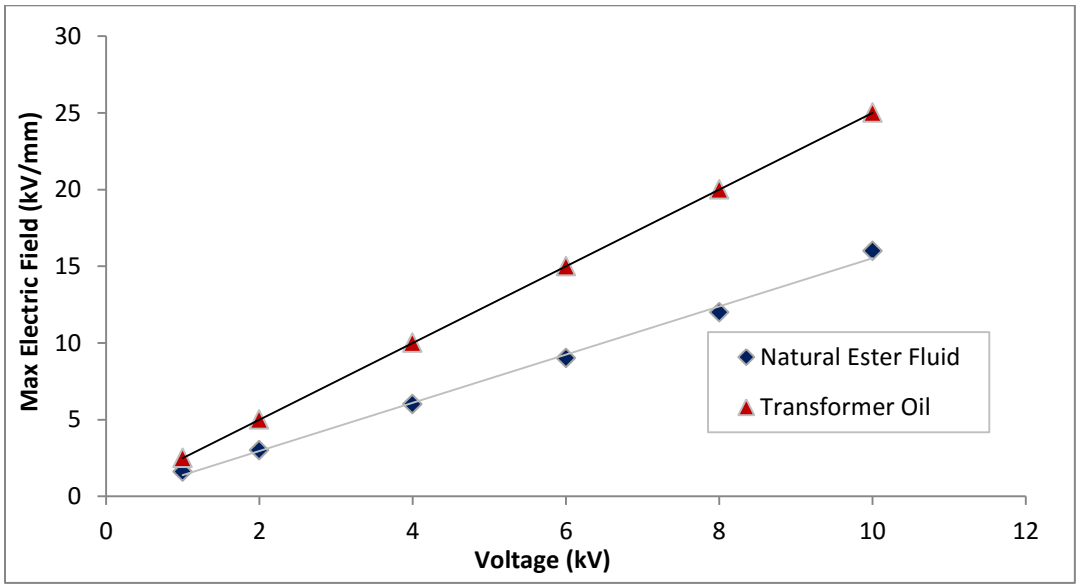


Figure 3: Relationship between Applied voltage and maximum electric field

Effect of Impurities

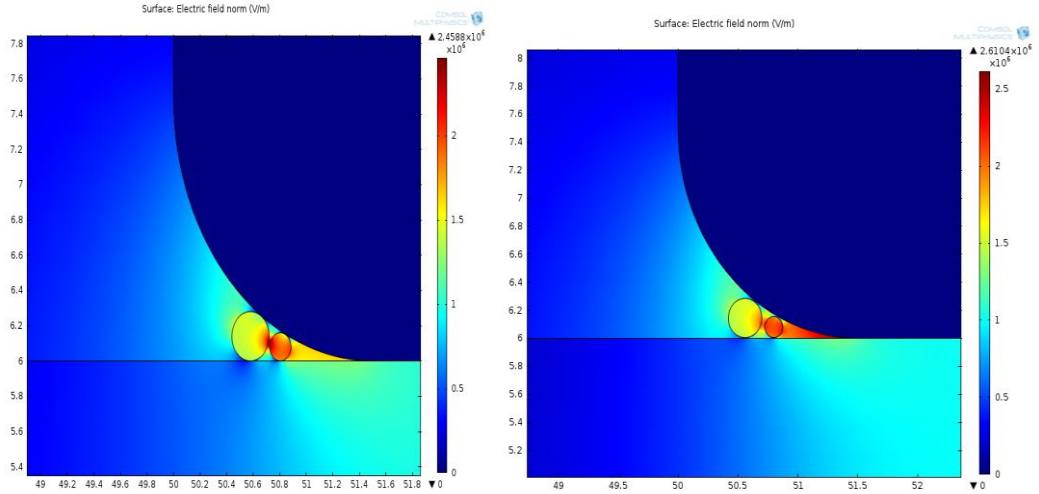
Impurities present in liquid electrical insulation under active operation and during the heating cycle can be present in the form of air voids or moisture. Micro air bubbles and water micro drops were simulated in the oil wedge regions of the electrical system.

Air micro-bubbles

Air micro-bubbles in the oil wedge region creates a field distortion, consequently resulting in the enhancement of the field strength in this region. As can be seen in figure 4, the bubble with smaller radius shows much more enhanced field strength than the bigger bubble.

Water micro-droplets

Natural ester fluid has high moisture absorption capability. Moisture absorption causes reduction in dielectric strength of natural ester but more in mineral oil (Lucas et al., 2001). The presence of moisture as impurities has less degradation impact in natural ester oil as compared with mineral oil (Mcshane et al., 2002).



igure 4: Electric field distributions in the presence of micro-bubbles of air (a) with natural ester fluid (b) with mineral oil; at wedge region at an applied voltage of 1 kV

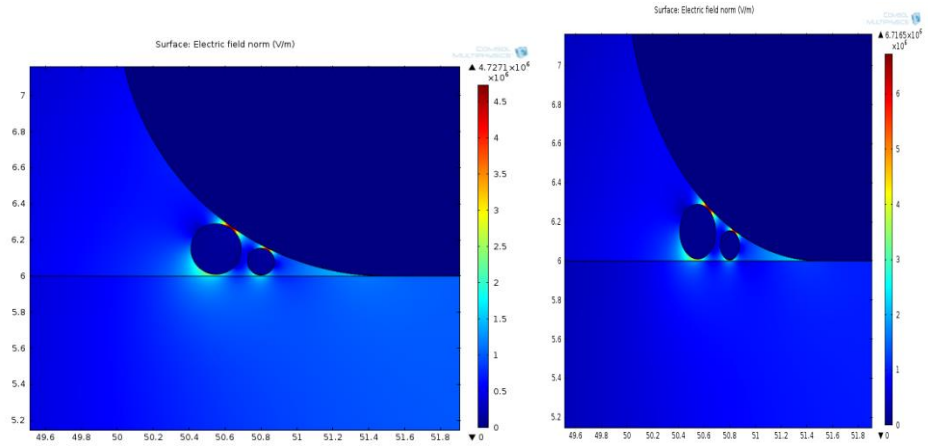


Figure 5: Injected water micro-droplets in the oil wedge region for (a) natural ester oil electric insulation system (b) mineral oil electric insulation system.

The effect of field intensity enhancement is more in the mineral oil insulation system than in the natural oil insulation system. In figure 5, at 1 kV, the maximum electric field strength outside the water drop is 4.74 × 106 V/m in the natural ester and 6.72 × 106 V/m in mineral oil.

DISCUSSION

Partial discharge initiation is associated with electric charge emission at electrically localized stress regions in the insulation system such as the oil wedge region due to the geometry of the system. In figure 2, as can be seen, maximum electric field intensities in the physical model were developed at the triple junction of the high voltage electrode and oil-impregnated paper insulation (i.e oil wedge region), consistent with results obtained by (Chen *et al.*, 2012). However, field intensity up to 2.57 kV/mm was developed in the mineral oil insulation system as against the 1.65 kV/mm in the natural ester oil insulation system. Hence, electrons are most likely to be emitted in the oil wedge region of the electrical system in figure 2 (b) than that in figure 2 (a). This is consistent with the literature (Chen *et al.*, 2012).

Figure 3 shows the correlation of the maximum electric field strength with the applied voltages. As seen, there is a linear relationship between them; the higher the magnitude of the applied voltages, the higher the magnitude of the maximum electric field intensities developed. And, for the same applied voltages, maximum electric field intensities of higher magnitudes are developed in the mineral oil insulation than in the natural ester insulation system.

Generally, sharp edges are prone to field enhancement and provide an area for discharge initiation and eventual system insulation breakdown (Wang & Santiago-avilés, 2007). The effect of geometry on field distribution patterns can be investigated so as to minimize likelihood of geometry associated field enhancement and to optimize the design of conducting components of high voltage facilities that may also reduce cost of design.

The presence of air voids or moisture in the insulation system creates great distortion of the local field distribution in the oil wedge region enhancing it and therefor accelerating partial discharge initiation. As can be seen in figure 4, presence of air micro-bubble created distortion of the field pattern and resulted in field enhancement. Maximum electric field strength occurs inside the air bubbles resulting in an increase of the maximum field intensity of the electrical insulation system from an initial value of 1.65 kV/mm to 2.46 kV/mm in the natural ester and from 2.57 kV/m to 2.61 kV/m in the mineral oil insulation system. Air has low permittivity and will breakdown at electric stress of about 3 kV/mm emitting high energy electrons in the oil-filled insulation. It is evident that electrical discharge initiation is more like in mineral oil insulation system than in natural ester insulation system.

The relatively high field intensity in the smaller micro air bubble in contrast to the bigger bubble is an indication that the presence of the bubble has effect on the dielectric strength of the insulation liquid. Studies have shown that, the maximum electric field in the inner part of the air bubble will result in field enhanced ionization and self-sustained local discharge in the bubble emitting free electron which impacts nearby molecule of the insulation liquid and eventual lead to breakdown of insulation (Chen *et al.*, 2012; Hwang, Zahn & Petterson, 2012).

Water or moisture content of a material is a very critical factor that determines breakdown

strength of insulation material. Water or moisture in high voltage facilities may become present either due to aging of the cellulose composition of paper insulation in it or through direct contact with ambient air through breathers (respiration). In figure 5, the presence of water droplets in the oil wedge region resulted in field enhancement up to a value of 4.73 kV/mm from an initial value of 1.65 kV/mm in the natural ester oil-filled insulation and from a value of 2.57 kV/mm to 6.71 kV/mm in mineral oil insulation. This is an indication that presence of moisture in electrical insulation promotes electric breakdown. However, research has shown that natural ester oil has resistance to aging effects of paper insulation due to its high moisture solubility and hydrolysis reaction in which acid is produced (Bandara *et al.*, 2016). This makes Natural ester oil better insulation fluid than mineral oil.

Although, a dielectric breakdown may be accelerated by bond breakage due to natural ester decomposition associated with aging or thermal effects from partial discharges. However, it is seen that for the same applied voltage, natural ester fluid shows better performance as insulating fluid than conventional mineral oil. This is because natural ester fluids have higher moisture absorption capability with less effect on dielectric strength compared to mineral oil and they are also relatively less susceptible to degradation due to partial discharge (Bandara *et al.*, 2016).

In conclusion, natural ester fluid from neem seed oil is a potential candidate for use as an insulation fluid in power equipment. And, considering the abundant availability of neem seed product in Nigeria, industrial-scale production of the oil can be actualized if research into the use of the oil for insulation applications is perfected

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Structural Studies and Determination of Depth to Magnetic Sources of the Area Around Parts of Central Nigeria

***Tawey M.D, Udensi E.E., Alhassan U.D. & Adetona A.A.**

Department of Chemistry, Ibrahim Badamasi Babangida University Lapai, Nigeria

*Correspondence: Email address: *taweymam@gmail.com;* Telphone: +2348065678908

ABSTRACT

Aeromagnetic datasets covering parts of the area around central Nigeria was acquired and processed to interpret the structural lineaments and their trends as well as the depth to magnetic source bodies in the area. First vertical derivative, tilt derivative, analytic signal, reduction to the pole and source parameter imaging were some of the mathematical algorithms used in enhancing the magnetic data with application of center for exploration targeting Plug-IN (CET) which is very good in extracting structures from potential field data automatically . These enhancements aided in locating the fractures and faults which may be favourable zones of mineralisation. The analysis of analytic signal reveals areas of low amplitudes and high amplitudes that range from 0.0024m to 0.2157m. Application of tilt derivative reveals structures that were mapped out and this is applicable to first vertical derivative map of the area where the structures that were manually mapped showed the usual NW-SE, NE-SW and minor EW and NS trends. Structural analysis using CET, ArcGIS and Rockworks produced a Rose diagram that showed that the area is dominated by same predominant NE-SW, NW-SE, and minor EW and NS trending structures. Depths to magnetic source bodies were estimated using source parameter imaging and these ranges from 136.01m depth to 960.66m depth.

Keywords: Analytical signal, Derivative, Structural Analysis and CET.

INTRODUCTION

Aeromagnetic data applications are well known in wide variety of geological studies and play an important role in tracing lithological contacts and for recognition of structures like faults, lineaments, dykes and layered complex (Reeves, 1990). These structures or Lineaments are linear, continuous features (fault, fold, joints and fractures) on a magnetic map that may reflect zones of deformation in rocks on maps and are invaluable tools for foundation and civil engineering constructions, detection of potential zones of oil-gas/minerals/water accumulation and earth quakes and natural hazards monitoring as such the present study aimed at identifying and delineating subsurface structures within part of central Nigeria by producing comprehensive structural maps of the study area which will improve information on the regional geology of the area and Nigeria.

METHODOLOGY

Location and extent of the study area

The study area is a rectangular block shape situated in the part of central Nigeria particularly in the northern Nigerian Basement Complex (Figure 1 A). It is bounded by latitudes $08^0 30^{'}$ N and $10^0 30^{'}$ N and longitudes $07^0 00^{'}$ E and $09^0 00^{'}$ E. The study area falls in three states namely Kaduna, Niger, Nasarawa and the Federal Capital Territory (FCT) (Figure 1A).

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Geology of the study area

The Basement complex of Nigeria is Precambrian to lower Paleozoic in age. Orogenic activities caused extensive sedimentation, folding, metamorphism, migmatisation and remobilisation of pre-existing basement rocks to produce suites of granitic rocks (McCurry, 1973). The predominant rock type in the area of study is the Migmatite which almost covered the entire area with an isolated occurrence of younger basalt, granite and granite porphyry, coarse Porphyritic hornblende granite, medium to coarse grained biotite granite, undifferentiated granite Migmatite and granite gneiss, Amphibolites schist and amphibolites, undifferentiated schist including philites granite gneiss and the Migmatite (Figure: 1B)

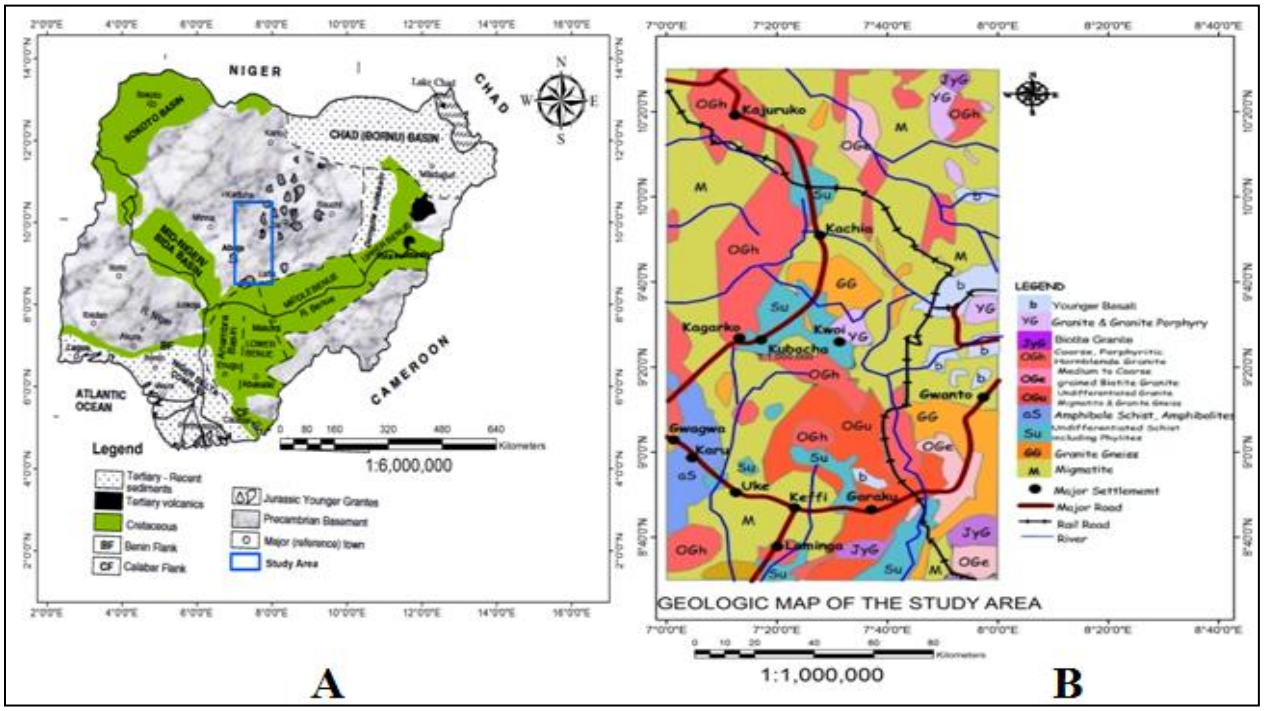

Figure 1 Location of study area on Nigerian Map (Obaje, 2009) and Geologic map of the area (NGSA,2006)

Source of Aeromagnetic Data

The Aeromagnetic Data used for this study which was acquired from the Nigerian Geological Survey Agency (NGSA) Abuja comprises of sheets (144, 145, 165, 166, 187, 188, 207 and 208). The survey took place between 2005 and 2009 and was financed by Nigerian Federal Government and the World Bank. The survey was conducted in two phases. Phase 1 was financed entirely by the Government of Nigeria while phase two was financed by both Nigerian government and the World Bank. All of the airborne geophysical work data acquisition, processing and interpretation, was carried out by Fugro Airborne Surveys.

Methods

The methodologies involved in this study include:

1. Production of Total Magnetic Intensity (TMI) map of the study area reduced to

magnetic pole in color aggregate using Oasis Montaj software

2. Production of Analytical Signal map to locate outcropping areas
3. Application of first vertical derivative (FVD) to manually map out structures in the area
4. Production of tilt derivative and source parameter imaging maps of the area
5. Analysis of structures at depths using Centre for Exploration Targeting Plug-IN(CET)

Theories of the Methods

Reduction to Magnetic Pole

Reduction-to-the-pole (RTP) is a useful and effective operation designed to transform a total magnetic intensity (TMI) anomaly caused by an arbitrary source into the anomaly that this same source would produce if it were located at the pole and magnetized by induction only (Li, 2008) and this was applied to the aeromagnetic data sets. When the Earth's field is inclined, magnetic anomalies due to induction have forms that are asymmetrically related to their sources, but when the inducing field is vertical, the induced anomalies are directly over their sources (Milligan & Gunn, 1997).

Analytical method

Analytical signal of TMI has much lower sensitivity to the inclination of the geomagnetic field than the original TMI data, and provides a means to analyses low latitude magnetic fields without the concerns of the RTP operator. Analytical signal is a popular gradient enhancement, which is related to magnetic fields by derivatives. Roest *et al.,* (1992), showed that the amplitude of the analytic signal can be derived from the three orthogonal gradient of the total magnetic field using

$$|A(X,Y)| = \sqrt{\left(\frac{\delta m}{\delta x}\right)^2 + \left(\frac{\partial m}{\partial y}\right)^2 + \left(\frac{\delta m}{\delta y}\right)^2} \qquad 1$$

Where A(x, y) is the amplitude of the analytical signal at (x, y) and m is the observed magnetic anomaly at (x, y). While this function is not a measurable parameter, it is extremely interesting in the context of interpretation, as it is completely independent of the direction of magnetisation and the direction of the Earth's field (Milligan & Gunn, 1997).

Vertical Derivative Filter

The first vertical derivative filter does the removal of the long wavelength properties of magnetic responses and most importantly enhances the quality of closely spaced and superposed responses (Keating, 1995). The application of the vertical derivative filter to a magnetic data is to improve the shallowest magnetic features and suppress the deeper anomalies in the data (Geosoft Inc., 1996). The vertical derivative emphasizes on the near surface geological features.

Tilt Angle Derivative

Since the amplitude of magnetic signature depends on magnetic field strength and to some extent the depth of magnetic sources, lower amplitude signature may be suppressed at the expense of higher amplitudes. For this reason, the edge-detection filters area normally applied for delineating linear features without necessary diminishing the long-wavelength anomalies (Oruc & Selim, 2011). The Tilt derivative filter, TDR (a very good edge-detection filter) brings out short wavelength and reveals the presence of magnetic lineaments. Verduzco *et al.,* (2004) showed in his work that tilt derivative filter also performs an automatic-gain-control (AGC) filter which tends to equalize the response from both weak

and strong anomalies. Hence, the filter provides an effective way to trace out along striking anomalies.

Source Parameter Imaging (SPI)

The source parameter imaging has been employed to determine the depth to basement rocks within the study area, and this is briefly discussed below:

Thurston and Smith (1997) and Thurston, Guillon, and Smith (1999 and 2002), developed the source parameter imaging (SPI) technique, based on the complex analytic signal, which computes source parameters from gridded magnetic data. The technique is sometimes referred to as the local wavenumber method. The local wavenumber has maxima located over isolated contacts, and depths can be estimated without assumptions about the thickness of the source bodies Smith, Thurston, Dai and MacLeod (1998).

Center for Exploration Targeting CET

Centre for Exploration Targeting (CET) is a suite of algorithms which provides functionalities for enhancement, lineament detection and structural complexity analysis of potential field data (Holden *et al.* 2008; Core *et al.* 2009). This technique automatically delineate lineaments and identify promising areas of ore deposits via outlining regions of convergence and also divergence of structural elements using several statistical steps that include texture analysis, lineation delineation, Vectorisation and Complexity analysis to generate contact occurrence density map. The CET Analysis system analyses also the texture of an image to detect areas of structural complexity (Geosoft, 2012).

RESULTS AND DISCUSSION

The total magnetic intensity map and total magnetic intensity reduced to pole (Figure 3A and 3B) reveals variation in magnetic intensity across the study area. For TMI (Figure: 3A), the map revealed high magnetic intensity region toward the southern part of the area from south eastern end to south western end of the area and when the TMI was reduced to Pole (Figure: 3B) the areas with highest magnetic intensity now is from the west of the pole reduced map through the center to the western end of the map (Figure: 3B). Application of analytical signal and first vertical derivative on the TMI reduced to magnetic pole produced the map (Figure 4: A and B).

The analytic signal map (Figure 4 A) produced revealed areas of high amplitude at the central portion of the area towards the western end. The map also reveals structures that were manually mapped out in black strikes. This is also applies to first vertical derivative map (Figure 4 B). This filter when applied to aeromagnetic data tends to sharpen the edges of the anomalies and revealed structures (lineaments) that are surface and subsurface and were mapped out also here using black strikes. The tilt derivative map, (Figure5 A) reveals most of the structures that were not revealed by the FVD and analytic signal maps. However, the depth to the top of the structure is a parameter that is commonly sought, and the source parameter imaging (SPI) method is one way of obtaining this depth estimate. One advantage of SPI method is that the depth can be displayed on an image. The source parameter imaging (SPI) module from Oasis Montaj software is a quick, easy and powerful method for calculating the depth of magnetic source and was applied to the residual data of the study area and the result shows a minimum

depth of 136.01 m to maximum depth of 960.08m.

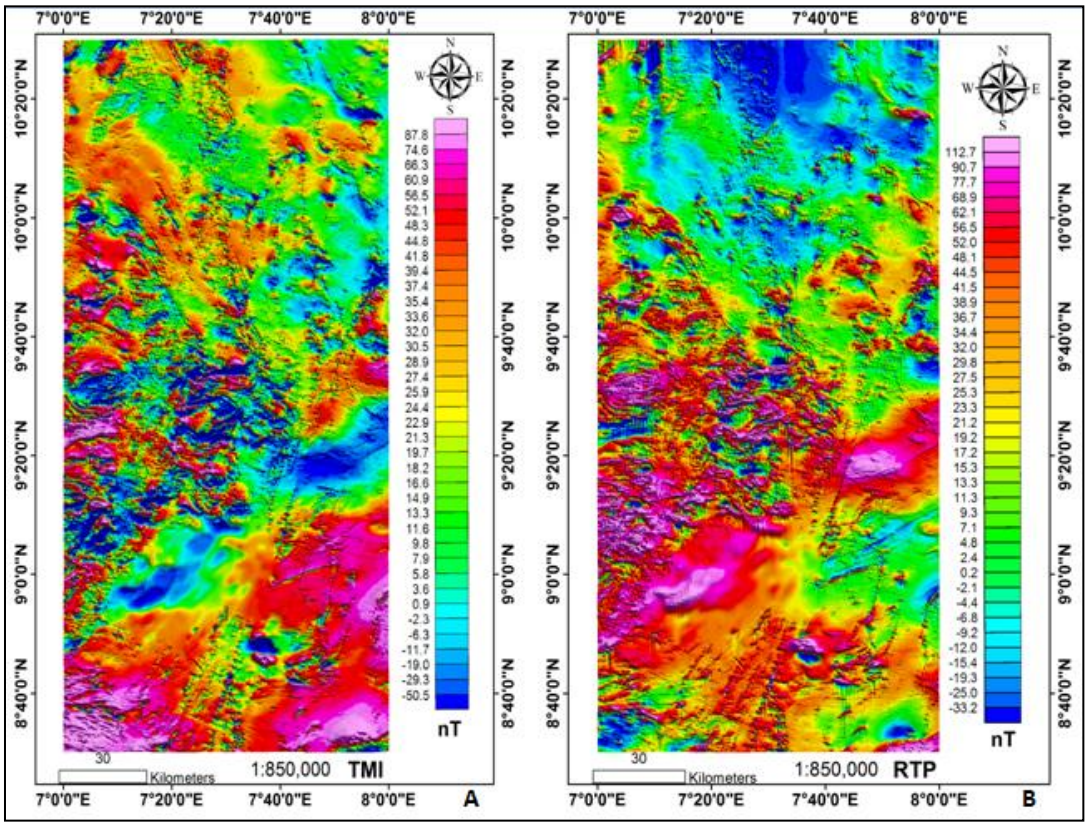

Figure 3 Total Magnetic Intensity and TMI Reduce to Pole maps

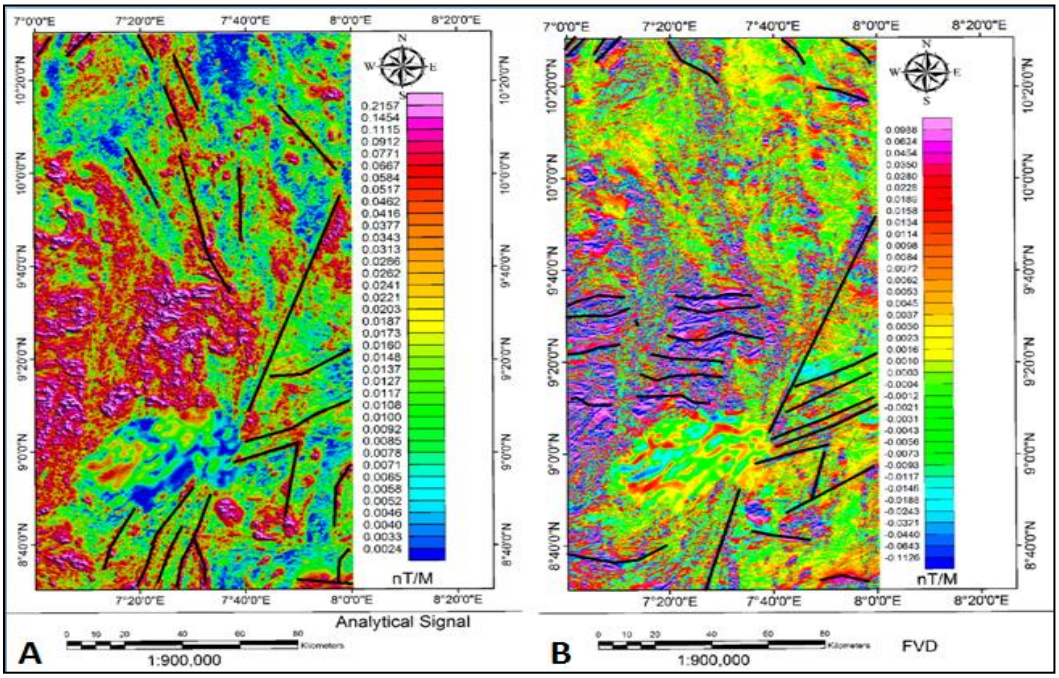

Figure: 4 Analytical Signals and First Vertical Derivative Map

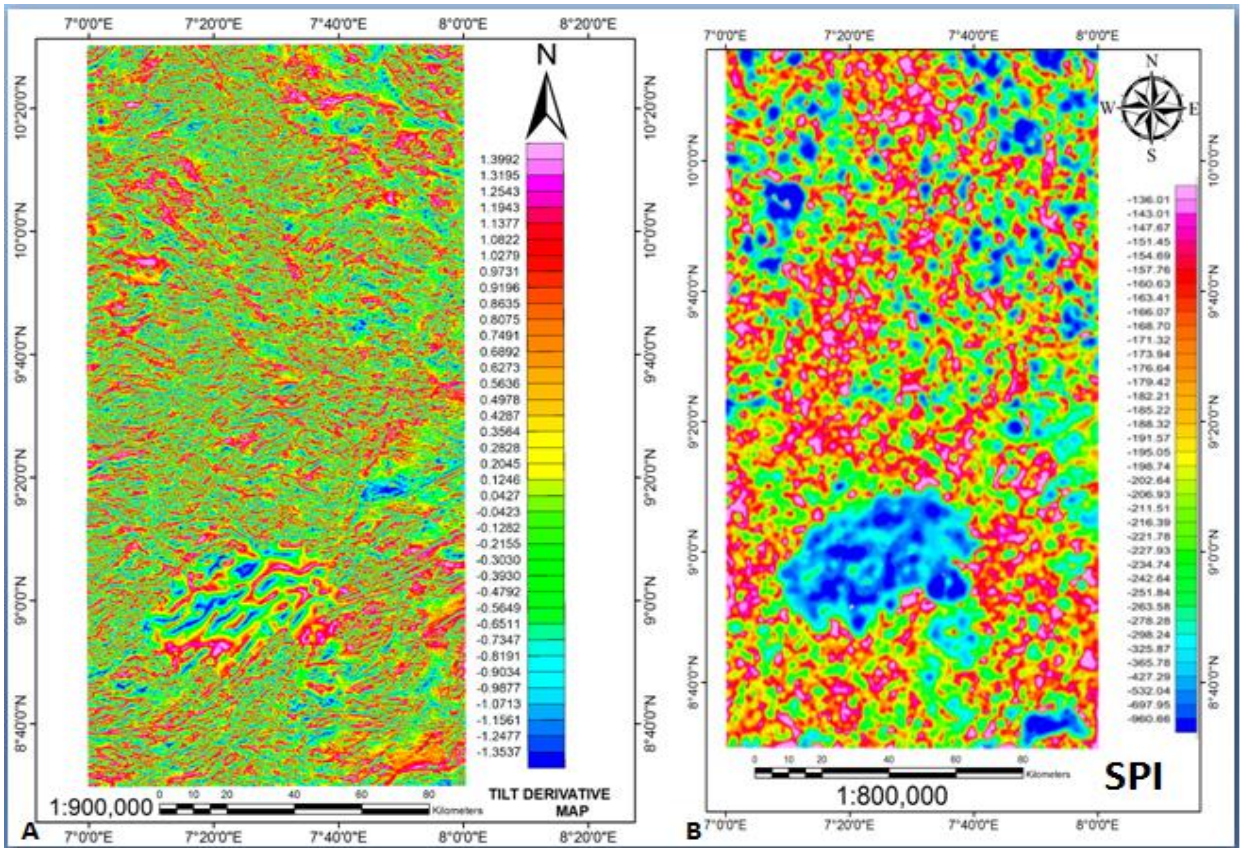

Figure: 5 Tilt Derivative and Source Parameter imaging (SPI) Maps

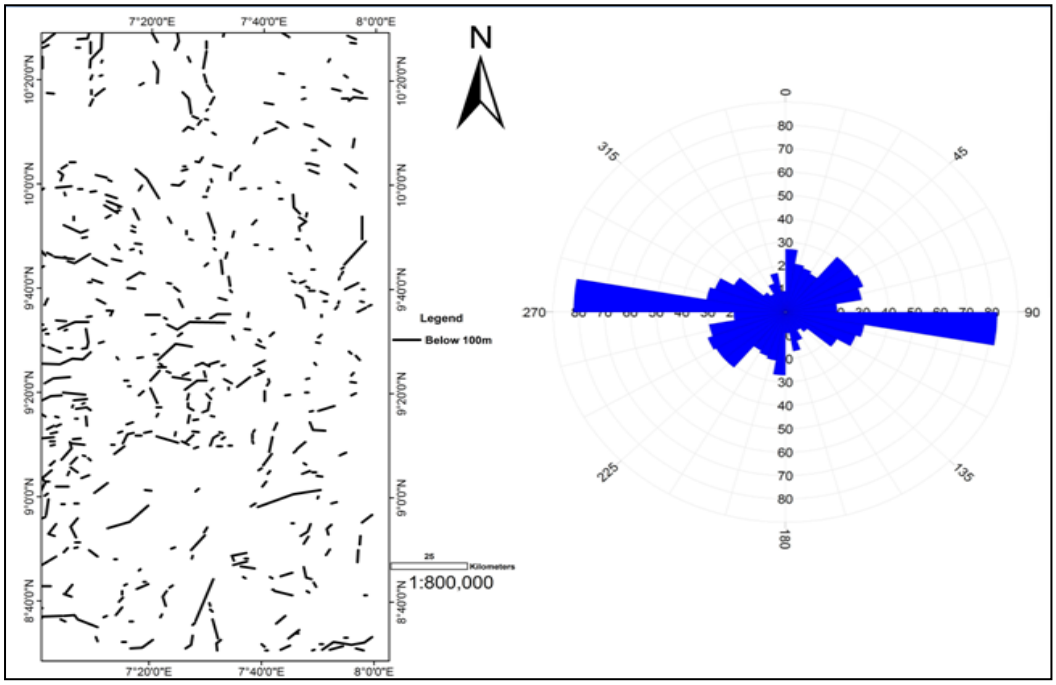

Figure: 6 Structural Map and Rose Diagram

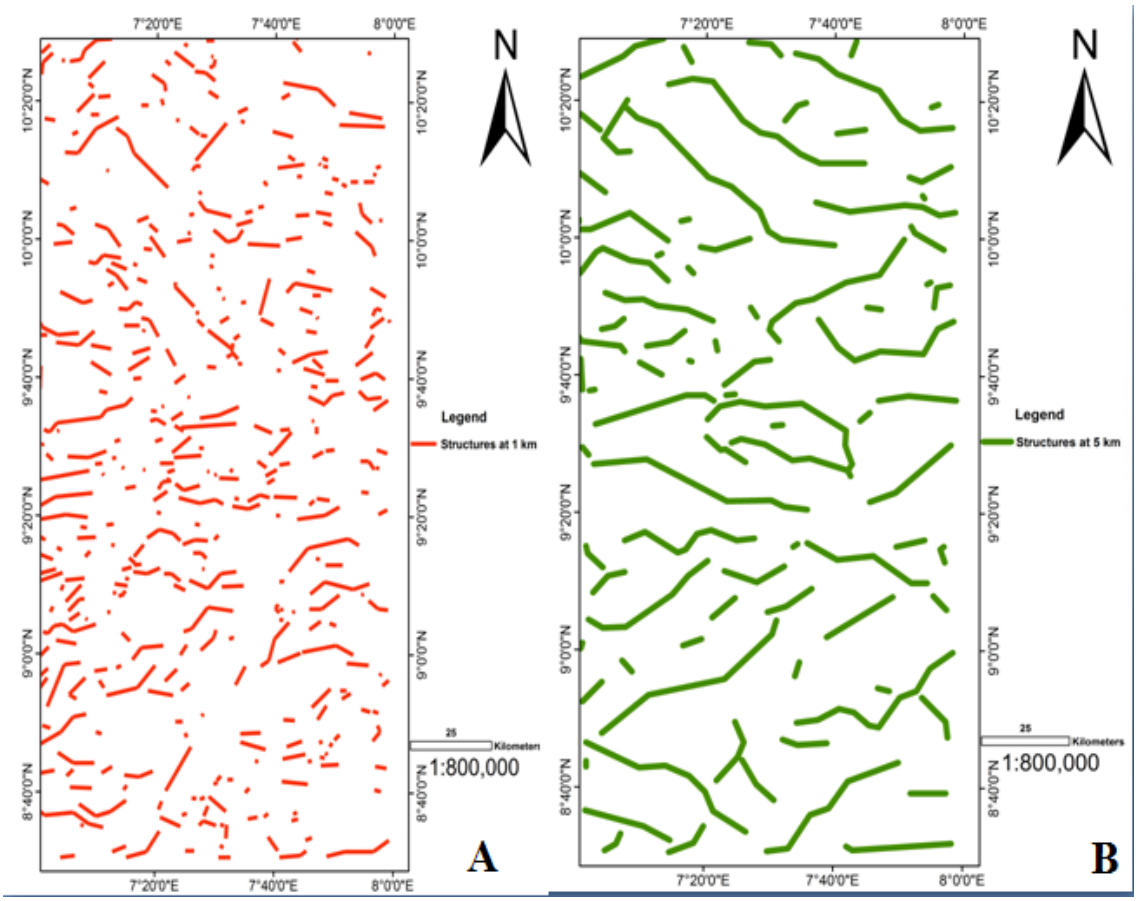

Figure: 7 Structures at 1000m and 5000m

Conclusions

From the analysis of analytical signal (Figure: 4 A), areas of high amplitude were observed mostly in portions from the center of the study area to the western end of it. Structures were also mapped out using black strike that trend NE-SW and NW-SE. These structures were also mapped out on the first vertical derivative map (Figure: 4 B), the structures were much more pronounce on the tilt angle derivative map of the area (Figure: 5 A). Source parameter imaging (SPI) map produced revealed areas around south central portion of the study area to have the maximum depth to magnetic source bodies while other part of the study area have minimum depth of 136.06m (Figure: 5 B).

Structural analysis using the CET, ArcGIS and Rockworks (Figure: 6) showed that the area have dominant structural trend of NE-SW, NW-SE, NWW-SEE with minor N-S and E-W trend. A deeper look into the basement where data sets were upward continued to 1000m and 5000m (Figure: 7 A and Figure: 7 B) compared to the near surface structures reveals the near surface structures to be of short dimension while structures at 1000m (Figure: 7 B) are longer and are of larger dimensions compared to the near surface structures. Structures at 5000m (Figure: 7 B) are longer than 1000m structures .They are broad and are of larger dimension compared to 1000m structures. This could be

attributed to activities or condition that lead to the formation of this structures that emanated from earth interior or from a deeper source where these structures are found. The effects of forces that lead to the formation of these structures also reduce with depth as it moves to the surface across the rock bodies as with structures created at each depth.

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Electric Double Layer Field Effect Transistor Using SnS Thin Film as Semiconductor Channel Layer and Honey Gate Dieletric

***[1,2]Daniel T.O., [2]Uno U.E., [2]Isah K.U. & and [2]Ahmadu U.**

[1]Department of Physics/Geology/Geophysics, Alex Ekwueme-Federal University Ndufu-Alike, Ikwo, Abakaliki, Ebonyi state, Nigeria

[2]Department of Physics, Federal University of Technology, Minna, Nigeria

*Correspondence: Email address: *danielojonugwathomas@gmail.com;* Telphone: +2348065732254

ABSTRACT

Electric double layer field effect transistor was assembled using tin sulphide (SnS) thin film whose thickness and annealing temperature were earlier optimised as semiconductor channel layer and honey as gate dielectric. A 0.4 µm semiconductor layer was deposited using Aerosol assisted chemical vapour deposition (AACV) and annealed in open air at 250℃ on an etched region about the middle of a 4×4 mm FTO glass substrate with the source and drain electrode region defined by the etching and masking at the two ends of the substrate. Iridium was used as gate electrode while a copper wire was masked to the source and drain region to create contact. The transistor operated with a p type channel conductivity in a depletion mode with a field effect mobility of 16.67 cm^2/Vs, cut-off voltage of 1.6 V, Drain saturation current of1.35µA, a transconductance of -809.61 nA/V and a sub threshold slope of -1.6 $Vdec^{-1}$ which is comparable to standard specifications in Electronics Data sheets. Positive gate bias resulted in a shift in the cutoff voltage due to charge trapping in the channel/dielectric interface. The Transistor may find application in portable electronics and synaptic electronics.

Keywords: Thin film, Transistor, Honey, Electric double layer field effect.

INTRODUCTION

Electric double layer can be regarded as a nano gap capacitor with superior characteristics of large specific capacitance which can modulate the semiconductor channel electrostatically by accumulating charges at the interface with very low gate voltage application (Du *et al.,* 2015). If one of the electrodes in EDLC is replaced with a semiconductor and an additional electrodes attached similar to the way they are attached in a solid capacitor, the device behaves as a FET called an electric double layer field effect transistor (EDFET) with an advantage of reduce operational voltage due to its large capacitance (Po-Jui *et al.*, 2014).

Electric double layer field effect transistors have attracted much attention due to their usefulness not only in known practical electronics application but also in fundamental emerging electronics applications such as mimicking of biological synaptic functions, electric control of ferromagnetism, signal processing and sensing in medical physics which requires a low operational voltage and higher carrier concentrations (Yaun *et al.*, 2011). The operation of a field effect transistor with a low operating voltage and higher carrier concentration is essentially dependent on the choice of semiconductor channel layer material and the gate dielectric among other parameters since the threshold voltage (V_{th}), which is the

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voltage required in switching a transistor is dependent on the semiconductor material(Du *et al.,* 2015) and also the minimum gate to source voltage differential that is needed to create a conducting path between the source and drain is dependent on the semiconductor channel layer in an EDLFET.

However, the reported EDLFET and the modulation of electronic states have been so far realised only on oxides, nitrides, carbon nanotubes and organic semiconductor. Therefore, the application of the electric double layer field effect transistor technique to other classes of semiconductor solids has become one of the emerging interests for novel electronic phenomena. This trend is increasingly important for the achievement and development of novel device concepts, applications and tuning of physical properties of materials (Yuan *et al*., 2011).

Metal chalcogenides (MX-where M denote metal (usually transition metal) and X denote chalcogen) such as Tin(II) sulphide (SnS) and metal dichalcogenides (MX_2, where M and X denote metal and chalcogen respectively) such as Tin(IV) sulphide (SnS_2) are of interest as potential candidates for the transport channel of EDLFET. Chalcogenides are materials containing a transition element and one or more chalcogen elements (Silicon, Selenium, Polonium and Tellunum). They found vital applic ations in solar cells, photoconductive materials, thermoelectric materials, rewritable memory, studying of dopant induced superconductivity, charge density formation and transistors. SnS is abundance in the earth's crust, SnS is one of the Tin chalcogenides layered semiconductors in group IV-VI, it possesses the layered orthorhombic structure with eight atoms per unit cell forming biplane layers normal to the largest c axis and the constituent elements are abundant in nature (Thiruramanathan *et al*., 2015). SnS does not contain any toxic or expensive elements; it is a p type semiconducting material with carrier concentration on the order of $10^{16}cm^{-3}$, hole mobility of 1.4 $cm^2V^{-1}s^{-1}$ and low resistivity. It has good air stability and high mobilities. Hence the SnS is a promising candidate to test the feasibility of the chalcogen family for transport channel of an electric double layer field effect transistor and to determine the optimum properties for its usage. The SnS is relatively unexplored for application in electric double layer thin film field effect transistor, as literatures pertaining to the application of SnS as semiconductor or transport channel of an electric double layer field effect transistor is relatively scanty or no report available.

Hence, the article focuses on the use of SnS as the channel layer in EDLFET and honey as the gate dielectric. Honey is produced via sugary secretions from bees which is harvested and packaged under various names for food consumption. Honey contains various amount and concentrations of minerals, glucose, water, fructose, amino acid, vitamins and sucrose that could be controlled via honey production and extraction mechanism (Ordonez, 2017). Honey formulates an ionic gel-like solution analogous to ion gels. Ion gels consist of room temperature ionic liguids and gelating triblock copolymers and have recently shown ideal performance characterics as electrolytic gate dielectrics for the rapid fabrication of Graphene field effect transistors with liquid metal interconnects (Ordonez, 2017). Honey was reported to possess the ability to produces high on-current and low voltage operation. The use of honey is of importance due to its commercial availability, non-toxicity, ability to regulate its ionic content to enhance altering of its dielectric

properties and quick mixing that reduces the time for honey preparation unlike the complexity present in the current usage of ion gel due to requirement of time of preparation in a controlled environment.

METHODOLOGY

A cleaned 4×4 mm FTO glass was masked (from both ends to create the drain and source contact respectively) and then etch about the middle using Zinc powder and Hydrochloric acid as the etchant in order to create the semiconductor channel region. A 0.40 µm SnS thin film was deposited on the etch region with a channel length of 70 µm and channel width of 4000 µm by AACVD to serve as the semiconductor channel layer. The SnS semiconductor channel layer was deposited using 0.1 M Tin chloride dehydrates and 0.2 M of Thiourea which was weighed in stoichiometric proportion and dissolve in ethanol solvent. The two solutions were mixed and stirred for 1 hour using a magnetic stirrer at room temperature, after which the resulting solution was filtered through a 0.22 µm syringe filter and then deposited on the substrate by aerosol assisted chemical vapour deposition (AACVD) at a constant substrate temperature of 258 ℃, nozzle distance of 6.8 mm, substrate to nozzle distance of 3 cm, spray volume of 0.2 mL and spray rate of 0.04 ml/min. With the Drain and Source region still masked, the deposited SnS thin film semiconductor channel layer was annealed in open air at annealing temperature of 250℃ for one hour after which it was allowed to cool to room temperature. The FTO drain and source contacts were bonded with a thin copper wire after which Honey which was commercially acquired was dispensed from a plastic dropper at a volume of 150.59 mm^3 and 136.90 mm^2 surface area of the SnS thin film semiconductor channel layer between the source and drain to act as the electrolytic gate dielectric after which an Iridium (Ir) electrode was suspended above the SnS channel layer, but inside the honey gate dielectric to act as the gate electrode. These were all placed in a transparent Glass container. The electric measurements was then carried out at room temperature using two variable power supplies (KOOCU DC Power supply 1502DD with voltage variation of 0-15 V and ATX-650W, hp invent with voltage variation of 0-12 V) and two digital multimeters (CHY VC 890C$^+$ digital Multimeter and DT9205ACE digital Multimeter). The V_G applied to the Ir electrode was scanned from 0 to 1 v by 0.2 v step. The carrier density, carrier mobility and carrier type were determined by an ECOPIA Hall Effect measurement system (HMS 3000 Hall measurement system) based on Van der pauw configuration. The quality of the EDLFET was determined by measuring the gate current Ig for a gate voltage Vg. A schematic of the SnS EDLFET is shown in figure.1.

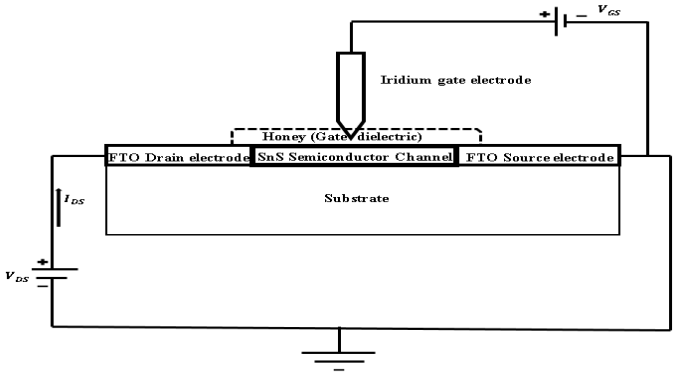

Figure 1: A schematic diagram of the SnS EDLFET

To evaluate the device performances, electrical parameters such as field-effect mobility (μ_{FE}),cutoff voltage, drain saturation current threshold voltage (V_{th}), transconductance and subthreshold swing (SS) were mainly considered as follows:

a. **Output characteristics**- The output characteristic was measured by applying a set of discrete drain voltages (V_D) at different fixed values of gate voltages (V_G) which, in turn produces a drain current (I_D). I_D was plotted on the vertical axis and V_D on the horizontal axis.

b. **Transfer characteristics-**It is a plot of I_D versus V_{GS} for a constant value of V_{DS}. For transfer characteristics, V_G is swept for fixed V_D. The response of I_D was plotted versus V_G. The key performance parameters were taken from the plot. The x-axis value at the intercept of the line gives the value of Gate to source cut-off voltage ($V_{GS\ (OFF)}$) while the y-axis intercept gives the drain saturation current(I_{DSS}). The transconductance (g_m) which is simply the slope of the transfer characteristics is given as the ratio of the change in drain current (I_D) to the change in gate-source voltage (V_{GS}) was calculated using:

$$g_m = \frac{\Delta I_D}{\Delta V_{GS}} \quad (1)$$

The field effect mobility μ_{FE} was also obtained from the transconductance at low V_{DS} by:

$$\mu_{FE} = \frac{Lg_m}{WC_iV_{DS}} \quad (2)$$

Where $C_i$is the capacitance per unit area of the gate dielectric, w is the device width while L is the device length. The mobility is the average drift speed of carriers under the unit electric field. The subthreshold swing (SS) which reflects the necessary V_G required to increase I_{DS} by an order of magnitude in the subthreshold region ($V_{GS} < V_{th}$) was determined as the inverse of maximum slope of the transfer curve. This is given as:

$$SS = \left(\frac{dlog(I_{DS})}{dV_{GS}}\right)^{-1} \quad (3)$$

RESULTS

Hall effect measurement for the SnS semiconductor channel layer

The measured electrical parameters of the SnS semiconductor channel layer from the Hall Effect measurement are given in table 1

The analytic signal map (Figure 4 A) produced revealed areas of high amplitude at the central portion of the area towards the western end. The map also reveals structures that were manually mapped out in black strikes. This is also applies to first vertical derivative map (Figure 4 B). This filter when applied to aeromagnetic data tends to sharpen the edges of the anomalies and revealed structures (lineaments) that are surface and subsurface and were mapped out also here using black strikes. The tilt derivative map, (Figure5 A) reveals most of the structures that were not revealed by the FVD and analytic signal maps. However, the depth to the top of the structure is a parameter that is commonly sought, and the source parameter imaging (SPI) method is one way of obtaining this depth estimate. One advantage of SPI method is that the depth can be displayed on an image. The source parameter imaging (SPI) module from Oasis Montaj software is a quick, easy and powerful method for calculating the depth of magnetic source and was applied to the residual data of the study area and the result shows a minimum depth of 136.01 m to maximum depth of 960.08m.

Table 1: Hall effect electrical parameters for the SnS semiconductor channel layer

Annealing Temperature (°C)	Bulk concentration N_b (cm^{-3})	Average Hall coefficient R_H (cm^3/c)	Carrier mobility μ (cm^2/Vs)	Resistivity ρ (Ωcm)	Conductivity σ $(\Omega cm)^{-1}$
250	3.167×10^{10}	1.971×10^{10}	1.619×10^{5}	1.025×10^{4}	9.756×10^{-5}

The SnS EDLFET assembly

Figure 2: Shows a picture of the assembled SnS semiconductor channel layer Electric double layer field effect transistor with Honey gate dielectric.

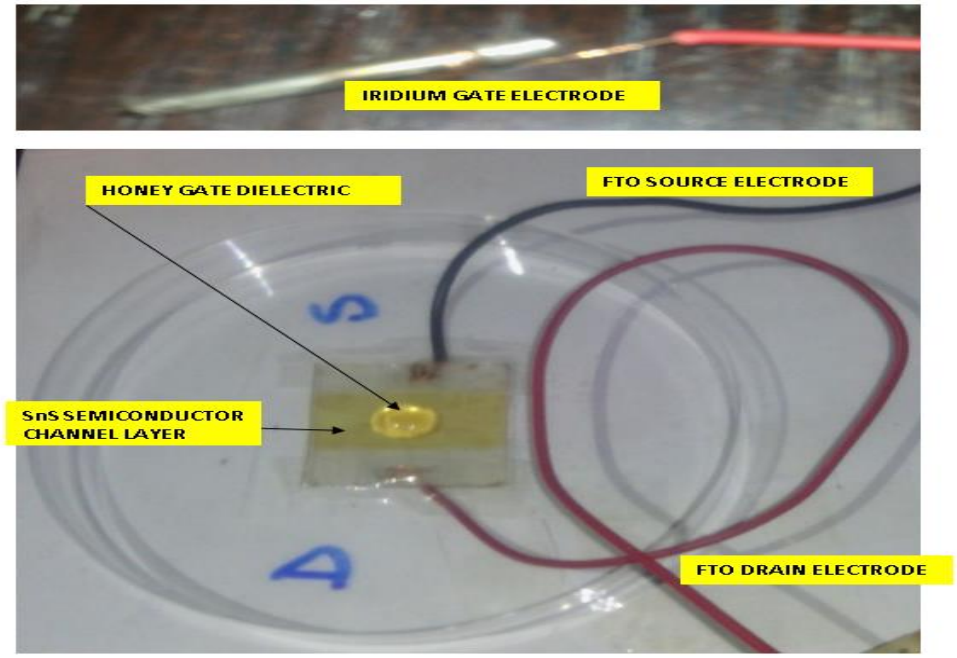

Figure 2: SnS semiconductor channel layer Electric double layer field effect transistor with Honey gate dielectric.

The thin layer forms an electric double layer which is a basic characteristics of ionic liguid contact with conductive materials and is as shown in figure 3.

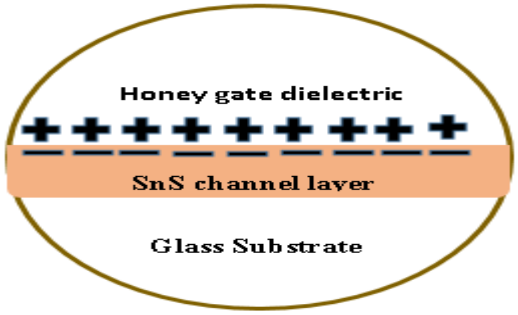

Figure 3: Representation of charge distribution in honey/ SnS layer interface

Transfer and output characteristics of SnS EDLFET

Figure 4. Shows the transfer characteristics (Source drain current I_{DS} as a function of gate bias V_G) of the SnS EDLFET gated by honey dielectric at room temperature. While Figure 5. Shows the output characteristics of the SnS honey gated EDLFET

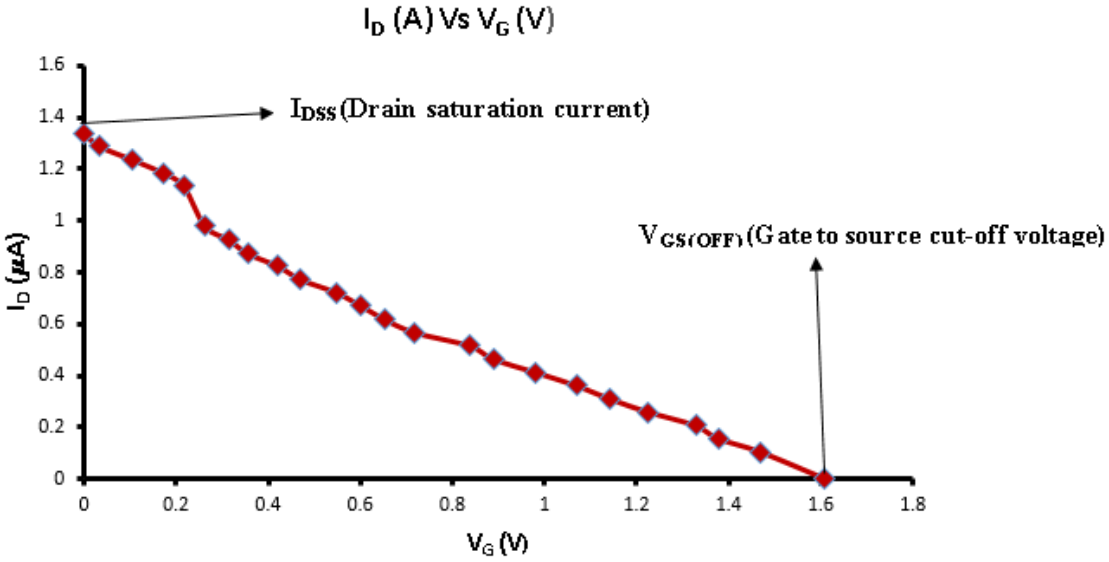

Figure 4: Transfer curve of SnS honey gated EDLFET

Figure 5, Shows the output characteristics (I_D-V_{DS}) of the SnS EDLFET transistor measured at gate voltage (V_G) of 0.2 to 0.8 V with a 0.2 v scan which also supports the field effect transistor operation.

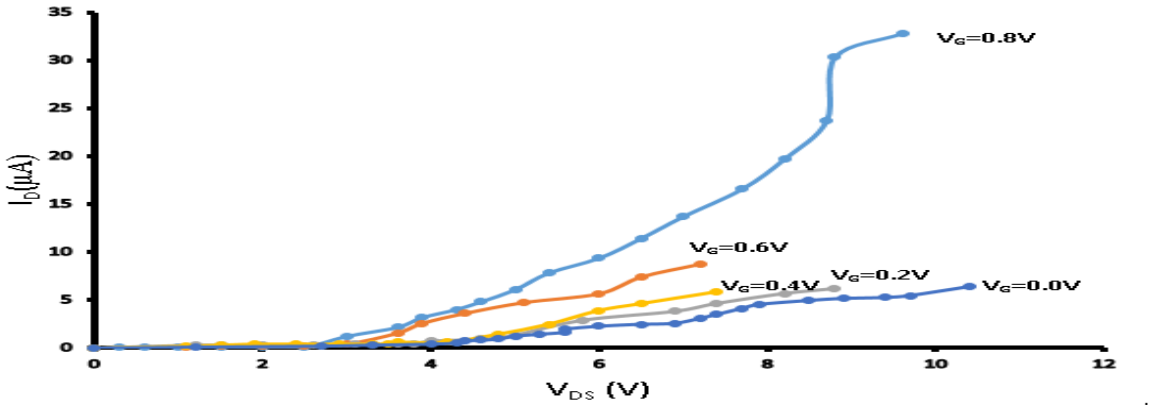

Figure 5: Output curve of SnS honey gated EDLFET

DISCUSSION

From table 1, the average hall coefficient of the annealed SnS semiconductor channel layer is positive which indicates that the semiconductor channel layer is of p type (with holes as majority carriers) conduction.

The measured gate capacitance of honey using LCR meter is 2.15 μF/ cm^2 while the dielectric constant is 20.50. Honey was used as a gel like electrolytic gate dielectric to generate an enhanced electric field response over SnS semiconductor channel layer. Due to the high polarizability of honey, a diffusion of charge is formed at the thin layer between SnS semiconductor channel and honey.

A large charge gradient was formed on the surface of the SnS layer due to the nanoscale separation distance of the electric double layer. Application of positive gate voltage will cause the gate electrode to be positively charged such that when it is submerged in honey, anions accumulate at the gate/honey interface and cations at the honey/SnS interface. The electric double layer form at the honey/SnS interface alters the SnS semiconductor channel layer conductivity. Increase in positive V_{GS} will deplete the SnS channel of its free holes thereby turning it off.

The channel length for the device is 70 μm while the channel width is 4mm. At all values of the Vg used the gate current $Ig \leq 10^{-3}Ids$ which signify that the fabricated device operates as a field effect transistor and specifically an electric double layer field effect transistor.

From Figure 5, it is evident that the SnS EDLFET is a P channel device since the channel conductivity decreases with increasing positive gate bias. The I_{DS} increased with decreased in V_{GS} scanning which indicates a typical p-type transistor operation in the device. The device also operates in depletion mode, i.e. appreciable drain current flows at zero gate voltage as evident from the V_{GS}=0V drain current which is judged from the zero bias current in the transfer curve. It is evident that the SnS EDLFET worked in a depletion mode with a normally "ON" state (i.e conducting without the application of gate bias voltage). This is because maximum current flows from source to drain when no difference in voltage exist between the gate and source terminals. Being a P channel device it will consist of holes as majority current/charge carriers moving through the channel when the EDLFET is on.

The drain saturation current I_{DSS} as determined from the transfer curve is 1.35 μA. This is the maximum limiting current that can flow between the drain and source and occurs at a drain to source constant voltage of 3V when the gate to source voltage is at zero (V_{GS}=0). At I_{DSS} the drain current was observed to increase linearly then began to be pinched off at the knee of the curve. I_{DSS} is temperature sensitive and has a negative temperature coefficient of approximately -0.5/℃. However with further application of voltage to its gate lead of the EDLFET, the drain source channel becomes more resistive such that the transistor completely shuts off. Application of a more positive V_{GS} than ($V_{GS\ (0ff)}$) will have an effect of "depleting" or shutting off the majority current carriers in the preformed channel by changing the size of the depletion region under the gate area, thus increasing the channel resistance.

From the transfer curve, the gate to source cut off voltage ($V_{GS\ (0ff)}$) was found to be 1.6 V. $V_{GS(0ff)}$ is the key characteristics specifying the voltage necessary to turn the device off. The gate to source voltage for a p channel device ranges from 0v for full conduction to several positive volts to turn it off. The 1.6V positive V_{GS} (less holes and less current) will deplete the SnS channel of its free holes thereby turning it "off". The low $V_{GS\ (0ff)}$ will enhance easy circuit design and low power dissipation. $V_{GS\ (0ff)}$ shifts with temperature and has a negative temperature coefficient of approximately -2mV/℃.

The transconductance which is the slope of the transfer characteristics was obtained as -809.61nA/V. The SnS EDLFET exhibit a sub-threshold slope (S), which is defined as the voltage required to increase the drain current by a factor of 10 was determined to be -1.6 $Vdec^{-1}$. This was gotten from the curve of Log (I_D) versus $V_{GS.}$ Sub-threshold slope could be attributed to a small number of interface traps in the SnS thin film which might not necessarily include the grain boundary. The small sub-threshold slope is suitable for switching devices in active matrix flat panel displays.

The field effect mobility obtained from the transconductance is 16.67 cm^2/v at low V_{DS}. The mobility value implies more carriers passing through the channel per unit time which is essential for small field effect transistors and has the advantage of enhancing higher screen luminance.

No appreciable hysteresis was observed in the output characteristics, indicating that the channel is stable once it is formed. The I_D curves are flat at low V_{DS} indicating that a condition of hard saturation is achieved due to complete pinch off of the channel. No clockwise hysteresis was observed which could be attributed to the non-availability or absence of continuous filling of traps by accumulated channel holes as V_{DS} is first increased from zero to a maximum of 0.8 V, then decrease back to 0 V. The fact that the I_D-V_{DS} characteristics curve do not show counter clockwise hysteresis indicates that ionic drift is not significant (Grover, 2008).

Conclusion

The Electric double layer field effect transistor was assembled using tin sulphide (SnS) thin film as semiconductor channel layer and honey as gate dielectric. The SnS EDLFET was assembled in a general laboratory setting not in a conventional clean room, however the device still gave comparable performance to the current available standard parameter values. The SnS EDLT using honey as gate dielectric operated in depletion mode with I_{DS} of 1.35 µA, $V_{GS\ (Off)}$= 1.6 v, Trans conductance of -0.80961 µA/v, field effect mobility of 16.67 cm^2/v and sub-threshold slope of 1.6 $vdec^{-1}$ which are applicable as load resistors in synaptic transistor network, biosensor, logic gate circuits and in depletion load logic circuits.

Conclusively, very scanty reports have been made or exist on the study of SnS thin film applied to electronics as a semiconductor channel layer. SnS EDLFET using honey as gate dielectric offers an opportunity for further research and innovation into other materials that are unconventional in the expectation discovering new semiconductor and dielectric materials that are readily available, has minimal time of preparation, biodegradable and non-toxic. To the best of our knowledge and within the limit of available literature, no SnS based EDLFET has been reported turning the results described here innovative to the scientific community.

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Comparative Study of Heavy Metals in Termite and Non-Termite Soils in Lapai,Niger State, Nigeria

***Elele U.E. & & Aliyu A.B.**

Department of Chemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

*Correspondence: Email address: *uuelele@yahoo.ca;* Telephone: +2348033803880

ABSTRACT

This study was aimed at the determination of seven heavy metals - Cd, Co, Cu, Fe, Pb, Mn and Zn from termite and non-termite soil samples collected from ten locations in Lapai, Niger State, Nigeria. At each location, termite soil samples (TSS) were collected from 20 randomly selected termite mounds to form a composite sample. The non-termite soil samples (NTSS) were collected 5 meters away from a termite mound at a depth of 0-20 cm. The soil samples were spread on plastic trays and air-dried at room temperature for 7 days. Then, the samples were pounded with wooden mortar and pestle, sieved (2 mm mesh) and packaged into labeled air-tight polythene bags for analysis. The samples were wet digested using USEPA 3050B Method and the digests were analyzed using AA500 Spectrophotometer. A standard reference material - SRM 2709 was analyzed using the same digestion and analytical methods. The results obtained for the analytes in the SRM had coefficient of variance of less than 10 %. The decreasing order of the concentrations of the heavy metals in both TSS and NTSS was Fe > Mn > Co > Zn > Cu > Cd > Pb. Cadmium (Cd) and Pb showed the lowest contents amongst the heavy metals studied. In general, the levels of the heavy metals were found to be higher in the termite soil samples (TSS) than in the non-termite soil samples (NTSS). None of the heavy metals except Co at some of the locations exceeded the world average contents and permissible limits in soils reported in literature implying that both the TSS and NTSS can be used for agricultural purposes.

Keywords: Termite soil samples (TSS), Non-termite soil samples (NTSS), heavy metals, Lapai.

INTRODUCTION

Heavy metals are a group of metals that have atomic density greater than 5 gcm^{-3}. They are natural constituents of soils that are usually present in trace amounts but are enriched through human activities. Heavy metals constitute a special group of metals in soil chemistry because of toxic effect exerted on plants when their concentrations are high, however, there is no common opinion on the hazard degree of any particular heavy metal in soils (Vodyanitskii, 2016). The biological effects of heavy metals are dependent on the level and duration of exposure (Wuana & Okiemien, 2011). The use of fertilizers in farming is one of the ways through which soils are enriched with heavy metals particularly Cd and Pb (Atafar et al, 2010**,** Malidareh et al, 2014). Monitoring of long-term impact of fertilization is necessary to assess metal accumulation in agricultural soils (Czarnecki & Düring, 2015). Some of the heavy metals are essential to plant growth while others are toxic even at low concentrations. Metals required by plants include Mn, Fe, Cu and Zn (Aziz et al, 2015, Czarnecki & Düring, 2015). Heavy metals are bio-accumulative and non-biodegradable. Heavy metals' contamination in agricultural soil is a potential environmental

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threat to the safety of agricultural food crops (Simon, Mtei & Kimanya, 2016).

Termites are social insects of economic importance that have a worldwide distribution (Alajmi, Abdel-Gaber & Alotaibi, 2019). Termite mounds are common sights in Lapai, which means that Lapai soils are infested with termites. Some of the mounds are as high as 3.50 m and as wide as 3. 90 m (see Figure 1). Mohammed, Abiodun, and Jibia (2014) in their study in Lafia Local Government of Nasarawa State of Nigeria reported termite mounds with mean height and width of 2.50 m and 7.88 m respectively

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Figure 1: Some of the termite mounds in IBBU, Lapai.

The mounds are features built from subsoils taken from a depth as low as 3 metres below the earth surface and the composition of mound soil is closely related to the nature of adjacent subsoil (Semhi et al, 2008). Their ability to collect particles from different soil depths and deposit them in mounds increases the organic Carbon, clay and nutrients, pH and microbial population in their mounds relative to adjacent soils (Ohkuma, 2003).

Termites can be major agricultural pests that cause crop losses (Faragalla et al, 2015). They are social insects of the order Isoptera with about 3,000 known species, of which 75 % are classified as soil-feeding termites (Vats & Sanjeev, 2011). Termite species are widely varied with a wide range of living, breeding, and feeding habits (Semhi et al, 2008). The termite species found in Lafia, Nigeria include: *Odontotermes, Callotermes, Eutermes* and *Termes* species (Mohammed, Abiodun, & Jibia, 2014). They are significant agents of ecosystem processes. Termites have peculiar activities in the soil, inducing significant changes in the soil properties (de Lima et al, 2018). According to Dangerfield, Mccarthy and Ellery (1998), they are predictable as "ecosystem engineers"

because they promote soil transformations by disturbance processes. The accumulated material is later redistributed by erosion causing changes in soil microstructure and fertility (Dangerfield, Mccarthy & Ellery, 1998). They are common biological agents that produce significant physical and chemical modifications to tropical and subtropical soils (Heikens et al, 2001, Semhi et al, 2008). They are undoubtedly key soil organisms in tropical and subtropical soils (Jouquet et al, 2016). Forest nurseries and plantations, roots of seedlings are damaged by different species of termites, particularly when the plants are about 1-3 years old (Sen-Sarma, 1974). Most farmers in Lapai neglect the effect of termites' infestation in their farms since they do not apply any chemical to reduce termites' population and their denudation effects.

The main aim of this study is to compare the quality of the termite and non-termites soils in Lapai, Niger State, Nigeria through the determination of the concentration of seven heavy metals namely, Cd, Co, Cu, Fe, Mn, Pb and Zn in the soils and compare the heavy metals contents with the average contents in world soils and some agricultural soil standards.

METHODOLOGY

Sample Collection

The soil samples were collected from ten different locations in Lapai, Niger State. These locations are: Cece (CC), Haske River Area (HRA), Ibrahim Badamasi Babangida University (IBBU), Government Secondary School (GSS), Kuso Gbogi (KB), Malle (M), Mashina (M2), Opposite (University) Main Gate (OMG), State Low Cost (SL), and Fifty-Fifty (FF). At each location, the termite soil samples (TSS) were collected from 20 randomly selected termite mounds at the top, center and base to form a composite sample. The non-termite soil samples (NTSS) were collected 5 meters away from a termite mound at a depth of 0-20 cm. The soil samples were placed in labeled polythene bags and taken to the laboratory for analysis. The soil samples were spread on plastic trays and air-dried at room temperature for 7 days. Then, the samples were pounded with wooden mortar and pestle and sieved using a sieve of 2 mm mesh. Conning and quartering were used to reduce the bulk of the soil samples, which were then packaged into labeled air-tight polythene bags for analysis.

Apparatus/Materials

The apparatuses / materials used in this study were AA500 Atomic Absorption Spectrophotometer (PG Instruments), Staut weighing balance (Model SPU402), beakers (100 cm^3), graduated measuring cylinders (5 cm^3, 10 cm^3, 100 cm^3), volumetric flasks (50 cm^3, 100 cm^3), Jenway 1000 hot plate and stirrer, pipette (1 cm^3, 5 cm^3), wash bottle, plastic sample bottles (120 cm^3), plastic trays, mortar and pestle, funnels, Whatman No.41 filter paper (ashless), and masking tape.

Reagents

The reagents used in this study were concentrated hydrochloric acid (HCl), concentrated nitric acid (HNO_3), 30 % Hydrogen peroxide (H_2O_2) and single-element standard stock solutions (1000 mg kg^{-1}) of Cd, Co, Cu, Fe, Pb, Mn and Zn produced by GFS Chemicals Inc., Columbia.

Preparation of solutions

HCl solution

250 cm^3 of conc. HCl was measured and transferred quantitatively into a 500 cm^3 volumetric flask containing distilled water. Distilled water was used to rinse the measuring

cylinder. The solution was made up to the mark with distilled water and was shaken to obtain a homogenous solution.

10 % HNO_3 solution

This solution was prepared by measuring 200 cm^3 of the acid into a 2 L volumetric flask containing distilled water. This was made to the mark with distilled water. The resulting solution was shaken to obtain a homogenous solution.

Sample Preparation

The soil samples were wet digested using United States Environmental Protection Agency (USEPA) (1996) 3050B Method. 1.00 g of each soil sample was weighed into a 100 cm^3 beaker. 10 cm^3 of 1:1 HNO_3 was added to the beaker and refluxed for 10 minutes on a hot plate without boiling. Then, 5 cm^3 of conc. HNO_3 was added, refluxed for 30 minutes and cooled. Another 5 cm^3 conc. HNO_3 was added followed by refluxing for 30 minutes so as to ensure complete digestion. The contents of the beaker were heated for about 2 hours until the volume was reduced to 5 cm^3 and cooled. 2 cm^3 of distilled water was added followed by the addition of 3 cm^3 of 30 % H_2O_2. Then, 1 cm^3 of 30 % H_2O_2 was added and the addition of 1 cm^3 of 30 % H_2O_2 was continued until bubbling subsided. The beaker with its contents was heated to reduce volume to about 5 cm^3. 10 cm^3 of concentrated HCl was added to the digest and refluxed for 15 minutes; cooled, filtered into 100 cm^3 volumetric flask using Whatman ashless filter paper No. 41 and made up to mark with distilled water.

For the analysis of Pb only, 1.00 g of each soil sample was weighed into a 100 cm^3 beaker. 2.5 cm^3 of conc. HNO_3 and 10 cm^3 of conc HCl were added to the beaker and refluxed for 15 minutes, cooled and filtered into a 100 cm^3 volumetric flask. Then, the filter paper was washed with 5 cm^3 hot conc. HCl followed by 20 cm^3 hot distilled water and filtered into the same volumetric flask. The filter paper together with the residue was removed and placed back into the digestion beaker. 5 cm^3 of conc HCl was added, refluxed for 15 minutes and cooled. This was followed by filtration into the same volumetric flask. 10 cm^3 of concentrated HCl was added to dissolve any precipitate formed and the digest was made up to mark with distilled water. Each sample was digested in triplicate and a blank determination was also carried out. The standard reference material - SRM 2709 (San Joachin) soil was also digested using the same method. Each sample solution was stored in a labeled plastic bottle ready for analysis using the AA500 Atomic Absorption Spectrophotometer. Each stock solution was serially diluted to prepare a working standard solution, which was in turn serially diluted to give the standard solutions of each analyte that was used in calibration of the AAS. Then, each sample solution (digest) was aspirated into the spectrophotometer following the working conditions of the spectrophotometer. The concentrations of the metals in each sample were calculated using the formula below:

$$\text{Concentration of metal, } mg\,kg^{-1} = \frac{(C_1 - C_2)\,x\,V\,x\,Df}{m}$$

Where:

C_1 = Concentration of metal in the sample, mg kg^{-1}

C_2 = Concentration of metal in blank, mg kg^{-1}

V = Total volume of sample digestion, cm^3

Df = Dilution factor

m = Mass of sample, g

Statistical Analysis

All statistical analyses were performed using Microsoft Office Excel 2007 on Windows 7 system. The data obtained were presented as mean ± standard deviation and subjected to one-tailed unequal variance t-test analysis at the $p < 0.05$ probability level to establish if there was any statistically significant difference for all the heavy metals under study in both the TSS and NTSS.

RESULTS

Table 1: Heavy metals contents of standard reference material - SRM 2709 (San Joachin Soil).

Heavy Metal	Certified Value,mgkg^{-1}	Measured Value*,mgkg^{-1}	Coefficient of Variance, %
Cd	0.371 ± 0.002	0.36 ± 0.10	2.8
Co	12.8 ± 0.2	13.12 ± 0.54	4.1
Cu	33.9 ± 0.5	33.47 ± 0.65	1.9
Fe	33600 ± 700	32880 ± 560	1.7
Pb	17.3 ± 0.1	16.95 ± 0.25	1.5
Mn	529 ± 18	538.70 ± 3.62	6.7
Zn	103 ± 4	105.25 ± 2.33	2.2

* Measured values are mean of triplicate determination ± standard deviation

Table 2: Concentration of heavy metals in termite soil samples (TSS) and non-termite soil samples (NTSS) in (mg kg^{-1})*

Sample	Cd		Co		Cu		Fe		Mn		Pb		Zn	
	TSS	NTSS	TSS	NTSS	TSS	NTSS	TSS	NTSS	TSS	NTSS	TSS	NTSS	TSS	NTSS
CC	0.0	0.9	52.2	80.2	20.8	6.1	362.5	279.2	52.2	80.7	0.1	0.1	32.8	30.7
HRA	0.9	0.0	59.3	73.3	41.4	35.4	27.2	404.2	59.3	13.6	0.5	0.5	29.6	31.8
IBBU	1.3	1.7	66.4	87.4	12.2	6.8	237.5	70.9	66.4	87.9	0.7	0.7	29.6	32.8
GSS	1.7	0.1	65.1	59.1	22.2	16.4	112.5	112.5	66.4	59.3	1.3	0.0	31.8	38.2
KB	1.4	0.5	45.0	87.0	5.6	1.8	195.9	154.2	45.0	87.9	0.0	0.4	40.4	32.8
M	1.6	0.5	73.6	87.6	35.4	26.8	237.5	154.2	73.6	87.9	1.4	0.1	27.5	30.7
M2	1.3	0.9	80.7	109.7	6.1	1.4	154.2	195.9	80.7	109.3	0.0	0.9	40.4	29.6
OMG	0.5	0.9	130.7	80.7	11.2	6.1	154.2	29.2	130.7	80.7	0.0	0.1	27.5	28.5
SLC	1.3	1.7	102.8	0.0	20.8	17.3	70.9	29.2	102.2	0.0	1.1	0.8	26.4	27.5
FF	0.0	1.7	0.0	0.0	10.6	6.1	195.9	0.0	0.0	0.0	0.9	0.7	26.4	65.1

* Measured values are mean of triplicate determination ± standard deviation.

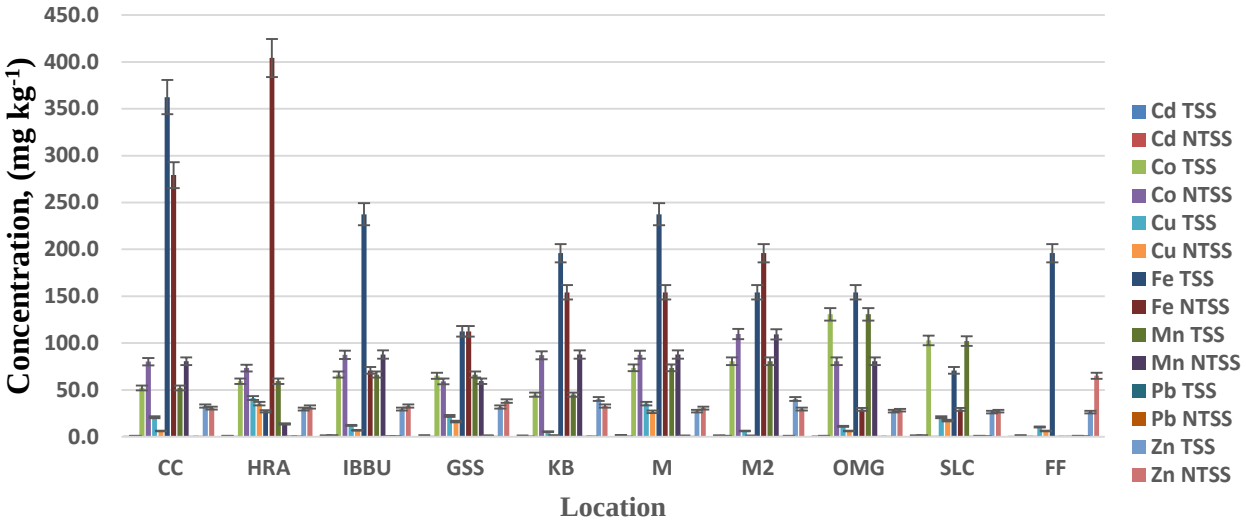

Figure 2: Heavy metal contents of termite and non-termite soils at all the locations.

Table 3: T-test of mean heavy metal contents (range of values in brackets) in mg kg^{-1} of the soil samples

Heavy Metal	Sample Size	Termite Soil Samples	Non- Termite Soil Samples	T-Test
Cd	10	1	0.9	0.36
Co	10	67.6	66.5	0.47
Cu	10	18.6	12.4	0.13
Fe	10	174.8	143	0.27
Pb	10	0.6	0.4	0.34
Mn	10	67.7	60.7	0.21
Zn	10	31.2	34.8	0.19
Degrees of freedom (df) = 18;		$p < 0.05$;	Critical Value = 2.10	

Table 4: Ranges of mean concentrations of heavy metals in TSS and NTSS versus world soil average (Kabata-Pendias, 2011), maximum permissible limits (MAL) Canadian Agricultural Soil Quality (Canadian Council of Minister of the Environment, 2007) and European Communities for Agricultural Soil with pH 6 - 7 (Commission of European Communities, 1986).

Heavy Metal	Concentration range, mg kg^{-1}		World soil average, mg kg^{-1}	Canadian MAL, mg kg^{-1}	European MAL, mg kg^{-1}
	TSS	NTSS			
Cd	0.0 - 1.7	0.0 - 1.7	0.41	1.4	1 - 3
Co	0.0 - 130.7	0.0 - 109.7	11.3	40	-
Cu	5.6 - 41.4	1.4 - 35.4	38.9	63	50 - 140
Fe	27.2 - 362.5	0.0 - 404.2	35,000	-	-
Pb	0.0 - 1.4	0.0 - 0.9	27	70	50 - 300
Mn	0.0 - 130.7	0.0 - 109.3	488	-	-
Zn	26.4 - 40.4	27.5 - 65.1	70	200	300

DISCUSSION

Table 1 shows that the precision of the heavy metals were 2.8, 4.1, 1.9, 1.7, 1.5, 6.7 and 2.2 % for Cd, Co, Cu, Fe, Pb, Mn, and Zn respectively, which shows that the USEPA (1996) Method 3050B used in the digestion of the soil samples was quite good. The concentration of sevenheavy metals in termite soil samples (TSS) and non-termite soil samples (NTSS) in (mg kg^{-1}) are shown in Table 2. The decreasing order of the concentrations of the heavy metals in both TSS and NTSS was found to be Fe > Mn > Co > Zn > Cu > Cd > Pb. However, the order found by Alajmi, Abdel-Gaber and Alotaibi (2019) in termite mound soils was Zn > Fe > Cu > Mn > Co > Pb > Cd while Addis and Abebaw (2017) reported the decreasing order of the heavy metals studied as: Fe > Mn > Zn > Co > Cu > Pb > Cd in soils used for cultivation of *Allium sativum* L.(garlic). The difference in order may be due to difference in the geologic composition of the rocks from which the soils were formed in the studies,

Cadmium (Cd) and Pb showed the lowest contents amongst the heavy metals studied. The Cd contents in both the TSS and NTSS had a range of 0.0 - 1.7 mg kg^{-1}. Only five of the locations, namely Haske River Area (HRA), Government Secondary School (GSS), Kuso Gbogi (KB), Malle (M) and Mashina (M2) had higher Cd contents. The range of Pb contents in the TSS was 0.0 - 1.4 mg kg^{-1} while in NTSS, it was 0.0 - 0.9 mg kg^{-1}. Amongst the TSS, higher concentration of Pb was obtained at GSS, M, SLC, and FF; same concentration at CC, HRA, and IBBU with lower concentrations at KB, M2 and OMG. Cadmium (Cd) and Pb are not essential to plants and animals but are potentially toxic to them when exposed to them (Kabata-Pendias, 2011). Cd and Pb are particularly recognized as toxic metals, even at

low concentrations and incidences of environmental pollution by these two heavy metals are well known (Adriano, 1986, Nriagu, 1990). The Co contents ranged from 0.0 - 130.7 and 0.0 - 109.7 mg kg^{-1} in the TSS and NTSS respectively, which was generally higher than the Cd and Pb concentrations.

The Cu contents obtained in this study in the ten locations in Lapai, Niger State, Nigeria ranged from 5.6 - 41.4 mg kg^{-1} for the TSS and 1.4 - 35.4 mg kg^{-1} for the NTSS while the range of Zn contents were 26.4 - 40.4 mg kg^{-1} and 27.5 - 65.1 mg kg^{-1} for the TSS and NTSS respectively. The Cu and Zn contents obtained in this study were generally higher than the values reported by Iyaka and Kakulu (2009) for urban agricultural soils of Bida and Minna in Niger State, Nigeria' that were Cu contents' range of 2.4 - 6.5 mg kg^{-1} for Bida and 12 - 89 mg kg^{-1} for Minna while Zn contents ranged from 0.57 - 36 mg kg^{-1} for Bida and 2.8 - 41 mg kg^{-1} for Minna. Copper and Zn are two important essential elements for plants, microorganisms, animals, and human beings (Wuana & Okiemien, 2011).

The Mn concentration ranged from 0.0 - 130.7 mg kg^{-1} for the TSS and 0.0 - 109.3 mg kg^{-1} for the NTSS. Higher concentration of Mn in the TSS than in the NTSS was obtained at HRA, GSS, OMG, and SLC; same Mn concentration at FF (0.0 mg kg^{-1}) since Mn was not detected at FF in both soil sample types and lower Mn concentrations at CC, IBBU, KB, M and M2. In a similar study, Eneji, Sha'Ato and Ejembi (2015) reported that Mn and Fe, are significantly higher in the termite mounds compared to the surrounding soils. Iron (Fe) had the highest concentration in the soil samples with a range of 27.2 - 362.5 mg kg^{-1} in the TSS and 0.0 - 404.2 mg kg^{-1} in the NTSS as evident in Figure 2. Seven (7) of the locations studied showed higher Fe contents in the TSS than in their corresponding NTSS. These locations were CC, IBBU, KB, M, OMG, SLC and FF while HRA and M2 locations showed lower Fe in the TSS than the NTSS. The Fe concentration in the TSS and NTSS at GSS was the same (112.5 mg kg^{-1}). Thus, the prominent bars in Figure 2 are mostly Fe and Mn contents at most of the locations. The findings of this study corroborate the findings of Abdus-Salam and Bello (2015) that Fe is a major constituent while Mn is a minor constituent in termite mounds. In general, the soil samples from the termite mounds contained more heavy metal contents than non-termite soil samples although not to ecotoxicological levels that will constitute problems to soil and crops. This is in contrast to the findings of Vats and Sanjeev (2011) that Mn, Co and Zn contents were lower in their termite soils.

Table 3 shows that there was no significant difference between the concentration of the heavy metals in the TSS and NTSS at $p < 0.05$ as the t-test values were all less than the critical value of 2.10. Also, the concentration of the heavy metals were below the average for world soils and maximum permissible limits set for Canadian agricultural soils and European communities agricultural soils with a pH of 6 - 7 reported in literature except Co at some of the locations (see Table 4). Addis and Abebaw (2017) also reported Co concentrations that were higher than the maximum tolerable levels in their study. The higher Co concentrations may be due to geologic factors.

Conclusion

The concentrations of seven heavy metals in soil samples collected from termite mounds and their surroundings from ten locations in Lapai in Niger State, Nigeria were determined. The decreasing order of the concentrations of the heavy metals in both TSS and NTSS was Fe > Mn

> Co > Zn > Cu > Cd > Pb. There was no significant difference between the concentration of the heavy metals in the TSS and NTSS. The concentration of the heavy metals were below the average for world soils and maximum permissible limits set for Canadian agricultural soils and European communities agricultural soils with a pH of 6 - 7 reported in literature except Co at some of the locations. Therefore, both the TSS and NTSS can be used for agricultural purposes.

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Dimensions of Household Water Insecurity in Under Served Communities of Minna Metropolis

***Sallau R.O. Umar P.F., Dangana, K., Bashir I.Y. & Olubadewo-Joshua O.**

Department of Geography, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

*Correspondence: Email address: *osesie@ibbu.edu.ng;* Telephone: +2347034207946

ABSTRACT

This study sought to understand water insecurity across underserved communities in poorly planned urban settlements of Minna metropolis, Niger State. The study applied a mixed methods approach. A household survey N=150 was used to obtain quantitative data. The data was analysed using SPSS version 21 software. Findings show that disparities exist in availability of functional water sources attached to residences based on accommodation ownership status. In the understanding of threats to water access, beyond proximity to water sources, the study shows that waiting times are equally critical. Social and health implications of water insecurity were also observed as some respondents reported having conflicts within and outside their homes over water related issues; and some respondents reported having illnesses which they attributed to their water insecurity experience. Coping mechanisms reported by the households in the study area were mainly carried out by individual households as opposed to community efforts which studies suggest may be more sustainable. Implications for research and policy were also discussed.

Keywords: water sources, water insecurity, poorly planned urban settlements.

INTRODUCTION

In sub-Saharan Africa, population growth and urbanization have led to an increasing concentration of people in poor urban and peri-urban informal settlements. Household access to portable water in Sub-Saharan Africa (SSA) continues to be a challenge; as evidenced by the statistics that show there are more people without access to water in 2015 than in 1990. (Scanlon *et al*., 2016). Water demand has continually intensified in the urban areas of SSA, while water supply capacity is constrained by old and weak infrastructure, (Rouse, 2014, pp. 21-23; Padowski, Carrera, & Jawitz, 2016) poor governance and weak institutions (Adams and Zulu 2015). Urban informal settlements have been found to be over ridden with poor sanitation, overcrowding, poverty and insecure housing tenure, poor water access which has resulted in the high prevalence of water related illnesses (Prüss-Ustün, *et al*., 201; Neelim, 2011). In a related study, Dos Santos, *et al*. (2017) highlighted the growing gap between the demand for water and available supply especially in urban areas, and further expatiated on how the problem of water insecurity is more prevalent in unplanned areas as compared to planned settlements within urban areas; despite the fact that these informal settlements are home to majority of SSA's urban population. Additionally, disparities in water access across urban/rural geographies rarely highlight the intra-urban disparities in water access and water insecurity. (Adams 2018; Smiley, 2013). It is against this

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background that this study seeks to understand the water insecurity dimensions within unplanned settlement of Minna Metropolis Niger State.

METHODOLOGY

The survey was carried out among 150 residents of two poorly planned settlements in Minna Metropolis. Data was gathered primarily using structured questionnaires which comprised of questions on Location, Demographic and Socio-Economic Characteristics, Water sources, Water access, Adverse social and Health implications of water insecurity and Coping strategies. Participant observations, Field notes and excerpts from household interviews were used to contextualize and discuss the findings. The target respondents were household heads, however in the event that they were unavailable, their spouses or a household member above age eighteen was interviewed. The questionnaires were administered in a face to face interview. The study was carried out during the dry season months (of December to March) thus the findings did not capture the influence of the rainy season on coping with water insecurity. Two residential communities within Minna metropolis were found suitable as they were underserved areas in terms of government provision of piped water and a such residents in these communities were responsible for their water provision. Thus they were suitable as respondents for this study. These communities were Kpakungu and Sauka ka huta. The survey data gathered was analysed using SPSS version 21 software. Descriptive statistics was used to summarize the data.

Water Availability and Accommodation Ownership

Figure 1 shows that more than half (57.14%) of the respondents who resided in their personally owned accommodations had water sources attached to their residence as compared to slightly above a quarter (28.57%) of the respondents in rented accommodations who reported having functional water sources attached to their accommodations.

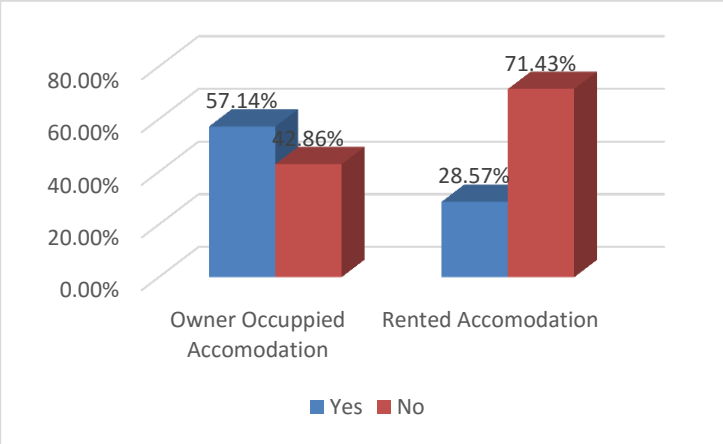

Figure 1: Functional Water Source Availability by Accomodation Ownership Status

Source: Author's Field Survey, 2018

Primary Water Source within Households

Table 1 showed that the respondents relied on multiple sources of water. The prevalent primary source of water reported by the respondents was the Borehole (47.85%), this was either attached to the respondent's accommodation, attached to a neighbor's accommodation or available in the community. This was closely followed wells (29.29%) either attached to their personal accommodation, attached to a neighboring accommodation or available in the community. The use of water supplied by water cart pusher's indigenously referred to as "mairuwa" accounted for 22.86%.

Table 1: Primary Water Source within Households

S.N.	Primary Water Source	Primary Water Source	Frequency	Percentage
1	Borehole	Public Borehole	17	47.85%
		Neighbor's Borehole	28	
		Borehole within Compound	27	
		Commercially Constructed Borehole	9	
2	Well	Well within Compound	23	29.29%
		Public Well	11	
		Neighbor's Well	19	
3	Cart Pushers "Mairuwa"	Mairuwa	40	22.86%

Water Sourcing

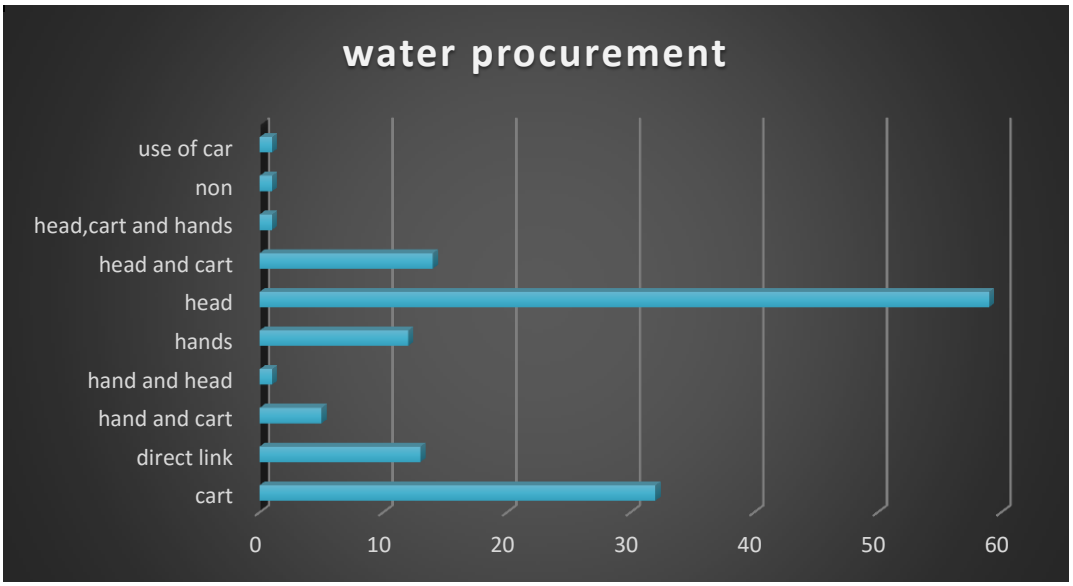

Figure 2: Method of water procurement

Source: Author's field survey (2018)

Nearly half (47.5%) of the respondents have one or more members of their households engaged in fetching water for their households. With 24.56% of the parents within the household reporting that they engage in fetching of water, 32.38% of the female children engaging in making trips to collect water; and 52.38% of the male child respondents reporting that they engage in making trips outside their homes to collect water. Thus indicating a greater burden of physically engaging in water collection on children.

Among the respondents that participated in fetching water 71.5% of the respondents reported that the water was sourced in the morning. The dominant mode of transporting this water was on the heads as reported by 56% of the respondents while the second dominant mode was by cart (35%) and the least reported mode was by hand (9%).

Average walking time is 5.6 minutes to water source, while the mean waiting time at the water source was 4.93 minutes. Totaling approximately 16.13 minutes as water

collection time per trip. Nearly three quarters (77%) of the respondents without functional sources of water reported that the water collected lasted within the household for a period of two days.

Water Costs

The monetary cost, of water used within the households on a monthly basis showed that nearly half of the respondents (48.6%) did not pay for water as they had boreholes or wells installed in the residences while others get to fetch from their neighbors at no financial costs.

Among the respondents that paid for water, the average cost of water spent monthly was ₦5332 per month paid to either mobile water vendors or at commercially borehole sales points.

Adverse Social and Health Implications of Household Water Insecurity

More than a third (35%) of the respondents had engaged in conflicts either at home or within their communities over water collection or water use. These disputes have ranged from the order of queuing for water at collection points in the community; the quantity of water an individual is entitled to per turn and disputes regarding the use of water within the household among others.

Adverse health experiences which the respondents attributed to their water insecurity experience include, Body pains 31.9%, Backache 9.9%, alongside other water related illnesses such as, typhoid fever 19%, diarrhea 8% and skin infections 2%.

Table 2: Social Implication of Water Insecurity

Type of Conflicts	Frequency		Percentage (%)
Conflict at point of collecting water (N=140)	Yes	86	61.4
	No	54	38.6
Conflict at home over the use of water (N=140)	Yes	87	62.1
	No	53	37.9
Reasons for conflict (N=53)			
Based on first come first serve		23	43.4
Fight over the number of containers an individual can fill		14	26.4
Locking of water sources, schedule made		7	13.3
Within the house as regard the use of water		9	16.9

Coping with water insecurity

Table 3: Coping Strategies

Coping Strategy	Frequency		Percentage (%)
Engage in water re-use (N=140)	Yes	57	40.7
	No	83	59.3
Migration (N=140)	Yes	55	39.3
	No	85	60.7
Water re-use (N=57)			
Re use of Laundry water to flush toilets		55	96.5
Re use of Laundry water for washing of yards		2	3.4

Beyond the reliance on alternative water sources, additional coping strategies include; Water reuse which was reported by more than a third of the respondents for sanitary activities such as washing their compound or flushing toilets. More than a thirds (39%) of the respondents reported either relocating or knowing persons that had resolved to residential mobility; by relocating from

residential community to another within the metropolis as a result water insecurity.

DISCUSSION

The study suggests that accommodation ownership status may be linked to availability of functional water sources attached to accommodations in locations underserved by government provision of water. This has been supported by similar studies which show that due to the high cost of investments occupants of rented housing do not engage in the installations of water supply facilities in their households as they believe it is the responsibility of the owner of the property. (Awunyo-Akaba *et al*., 2016). The study equally points to other dimensions of water insecurity that could result from the wide patronage of water vendors. As these vendors still remain part of the informal sector, and the quality of the services they render still go unregulated. (Ezekwe *et al.,* 2016). In Sub Saharan Africa, approximately 70% of the population must leave their home to fetch water. (Pickering & Davis, 2012). Despite the fact that studies have highlighted distance as an important metric for rural water access (Ho *et al.,* 2014), especially as rural households tend to be more dispersed, and this study demonstrates that beyond distance, another significant contributor to understanding water insecurity is the time taken to access water. Particularly that not all respondents have abilities to estimate distance, and more importantly even in cases where water sources are in close proximity to the household, the waiting time could still span well over an hour 30 minutes. The time burden can be further explored along the metrics of walking time, or waiting time. Studies have shown that even in cases where water sources are close to the respondent's residence, limitations in water access have been perceived to a greater extent to result from the long waiting times (Adams, 2018; Smiley, 2016). The inclusion of the number of trips required to meet daily household water needs in informal settlements, is also important to better understand the full burden of time, especially on the women and children who predominantly engage in water fetching activities in African communities and engage in multiple trips per day (Adams, 2018) and this is equally supported by the findings of this study.

The findings further show that the implications of water insecurity are highly gendered and contentious (Das & Safini, 2018,). Considering the adults within the household, women /mothers were more likely to be responsible for fetching water as compared to men/fathers. However, considering the family unit as a whole, the burden of fetching of water is still predominantly on children, predominantly in the morning hours and this can affect punctuality and school attendance for in-school children. The average time used to access water compared favorably and was much lower in the study area as compared with similar studies in Malawi which reported average waiting times of 38 minutes (Adams, 2018). The study suggests that water insecurity may have adverse implications on health, social cohesion along with residential mobility. Coordinated Community provisioning for water within the study area still holds great promise, as more than half of the households in the study area pay for water provisioning services, alongside relying on community relationships for their water supply. The need for research in this area, is further highlighted as similar studies point to coordinated community responses as a future direction to increasing portable water access to

households in a sustainable manner. (Dos Santos, *et al*, 2017).

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Noise Pollution in Nigeria: Causes, Effects, Policies and Government Regulations

[*1]Olubadewo-Joshua O., [1]Sallau R.O. & [2]Joshua O.F.

[1]Department of Geography, IBB University, Lapai, Niger State, Nigeria.
[2]Federal Fire Service Training College. Minna, Niger State, Nigeria

*Correspondence: Email address: oluwaseuno@ibbu.edu.ng; Phone number: +2348032202204

ABSTRACT

Noise is any form of unwanted sound. Exposure to excessive noise can cause a number of deleterious effects on humans, yet, noise generation is one of the inevitable features of development. Electric power generating plant, which exists in their millions across the country have been identified as a major source of noise pollution in Nigeria. Loud noise from music and radio sets, human conversation, various types of advertisement and hawking among others have been identified as sources of noise in Nigeria. There are guidelines for the control of industrial noise and there are reasons to believe that they are being implemented. Unfortunately, the noise in the streets and residential areas to which the greater majority of Nigerians are exposed, appears not to be subject to any guidelines or any form of control. This is a major environmental problem in Nigeria that requires urgent attention. The solution is to enact laws on noise in the streets and residential areas and ensure strict enforcement.

Keywords: Environment, Generator, Noise, Noise pollution

INTRODUCTION

Noise is defined as any unwanted signal and in most cases the signal is nothing but sound. The extent to which noise isannoying depends on many factors such as the pitch irregularities, duration, rhythm and unexpectedness or whether thenoise has any meaning for the particular observer (Ebeniro and Abumere 1999).Noise as pollution is said to occur when the noise level is above the maximum permissible level for a givenenvironment (WHO, 1980 and FEPA, 1991). It is also defined as the addition of sound to the environment beyond the natural sources and measured in intensity, duration and frequency of occurrence (Miller, 1979 and Burtz 1977). Noiseas a polluting agent in the environment has been recognized for some time as a serious threat to the quality of lifeenjoyed by the populace (Abumere, Ebeniro and Ogbodo, 1999). The most important measurement of noise is its loudness. Thisloudness depends on the physical sound pressure that is measured on the sensitivity of the human ear to it. Thesensitivity of the human ear depends on the frequency of the sound (Levitt 2001).

Noise pollution have been proven to be associated to physiological effects such as hearing damage (WHO, 1999), cardiovascular (Babisch, 2000), childhood hypertension (Evans and Lepore, 1993) and hormonal disturbances. Exposure to high levels of noise, even for a short period of time may lead to a temporary loss of hearing. For example, when employees are exposed to high levels of noise every

Olubadewo-Joshua O., Sallau R.O. & Joshua O.F.(2019). Noise Pollution in Nigeria: Causes, Effects, Policies and Government Regulations. 1st Faculty of Natural Sciences Annual Conference. IBB University Lapai held between 6th to 9th May 2019. Pp 237-243

working day for many years, they gradually develop a permanent hearing loss. Noise pollution has a longer lasting negative effect on people. In developing countries the level of noise pollution is not checked, hence, individuals are exposed to it all day long. The most common classes of noise is the community noise and the industrial noise. Community noise is emitted from all sources except the industrial workplace. Community noise emanates primarily from road networks, rail, traffic congestions, industries, construction, and the neighborhood (WHO, 1999). Also, noise can be classified with respect to frequency. For example, noise with low frequency from 200Hz and below; noise with medium frequency ranges between 200Hz and 2000Hz; noise with high frequency ranges from 2000Hz and above. Amazingly, noise cannot be measured directly, what can be measured is the sound level. Sound level is measured in decibels (dB), by a device called a sound level meter.

Generally, the human level of discomfort starts from about 100dB; pain from about 120DB; and damage to the ear drum will occur at 160dB. Different nations adopt different safety sound levels in different environment, such as the industrial work place, open street and residential areas.

Noise is an unwanted sound and one of the most disturbing of modern civilizations is its ever-increasing noisiness. High noise levels can contribute to cardiovascular effects in humans and an increased incidence of coronary artery disease. In animals, noise can increase the risk of death by altering predator or prey detection and avoidance, interfere with reproduction and navigation, and contribute to permanent hearing loss. While the elderly may have cardiac problems due to noise, according to the World Health Organization (WHO), children are especially vulnerable to noise, and the effects that noise has on children may be permanent. Noise poses a serious threat to a child's physical and psychological health, and may negatively interfere with a child's learning and behavior.

Ising (1980) showed that decrease of working quality, increase of psychical tension, increase of blood pressure and pulse frequency are associated with a noisy work environment. People have different levels of discomfort for noise.Noise pollution is becoming a serious threat in our cities and it is increasing on a daily basis as the emergence of industries, companies and factories increases. The one being generated by our household appliances such as theelectric power generator, home musical set is contributing a great nuisance to the environment to the detriment of the next neighbour. Furthermore, noise generated by roadside music/movie disc sellers, car mechanics and other artisans who power their businesses using electric power generator makes hearing difficult when you are within the vicinity. As a result of the state of electric power supply in the country, most homes have switched to the use of electric power generator commonly known as "I *better pass my neighbour"* as an alternative to the electricity instability. This electric power generator does not only cause noise pollution, but also pollute the air as a result of the fumes (carbon dioxide) from the exhaust pipe which has led to the death of scores inhaling it.

THE STUDY AREA

Nigeria lies between latitudes 4° and 14°N, and longitudes 2° and 15°E. It is located in Western Africa on the Gulf of Guinea and has a total area of 923,768 km2 (356,669 sq mi). Its borders span for 4,047kilometre (2,515 mi), and it shares borders with Benin (773 km or 480 mi), Niger (1,497 km or 930 mi), Chad (87 km or 54 mi), Cameroon (1,690 km or 1,050 mi), and has a coastline of at least 853 kilometres (530 miles).

Figure 1: Map of Nigeria

Source: Wikipedia

EMPIRICAL CLARIFICATIONS

Sources of Noise Pollution

The main sources of noise pollution in Nigeria are electricity generating plants, vehicular traffic noise (engine and pressure horns), construction/industrial noise, machinery noise, noise from religious worship, institutions and household noise.

1. Electricity Generating Plants: Electric energy occupies the top grade in energy hierarchy as it is in innumerable use in homes, industries, agriculture, and for transportation purpose. Nigeria's electricity power situation is very poor because of erratic power supply (Akinbulire, Oluseyi, Awosope, & Okoro, 2008). There is an upsurge in the use of electricity generating plant with its attendant noise pollution on the environment and human health. Most workplaces and homes use generating plants 24 hours in alternative to power supply.

2. Vehicular Traffic Noise (Engine and Pressure Horns): Increase in vehicular traffic and engine noise is a major source of noise pollution in most urban cities in Nigeria. The situation is alarming with increase in traffic density in the

cities. The emissions of smoke from cars are of great concern to the changes in climate.

3. Construction/Industrial Noise: The construction of buildings, highways and city streets causes a lot of noise. Pneumatic hammers, air compressors, bulldozers, loaders, dump trucks and pavement breakers are the major sources of noise pollution in construction sites.

4. Machinery Noise: Motors and compressors used in the industries create a lot of noise which adds to the detrimental state of noise pollution in Nigeria. Plumbing, boilers, generators, air conditioners and fans creates a lot of noise in the buildings and add to the prevailing noise pollution (Despak, 2009).

5. Noise from Religious Worship Institutions: Nigeria is a multi-religious society and is therefore prone to religious activities. These congregational worships are held in mosques, churches and other nonconventional areas like residential and workplaces, in day time and even throughout the night.

6. Household Noise: Household equipments such as vacuum cleaners, mixers and some kitchen appliances are noise makers of the house. Also noise can be generated from neighborhood.

Effects of Noise Pollution

1. Effects of Noise Pollution on Human Health: Noise damages the ear and causes temporary or permanent noise induced hearing loss. Auditory effects may lead to temporary hearing loss or permanent hearing loss. Many people complain that noise makes them mentally ill. Doctors and scientists have now medically confirmed that noise disturbs the biological organisms and their respective functions of the humans. Fire crackers and other excessive and continuous explosives become physically painful giving rise to neurosis, mental illness, cardiovascular diseases, stomach ulcers and respiratory disorders reducing human life (Kaur, 2007). Psychiatrists and psychologists have recently observed that noise has certain relation with physical health causing tension resulting in problems such as speech interference, annoyance, fatigue, sleep interference and emotional distress. Noise levels in industries causes interference in efficiency and communication and raises possibilities of accidents.

2. Effects on Animals and Other Living Things: The effect caused by industries, railways, explosions and commotion in the cities, aircrafts etc can be felt on animals and birds. Noise emissions caused by supersonic aircraft, railway noise emissions etc may cause miscarriage in mammals, fishes and birds. Intense noise levels also affect non-living things too. The sonic booms cause cracks in national and archaeological monuments as well as very high levels of noise are the cause of cracks in hills. High intensity explosions can break glass panes and vibrations in buildings.

GOVERNMENT POLICIESAND REGULATIONS

Looking to the serious and disastrous effects of noise pollution on human life, it is essential to take preventive and protective measures. There is no doubt; the Nigerian government has taken bold steps to curtail noise especially in the cities. As regards the statutory control of noise, it is surprising that there exists no law under the Nigerian legal system exclusively, dealing with the problem of noise or its control. Nigeria does not have specific legislations on noise pollution as is the case in countries like the United States of America and United Kingdom. The relevant policies on noise pollution are:

1) The National Environmental Standards and Regulations Enforcement Agency (Establishment) Act. The National Environmental Standards and Regulations Enforcement Agency (Establishment) Act of 2007 is the major law on noise pollution in Nigeria. The law states that:a) The Agency shall on the commencement of this Act, and in consultation with appropriate authorities: i) identify major noise sources, noise criteria and noise control technology; and ii) make regulations on noise, emission control, abatement, as may be necessary to preserve and maintain public health and welfare. b) The Agency shall enforce compliance with existing regulations and recommend programs to control noise originating from industrial, commercial, domestic, sports, recreational, transportation or other similar activities.

2) National Policy on the Environment and Noise Pollution. The National policy on the Environment of 1988 provided that programmes will be established to: a) set up standards including acoustic guarantees; b) prescribe guidelines for the control of neighbourhood noise especially with respect to construction sites, market and meeting places. c) prescribe permissible noise level in noise-prone industries and construction sites and to ensure the installation of noise dampers on noise equipment; d) set up quiet zones especially within game parks, reserves and recreational centres; e) ensure compliance with stipulated standards by conducting periodic audit checks.

3) National Environmental Protection (Pollution Abatement in Industries and Facilities Generating Wastes) Regulations and Noise Pollution. The National Environmental Protection (Pollution Abatement in Industries and Facilities Generating Wastes) Regulation of 1991 enjoined designated industrial layouts separate from residential areas and to create buffer zones separating industrial areas from residential areas.

4) National Guideline and Standards for Environmental Pollution Control in Nigeria. The National Guidelines and Standards for Environmental Pollution Control in Nigeria of 1991 were meant to monitor and control industrial and urban pollution.

The Federal Environmental Protection Agency (FEPA) came into being in 1988 following the Koko toxic waste dump episode. In 1991, FEPA issued guidelines on industrial and work place noise. These guidelines among other things, give the maximum permissible noise levels for different hours spent at work as presented in the Table 1.

In order to ensure that these limits are not exceeded, the maximum noise level adopted in the code of practice by each organization is often lower than the national permissible levels. The Federal Ministry of Environment which took over FEPA is expected to conduct a periodic audit checks to ensure compliance with the stipulated limits. The objectives of such checks are to determine the noise levels at all area of operations and to assess the status of measure taken to ensure the protection of personnel in places where the limits are exceeded. Ear muffs are used where the noise level exceeds 90dB. This is in compliance with the national guidelines in Table 1.

Table 1: Noise exposure limits for Nigeria

Duration per day (Hr.)	Permissible exposure limit, dB (A)
8	90
6	95
4	96
3	97
2	100
1.5	102
1	105
0.5	110
0.25 or less	115
Impulsive or impact noise	140

Source: Okorodudu-Fubara (1998).

Conclusion

Proper laws must be enacted to check noise nuisance in our environment and anybody found guilty must be adequately sanctioned without regard to status or achievement, this is the best way to fight this menace. A noisy area is never conducive and it impairs hearing that is why it must be curtailed.Government should try as much as possible to see to the problem of power outage in the country so as to cut down the use of all the noisy and air polluting power plants by providing environmentally friendly source of power such as solar energy, hydro-electricity etc.

As a matter of priority, therefore, comprehensive national laws on noise control in the streets and in the residential areas should be promulgated and enforcement should be ensured. From past experience on issues, compliance and enforcement will require very firm monitoring and compliance mechanism. Public sensitization on the consequences of excessive noise to humans and the environment is most necessary to ensure noise control.There is the urgent need for review or strategic assessments of government policies, plans and programmes for noise pollution and environmental health. There is also need for public enlightenment, education and sensitization on the hazards, dangers and human health problems associated with noise pollution.

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Natural Radioactivity Of Some Selected Economic Minerals From Quarry Sites In Igarra Area, Edo State, Nigeria

[*]Ojo E.O., Kolo M.T. & Olarinoye O.I.

Department of Physics, Federal University of Technology, Minna, Nigeria.

*Correspondence: Email address: emmacyosy13@gmail.com; Phone Number: 08027476327

ABSTRACT

Activity concentrations of radionuclides in rocks and minerals are of vital importance in geosciences and health physics especially that primordial radionuclides 226Ra, 232Th and 40K constitute the main source of radiation exposure risks externally in soil, rocks and minerals.In this study, activity concentrations of 226Ra, 232Th and 40K in three economic minerals were evaluated using gamma ray spectrometric technique. Mean activity concentrations of 226Ra, 232Th and 40K were 3.15, 1.85, 291.59Bq/kg in dolomite, 1.01, 0.78, 197.89Bq/kg in feldspar and 5.41, 4.02, 427.03Bq/kg in calcite respectively. Computed average absorbed dose rate at 1m above ground and annual effective dose equivalent were 14.73, 9.19, 22.73nGy/h and 0.02, 0.01, 0.03mSv/y respectively for dolomite, feldspar and calcite. Other computed hazard indices for the studied economic minerals were below their respective safety limits stipulated in the UNSCEAR report.

Keywords: Environment, Generator, Noise, Noise pollution

INTRODUCTION

The earth and the atmosphere contains various levels of radionuclides, but only those with half lives similar with the age of the earth and their corresponding decay products active in terrestrial materials, such as ^{40}K, ^{226}Ra and ^{232}Th are of great interest, since gamma radiation from these represents the major external source of human exposure. The level of natural radioactivity in soils, rocks and in the surrounding environments as well as the associated external exposure due to gamma radiation depends primarily on the geological and geophysical conditions of the region (UNSCEAR, 2000). Since these radionuclides are not uniformly distributed, the knowledge of their distribution in soils, rocks, minerals and their measurements is very important, not only to estimate the amount of change of the natural background activity with time, but also for radiation protection issues (Kolo *et al*, 2012). These elements are widely distributed through the environment, generally in a trace amount in sediment, air, soil and others (Dinh Chen et al, 2011; Fasae & Isinkaye, 2018). Natural occurring radionuclides are present in every human environment; earth materials, water, air, food and even our body contain natural occurring radioactive (Najeba, 2008). These economics minerals (dolomite, feldspar and calcite) taken from the environment are used as raw material and product for buildings road, playground and for land filling (Najeba, 2008). These rocks used

Ojo E.O., Kolo M.T. & Olarinoye O.I. 2019). Natural Radioactivity Of Some Selected Economic Minerals From Quarry Sites In Igarra Area, Edo State, Nigeria. 1st Faculty of Natural Sciences Annual Conference. IBB University Lapai held between 6th to 9th May 2019. Pp 244-251

for construction of building, road, playground and land filing is one possible way of exposure to radiation in the environment, radiation cannot be felt using our senses organs, hence it is very important that the total amount of radiation emitting NORMs in the quarry site area are accurately known and kept to a level as low as reasonably achievable, in other to safeguard human lives and ensure that the environment is free from radiation exposure (Aborisade *et al*, 2018). However, the environmental impacts, human health challenges and issues associated with mineral exploration and utilization demands for urgent attention (Kolo *et al*, 2016). Quarry site studies to access radionuclides has been reported in literatures (Aborisade *et al*, 2018; Essien & Akpan, 2016; Shittu *et al*, 2015), Nimat et al, 2017) but not so much has been reported from the study area even though quarry activities has been around the study for more than two decades. Therefore, this study seek to estimate the concentration level of the radionuclide at the quarry sites, to estimate the radiological hazards indices posed by the activities of the quarry and for this study to serve as baseline for the public.

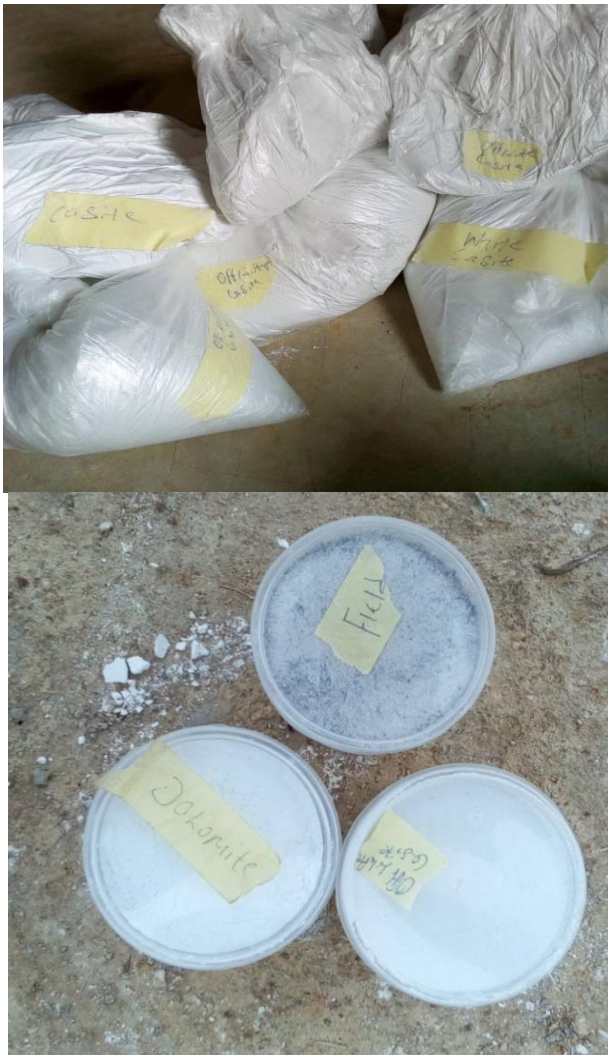

Figure 1: Samples of the collected minerals, labelled and packaged

METHODOLOGY

Sample Collections, Geology of the Area and Preparation

Five samples each of dolomite, feldspar and calcite, totally fifteen samples (15) were collected randomly from selected quarry sites in Igarra area. The samples collected were labelled and sealed at the point of collection (figure 1).

The location of the study area is Igarra in Edo State of Nigeria (figure 2). The Igarra area lies within Latitudes $7^{o}24'5''$N-$7^{o}30'$N and Longitudes $6^{o}00'$E-$6^{o}10'5''$E at the northern fringe of Edo State, Nigeria and underlain in the north by Precambrian Basement Complex, and in the south by Cretaceous and Tertiary sediments. Igarra lies in the northern part of Edo State and is the headquarters of Akoko Edo Local Government Area .The major highway in the area runs from Auchi through, Sobe Ogbe, Ikpeshi, Igarra to Ibillo (Oloto & Anyanwu, 2013).

The collected was air dried and filtered before transmission to the Laboratory (figure 1).

The Study Area

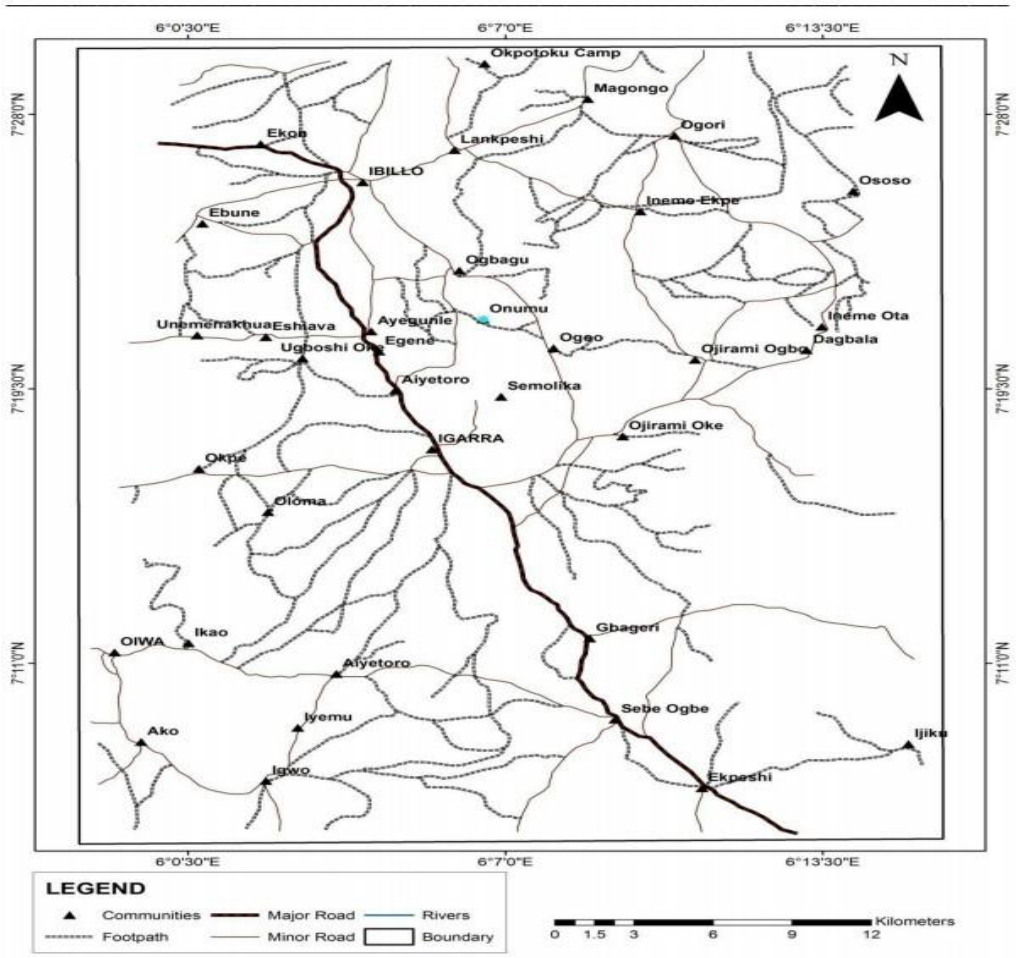

Figure 2: the map of the study area (Oloto& Anyanwu, 2013).

Radioactivity Measurement

The gamma ray spectrometry system used for the measurement consist of 3x3 inch NaI(Tl), detector by Scintillation Technologies USA located at Ladoke Akintola University of Science and Technology Ogbomosho (LAUTECH), Nigeria. The detector is housed in a 6cm thick lead shield so as to reduce the background radiation levels many times. The inside of the detector is also lined with cadmium and copper sheets, which according to El-Ayadarous (2007), help to absorb the emitted x-rays from lead which may contain radioactive impurities due to antimony impurities. The detector assemblage is coupled to a computer based multichannel analyzer (MCA) with ACCUSPEC computer program used for data acquisition and analysis of gamma spectra. The efficiency and energy calibration of the detector were done over energy range using 137Cs and 60Co standard isotopic sources over energy range of 200keV to 3MeV, being the energy range of radionuclides

to be determined. Also, the IAEA gamma spectrometric reference materials, RGK-1, RGRa-1 and RGTh-1 were used to recalibrate the system for quantitative determination of ^{40}K, ^{226}Ra and ^{232}Th in the economic mineral samples. The activity concentration of ^{226}Ra was evaluated from 1764keVgamma line of 214Bi, while 2614keV gamma line of 208Tl was used to evaluate the activity concentration of ^{232}Th. The single 1460keV gamma line of ^{40}K was used for its content evaluation. Each sample was counted for 10hours

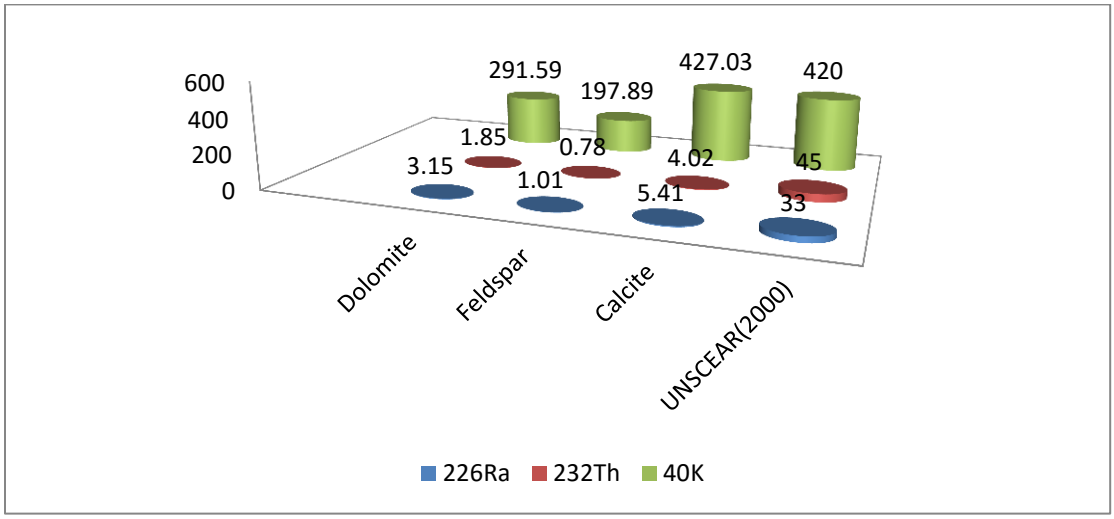

Figure 3; Mean activity concentrations of the economic minerals compared with UNSCEAR limits

RESULTS AND DISCUSSION

Activity Concentration

Descriptive statistical results of activity concentrations of ^{40}K, ^{226}Ra, and ^{232}Th, in the economic minerals from Igarra Area are given in Table 1. Mean activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K were 3.15, 1.85, 291.59Bq/kg in dolomite, 1.01, 0.78, 197.89Bq/kg in feldspar and 5.41, 4.02, 427.03Bq/kg in calcite respectively. The mean values were below their respective world medians of 30, 35 and 400Bq kg^{-1}(figure 3) except calcite which has its ^{40}K exceeded the recommended limit slightly as documented by UNSCEAR (2000).

Table 1. Mean activity concentrations and radiation hazard indices of three economic minerals from quarry sites in Igarra area, Edo State, Nigeria

Minerals	No of samples	Activity concentrations (Bq/kg)				D_R (nGy/h)	AEDE (mSv/y)	Hex ≤ 1	Iγr ≤ 1	ELCR (× 10^{-3})
		^{226}Ra	^{232}Th	^{40}K	Ra_{eq}					
Dolomite	5	3.15	1.85	291.59	28.24	14.73	0.02	0.08	0.23	0.06
Feldspar	5	1.01	0.78	197.89	17.36	9.19	0.01	0.05	0.15	0.04
Calcite	5	5.41	4.02	427.03	44.04	22.73	0.03	0.12	0.36	0.10

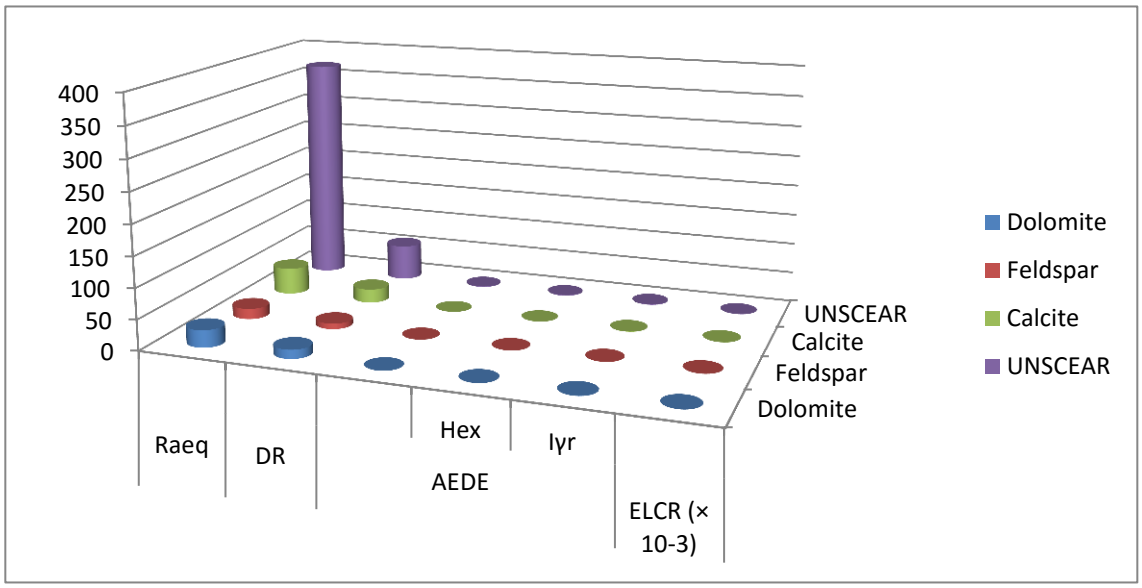

Figure 4: Hazard Indices compared with the UNSCEAR report

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Hazard Indices

Gamma Dose Rate

From the measured activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K in the economic minerals samples, the external gamma absorbed dose rate in air 1m above ground level was estimated following the equation (UNSCEAR, 2000)

$$D\ (nGyh^{-1}) = 0.462C_{Ra} + 0.621C_{Th} + 0.417C_K \quad (1)$$

Where D is the dose rate in $nGyh^{-1}$, C_{Ra}, C_{Th}, C_K (Bqkg-1), and 0.462, 0.621, and 0.417 ($ngyh^{-1}$ per $Bqkg^{-1}$) are the activity concentrations and dose conversion factors of ^{226}Ra,^{232}Th and ^{40}K respectively. In equation (1) above, it is assumed that all decay products of ^{226}Ra and ^{232}Th are in radioactive equilibrium with their precursors (UNSCEAR, 2000).

Radium Equivalent

A common index with respect to radiation exposure, called the radium equivalent activity (Ra_{eq}), is defined to represent the specific activities of ^{226}Ra, ^{232}Th and ^{40}K in different combinations in economic mineral samples. This index is calculated using the relation in these literatures as shown in equation (2) below (Beretka & Matthew, 1985; Yu et al, 1992; UNSCEAR, 2000).

$$Ra_{eq}\ (Bqkg^{-1}) = C_{Ra} + 1.43C_{Th} + 0.077C_K \quad (2)$$

Where C_{Ra}, C_{Th}, and C_K are activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K in $Bqkg^{-1}$ respectively. Ra_{eq} is defined according to equation (2) with the assumption that $1Bqkg^{-1}$ of ^{226}Ra, $0.7Bqkg^{-1}$of ^{232}Th or $13Bqkg^{-1}$ of ^{40}K yields the same gamma ray dose (Malanca et al, 1993; OECD, 1979).

Annual Effective Dose Equivalent

Annual effective dose equivalent for the studied economic minerals was evaluated from the absorbed gamma dose rate.

$$AEDE_{out}(mSvy^{-1}) = D_R \times 1.23 \times 10^{-3} \quad (3)$$

outdoor occupancy factor of 0.2, and conversion coefficient of 0.7SvGy-1, provided in the UNSCEAR (2000) report was used in equation (3). AEDE was calculated from the equation (3): Average AEDE for the economic minerals samples were 0.02, 0.01, and 0.03mSvyear^{-1}respectively for dolomite, feldspar and Calcite (Table 1 and figure 4). These values were below the average annual effective dose of 0.460mSv year^{-1} from terrestrial radionuclides in normal background areas as acknowledged in UNSCEAR (2000) report.

External Radiation Hazard Index

The external hazard index (H_{ext}), which is an estimate of radiation risk resulting from exposure to gamma rays of the primordial nuclei is evaluated from the equation (UNSCEAR, 2000)

$$H_{ext} = \frac{C_{Ra}}{370} + \frac{C_{Th}}{259} + \frac{C_k}{4810} \leq 1 \quad (4)$$

Where C_{Ra}, C_{Th} and C_K are activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K (Bqkg^{-1}) respectively. The maximum value for H_{ext} is equal to unity, which corresponds to 370Bqkg^{-1}, the upper limit of Ra_{eq}. As shown in table (1) H_{ext} for the studied minerals were 0.08, 0.05 and 0.12 for dolomite, feldspar and calcite respectively (figure 4). Hence the values of these indices are less than unity so the radiation hazard indices of the area are insignificant.

Gamma Index

Other key hazards that were considered in this study are gamma (Iγ) index. This index was estimated based on the European Commission standard. Gamma index (Iγ) is the factor that assesses the γ-radiation hazard(s) associated with the naturally occurring radionuclides in a material. The Iγ is determined based on Eq. (5) as given by (OEDC 1979; Bouhila et a*.,* 2017].

Iγ = 0.3333ACRa + 0.0050ACTh + 0.0003ACK (5)

Where AC_{Ra}, AC_{Th} and AC_K are the same as for other estimated hazards. The permissible range of the outdoor annual effective doses' contributions to the γ-radiation is 0.3 to 1mSv y^{-1}. Any material or sample that poses the AEDOutdoor> this range should be exempted from use as raw materials or finished products. If the $I\gamma \leq 1$, it corresponds to an outdoor dose of 1mSv y^{-1}. However, if the $I\gamma \leq 0.5$, it corresponds to an outdoor dose of 0.3mSvy^{-1}(Adagunodo et al*,* 2018)). From Table 3, the Iγ ranged from 0.42 to 0.61, with a mean of 0.48. These results correspond to $I\gamma \leq 0.5$, which gives the outdoor effective dose (Adagunodo et al, 2018).

Excess Life Cancer Rate

Excess lifetime cancer risk ELCR is defined as the probability that an individual will develop cancer over his lifetime of exposure to radiation and it is given as equation (5)

$$ELCR = AEDR \times DL \times RF \quad (5)$$

Where DL is the average lifespan (70 years) and RF is risk factor (Sv−1) which is 0.057, for stochastic effects from low-dose background radiation ((Kolo et al, 2015; Kolo, 2014), from the studied minerals, ELCR fell below the standard level which shows that the quarry sites is safe.

Conclusion

In view of environmental and human health impacts of these economic minerals (dolomite, feldspar and calcite) quarry activities around the world and particularly the studied area, specific activities of ^{40}K, ^{226}Ra, and ^{232}Th in the economic minerals collected from selected quarry sites in Igarra Area, Nigeria, were

measured and analyzed. Results of this studied indicated that mean activity concentrations of primordial radionuclides were far below their respective world average values. Calculated values for all radiation hazard parameters were below their respective recommended limits from radiation protection perspective. The results of this investigation showed that quarry activities in the selected Area of the Igarra do not constitute any immediate radiological risk to the workers, the environment, and the public in general (see Table 1, Figure 3 and Figure 4).

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Study of the Benthic Ecological Characteristics of Antau River Keffi, Nasarawa State

[*]Dominique P.K., Kolo, R.J. & Ojutiku R.O.

Department of Water Resources, Aquaculture and Fisheries, Federal University of Technology, Minna, Nigeria

*Correspondence: Email address: princekennedydominique@gmail.com; Phone Number: 08036540391

ABSTRACT

Benthic ecology is the study of aquatic organisms and sediments that make up the bottom characteristics of an aquatic ecosystem, for the purpose of identifying environmental conditions and conduct ecological impact surveys. The research was conducted to study the grain size distribution and the benthic macrofauna present in Antau River in Keffi, Nigeria. Benthic sediment and macrofauna samples were collected from five stations from upstream towards downstream of the River to determine the grain size distribution and identify the Benthic macrofauna. Grain size distribution between stations showed slight variations; as station three contained the highest amount of gravel at 37%. All the stations had significant percentage of sand, with Station five having the highest percentage at 92.6%. Mud was present in all stations with station one with the highest Percentage at 7.8%. Station three showed the highest percentage of very fine gravel, very coarse sand and coarse sand at 37.4%, 16.2% and 18.1% respectively. Medium sand was at the highest percentage of 21.2% at Station four. Fine sand was at the highest percentage of 54.4 % at Station five and very fine sand was highest at 38.3% in Station one. The composition of macrofaunas comprises of; Arthropoda, Annelida and Chordata. Total number of species recorded was 4411. *Bufobufo* of the group of Anura had the highest number of individual species at 25.4% abundance. *Acroneuria sp* of the Plecoptera group yielded the smallest number of individual species of at 2.6% abundance. Other group are Diptera (16.6%), Coleoptera (6.2%),Trichoptera (8.3%), Ephemeroptera (4.3%), Hemiptera (9.2%), Odonata (13.9%) and Arhynchobdellida (3.9%) abundance. The relationship between some grain size distributions and benthic macrofauna showed significant correlation. The research however indicates that the river has high biodiversity and wide-raging grain size distribution.

Keywords: Benthic macrofauna, Benthic sediments, Grain size Distribution, Antau River

INTRODUCTION

Benthic ecology is the study of the of aquatic organisms and sediments that make up the bottom structure, communities and characteristics in an aquatic ecosystem, to determine environmental conditions and conduct ecological impact surveys (Babbe and Culter, 2010).

Benthic macrofaunas are invertebrates' aquatic animals that are generally visible with the naked eyes. They are benthic organisms, living underneath rocks and lower areas of Rivers and streams (Shailendra *et al.,* 2006). Benthic macro faunas are also referred to as macro benthos or macroinvertebrates (Obot *et al.,* 2014). They

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play an essential role in aquatic ecosystem, as they maintain several levels of interaction between their community and the aquatic environment (Adakole *et al.,* 2001). They aid in recycling of organic matter, mixing of sediments, influx of oxygen into sediments and mineralization (Gorge *et al*., 2009). They also filter phytoplankton and are food source for larger aquatic life forms, such as fish. Therefore, they link primary production with higher tropic level (Tagliapietra and Sigovini, 2010). In freshwater sediments, they are diverse and abundant, but are patchily distributed and are not readily accessible to sample, especially when they live in deep subsurface sediments (Alan *et al.,* 1999). Benthic macro faunas are often used as biological indicators to determine the condition of an aquatic environment. This is as a result of different species sensitivity, tolerance level and reaction to the different environmental stressors such as sediment loading, pollution and habitat change or degradation (Castelli *et al.,* 2004).

Sediments are parts of a waterbody that can be easily overlooked, but they are in fact a very important structure of a waterbody. Sediments give structure to benthic communities because of grain size preference by various organisms. For example, grain size makes a difference in the ability of flatfish to bury themselves in sediments (Canfield *et al.,* 1998). Sediments act like sinks or catchment for trace pollutants, like heavy metals and can directly influence the water quality of its overlying water when disrupted as a result of a result of anthropogenic disturbance or change of environmental conditions. Sediments are natural habitat for benthic aquatic organisms such as snails, mussels or aquatic plants (Reuther, 2002). Monitoring sediments are integral part of water management and protection programs, as they aid to access the ecological status of a waterbody, detect toxic contents and to control their effects on benthic Faunas (EOLSS, 2002).

METHODOLOGY

The Study Area

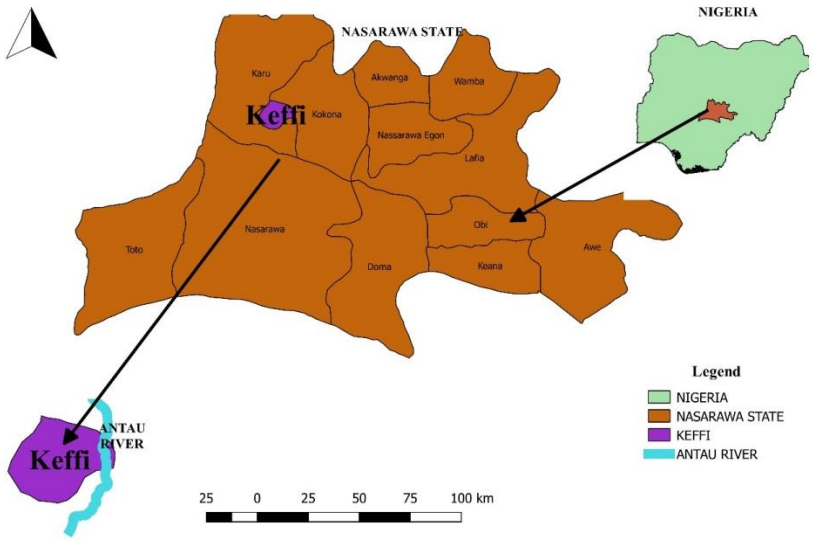

Figure 1: Study Area, Keffi L.G.A, Nasarawa State

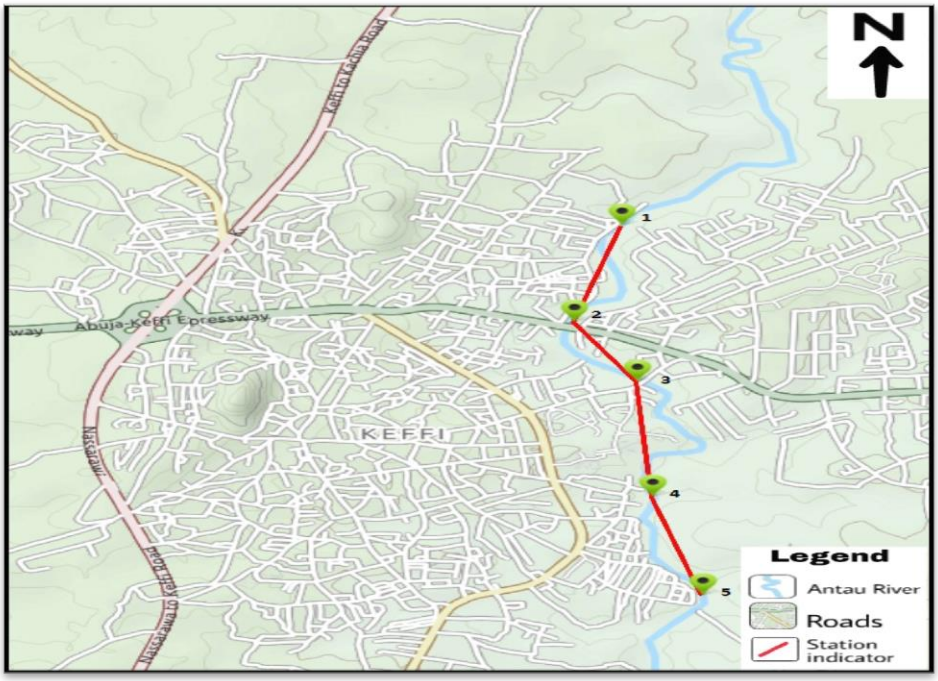

Figure 2: Study Area indicating sample stations

Table 1: Description of sample Stations

Stations	Descriptions	Geocordinates
1	Located in Dadikowa area in Keffi. Observable features include; high collection of residential buildings and scattered shrub vegetation.	latitude 8.862767^{o} N and longitude 7.887218^{o} E
2	Located at keffi-akwanga high way under a bridge locally called "Mannu" bridge. High shrub vegetation cover can be observed in this area	latitude 8.854939^{o}N and longitude 7.885105^{o} E
3	Located at another bridge, named after Antau river; Antau bridge. Observable features include; high vegetation cover and high waste disposal sites.	latitude 8.850156^{o}N and longitude 7.889091^{o} E
4	Located at a neighborhood called Agwan Nepa. it is characterized by high residential buildings and sparse vegetation cover	latitude 8.840867^{o}N and longitude 7.889091^{o}E
5	Located at in an area called Agwandadi, behind the keffi stadium. observable characteristics include; sparse vegetation cover	Latitude 8.8298446^{o}N and longitude 7.8991445^{o} E

Benthic macrofauna Sampling

Sampling was carried out in five (5) stations monthly, for a period of twelve (12) months. Time of Sampling was around 7am to 10am, a total of sixty (60) samples were collected all through the research period. Macro fauna samples were collected in the river using a scoop net, dip net and samples were also manually sampled by hand at the river bank. Sample collected were emptied inside a labeled plastic container and taken to the laboratory of department of Zoology, faculty of sciences, Nasarawa state university Keffi, for identification. Samples were rinsed gently and macro faunas were gently sorted out from debris and were identified using hand lens and

microscope with the aid of freshwater macroinvertebrates taxonomy identification guide (Gill, 1999), aquatic macroinvertebrates identification key (Chessman, 2003), the Atlas of common Freshwater macroinvertebrates (Kautz *et al.,* 2018). Macro fauns were stored at 5% formalin to enable further identification.

Sediment Sampling

Sediments were collected using a van veen grab-like instrument. Sampling was carried out in five (5) stations at the first month of sampling during the dry season at February and at September in wet season. Samples collected were taken to the geology laboratory of Nasarawa state university Keffi. Samples were air-dried to reduce moisture and weighed to get an initial weight of 500g on a sensitive weighing scale and passed through a nine (9) stacked graded sieve plus its receiving pan. The graded sieve was stacked from the biggest mesh size to the smallest mesh size available. The mesh sizes of sieve used are; 2812µm, 1680µm, 1405µm, 600µm, 250µm, 150µm, 105µm, 53µm and 37µm. Sediments retained at each sieve and pan was collected and weighed (Callaway *et al*., 2016).

Statistical Analysis

Species richness and diversity were calculated using PAST (version 1.0). Sediments were analyzed using GRADISTAT (version 8.0) to determine the grain size distribution (Blott, 2010).

RESULTS

Table 4.1**:** Grain size distribution of Sediments in Antau River

Grain Size Distribution	Station 1	Station 2	Station 3	Station 4	Station 5
Textural Group	Slightly Gravelly Sand	Gravelly Sand	Sandy Gravel	Gravelly Sand	Slightly Gravelly Sand
Sediment Name	Lightly Very Fine Gravelly Fine Sand	Very Fine Gravelly Fine Sand	Sandy Very Fine Gravel	Very Fine Gravelly Medium Sand	Lightly Very Fine Gravelly Fine Sand
Gravel:	1.5%	5.7%	37.4%	29.3%	2.6%
Sand:	90.7%	92.6%	62.0%	70.1%	92.6%
Mud:	7.8%	1.7%	0.6%	0.5%	4.8%
Very fine gravel:	1.5%	5.7%	37.4%	29.3%	2.6%
Very coarse sand:	0.6%	5.7%	16.2%	15.5%	4.3%
Coarse sand:	0.7%	12.3%	15.2%	18.1%	5.6%
Medium sand:	1.3%	27.4%	16.4%	21.2%	8.4%
Fine sand:	49.8%	41.1%	11.1%	13.0%	54.4%
Very fine sand:	38.3%	6.2%	3.0%	2.3%	19.9%

Table: 4.2 The Composition and Abundance of Benthic Macro fauna Species in Antau River

Phylum	Order	Taxa/Species	Species Abundance	Group Abundance	Percentage (%)
Arthropoda	Diptera	*Forcipomyia sp.*	227	732	16.6%
		Culex quinquefasciatus	505		
	Coleoptera	*Gyrinus* sp	168	275	6.2%
		Phanaeus difformis	107		
	Trichoptera	*Hydrophilus sp.*	190	367	8.3%
		Psychomyia sp.	177		
	Ephemeroptera	*Baetis sp*	188	188	4.3%
	Hemiptera	*Lethocerus cordofanus*	162	407	9.2%
		Paraplea sp.	245		
	Odonata	*Macromia magnifica*	304	614	13.9%
		Paragomphus linearus	310		
	Plecoptera	*Acroneuria sp*	113	113	2.6%
Annelida	Haplotaxida	*Helodrilus caliginosus*	424	424	9.6%
	Arhynchobdellida	*Hirudidae medicinalis*	170	170	3.9%
Chordata	Anura	*Bufo bufo*	1121	1121	25.4%
	Total		4411	4411	100%

Table 4.3: Biodiversity index of Benthic Macrofauna in Antau River.

Total number of organisms	Average population	Total of stations	Total number of species	Simpson index	Shannon index	Equitability index
4411	294.1	5	15	0.113	3.549	0.9684

Table 4.4: Correlation Coefficient between Benthic Macrofauna and Grain size distributions in Antua River

Species	Gravel	Sand	Mud	Very fine Gravel	Very Coarse sand	Coarse sand	Medium sand	Fine Sand	Very fine sand	Very coarse silt	Coarse silt	Medium silt	Fine silt	Very Fine silt
Acroneuria sp.	0.450	-0.415	-0.482	0.450	0.413	0.337	0.306	-0.337	-0.494	-0.483	-0.450	-0.450	-0.450	-0.450
Baetis sp.	-0.348	0.262	0.645	-0.348	-0.409	-0.409	-0.600	0.283	0.686	0.684	0.528	0.528	0.528	0.528
Bufo bufo	0.089	-0.050	-0.243	0.089	0.047	0.245	0.507	-0.245	-0.194	-0.348	0.072	0.072	0.072	0.072
Culex quinquefasciatus	0.810	-0.828	-0.0515	0.810	0.822	0.615	0.081	-0.767	-0.475	-0.367	-0.777	-0.777	-0.777	-0.777
Forcipomyia sp.	0.358	-0.341	-0.350	0.358	0.482	0.397	0.060	-0.288	-0.382	-0.238	-0.602	-0.602	-0.602	-0.602
Gyrinus sp.	-0.503	0.605	-0.104	-0.503	-0.316	0.084	0.541	0.360	-0.163	-0.231	0.166	0.166	0.166	0.166
Helodrilus caliginosus	0.113	-0.069	-0.300	0.113	0.263	0.431	0.365	-0.246	-0.298	-0.282	-0.299	-0.299	-0.299	-0.299
Hirudidae medicinalis	-0.603	0.593	0.481	-0.603	-0.491	-0.447	-0.390	0.628	0.416	0.496	0.347	0.347	0.347	0.347
Hydrophilus sp.	0.884*	-0.835	-0.879*	0.884*	0.962**	0.902*	0.546	-0.859	-0.866	-0.788	-0.993**	-0.993**	-0.993**	-0.993**
Lethocerus cordofanus	0.894*	-0.860	-0.809	0.894*	0.937*	0.806	0.412	-0.820	-0.797	-0.704	-0.964**	-0.964**	-0.964**	-0.964**
Macromia magnifica	0.719	-0.669	-0.754	0.719	0.789	0.695	0.410	-0.612	-0.774	-0.673	-0.886*	-0.886*	-0.886*	-0.886*
Paragomphus linearus	-0.724	0.708	0.602	-0.724	-0.663	-0.666	-0.510	0.827	0.519	0.592	0.495	0.495	0.495	0.495
Paraplea sp.	0.030	-0.003	-0.153	0.030	0.182	0.160	-0.016	0.050	-0.217	-0.078	-0.364	-0.364	-0.364	-0.364
Phanaeus difformis	0.556	-0.537	-0.504	0.556	0.663	0.618	0.243	-0.575	-0.493	-0.395	-0.692	-0.692	-0.692	-0.692
Psychomyia sp	0.208	-0.225	-0.083	0.208	0.299	0.184	-0.176	-0.165	-0.096	0.038	-0.364	-0.364	-0.364	-0.364

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed

DISSCUSION

The sediments grain size distribution of Antau river showed variations across sampling stations, as stations one and five showed the same textural group; Slightly Gravel Sand. Stations two and four also showed the same textural group of gravely sand, while station three was sandy sand (Table 4.1). All the stations had significant percentage of sand, with Station two and station five having the highest percentage at 92.6% and Station three with the lowest percentage of 62.0%, Station three contained the highest percentage of gravel at 37%, Mud was present in all stations with station one having the highest Percentage of 7.8%, Station three had the highest percentage of very fine gravel, very coarse sand and coarse sand at 37.4%, 16.2% and 18.1% respectively. Medium sand was at the lowest percentage of 1.3% at station one and the highest percentage of 21.2% at Station four. Fine sand was at the highest percentage of 54.4 % at Station five (5), Very fine sand was highest at 38.3% in Station one (1) (Table 4.1). This distribution aligns with researches that indicate that sediments are widespread with various grain size distributions (Cahoon *et al.,* 1999).

The composition of benthic macrofaunas in Antau River comprises of Three phylums; Arthropoda, Annelida and Chordata, with an Order that consist of Diptera, Coleoptera, Trichoptera, Ephemeroptera, Hemiptera, Odonata, Plecoptera, Heplotaxida, Arhynchobdellida and Anura (Table 4.2).

Total number of species recorded in Antau River was four thousand four hundred and eleven

(4411). *Bufo bufo* of the group of Anura had the highest number of individual species of 1121 that is 25.4% of total species. *Acroneuria sp* of the Plecoptera group yielded the smallest number of individual species of one hundred and thirteen (113), which amount to 2.6% of total species. Other groups are Diptera(732) at 16.6% , Coleoptera (275) at 6.2%, Trichoptera (367) at 8.3%, Ephemeroptera (188) at 4.3%, Hemiptera (407) at 9.2%, Odonata (614) at 13.9%, and Arhynchobdellida (170) at 3.9% abundance (Table 4.2). The abundance of benthic macrofauna indicates the River is suitable of aquatic life and that the level of anthropogenic inputs has not gravely damaged the health status of the waterbody, as quantifying the density and diversity of certain macro benthos at a given site are used to determine the condition of a waterbody (Pires *et al.*, 2000; Castelli *et al.,* 2004 & Sporka *et al*., 2006).

A total number of fifteen (15) species were recorded with a Simpsons index of 0.113, Shannon index of 3.549 and Equitability index of 0.9684. However, with a Shannon index of 3.5, it supports the theory that Shannon index rarely exceeds 4.0 (Clarke and Warwick, 2001; Magurran, 2004 & Shannon and Weaver, 1949). And at 3.5 Shannon index, it indicates high diversity (Clarke and Warwick, 2001) (Table 4.3).

The correlation between benthic macrofauna and grain size distribution showed a widespread relationship between bethic macrofaunas and benthic sediment grain size distribution in Antau River. Though the correlation was not very strong across sampling stations, the study corresponds with research establishing the relationships between macroinvertebrates and grain size distribution (King and Potyondy, 1993). Different species showed a relationship with different grain size distribution, some showed negative relation, while others showed significant negative relationship (Table 4.4). *Acroneuria sp* showed a negative relationship with sand, mud, fine sand, very fine sand, very

coarse silt, coarse silt, medium silt and very fine silt. *Baetis sp* showed negative correlation with gravel, fine gravel, very coarse sand, coarse sand and medium sand. *Bufo bufo* showed a negative correlation with sand, mud, fine sand, very fine sand and very coarse silt. *Culex quinquenfaciatus* and *Forcipomyia sp* had a negative correlation with sand, mud, fine sand, very fine sand, very coarse silt, coarse silt, medium silt, fine silt and very fine silt (Table 4.4). The negative relationship found in most very fine and fine sediment groups could be observed across species is in agreement with reports of other author (Castro and Reckendorf, 1995). Species with negative relationship with a particular grain size indicates that as that grain size increases, the species reduces or as it reduces the species increases, however the relationship can be significant or not significant (Cahoon *et al.,* 1999).

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GIS Based Flood Modelling For Sustainable Development In Suleja, Niger State

[*]Liman Y.D. & Jiya S.N.

Department of Geography, Ibrahim Badamasi Babangida University, Lapai, Nigeria

*Correspondence: Email address: limanyahaya322@gmail.com; Phone Number: 07036048858

ABSTRACT

In recent times, flood occurrences have become an annual phenomenon resulting to irreplaceable lost of lives and properties. Associated with post flood events, is the outbreak of diseases, pollution of the human environment and destruction of basic infrastructure. This research utilized the potential integration of soil parameters, remote sensing and climatic data to effectively predict fluid dynamics and flood events over time space. The major factors considered as flood initiating factors are rainfall, elevation, Land use land cover type, drainage density; soil type and slope contribute to flood occurrences at different level. This research aim is to evaluate the contribution of multiple factors to flood occurrence flood using modified analytical Hierarchy Process (AHP) in GIS environment for prospecting and mitigating a highly dynamic system with the following specific objectives: To Identify flood initiating factors and criteria using remote sensing and other auxiliary data, To Evaluate the identified factors impact using Saaty's Scale for subjectivity analysis, Determine spatiotemporal intensity of flood using AHP for objective decision making, Development of flood risk map using result from objective three in GIS environment. The research addresses among others, flood vulnerability areas to predict flood extend and magnitude of flood in Suleja. The identified parameters were rated and validated by experts in the field of hydrology, geology and soil science. A pair wise comparison matrix was excel base matrix for better decision making to determine the consistency index and ratio. Analytical Hierarchy Process (AHP) was used in GIS environment for vulnerability mapping. The result indicated the existence of five major flood vulnerability zones; the very high prone zone, high prone zone, mild potential prone zone, low zone and very low prone zone. Vulnerability flood map of highly prone area and low prone area are precisely estimated than the moderately prone area. The result from the Multi-criteria Decision Analysis attest for acceptable to accurate excellence of the model performance due to the incorporation of many intricate factors which enhance flood predictive capability and efficiency performance. This model will provide a tool for effective decision making and planning for policy makers and stake holders in environmental hazards mitigation. The study recommend the need for the implementation of green zone in urban planning and afforestation to reduce the modification of the subsurface storm water mechanisms which influences flood events. This method can be applied in area of similar geology and climate.

Keywords: Geographical Information System, Analytical Hierarchical Process, flood prediction

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INTRODUCTION

Flood occurrences have become an annual phenomenon globally resulting to irreplaceable lost of lives and properties. Associated with post flood events, is the outbreak of diseases, contamination and, pollution of the human environment and destruction of basic infrastructure. In developing world like ours, flood is estimating to have resulted to destruction of many lives and properties running into billions of naira annually (Schachter *et al.*, 2017). The most devastating part of the scenario is that flood preparedness takes proactive measures rather than financial input after it occurrences.

Turner *et al.* (2003), Brouers and Al-Musawi (2018), Zhang *et al.* (2018), have attributed flood occurrences to the growing incidence of climate change scenario and lack of sustainable urbanization. In an attempt to mitigate the effects of flood occurrences, Izham *et al.* (2008),Thakur *et al.* (2017), Kumar *et al.* (2017), Izham *et al.* (2008) explored Remote Sensing (RS), GIS, insitu based measurements and other auxiliary data that has direct implication on flood events. Izham *et al.* (2008),Thakur *et al.* (2017), Kumar *et al.* (2017), Izham *et al.* (2008) offered useful conceptual framework for flood mitigation but severity, duration and quantitative volume estimation in term of flood were grossly lacking thus, making complex decision making difficult to come by. According to Exner *et al.* (2012), the designed drainage capacity of the Nigeria cities become so difficult to expand or redesign to suit unforeseen weather phenomena due to level of unplanned or unstructured urbanization that has taken place. Thus, flood preparedness becomes highly unrealistic.

In flood mitigation, knowledge regarding the complex parameters that do not only indicate the likely occurrences of flood but also provide inference regarding it magnitude and potential risk areas with detail evidence that shows severity based on evidence indicators parameters is required. Bobryk *et al.* (2018), integrate weather, pedeology, elevation and auxiliary data in a GIS environment as a hybrid approach to enable sustainable decision making due to the versatile nature of GIS in handling multi layer information from different sources and regional generalization of result.

Mojaddadi *et al.* (2017), Liu *et al.* (2018), Singh *et al.* (2019),have utilized remote sensing, GIS and machine learning techniques to study floods. These researches are often deficient in reliable technique that is capable of addressing the sustainability in term of flood management although the spatial extend has been effectively managed in GIS environment. Thus, issues regarding spatiotemporal variation of rainfall intensity and duration within a local environment as well as clear definition of parameter contributions to flood events are not clearly defined resulting to ambiguous generalization which result to model applications

This research therefore, utilizes the potential of GIS in the integration of heterogeneous data to effectively predict fluid dynamics resulting to flood events over time and space. The effectively achieved spatiotemporal evaluation, multi layer information's regarding flood is been quantify and evaluated based on their perceived magnitude to flood initiation, where issues regarding the magnitude is highly considered.

METHODOLOGY

Study Area

Suleja is located on Longitude.7° 08'to 7° 14'E and Latitude.9° 05'to 9° 17'N Mailafia *et al.* (2017) (Figure.1.1). Suleja is boarded by Gurara L.G.A by the North and West and by the East Tafa L.G.A and by the South, Abuja (Federal capital territory). The landmass of Suleja which can be accessed through major roads covers the emirate's wooded savanna area of about 2,980 square kilometers (1,150 sq mile)

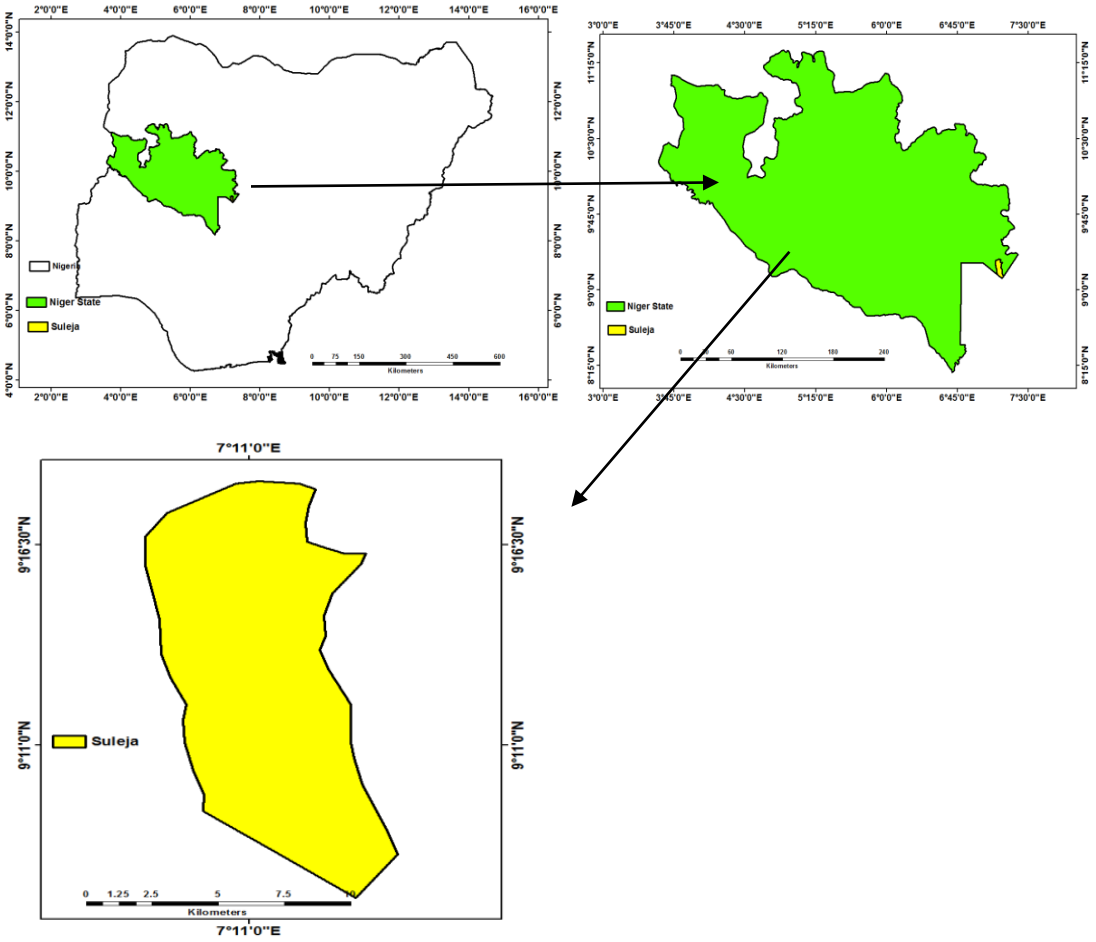


Figure1: Map of Suleja Inset Niger state and Nigeria

Identification of flood initiating factors and criteria using remote sensing and auxiliary data

To achieve this objective, Land use Land Cover Types of Suleja was developed from Land Sat 8 thematic Mapper (LandSat TM). The LULC type date enable the identification of different land use types as each LULC types present a unique infiltration and subsequent flood events Precipitation inform of rainfall were sourced from Nigerian meteorological agency and Agricultural Development Agency (ADP). Due to the need for accuracy, a 15m resolution DEM downloaded from SRTM was processed to generate the stream network of Suleja due to

the versatile nature of spaced based DEM. In addition, soil type information was gotten from geological map of Niger State in order to determine the characteristic of available soil types it relate to moisture and soil saturation.

Data Analysis Technique for the Identification of Flood Initiating Factors

The generated Remote Sensing Data for the LULC type analysis was subject to image correction in order to remove dust haze, precipitation and other aerosols that can significantly influence image quality to avert ambiguous classification of object or features, in addition, the preprocessing allowed for the removal of projection related issues through coordinate transformation. The selection of criteria that has spatial reference is an important step in multi-criteria decision analysis(Charabi & Gastli, 2011). The criteria used in this study were selected due to their relevance in the study area; they are as follows Rainfall, Drainage density, Elevation, Slope of the basin, Soil type and LULCT.

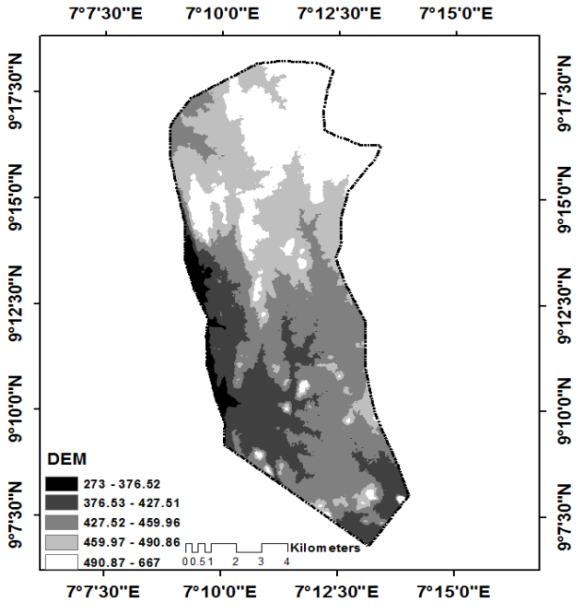


Figure 2 Digital Elevation Model

Figure 2 (DEM) was downloaded from SRTM and used to generate two major data sets; the drainage network characteristics and, areas of low and high elevations. The generated stream networks were converted into a data base file hosting information regarding length and the cross-sectional area. The obtained result was used in producing a raster layer map showing the drainage network density per square kilometers. The raster layer is more versatile to handle in geodata base and allowed for easy rating in analytical analysis of this nature(Van Niekerk, 2008)

Elevation generated from the DEM on the other hand was classified into four classes each corresponding to: Low Medium, High and Very High elevated areas with each having a unique score based on it inferred inference to flood occurrences. From the classification of the acquired and preprocessed LULC image, features such as surface water, bare surface, rock outcrop, vegetation, built-up affect the rate at which flood occurrence take place because these features has different infiltration rate and capacity which predetermine the intensity of flood in a particular place in the study area after heavy rainstorm.

Evaluate the Identified Factors Impact using Saaty's Scale for Subjectivity Analysis.

In achieving this objective, a decision making framework was used for large-scale, multi-criteria decision analysis, and of the Analytic Hierarchy Process, its generalization to decision with dependence and feedback. MCDA technique allows parameters to be weighted in order to reflect their relative influence/ importance. Saaty, T.L (1980) AHP is used to determine the weights of the factors/criteria with successful evident in the work of; Ishizaka and Labib (2011) as a methodology for studying

prioritized vectors and predictive model optimization. This is based on scores of relative importance for each factor/criterion in pair-wise comparison.

Nine-point intensity of importance scale modified from, (Schoenherr *et al.*, 2008) as presented in Figure 3.2 was developed for parameter rating. The inferred impact of the identified parameters/factors is evaluated using a Saaty's scale that enable the relative description each factor proportional to it's influence to flood occurrences.

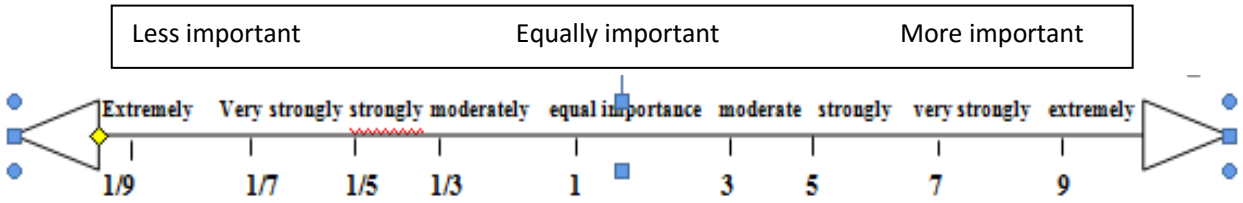


Figure 3: Saaty's Scale

Determine spatiotemporal Intensity of Flood using AHP for Objective Decision Making

The process of AHP is summarized into Four steps: the first steps involves the construction of decision hierarchy where each of the factors is evaluated according to it inferred influences. Sequel to the construction of decision Hierarchy is the determination of the relative importance of attributes and sub-attributes using the Saaty's scale values of importance from 1-9 indicating relative influences. The evaluated alternatives are calculated to generate the consistency ratio of the assigned weight for the criteria. The overall weight regarding each attribute is evaluated to check the consistency of the subjective evaluations.

Determining criteria consistency ratio

The quality of the output of the AHP is strictly related to the consistency of the pairwise comparison judgments. The consistency is defined by the relation between the entries of the matrix.

The consistency index CI is given by Equation (3.1):

$$\text{Consistency index } CI \ \frac{(\lambda - \mathrm{n})}{(n-1)} \qquad 3.1$$

Where, n= number of factors

λ= average value of the consistency vectors

The consistency ratio (CR), which give room for conclusion whether the evaluations are sufficiently consistent, is calculated as the ratio of the CI and the random index (RI), as expressed in Equation (3.2)

$$\text{Consistency ratio } \boldsymbol{CR} = \frac{CI}{RI} \qquad 3.2$$

Where, RI is the random index

The row average provides an approximation of the eigenvector of the square reciprocal matrix. The eigenvector is an estimate of the relative weights of the criteria been compared.

Because individual judgment will never agree perfectly the degree of consistency achieved in the ratings is measured by a Consistency Ratio (CR) indicating the probability that the matrix ratings were randomly generated. The rule of thumb is that a CR less than or equal to 0.1 indicates an acceptable reciprocal matrix, a ratio over 0.1 indicates that the matrix should be revised(Nahayo *et al.*, 2019). Revising the matrix entails, finding inconsistent judgments regarding to the importance of criteria, revising these judgments by comparing again the pairs

of criteria judged inconsistentlyZardasti *et al.* (2018).The suggested value of the CR should be no higher than 0.1 The value of CR=0.0964 falls below the threshold value of 0.1 and it indicates a high level of consistency. Hence the weights can accepted(Saaty, Thomas L, 1977)

The criterion weights were calculated as 0.36, 0.23, 0.13, 0.12, 0.10, 0.06, for rainfall, drainage density, slope, soil type, elevation and LULCT respectively. With the input values in pairwise comparison and weights calculated, consistency ratio (CR) was found as 0.0964. This indicated a reasonable level of consistency in the pairwise comparison of the factors. GIS act as the interface between technology and the decision maker with integrating MCE methods into the GIS

Table 1: pairwise comparison matrix

factors	rainfall	drainage den	slop	soil type	eleva	lulct
rainfall	1.00	3.00	2.00	3.00	4.00	4.00
drain den	0.33	1.00	2.00	3.00	4.00	2.00
slop	0.04	0.50	1.00	2.00	2.00	3.00
Soil type	0.33	0.33	0.33	1.00	3.00	3.00
elevation	0.25	0.25	0.50	0.25	1.00	5.00
lulct	0.25	0.50	0.33	0.33	0.25	1.00
Total	2.20	5.58	6.17	9.58	14.25	18.00

Table 2: Normalised and Consistency Ratio Table

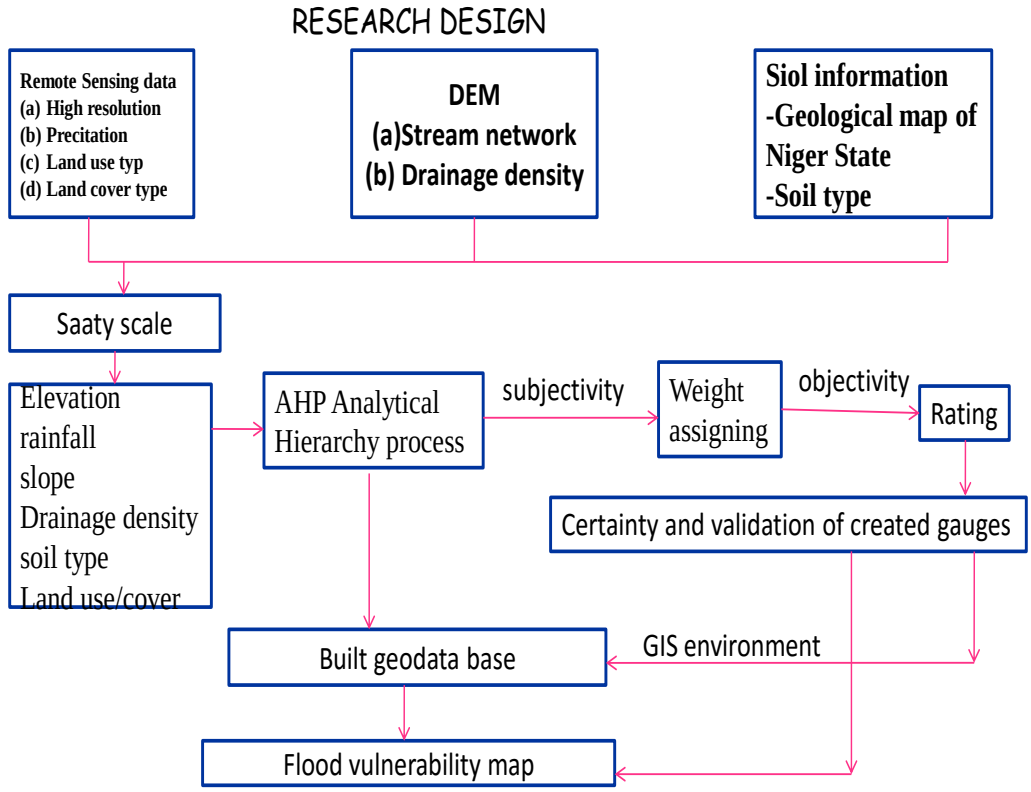
Factors	Rf	DD	S	ST	E	lulct	weight	egen value	CI	CR
Rf	0.45	0.54	0.32	0.31	0.28	0.22	0.36	0.78	0.11958	0.09643271
DD	0.15	0.18	0.32	0.31	0.28	0.11	0.23	1.27		
S	0.02	0.09	0.16	0.21	0.14	0.17	0.13	0.81		
ST	0.15	0.06	0.05	0.10	0.21	0.17	0.12	1.19		
E	0.11	0.04	0.08	0.03	0.07	0.28	0.10	1.46		
lulct	0.11	0.09	0.05	0.03	0.02	0.06	0.06	1.09		
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	6.60		

Note: Rf= Rainfall, DD= Drainage Density, S= Slope, ST= Soil Type, E= Elevation, LULCT= Land Use Land Cover Type W= Weight, Ev= Eggen value, CI= consistency index, CR= Consistency Ration. Thus, the column average must sum up to 1 approximately

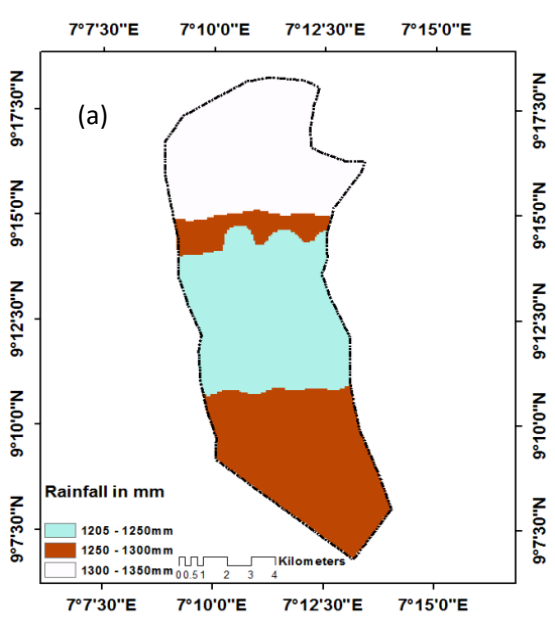
Development of Flood Risk Map Using Result from above in GIS Environment

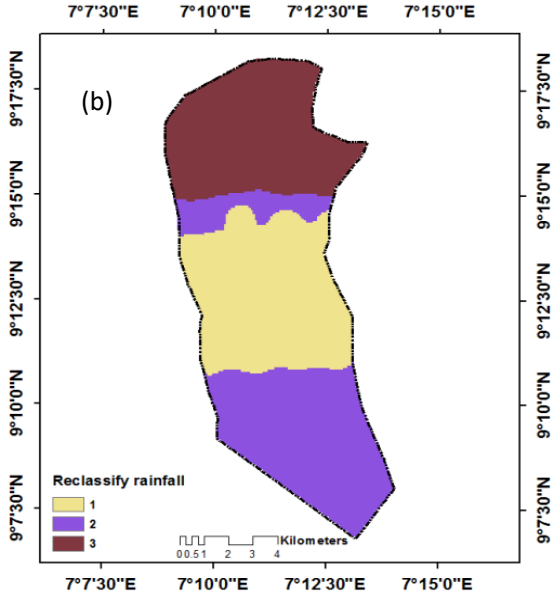
The generated results from the preceding sections were then incorporated into GIS environment to enable the Flood vulnerability map development. Prior to the development of the vulnerability map was, the design of evidential layer polygon to enable effective and accurate analysis of result from local to regional scale. In achieving this desire criterion, the map of the study area developed was subjected to grid construction in GIS environment using the grid tool in Arc catalog toolbox. The center of

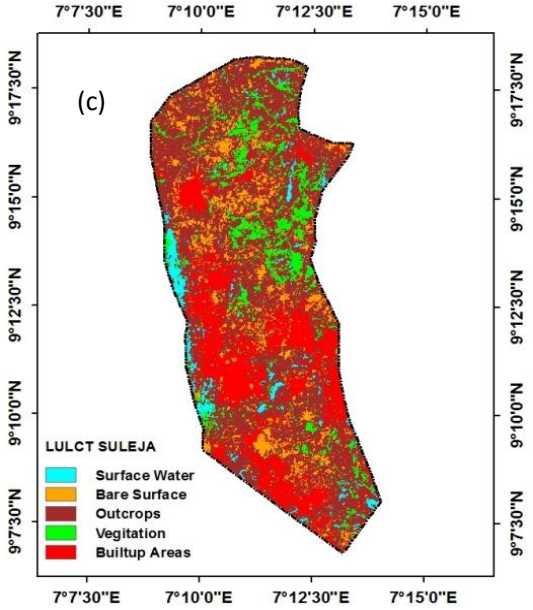
each grid was determined and coded as dbase file. The dbase file contains; X, Y and Z data.

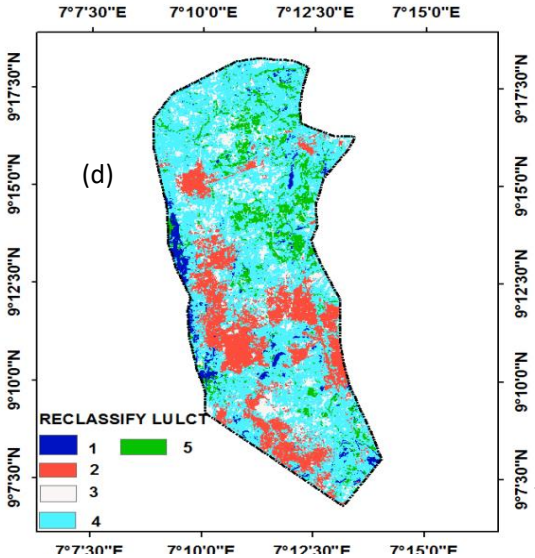


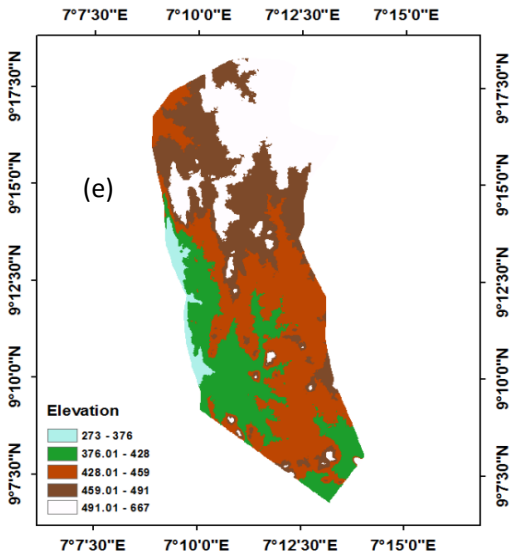
Classified and reclassified maps for the criterion in concerned flood’

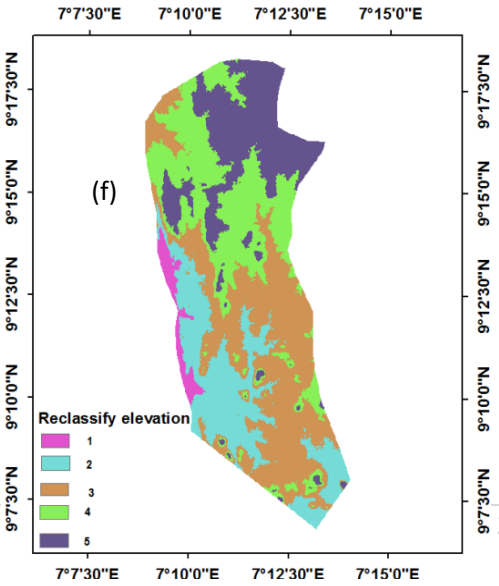












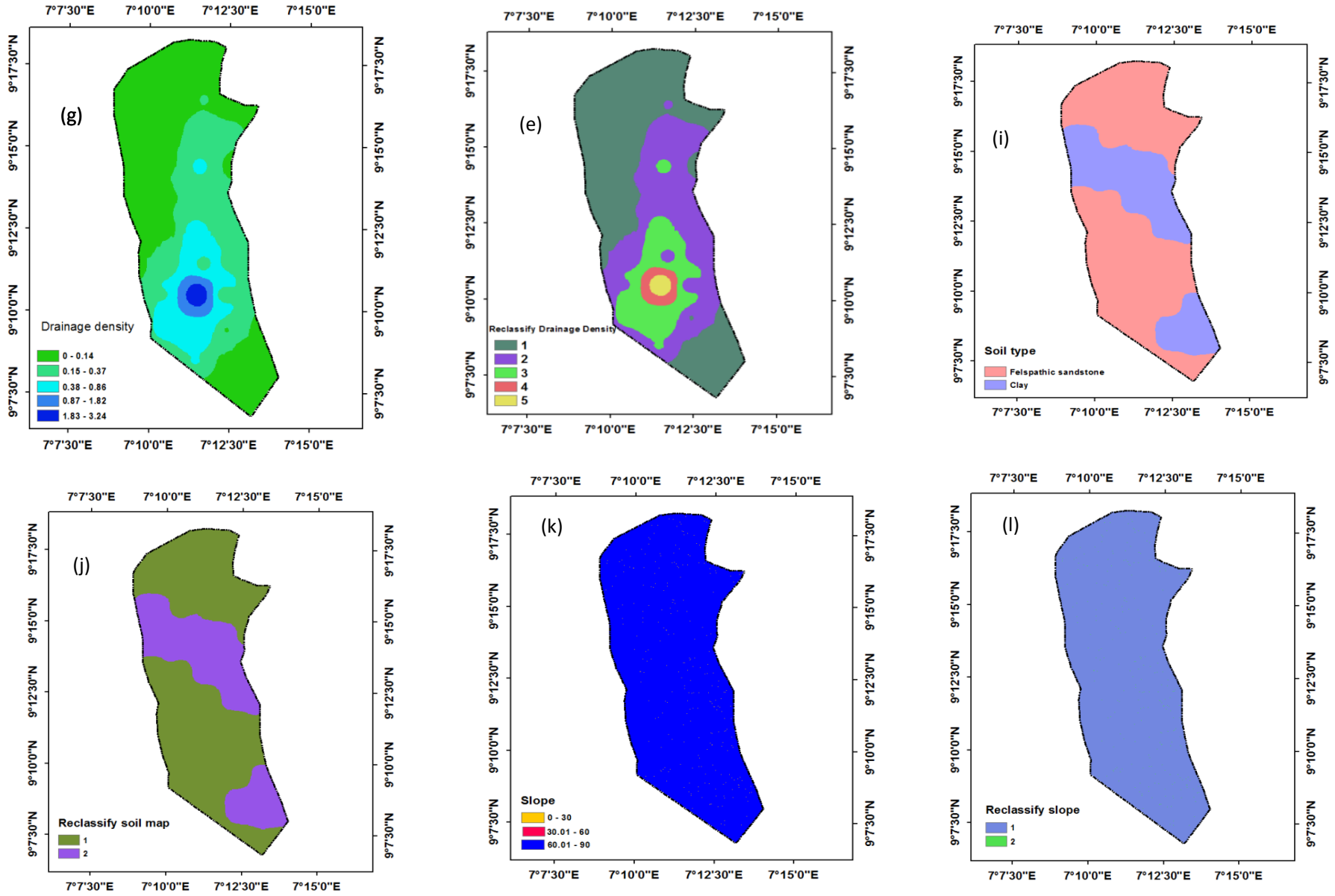


figure 5(a) rainfall map, (b)Reclassified rainfall, (c) LULCT Map, (d)Reclassified LULCT, (e)Elevation map, (f)Reclassified Elevation, (g)Drainage densitymap, (h)Reclassified drainage density, (i)soil map, (j) reclassified soil map,(k) slopemap (l)Reclassified slope map

RESULTS AND DISCUSSION

Figure 6 is the composite map showing the flood vulnerable areas that was created using multicriteria evaluation methods with GIS. From the pairwise comparison rainfall is the highest in term of weight with 0.36 follow by drainage density indicating rainfall as the highest initiating factor amongst selected criteria.

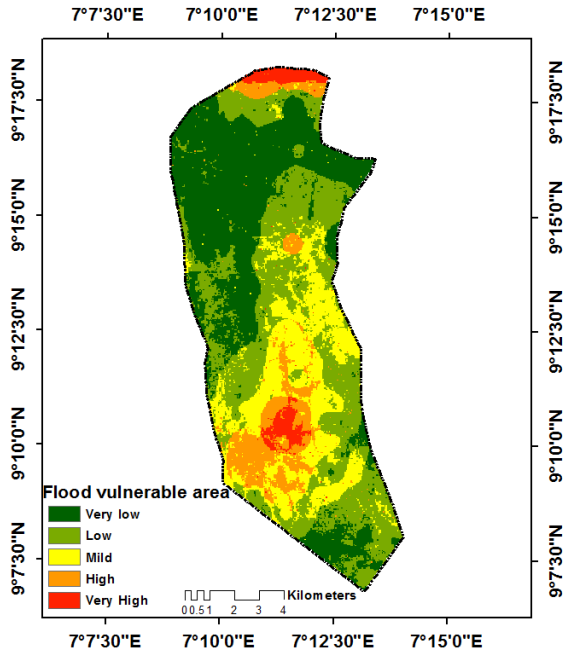


Figure 6. Flood Vulnerable Map Of Suleja

The criterion maps were combined using raster calculator to multiply each criterion by the weight gotten from pairwise comparison in order to delineate different prone zones to flood occurrences. The weight of each criteria is been multiply by the reclassified map figure 6 in raster calculator and all add together by logical operation to depict the flood vulnerability map that show area highly liable, medium and low liable to flood.

In GIS environment, the range numbers are designated as very high, high, Medium and Low and very low on the output map depicting the level of flood vulnerability of the area. Figure 5 (h) and (a) depict the rainfall distribution in the study area when compared with figure 6 shows clearly that the areas of very high prone zone corresponded to area of high rainfall especially extreme northern part of suleja moving close to Abuja, therefore, indicating how influential rainfall is as an initiating factor to flood occurrence. Drainage density as one of the criteria considered has high influential power as an initiating factor of flood occurrence in the study area with proof comparing figure 5(d) with figure 6 showing the area with high drainage density to be very high flood prone zone, This means that the higher the density, the higher the catchment area is susceptible to flood, resulting in concentration of flood water at the lower grounds.

Considering LULCT as flood initiating factor and how much the land use and land cover type influences flood occurrences because it play a key role in relation to soil stability and infiltration of water into the soil and at a time ability of soil to act as a water store, runoff of rain water is much more likely on bare surface than those with vegetation cover, from figure5(b) and (c) it is noticed that presence of thick vegetative cover slow down water movement into the soil and reduce amount of runoff. On the other hand impermeable surfaces such as concrete, road, buildings, rock outcrop generate more runoff making developed areas of suleja liable to flood as depicted in figure 6.

Conclusion

The results of this study confirm that the integration of AHP and GIS techniques provides a powerful tool for decision making procedures in flood hazard mapping, as it allows a coherent and efficient use of spatial data. The use of multi-criteria evaluation for different factors is also demonstrated to be useful in the definition

of the risk areas for the flood mapping and possible prediction. In overall, the case study results show that the GIS-AHP based category model is effective in flood risk zonation..

Recommendation

Since flood is inevitable in as far as the initiating factors remain constant, there is need for mitigating the effect of flood both on life, properties and immediate environments. From the study the following recommendation are made to reduce the advance effect of future occurrences and for further studies**:** Afforestation should be encouraged on areas highly prone to flood; this is a measure to reduce the risk. More studies should be undertaken to discover new techniques for evaluating more initiating factors of flood. Developmental projects on flood prone areas should be critically analyzed based on the effective factor causing flood in order to mitigate the effect of occurrences.

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Sustainable Urbanization in Minna, Niger State Nigeria using Geospatial Technique and Remote Sensing Data

[*]Bashir Y.I., Muhammad H.K., Hassan S.M., Sallau R.O. & Suleiman H.

Department of Geography, Ibrahim Badamasi Babangida University Lapai, Nigeria

*Correspondence: Email address: ishakubashiryakubu@gmail.com; Telphone: +2349060995569

ABSTRACT

This study examine the potentiality of RS and GIS in detecting Land Used and Land Cover (LULC) types changes in Minna between 1976 and 2016 (50 years period of time). Therefore, this was aimed at understanding LU and LC types in Minna. Satellite images of landsat 8 TM of 1976,1986,1996,2006 and 2016 were obtained, processed using image correction to enhanced image quality. The corrected imageries were then subjected supervised classification using maximum likelihood technique and LULC Change was detected and a trend of LULCC was computed. Literature of review was carried out to understand feature occurrence and other relative information. Analysis of the satellite data was carried out by using ARCGIS 10.3 and ERDAS EMAGINE 2014 software package with Microsoft office analytical tools. The maximum likelihood classifier was used to classify the satellite images and six major land use land cover (LULC) classes were observed and mapped which include; vegetation, bare surface, developed area, farm land, water body and rock outcrop. The result identifies a rapid growth in the developed area between 1976 to 1986 to be 10% (51.4ha), further increase to 17% (68.0ha) between 1986 to 1996 with decrease in the subsequent years. Decrease in bare surface of 50% (255.9ha) between the year 1976 to 1986 and increase to about 56% (227.2) between the 1986 to 1996, with increase in farm land of about 18% between the year 1976 to 1986 and further decrease respectively in the subsequent years..

Keywords: Sustainable Urbanization, Remote Sensing, LULC, GIS.

INTRODUCTION

The global population has been on the increase which according to Bashir et al. (2018) is due to; increase in birth rate, low mortality rate, advancement in science and technology, improvement in health care delivery among several factors. The global population has been projected by the Dos Santos et al. (2017) to be over six (6) billion with over two (2) billion living in the urban centers with a projected increase rate of 34 percent. This large number of urban dwellers will required shelter, food and employment to enable them carried out their daily activities. However, due to economic disparities, rise in different kind of urban structure begins to develop with significant implication on planning (Dos Santos et al., 2017). In a developing countries like ours where resources is grossly in adequate, provision of shelter to meet the number of urban dwellers becomes a challenge. Thus, the need to determine the trend of urbanization that can guide policy makers for effective planning and sustainable development while maintaining global standard best practices

RS provide access to information about the earth using sensor by detecting the electromagnetic response pattern emitted by a

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target, analyze the received response, visualize and classify for effective decision making regarding the surface condition. RS has found wide application in varieties of field including but not limited to; geographical application (Blaschke et al., 2008, Clifford et al., 2016, Frohn and Lopez, 2017, Tiner et al., 2015), biodiversity conservation (Mather and Tso, 2016, McCord et al., 2018, Smyrnakis et al., 2015, Vale et al., 2016), infrastructural monitoring and management (Behnam et al., 2016, Hegazy and Kaloop, 2015, Jalbert, 2016, Repetto et al., 2018)

Although remote sensing has effectively provide access to remote areas, it has widely been limited by it total dependence on spectral characteristics of the energy emitted by a target. Energy emitted by a target might not necessarily reflect the target condition due to the presence of spectral variability arising from differences in the spectral response pattern of different features on the earth surface which results to ambiguity with high level of uncertainty (Camacho et al., 2019, Dong et al., 2019, Gordon et al., 2019) .

GIS stand being distinctive through this abilities among other information system tools, and make it invaluable to various private and public enterprises for predicting outcome, planning strategies, and explaining events (Musa et al., 2017). The rapid rate of urbanization as an increase in population and migration of people from rural environment in search of opportunities has becomes a source of concerned to policy makers and other stake holders globally. The immediate effect of the large number of urban dwellers is the inability to secure accommodation in addition to inadequate financial capability to pay for available shelter. This brings about poor and ineffective development of urban centers, affecting proper planning and absent or inadequate shelter.

In light of this challenges; Schwarz et al. (2018) among several others developed a remote sensing approach that is capable of integrating multi layer information regarding; population sizes and both source and destination, push and pull factors, employment opportunities among other factors were considered. These studies offered a useful conceptual frame work through which remote sensing can be applied in urban studies but are deficient in predictive capability, thus sustainability becomes difficult. The needs for a more realistic approach form monitoring the trends of urbanization, it driving forces and inference regarding sustainable urbanization with emphasis on the socioeconomic livelihood of it citizen.

This research is aimed at to explore the potential of remote sensing and demographic data using GIS technique to assess the trend of urbanization in Minna Niger State by; (a) identifying the different land used land cover types in Minna using remote sensing data of 1976 to 2016 at interval of 10 years, (b) classify the different land cover types from remote sensing data using supervised classification in ERDAS application and determine the rate of change in both population and urbanization from 1976 to 2016.

METHODOLOGY

Location of the Study Area

Minna is located on longitude ⁹ 32’ 30” to ⁹ 42’ 30” N and latitude ⁶ 20’ 00” to ⁶ 37’ 30” E as shown in Figure 1. with a geological base of undifferentiated basement complex with mainly gneiss and magnetite. It occupies about 884 hectares of land with total population of 304.127 people as at 2006 population censors.

The study is bounded to the North by Bida and Lapai local government, to the south is Shiroro Local Government, to the West is Wushishi Local Government and to the East is Paikoro Local government area of Niger State of Nigeria..

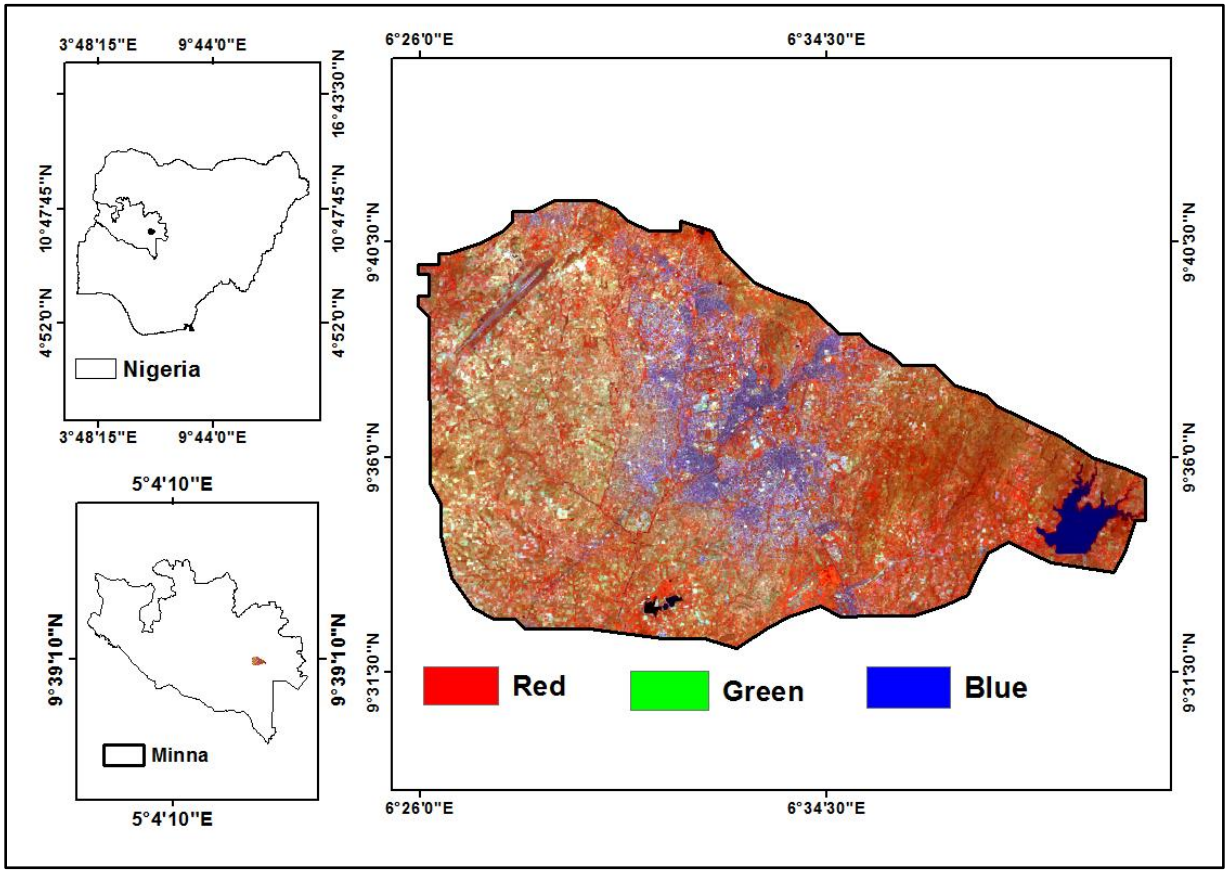

Figure 1: Map of the study area.

Identification of LULC Type of Minna

Landsat Multispectral Scanner (MSS), Thematic Mapper (TM) and Enhanced Thematic Mapper Plus (ETM+) were acquired at different epoch from the USGS satellite operated by (US) government for 1976, 1986, 1996, 2006 and 2016. The acquired images were subjected to geometric corrections and enhancement to remove cloud, smoke and dust haze effects, which might influence the result prior to LULCT and LULCC dynamics. Acquired images were classified using supervised classification. The different image bands were layer stacked and band 4, 3 effective throughout the composite. Ground base control points of variable resolutions and scale where used for both training area selection and evaluation of classification accuracy, and established for both training area selection accuracy assessment of the classified images.

Table 1: Data types used in the research

S/N	Data Type	Details	Year
1	Landsat MSS	60m	1976 &1986
2	Landsat TM Image	30m	1996 & 2006
3	Landsat ETM+ image	15m	2016
4	Spot Image	1m	2015
5	Google Earth Image	6m	2018
6	Street Guide Map	1.50,000	1991

Image Processing

Each feature on the images were given a unique signature as inferred from the composite and the ground based control points used in developing training samples. The processed satellite images where subject to qualitative evaluation using their spectral characteristics and properties to ascertain the uniqueness of different LULC types in the study area and a supervised maximum likelihood classification algorithm was employed for data classification and defined as presented in Table 2

Table 2: Land used land cover classification system employed in the research

Land-cover types	Description
Developed Area	All residential and industrial areas, settlement and transportation infrastructural network.
Water body	River, permanent open water, lakes, ponds, canals, and reservoir.
Vegetation	Trees, shrubs and semi-natural vegetation, deciduous coniferous and mixed forest, palms, orchards, herbs, climbers' gardens, inner-city re-creational areas, parks, playgrounds.
Farmlands	All agricultural lands both on small and large scale, it involves grass-land and ridges.
Rock outcrop	This referred to exposed rock either due to denudation or human activities.
Bare surface	This referred to sand land, , and areas which has no vegetal covered.

Change detection analysis

Post classification change detection technique that detects nature, rate and location of change was used in this research, using an overlay procedure in GIS, spatial change in LULC between 1976 to 2016 were obtained.

Determination of LULCC trends using information generated from 1976 to 2016

The land used trend it the study area was determined using the moving averages for the period under review. The LULCC of 1976, 1986, 1996, 2006 and 2016 where determined and added to the previous epoch which gives the trend for the current year. The trend pattern is illustrated in equation 3.1

$$\sum_{LUC}^{t} x_1 + x_2 + x_3 + x_4 \ldots . + x_n \qquad (3.1)$$

Where $t = time\ under\ review$,

$LUC = the\ amount\ of\ land\ used\ land\ cover\ changes\ and$

$x = \ the\ obtained\ land\ used\ changes\ amount$

RESULTS AND DISCUSSION

LULC Types of Minna

The LULC types of Minna are presented in Figure 4a Based on the remote sensing and ground control points, six (6) to Seven (7) different land cover types can be identify as developed area, areas covered by water bodies, farm land, bared surface, vegetation, exposed rock outcrops and wet lands. The developed region includes; building, Educational centers, constructed road networks and other adjourning structures like market, industrial layout and mechanical workshops. Other developed areas are the farmlands which become flexible to conversion into developed regions. It is on this region that an agricultural activity such as crop cultivation and irrigation agriculture takes place. Vegetation is a transitional zone from the farm lands to a relatively dense vegetation containing trees,

shrubs, grasses among several other vegetation compositions.

The vegetation is often characterized by scattered trees that shade their leaves during the dry seasons; with little or no climbing and under growth, as inferred during reconnaissance survey as often associated with guinea savanna type of vegetation. Bared surface in Minna arises as a result of population growth, urbanization and increase in subsistence agricultural practices. Agricultural practices such as; farming result to loose of large hectares of vegetation through clearing and bush burning. In addition to farming, construction on the other hand greatly result to the development of bared land as the top and lateritic soils are often excavated for sand filling and building of structures. The wetland in this research is due to similarity in spectral sensitivity, and was merged with water body in most of the images classified. Though, water body cover substantial hectares of land as reviled from the remote sensing technique employed. It comprises of small pound water, streams, channels and rivers that runs with direction in response to precipitation, elevation and prevalent geology. Rock outcrop on the other hand becomes visible from their spectral response pattern; however, visibility amount of rock features resulting from the maximum likelihood classification scheme employed varies according to season.

LULCC of Minna between 1976-2016

The remote sensing data used in this research observe 6 different land cover types as shown in Figure 4b which include vegetation, bared surface, water body, developed area, farm land, and rock outcrop. As a result of abundant fertile land agricultural activities were predominantly practiced by both the indigenous land owners and the migrants; therefore bring about increase in population and change in LULCT from what it is used to be to agricultural land. Population tend to increase gradually with increase in demand for shelter, and other social amenities which causes urbanization and also change the land use land cover type to a developed area as such sustainable urbanization has taken place

Land use Land Cover Change between1976-1986

Figure 4c shows land cover changes during the first era (1976) the most dominant land Cover Types (LCT) were vegetation, bared surface and water bodies. By 1986 most of the vegetation and bared surface were replaced by developed area and farm lands. The farm lands were more dominant at the northern part of the study area. The rapid declined in the available vegetation is coherent with the creation of Niger State from Sokoto State and the making of Minna as the State Capital. It was during this period that Minna experience rapid expansion and construction of structures to meet the demand of change in function to administrative function. At the periods of 1986 to 1996, the expansion rate relatively stabilized however, construction of road networks and channelization of Minna drainage system received a significant facelift there by increasing the expansion rate slightly. The increase in vegetation cover and farm land is due to the abundant rain which results to minimal rock outcrop and developed area.

The creation of more administrative centers including Local Government and development areas have contributed immensely to the rapid expansion rate of Minna settlement. The pattern and area of development in the second epoch varies proportionally compared to the

first epoch: while development during the first epoch was more around Minna city centre except for some few isolated settlement, the second epoch show more rapid development at the buffer zones. Maikunkele, Bosso, Barikin Sale and Chanchaga begin to develop rapidly in terms of infrastructure, housing and basic amenities. Availability of basic amineties includig electricity, pipe born water, hospital and improvement of income are some of the most critical driving forces of rapid urbanization.

LULCC of Minna between 1986-1996

During the third decade (1996-2006) as presented in Figure 4d, developed area expanded in all direction placing a greater constrain on vegetation and water body while exposing most of the rocky outcrops. Due to this development, farm land begins to decline primarily at the expense of developed area. Available data sited during the course of this research shows that, selling of land at the outskate of Minna became pronounced during this period. Many of the farm land at the fringes of the town were sold and converted to; residential, commercial and educational institutions.

LULCC of Minna between 1996-2006

Between 2006-2016 (Figure 4e), mark the era of rapid expansion of Minna metropolis. Historical document indicated the development of two major housing estates (M.I. Wushishi and Talba Housing estate) along the western and eastern bypass respectively. In addition, other private properties developers and individual buildings received tremendous increase. This period also coincide with the relocation of Federal University of Technology Minna to it permanent site and the completion and relocation of National Examination Council (NECO) national head quarter along Minna Bida Road, thereby resulting to increase in higher demand for shelter and proximity to place of work. The successful completion and relocation of central Market to the Old Airport field developed by Urban Shelter Clay and building of the timber shade at the extreme of eastern directly covert the grassland and some minute farmlands to a permanent developed area thus, conversion of vegetation and farmlands to commercial area.

LULUC of Minna between 2006-2016

The rapid development of developed area as reviled by this research can be attributed to rapid increase in population, rise in public, private sector and individual household sectors. This result is coherent with the work of (Li et al., 2018). Most of the development projects were undertaking through individual and the public sector to meet societal demand for housing, education, commercial and industrial activities. This pattern of development at the later period (between 1999 till date) was further excoriated by the Public Private Partnership Initiatives (PPPA) where development need considered critical can be initiated by the society while it development can be financed by the private sector and government provide the enabling environment.

LULCC of Minna for 2016

Land speculation was observed among the suburban areas in response to increase in land prices due to increase in demand for housing, educational and infrastructural development. The agricultural land at the city fringes are becoming rapidly developed by public and individual property developers. Most of the recently developed properties appeared to have taking into cognizance the accessibility needs thus, most of the layout were provided with access network as opposed to their previous

developed properties especially among individual properties owners.

Driving forces of Urbanization in Minna,

The spatio-temporal change analysis presented in Table 4 reviled that changes has taking place in the different land cover types over times. Vegetation in 1976 covered 299.85 ha considerably declined by 43.99ha as at 1986. Between 1986-1996, 28.63 were lost and, by 2006, vegetation lost was now 84.52ha.Overal evaluation of change between 1976-2016 shows that 219.93ha of vegetation was lost and change into other land used types. Water body on the other hand exhibits an oscillating pattern of change between 1976 to 2016 with some years showing increment while other years shows declining in amount of area covered by water body. For example in 1976, water body covered 74.82ha and declined by 40.23ha by 1986 and by 1996 increase by 8.90ha. Between 1996 to 2006 it also rose to 25ha while between 2006 to 2016 the value declined by 66.30ha. The nature of decline cleared represents a pattern of growth in the developed area.

Developed area in this study had been on the increase in 1976 developed area was just 34.71 ha. This value however, increased to 51.36ha by 1986, 68.02ha by 1996, 72.75ha by 2006 and 116.58ha by 2016. Changes in the developed area pattern between 1967 to 2006 were on the average rate of 12.68ha per decade amounting to increase of this land used type by 38.04ha. Statistical evaluation of the years 2006 to 2016 correspond to the decade with highest urbanization value of 116.58ha more than three times greater than 1976-2006 values. This rapid urbanization period coincide with period of many PPPA by the government resulting to increase in infrastructural development, housing, education, commercial, industrial, small and medium scale business, demographic changes, immigration from the surrounding rural areas in addition to availability of social and basic amenities in the suburb also influences this development rate.

The geospatial analysis from the Landsat images reviled that expansion from 1976 to 2006 did not occur proportionally to each other. Vegetation for the period under review shows a declined from 54.03% to 18.71% in 2016. Water body on the other hand declined from 13.48% to less than 1percent in 2016. While vegetation and water body shows a tremendous declined in area coverage, farm land and developed areas indicate a significant growth from 0.41% to 10.87% and 2.25% to 27.20% between 1976 to 2016 respectively. A slight increase was also observed in rock outcrop from 4.22% in 1976 to 4.75% in 2016.while bared surface increases from 21.62% to 37.76% for the same period. The high value of change in bared surface was largely due to change in farm land to development of road network and open space development.

The study revealed that the rapid rate of urbanization of Minna has been relatively more rapid during the last decades compared to the 1976-2006 era. The rapid urbanization rate has resulted to the significant changes in LULC pattern with adverse effects on the environment. The expansion rate for the period under study shows that in the last 10years Minna has developed by more than 110ha.

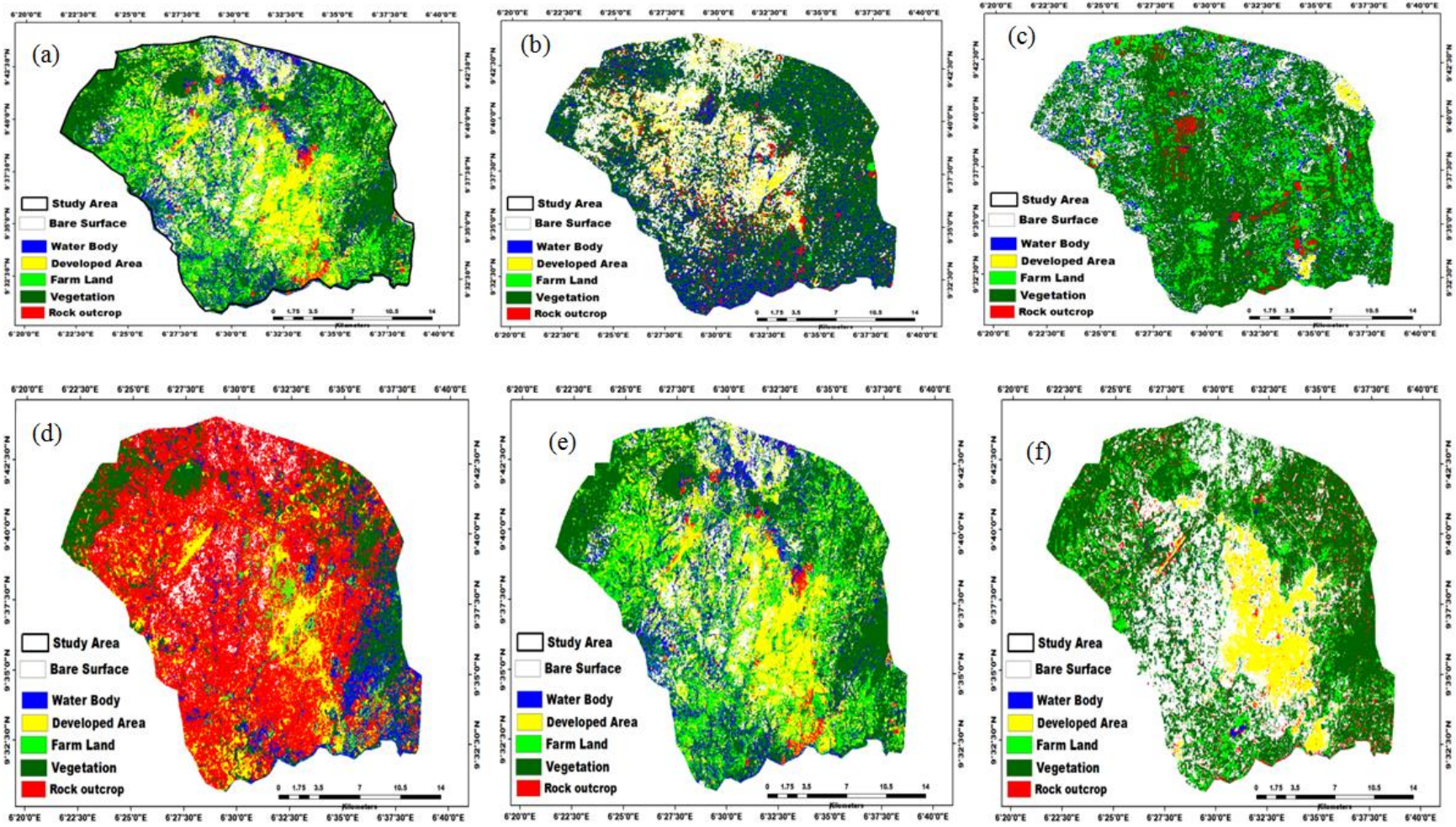

Figure 2: (a) LULC types of Minna from 2017 landsat TM+ image (b) Classified 1976 landsat image, (c) Classified 1986 landsat image, (d) Classified 1996 landsat, (e)Classified 2006 landsat image and (f)Classified 2016 landsat image

Table 0.3: LULCC pattern of Minna Niger State between

	1976		1986		1976-1986	1996		1986-1996	2006		1996-2006	2016		2006-2016
LULC	Area (ha)	%	Area (ha)	%	Change area (ha)	Area (ha)	%	Change area (ha)	Area (ha)	%	Change area (ha)	Area (ha)	%	Change area (ha)
V	299.9	54	255.9	50	-43.99	227.2	56	-28.63	142.7	31	-84.52	79.9	19	-62.8
WB	74.8	13	34.6	7	-40.23	43.5	11	8.90	68.9	15	25.43	2.6	1	-66.3
DA	34.7	6	51.4	10	16.65	68.0	17	16.66	72.8	16	4.73	116.6	27	43.8
FL	2.2	1	95.4	18	93.20	15.9	4	-79.57	123.4	26	107.52	46.5	11	-76.9
RO	23.4	4	13.2	3	-10.24	28.6	7	15.42	10.7	2	-17.89	20.31	5	9.6
BS	120	22	61.97	12	-58.03	21.1	5	-40.87	47.6	10	26.53	161.3	37	113.68
Total	555.02	100	512.4	100		404.2	100		466.1	100		427.2	100	

Note: V: Vegetation, WB: water Body, DA: Developed Area, FL: Farm Land, RO: Rock Outcrop, BS: Bared Surface

Conclusion

This research has identified, classified the LULC type and examines the Spatio-temporal changes in LULCC of Minna during the years under review using remote sensing data, geospatial technology and other auxiliary variables. Analysis reviled that developed area increase from 74.82ha in 1976 to 116.58ha in 2016. Farmlands increased from 2.23 ha to 46.45ha and bared surface increases from 120.00ha to 161.31ha from 1976 to 2016. The study revealed that this growth rate resulted to substantial reduction in vegetation and water body including wetlands. The transformation of vegetation and water body to build up area and farmlands has resulted to a severe environmental degradation with adverse vulnerability to flood occurrences, growth of slums and ghettos.

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Overview of the Effects of Climatic Variables on the Proliferation of Pathogenic Microorganisms

[*1]Enemanna C.E., [1]Raji R.O., [2]Sadiq U.M., [1]Okwor C.R., [1]Shaibu B.O. & [3]Balogu T.V.

[1]Department of Microbiology, Federal University of Technology Minna, Nigeria.
[2]Department of Biological Science, Federal Polytechnic Bida, Nigeria.
[3]Department of Microbiology, Ibrahim Badamasi Babangida University Lapai, Nigeria

*Correspondence: Email address: chineduegbelu@gmail.com Phone number: +2348063397700

ABSTRACT

Over the years, experts have long predicted the sway of climatic parameter on the epidemiology of pathogenic microorganisms, the proliferation of public health important microorganisms such as *Vibrio cholera, Salmonella enterica, Plasmodium* spp, and *Candida albican*, have become an emerging concern. These could be directly due to varying climatic variables as a result of varying climatic variables, notable among these climatic parameters are precipitation/rainfall. Humidity, sunshine, temperature, dust and global warming. Optimum conditions of these variables are required for their survival, proliferation and distribution. Temperature is one of the predominating factors that affect the proliferation of microorganisms. Common pathogenic organism, *Salmonella* spp is capable of increasing its population as temperature rise at range of 7°C and 37°C. *Plasmodium falciparum* responsible for malaria predominates in most tropical and sub-tropical regions because of their tolerance to warm condition. Rainfall plays an important role in the development of water-borne disease pathogens providing a condition favourable for the proliferation of fecal pathogens. The air-borne pathogens such as *Influenza*, are active in humid condition. For example, absolute humidity and temperature were found to affect *influenza* virus survival, proliferation and transmission. Direct transfer of meningococcal meningitis appears to be related to temperature, rainfall and other environmental which include winds and dust. Climatic variables have profound effect on the proliferation of pathogenic microorganisms, therefore, improvement in public health training, emergency response and prevention is highly needful. Also, more awareness campaign is needed on the dangers of climatic variables on pathogenic microorganisms.

Keywords: Climate change, pathogenic microorganism, temperature, infectious disease.

INTRODUCTION

In the last few decades, the modulating potentials of climate variables, with respect to epidemiology of pathogenic microorganisms, human health, as well as disease transmission and food yields, has been a subject of discussion. Before the nation of infectious agents was understood, man is aware of the effects of climates variables on pathogenic microorganisms. Late in the19th century, For instance, malaria incidence have been prevented during summer by the Roman aristocracy (Patz*et et al*., 2003). Similarlarly, in Southern Asia, curried foods are less prone to induce diarrhea diseases during the summer.

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While influenza epidermic mostly occurs during mid-winter in developed countries (Patzet *et al*.,2003).

According to Intergovernmental Panelon Climate Change (IPCC) 2014, a statistically significant variation in either the mean state of the climate or in its variability, persisting for an extended period (typically decades or longer) is known as climate change. It include elevated mean temperature, extreme humidity/precipitation, drought frequency, ocean acidification, water quality and seasonality (Milena *et al*., 2013).

Optimum climatic setting are required for the continued existence, proliferation and distribution of microorganisms (Field *et al*.,2014). Climate change can have adverse effects on the pathogens, this can come directly by influence their survival, reproduction as well as the life cycle of the pathogens, while some may affects them indirectly through their natural habitat, their environments. As a results of this, even the geographical and seasonal distribution of the pathogens may change (Patz *et al.,* 2014.). There is therefore need to review Transmission of pathogenic microorganisms within an ecological framework.

CLIMATIC VARIABLES THAT AFFECT MICROBIAL PROLIFERATION

Climatic parameters act as environmental filter amid direct influence on the geographical allotment and survival of pathogens thereby influencing disease dynamics (Milena *et al.*,2013).

For instance, change in weather setting lead to decrease in the continued existence of pathogens in the surroundings which directly affect the spread of infectious diseases. Pathogens are likely to be pretentious by some aspect(s) of climatic conditions which include; elevated mean temperature, extreme humidity/ precipitation, drought frequency, atmospheric pressure, wind, sunshine, atmospheric particle count, ocean acidification, water quality and seasonality.

Temperature:

Different microorganisms have different optimum temperature ranges at which they grow. Most human pathogens encompass an most favorable temperature of 37^{o}C (98.6^{o}F). Most bacteria species do not grow well at temperatures higher than 45^{o}C while several others can grow in hot springs and even at high temperatures below circumstances of high pressure (Xiaoxu *et al.,* 2016). On the other hand, some microbes can be able to survive at 0^{o}C or even lesser temperatures. In general, all organisms have an optimum temperature in which the can grow and this range of temperature under which it is capable of growth. Psychrophiles can grow between 0^{o}C - 25^{o}C, mesophiles between 10 ^{o}C-45^{o}C, and thermophiles between 25 ^{o}C-80^{o}C (Hervell *et al*.,2002).

Plasmodium falciparum which causes greater death compare to *Plasmodium vivax* requires warmer environment that predominate in the majority of tropical and sub-tropical regions. Various studies assumed that the temperature requirements for *P. vivax* for its developmental threshold is around 14^{0}c-16^{0}C which is lower than the 17^{0}c-19^{0}C that is needed by *P. falciparum* (Dudley *et al*., 2015).

Most pathogens survived well in warmer temperature while others prefer colder temperature. For example, rotavirus infections (which cause diarrheal disease in children) happen at much higher rates in winter than in

summer (Turcios *et al*., 2006). Increases in respiratory tracts infection among children during cold season is commonly reported in temperate climates (Chan *et al*., 2002; Al-Khatib *et al*., 2003). However, Africa, Asians, and American countries have seasons for this respiratory syncytial virus, which normally happened during the rainy season (Shek and Lee, 2003). This suggests that factors other than or in combination with temperature may be important in the transmission of this virus.

Salmonella, and other bacteria that is responsible for food poisoning, proliferate more rapidly at higher temperatures. The rate at which *Salmonella* species multiplies is directly related to temperature within the range 7.5 ºC–37ºC (Xiaoxu *et al.,*2016*).*

Cholera, one of the most feared disease in the world is also sensitive to environmental temperature. This have led to diverse evidence of the proliferative response of *Vibrio cholera* to warmer water in lakes, estuaries, and coastal waters (Long *et al*., 2005; Wilcox and Colwell, 2005). Meningococcal meningitis in the Sahel region ('meningitis belt') of West Africa provides a tantalizing example of an epidemic infectious disease possibly related to climatic conditions. The fact that outbreaks occur approximately periodically may reflect cyclical fluctuations in climatic conditions (Sultan *et al*., 2005).

Precipitation/Rainfall

Shift in precipitation that usually affects the dissemination of water borne pathogens is as a results of change in the climate. Rainfall is an important factor in the growth of water-borne disease pathogens, because during rainy season, there is usually increases of fecal pathogens as heavy rain may stir up sediments in water, therefore, leading to the accumulation of fecal microorganisms (Jofre *et al.,* 2010).

Hofstra (2011), reported that unusual precipitation after a long drought can result in an increase of pathogens, cause a disease epidemic. Low rainfall, as well as drought can lead to low river flows, causing the concentration of effluent water-borne pathogens and also variability in precipitation can have direct consequences on the infectious diseases outbreak.

In addition, increased in precipitation may also cause increase in the presence of disease vectors by expanding the size of existing larval habitant and also may lead to creating new breeding vector-borne pathogens (Hofstra,2011). Increase in rainfall will lead to outbreak of most diseases like small pox, polio among others. Hoover and Barker (2016), reported that increase in rainfall causes the outbreak of *Aedes aegypti* in 1947 in America that causes dangue and yellow fever but was letter eradicated with the help of Pan-American Health Organization (PAHO), In addition, increased in precipitation may support a growth in food supplies which in turn support a greater population of Vertebrate reservoirs. Unseasonable heavy rainfall may cause flooding and decrease vector population by eliminating larval habitant and creating unsuitable environment for Vertebrate reservoir. Alternatively, flooding may force insects or rodents vectors to seek refuge in houses and increase the likelihood of vector-human contact (Hoover and Barker, 2016).

For instance, because of several flooding that occurred in Brazil, epidemics of *leptospirosis*, a rodent-borne disease were documented (Galway *et al*.,2015).

Humidity

Humidity also have an impact on the pathogens of infectious diseases. infleuenza virus one of the pathogens of air-borne infectious diseases tend to be responsive to humidity conditions but although different researcher have found that absolute humidity and temperature tend to affects infleuenza virus survival, proliferation and transmission (Molesworth *et al*., 2003)

Direct transfer of meningococcal meningitis happens to be linked to temperature, rainfall and other environmental (particularly winds and dust) conditions. Also, the uncommonness of outbreaks in humid, forested, or geographical areas may be as a result of high continuous humidity impair transmission (Molesworth *et al*., 2003).

Lowen *et al.,* (2007), projected that freezing temperature and small relative humidity are encouraging to the increase of influenza virus. Changes in humidity also affects viruses in the water borne diseases. Additionally, it have also been reported that water borne viruses in surface water do not survived due to limiting dryness of the surface water. Moreover, due to change in humidity, viruses of vector-borne disease are affected. Also changes in humidity have been reported to affect the developments of malaria parasite in *Anopheles* mosquitoes, but in Yangon and Singapore, temperature and humidity favors Dengue virus propagation in mosquitoes and this have contributed in the outbreaks of Dengue hemorrhagic fever in these regions (Xu *et al*., 2014).

Sunshine

One of the important climate variables that affect pathogens of infectious diseases is sunshine. during sunshine periods, temperature act synergistically during cholera periods, and this help to create a favorable condition for the multiplication of *Vibrio cholerae* in aquatic environments (Chen *et al*., 2010).

Dust particles

According to Galway *et al*.(2015), various studies have reported a positive correlation that exist between dust particle association or attachment and virus survival or their transportation. Microorganisms do not grow in the air but some type are found in it (Chen *et al*. (2010). Usually this microorganisms in the air are passengers through aerosols (tiny particles of liquid) or dust particles. We make microorganisms bearing aerosols when we cough, sneeze,or talk. Any of this or other agitation of water such as waves breaking, river rapids and sprays, also create aerosols which effects pathogenic microorganisms ((Griffin, 2007).

Chen *et al*. (2010), reported that flaming a wet inoculating loop produces aerosol bearing Microorganisms. According him, aerosols and dust are the major principal means of transporting respiratory disease.

Global warming/Climate change

This is the variation in global or geological change over temperature in region which effects microbial activities. It could be a shift of the weather including its average. It's reflect the change in variability of the atmosphere over time ranging from millions of years. Climate change or global warming can be caused by the process internal to the earth (Xiaoxu *et al*.,2016). Human changes is mainly cause due to greenhouse gases which can come from chemical or biological processes contracting from the earth that lead to global warming. In other hand, human activities such as production of carbon dioxide ,mathene , nitrous oxide

through agriculture and deforestation can also increase the release to green house gases which effects microbial activities (Lubchenco and Karl, 2012) . Also stable gases such as methane, CO_2, nitric oxide , nitrous oxide influence microbial activities. Other climatic activities that effects Microorganisms include landfill and Rice paddies because they aid in the release of methane to the atmosphere over a long period of time. Also the use of natural gases. Forest distortion (deforestation) cutting down of trees cement production by man required burning of gases at high temperatures and it's results in the emission of gases to the atmosphere which effects Microorganisms. Minning of copper and other element used for electricity wiring, among others, contribute to global warming which in other hand have a negative impact on the proliferation of microorganisms (Patz *et al*., 2003).

Also, since the beginning of the industrial revolution in the early mid-eighteenth century, humans have been burned fossil fuels at an ever increasing rate which in one way or the other affects the proliferation of pathogenic microorganisms (Sonne *et al.,* 2017).

This factors along with a decline in photosynthesis have change carbon cycle and when carbon cycles is distorted, the proliferation of microorganisms, will be drastically reduced

CONCLUSION

Climatic conditions have profound effect on the proliferation of pathogenic microorganisms which could either be positive or negative. However, organisms have optimum conditions at which they perform best. The proliferation of pathogenic microorganisms are consequent on several climatic conditions/variables. These variables could either enhance or impede the growth of pathogenic microorganisms. Complex interaction in public health triad (environment, human host and illness) have been influenced by these factors.

RECOMMENDATIONS

Improved in public health infrastructure mainly in public health training, emergency response and prevention as well as programs is highly needed because it will help in the understanding the need of the adaptive capacity of those individuals as affected by climatic change.

More study needs to be carried out on the effect of climatic variables on pathogenic microorganisms.

Also, enlightenment campaigns needs to be carried out because this will help educate the people on the dangers pose by this climatic variables on pathogenic microorganisms as well as man in his environment.

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Gene Technologies and their Applications in Livestock Production- A Review

[*1]Idris, I.A. & [2]Egena S.S.A.

[1]Department of Animal Production, Ibrahim Badamasi Babangida University Lapai, Nigeria.
[2]Department of Animal Production, Federal University of Technology Minna, Nigeria

*Correspondence: Email address: idib2288@gmail.com; Phone number: +2348032562607

ABSTRACT

This paper discusses the use of genetic technology application in animal production, including the basic steps involve in genetic modification, its potentials, current uses and ethical issues. Genetic Technology (GT) is often termed as Genetic Engineering (GE), gene manipulation or recombination DNA, with all four often used interchangeable. It is the combination of techniques used for identification, replication, modification and transfer of genetic material. This technology has been in place since the 1980's and has found it is application in fields such as agriculture, pharmaceutical, health, environment, and the industry. The application in animal production including direct transfer of specific gene/alleles between individuals, species or even kingdoms in order to change the phenotypic expression in the recipients and for biomedical research. GT is also making strides in increasing disease resistance, productivity and enhancing animals' interactions with humans (hypo-allergenic pets). Various regulations for GT have been introduced. The level of acceptance depends on religious and ethical groups as well as the public, as some have come to see genetically modified foods as being unnatural which create a reverse halo effect including all the negatives adduced so far to Genetically Modified (GM) or Genetically Modified Organisms (GMOs).

Keywords: Genetic technology/engineering, RNA, DNA, Marker Aided Selection, GMA/GMO.

INTRODUCTION

Genetic technology is the direct manipulation of an organism's gene using biotechnology. It is a process of inserting new genetic information into existing cells in order to modify a specific organism for the purpose of changing its characteristics (Vert *et al*, 2012). Gene is any discrete locus of inheritable genomic sequence which affects organisms' traits by being expressed as a functional product or by regulation of gene expression (Pearson, 2006; Pennise, 2007). According to Gericke and Hagberg (2006), during gene expression, the DNA is first copied into RNA. The RNA can be directly functional or be the intermediate template for a protein that performs a function. The transmission of genes to an organism's offspring is the basis of the inheritance of phenotypic traits.

Identifying genes and their function is an important application of gene technology and can lead to move efficient conventional breeding processes. Marker aided selection breeding is an example. Marker Aided Selection (MAS) in plant or animal is an indirect selection process where by a trait of interest is selected based on a marker (could be morphological,

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biochemical or DNA/RNA variation) linked to a trait of interest (e.g. productivity, disease resistance, abiotic stress tolerance, and quality), rather than on the trait itself (Ribuat, 2001; Ribuat and Hoisington 1998; Rosyara, 2006).

GENE TECHNOLOGY

According to Royal Society (2019), genetic engineering is the process that alters or changes the genetic makeup of an organism by either removing or introducing a DNA. Unlike traditional animal and plant breeding, which involves doing multiple crosses and then selecting for the organism with the desired phenotype; genetic engineering takes the gene directly from one organism (even ones from different species) and prevents other undesirable genes from also being added. Erwin *et al*. (2015) observed that, it potentially fixes severe genetic disorders in humans by replacing the defective gene with a functioning one. It is an important tool in research that allows the function of specific genes to be studied (Alexander, 2003). Drugs, vaccine and other products have been harvested from organism genetically engineered to produce them (Nielsen, 2013). Crops have also been developed that aid food security by increasing yield, nutritional value and tolerance to environmental stresses (Qaim and Kouser, 2013).

According to European Parliament and the council of the European Union (2001), genetic engineering does not normally include traditional breeding, *in vitro* fertilization, induction of polyploidy, mutagenesis and cell fusion techniques that do not use recombinant nucleic acids or a genetically modified organism in the process. However, some broad definitions of genetic engineering include selective breeding. Van Eenennaan (2008) stated that, cloning and stem cell research are not also considered as genetic engineering, while Suter *et al.* (2006) were of the opinion that they are closely related and genetic engineering can be used within them. Synthetic biology is an emerging discipline that takes genetic engineering a step further by introducing artificially synthesized material into an organism (Andrianantoandro *et al*, 2006).

STEPS INVOLVE IN GENE MODIFICATION

The creation of genetic modified organism (GMO) is a multi-step process. Genetic engineers must first choose what gene they wish to insert into the organism. This is driven by what the aim is for the resultant organism and is built on earlier research. Gene screening is carried out to determine potentials gene and further testing is used to identify the best candidates. The use of microrrays, transcriptomic and genome sequencing has made it much easier to find suitable genes (Koh *et al*, 2015). The next step is isolating the candidate gene. The cell containing the gene is opened and the DNA is purified (Nicholl, 2008). The gene is then separated by using restriction enzymes to cut the DNA in fragments (Albert *et al*, 2002) or by polymerase chain reaction (PCR) to amplify up the gene segment (Kaufman and Nixon, 1996). These segments can then be extracted through gel electrophoresis. If the chosen donor organism's genome has been well studied it may be accessible from the genetic library. If the DNA sequence is known, but no copies of the gene are available, it can also be artificially synthesized (Liang *et al*, 2011). Once the isolated gene is ligated into a plasmid then insertion will take place. The plasmid is replicated when the bacteria divided; ensuring unlimited copies of the gene are available. According to Berg and Mertz (2010), before the gene is inserted into the target organism it must

be combined with other genetic elements. These include a promoter and terminator region, which initiate and end transcription. A selected marker gene is added, which in most cases confers antibiotic resistance, thus, make it easier to be determined which cells have been successfully transformed.

APPLICATION OF GENETIC TECHNOLOGIES TO ANIMAL PRODUCTION

Genetically modified mammals are used in research to investigate changes in the phenotype when specific genes are altered. This can be used to discover the function of an unknown gene, any genetic interaction that occurs or where the gene is expressed. It can also produce mammals that are susceptible to certain compounds or stressors for testing in biomedical research (Sathasivlan *et al*, 1999). Some genetically modified mammals are used as models of human diseases and potential treatments and cures can first be tested on them (Vollrath and Knight, 2001). For instance, in 2011 a Japanese-American scientist created genetically modified green-fluorescent cats in order to find therapies for HIV/AIDS and other diseases (Wongsrikeao *et al*, 2011) as Feline immunodeficiency virus (FIV) is related to HIV (Staff, 2012). Other mammals such as pigs expressing human antigens are being used to increase the success of xenotransplantation (Edge *et al*, 1998). Similarly, the enviropig is the trademark for a genetically modified line of Yorkshire pigs with the capability to digest plant phosphorus more efficiently than ordinary unmodified pigs that was developed at the University of Guelph. Enviropigs produce the enzyme phytase in the salivary glands that is secreted in the saliva (Cooke, 2011).

PROBLEMS ASSOCIATED WITH GENETICALLY MODIFIED ANIMALS

Religious groups have raised concerns over whether genetically modified food will be acceptable. Consumer concerns about food quality however first became prominent long before the advent of GM foods. The 1906 Pure Food and Drug Act for instance was the first major US legislation on the subject of food quality (Swann, 2013). This began an enduring concern over the purity and later "naturalness" of food that evolved from a single focus on sanitation, to include other issues such as added ingredients like preservatives, flavours and sweeteners, residues such as pesticides, the rise of organic food as a category and, finally, concerns over GM food. The public came to see the latter as "unnatural", which created a reverse halo effect including all the negatives adduced so far to GM or GMOs (Konnikova, 2013).

REGULATION OF GENETIC TECHNOLOGY

The regulation of genetic engineering concerns the approaches taken by governments to assess and manage the risks associated with the use of genetic engineering technology and the development and release of genetically modified organisms (GMO), including genetically modified crops and genetically modified fish. There are differences in the regulation of GMOs between countries, with some of the most marked differences occurring between the USA and Europe (Gaskell *et al*, 1999). Regulation varies in a given country depending on the intended use of the products of the genetic engineering. For instance, regulation (EC) No. 1829, 2003 of the European Union for instance stated that one of the key issues concerning regulators is whether GM products should be labelled. The European

Commission wants mandatory labelling and traceability in order to allow for informed choices by consumers, avoid potential false advertising and facilitate the withdrawal of products if adverse effects on health or the environment are discovered. According to American Association for the Advancement of Science and the American Medical Association (Hallenbeck, 2014) even the absent of scientific evidence of harm or voluntary labelling could be misleading and will falsely alarm consumer. Labelling of GMO products in the market place is now required in 64 countries. Labelling can be mandatory up to a threshold GM content level (which varies between countries) or voluntary. In Canada and the USA for instance, labelling of GM food is voluntary. In Europe however, all food (including processed food) or feed which contains greater than 0.9% of approved Genetic Modified Organisms must be labelled (Davison, 2010). Similarly, the Cartagena Protocol on Biosafety, an internationally accepted treaty that governs the transfer, handling, and use of Genetic Modified Organisms, was adopted on 29 January 2000 (Redic, 2007). One hundred and fifty-seven countries are members of the Protocol and many use it as a reference point for their own regulations. Africa is also emerging as one of the frontlines in the battle for acceptance (or otherwise) of agricultural biotechnology. A series of regional conference has being held on animal agriculture, initially focusing on Eastern and Central Africa, was conceived by a small group of members of the Animal Production Society of Kenya in 1988. This culminated into an Eastern and Central Africa regional conference on animal agriculture which was held in 1989 in Nairobi and was the precursor of the first All Africa Conference on Animal Agriculture held in Nairobi in 1992. It was at that first conference that a decision was made to have such a meeting of livestock sector scientists and stakeholders every 4 years; the second one was held in Pretoria, South Africa in 1996 and the third one in Alexandria, Egypt in 2000. Thus the conference has been held in eastern, southern, and northern Africa. Similarly, in 2005 a conference was organized by the All Africa Society for Animal Production (AASAP) in association with the Tanzania Society for Animal Production (TSAP) with the overall objective of the conference was to provide an opportunity for African scientists and the broader stakeholder groups of the livestock sector to discuss the potential role of biotechnology in animal agriculture to improve the livelihoods of African people. The conference aimed to attempt, through discussions of a series of papers, to answer the questions: Is Biotechnology a menace or an opportunity to address the pressing needs for sustainable livelihoods of poor people? (Tanzania Society for Animal Production, 2005).

CONCLUSION AND RECOMMENDATION

Despite all these concerns, the importance of gene technology is tremendous. However, further laboratory testing and research will be required to educate the public on the pros and cons of gene technology. There is no doubt that this technology will continue to present intriguing and difficult challenges for 21st century scientist and ethicists; education and meaningful respectful discourse are just the starting point of what is required to tackle such complex ethic issues.

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Evaluation of Naturally Occurring Radionuclide in Soil Samples from Kutayi Mining Sites in Niger State, Nigeria

***Suleiman, I. K & Muhammad, A.**

Department of Physics, Ibrahim Badamasi Babangida University Lapai, Nigeria.

*Correspondence: Email address: ibsguto@gmail.com, Phone number: +2348037000369

ABSTRACT

This study presents results of Activity Concentrations, Absorbed dose rate and the Annual Effective dose rates of naturally occurring radionuclides (^{40}K, ^{232}Th and ^{226}Ra) absorbed in 8 soil samples collected from different areas within the Kutayi mining sites in Niger State, North Central Nigeria. A laboratory γ-ray spectrometry NaI (Tl) was used to carry out the analysis of the soil samples. The values of Activity Concentration for ^{40}K ranged from 428.9269 ± 1.4308 to 93.6236 ± 3.7325$BqKg^{-1}$; for ^{226}Ra it ranged from 50.1738 ± 8.2271 to 13.7891 ± 3.8238$BqKg^{-1}$ and for ^{232}Th the ranged from 52.1095 ± 1.8244 to 18.7457 ± 3.9909 $Bq.Kg^{-1}$. The Absorbed Dose for ^{40}K ranged from 17.8863 ± 0.0597 to 3.9041 ± 0.1556 $ŋGy.h^{-1}$, for ^{226}Ra the range from 23.1803 ± 3.8009 to 3.1614 ± 1.2152 $ŋGy.h^{-1}$ and for ^{232}Th it ranged from 31.4741 ± 1.1019 to 11.3224 ± 2.4105 $ŋGy.h^{-1}$. The total average Absorbed Dose rate of the 8 soil samples collected was 42.5341 $ŋGy.h^{-1}$ and the estimated Annual Effective Dose for the sampled areas range from 0.0355- 0.0756$mSvy^{-1}$ (i.e. 36 – 76 $\mu Sv.y^{-1}$), with an average Annual Effective Dose of 0.0522 $mSv.y^{-1}$ (i.e. 52.2 $\mu Sv.y^{-1}$). These results show's that the radiation exposure level reaching members of the public in the study areas is lower than the recommended limit value of 1 $mSv.y^{-1}$ (UNSCEAR, 2000). Also the mean Radium Equivalents obtained ranged from 64.0280 $BqKg^{-1}$ (KT6) to 135.3245 $BqKg^{-1}$ (KT8). These results show that the recommended Radium Equivalent Concentration is $\leq$ 370 $BqKg^{-1}$. The mean External Hazard Index (H_{ext}) ranged from 0.1730 $Bqkg^{-1}$ (KT6) to 0.3438 $Bqkg^{-1}$ (KT8), while the maximum allowed value of (H_{ext} = 1) corresponded to the upper limit of Ra_{eq} (370 $BqKg^{-1}$) in order to limit the external gamma radiation dose from the soil materials to 1.5 mGy y^{-1}. Furthermore, the mean Internal Hazard Index (H_{ext}) ranged from 0.2250 $Bqkg^{-1}$ (KT6) to 0.5425 $Bqkg^{-1}$ (KT2) .Finally, the mean value of the Excess Alpha Radiation (I_α) ranged from 0.0690 $Bq.Kg^{-1}$ (KT3) to 0.2509 $Bq.Kg^{-1}$ (KT7). All these values for I_α are below the maximum permissible value. It can therefore be said that no radiological hazard is envisaged to dwellers of the study areas and the miners working on those sites.

Keywords: Radionuclides, Soil, Mining, Activity Concentration, Absorbed Dose.

INTRODUCTION

Radionuclide of natural origin is present in both working and public environments, although their activity concentrations vary considerably. Exposures to natural sources are in most cases not a matter for regulatory concern. However, there are situations where exposures to natural sources may warrant consideration as to whether controls should be applied. One such

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situation is where the conditions are conducive to the buildup of elevated concentrations of radon in air. Another situation is the mining and/or processing of material where the activity concentrations of radionuclide's of natural origin in the material itself, or in any material arising from the process, are significantly elevated — such material, has come to be referred to as Naturally Occurring Radioactive Material (NORM). (IAEA-TECDOC-1472 – (2004)). In the past, regulatory attention has been focused mostly on exposures arising from the mining and processing of uranium ores because such activities are part of the nuclear fuel cycle. More recently, attention has been broadened to include exposures from other industrial activities involving NORM, in recognition of the potential for such activities to also give rise to significant exposures of workers and members of the public if not adequately controlled. More and more countries are now including provisions in their national legislation and regulations for the control of exposures to natural sources, and the body of radiological data on such exposures is growing rapidly. In recent times, there has been increase in the solid minerals mining in Niger State where the miners have been operating in recent time are in the exploitation of solid minerals such as copper, gold, quartz, limestone, diamond, tale, gypsum, calcite topaz apatite and a host of other minerals. This work examines the Kutayi mining sites with a view of assessing the activity concentration and effective dose rate of naturally occurring radionuclides materials in these sites. The exploration activities are also associated with a number of environmental degradations. One of such degradation is increase in radiation levels as a result of drilling the earth's crust in search of minerals, thereby stimulating major naturally occurring radioactive nuclei to release more radiations into the environment. Minerals are naturally occurring, solid chemical substances found in –situ in the earth's crust. A rock for example is an aggregate of several minerals. Therefore, it is of significance that the total amount of radioactivity in an environment is accurately known and kept to a level as low as reasonably achievable (ALARA) in order to safeguard the lives of the people, and ensure radiation- pollution free environment. Hence this work is an efforts geared towards protecting people and the environment from accumulation of higher doses of radiation. In the work, measurement of gamma radiation level in the mining sites of the selected areas was performed in the environmental laboratory using gamma spectroscopy system at the Centre for Energy Research and Training (CERT) Ahmadu Bello University (ABU), Zaria, Nigeria. This was used to assess the concentrations of NORM i.e. three most prominent primordial radionuclides, potassium, thorium and radium by determining the base line radioactivity associated with their occurrences in 8 soil samples collected from the Kutayi mining sites of in Muya Local Government area in Niger State, North Central Nigeria. We also analyzed their possible effects on human lives due to occupational and settlement exposures from the mining sites. Finally, given the results obtained, we made some recommendations.

MATERIALS AND METHODS

i. **Sodium Iodine Scintillation Detector:** for detecting the type of radiation and activity concentration.
ii. **Gamma Spectrometer:** Used to determine the energy of gamma rays emitted by radioactive substances
iii. **Multichannel Analyzer (MCA) Device:** used to provide for simultaneous

recording of radiation events in the multiple energy windows

iv. **Masking Tape:** for proper labeling of samples
v. **Weigh Balance:** for weighing samples to know how heavy they are.

Sample Collections and Preparation

The methodology employed in carrying out this work includes careful collection of soil samples (of about 1 kg each) from the mining site as shown in Figure 1, initially filled into polyethylene bags separately from respective points in equal measures sealed and labeled for easy of identification and transporting same to CERT ABU Zaria, Nigeria, for laboratory analysis. In the laboratory, the soil samples were put in an oven at a temperature of 105°C to allow for drying overnight in order to remove any available moisture. The dried samples were crushed and sieved with a mesh having holes each of diameter of 2mm in order to remove organic materials, stones and lumps. Thereafter, the homogenized samples were packed to fill cylindrical plastic beakers of 7cm by 6cm diameter which is the same as geometry of the counting detector. This satisfies the selected optimal sample container height.(Ibeanu, 2000). The samples were carefully sealed using vaseline, candle wax and masking tape in order to prevent trapped radon gas from escaping.They were then weighed on a digital weighing balance with a precision of ±0.01g . Each plastic beaker accomodated approximately 300g of the soil sample. The sealed samples were kept for a minimum period of 30 days so as to allow for ^{226}Ra and its short-lived progenies to reach secular radioactive equilibrium before gamma counting (Okeyode and Akanni (2009). The samples taken from Kutayi were labeled as KT1- KT8.

Figure 1: Kutayi Mining Site.

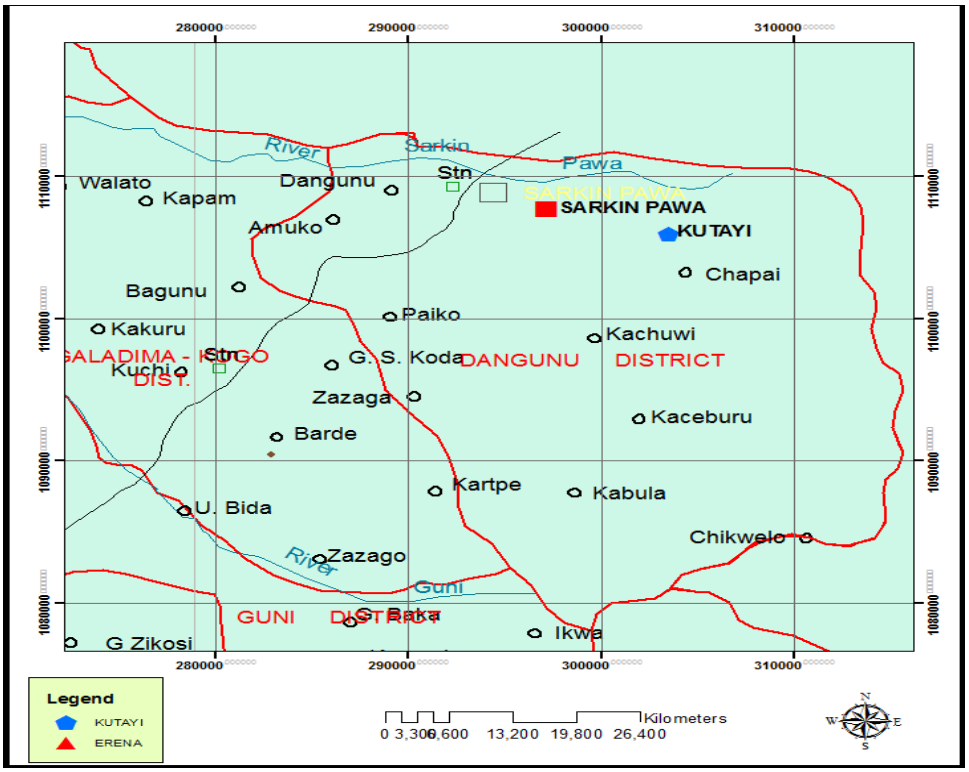


Figure 1a: Map of Kutayi Mining Site (Adapted from Abdulfatai *et al.,* 2014)

The Experimental Set-up and Procedures for Sodium Iodine Thallium (NaI(Tl)) Detector Gamma Spectroscopy System.

The gamma-ray spectrometry operation of the NaI(Tl) system was done in four procedures. The initial procedure was to ensure equipment settings were adhered to in terms of voltage supply to the equipment regulation as specified, however the initial high voltage supply was switched off.

The startup procedure was to ensure that the operator was consciously starting the experiment by turning on the set up from the power button to booting of the computer according to laid down regulations. The operating voltage for this equipment which was given as 900 volts was attained by turning the control knob in steps of 100, until the desired level of 900 Volts was attained.

The spectrum acquisition procedure which puts the set up in the spectra acquisition mode was carefully executed. After the computer booting process, the acquisition command was preset by setting the live time limits (which was about 29000 seconds), then the analog –to- digital converter (ADC) set up and the manual control to adjust the amplifier gain was setup according to specification and finally the startup command was given to commence counting for the background of the sample, for a specified time limit. The acquired spectrum and values of the live time were duly recorded in the already created save medium.

After the completion of the experiment, the shutdown procedure allowed for proper demobilization of the equipment, in accordance with the specified protocol, most especially the stepwise reduction of the voltage level from the highest operating point of 900 volts down to the 0 level. Then the computer was shut down.

The peak area of each energy in the spectrum was used to compute the activity concentrations in each of the soil sample by the use of the equation (1):

$$C\ (Bq.kg^{-1}) = Cn/C_{fk} \quad (1)$$

where C = activity concentration of the radionuclides in the sample given in $BqKg^{-1}$, Cn = Count rate (count per second) = count per second (cps) = Net/Live time, C_{fk} = calibration factor of the detecting system.

Calibration and Efficiency Determinations

Calibration of the system for the energy and efficiency were done with two calibration point source, Cs-137 and Co-60. These were done with the amplifier gain that gives 72% energy resolution for the 66.16 Kev of Cs-137 and counted for 30 minutes.

Standards to Check for the Calibration

The standards used to check for the calibration are the IAEA Gamma Spectrometric reference materials RGK-1 for K-40, RGU -1 for the Ra-226 (Bi – 214 peak) and RTG -1 For Th-232 (Ti - 208). Background area counts corresponding to the three radionuclides (i.e. ^{40}K, ^{226}R &^{232}Th) were measured and the evaluation of the results evaluated. The background count rate was done for 29000 seconds and the results obtained are given Table (1):-

The gross area count G_c is related to the area count by through the expression (Okeyode and Akanni-2009).

$$N_c = G_c - B_c \quad (2)$$

where B_c is the background area count, (area count recorded by the detector in the absence of the samples). Using equation (2), the net area counts N_c was calculated from the gross area counts G_c generated by the gamma spectroscopy system. Consequently, the net count per second (cps) was also calculated for the three radionuclides (^{40}K, ^{226}R &^{232}Th).

Table 1: Background Count Rate used in the Evaluations of the Samples

Serial	Isotope	Background Count (CPS)	Background Count (Bq/kg)
1	^{40}K	0.2219± 0.017	345.1011±25.5940
2	^{226}R	0.0229± 0.0109	26.5353± 12.6304
3	^{232}Th	0.01202± 0.0078	137.0582± 8.8940

Activity Concentrations

The activity concentrations for the natural radionuclides in the measured samples were computed using the following relation (Okeyode and Akanni, 2009).

$$A_c = \frac{Nc}{Lt}\sigma - 1 \qquad (3)$$

where L_t is the lifetime of the counting, and σ is the conversion factor. It is constant for each radionuclide at a constant geometry and it is the characteristics of the efficiency of NaI(Tl) detector assembly used in the analysis of the sample.

In Table 2, we presents the values of the conversion factor (σ) for the ^{40}K, ^{226}R and ^{232}Th (Umar *et al.*, 2012).

Table 2: Presents the Values of the Conversion Factor (σ) for the ^{40}K, ^{226}R and ^{232}Th.

Serial	Nuclides	CPS/Bq-kg^{-1}	Gamma ray line (KeV)
1	^{40}K	0.000643	1460
2	^{226}R	0.000863	1764
3	^{232}Th	0.000877	2614.5

Absorbed Dose Rates (D)

The Absorbed Dose (Equation 4) is the energy imparted by radiation per unit mass of irradiated material. The gray **(Gy),** which has units of (j/kg), is the SI unit of absorbed dose, and is the amount of radiation required to deposit 1 joule of energy in a kilogram of any kind of matter

$$D(\text{ŋGy.h}^{-1}) = 0.0417A^{40}_{K} + 0.462A^{226}_{Ra} + 0.604A^{232}_{Th} \qquad (4)$$

where $A^{40}K$, $A^{226}R$ and $A^{232}Th$; are the activity concentrations of ^{40}K, ^{226}R and ^{232}Th respectively in Bq.kg^{-1}. The conversion factors 0.0417, 0.462 and 0.604 are expressed in ŋGy.h^{-1}/ Bq.kg^{-1}**.** The absorbed dose rates in air are usually related to human absorbed dose in order to assess radiological implications.

Annual Effective Dose Rates (E_d)

To estimate the annual effective dose rates, the conversion coefficient from absorbed dose in air to effective dose (0.7Sv.Gy^{-1}) and outdoor occupancy factor (0.2) proposed by (UNSCEAR 2000; Harb *et al*., 2010 and Agbalagba and Onoja, 2011) as shown in equation 5 were used.

Annual effective dose rate (mSv/yr) (E_d) = D (ŋGy.h^{-1})× 8760 (hr.y^{-1}) ×0.2 × (0.7 x 10^{3}mSv) x (10^{9}ŋGy)$^{-1}$ (5)

where 8760 (i.e. 365 x 24 hours of the day) is the numbers of hours in one year.

Equation (5.) simplifies to:

$$E_d = D \times 1.21 \times 10^{-3}\,(\text{mSv/yr}) \qquad (6)$$

where E_d is the annual effective dose rate in $(\text{mSv.y}^{-1})$and D is the value of absorbed dose rate earlier calculated from equation (4). Table (5) and figure (4) present the calculated Annual Effective Dose Rates (E_d) (mSv.y^{-1}) for the investigated soil samples.

Radium Equivalent

The magnitude of radiation exposure from natural soil materials is strictly connected with the Radium, Thorium and Potassium contents in the soil material and also on ventilation

conditions; hence the Ra-equivalent concentration Ra_{eq} is a useful and instructive quantity which is internationally accepted parameters that is applied to describe the suitability or otherwise of a soil material for construction or farming purposes. The radium equivalent in the samples was estimated using equation (7):

$$Ra_{eq} = C_{Ra} + (C_{Th} \times 1.43) + (C_k \times 0.077) \leq 370 \text{ Bqkg}^{-1} \quad (7)$$

The value of this parameter should be less than 370 $Bqkg^{-1}$ so as to keep the annual radiation dose below 1.5 mGy y^{-1} (UNSCEAR 2000).

External Hazard Index

The external hazard index (H_{ext}) is a criterion used for evaluation of external exposure to gamma radiation in the air. This has served as safety criterion in many countries of the world. It was proposed by Krisiuk *et al.* (1971) and supported by Stranden (1976) and was used by Beretka and Mathew in 1985. In order to limit the external gamma radiation dose from the soil materials to 1.5 mGy y^{-1} this index should be equal to or less than unity ($H_{ext} \leq = 1$). The maximum allowed value (H_{ext} = 1) corresponds to upper limit of Ra_{eq} (370 $BqKg^{-1}$). (Beretka and Matthew), A widely used hazard index (reflecting external exposure) called the external hazard index Hex is defined by equation 8 (UNSCEAR 2000).

$$H_{ex} = C_{Ra}/370 + C_{Th}/259 + C_k/4810 \quad (8)$$

Internal Hazard Index

Radon and its short lived progeny are also hazardous to the respiratory organs. Thus in addition to the external hazard index, internal exposure to radon and its daughter progenies is quantified by the internal hazard index H_{in} which is given by the equation (UNSCEAR 2000) :

$$H_{in} = C_{Ra}/185 + C_{Th}/259 + C_k/4810 \quad (9)$$

The values of the indices (H_{ex}, H_{in}) must be less than unity for the hazard to be negligible (Agbalagba and Onoja, 2011).

Excess Alpha Radiation

The use of soils from and around these mining sites may pose external radiation and internal hazard to the dwellers and miners as a result of inhalation of radon and its decay products, which are predominantly alpha emitters. The excess alpha radiation due to radon inhalation originating from soil materials is estimated using equation 10 (Isinkaye and Shitta, 2009):

$$I_\alpha = C_{Ra}/ 200 \quad (10)$$

Recommended Dose Limit Exposure to Natural Radiation Sources.

Table 10 gives an average worldwide exposure to natural radiation sources for occupational persons and members of the public.

Table 10: Recommended Dose Limit Exposure to Natural Radiation Sources. (HRD-WHS-GUI-144.6)

APPLICATION	DOSE LIMIT	
	OCCUPATIONAL EXPOSED PERSON	MEMBER OF THE PUBLIC
Effective Dose	20 mSv per year average over 5 consecutive calendar years	1 mSv in a year
Equivalent dose to:		
1. Lens of the eye	150 mSv in a year	15 mSv in a year
2. Skin	500 mSv in a year	50 mSv in a year
3. Hands and Feet	500 mSv in a year	No limit specified

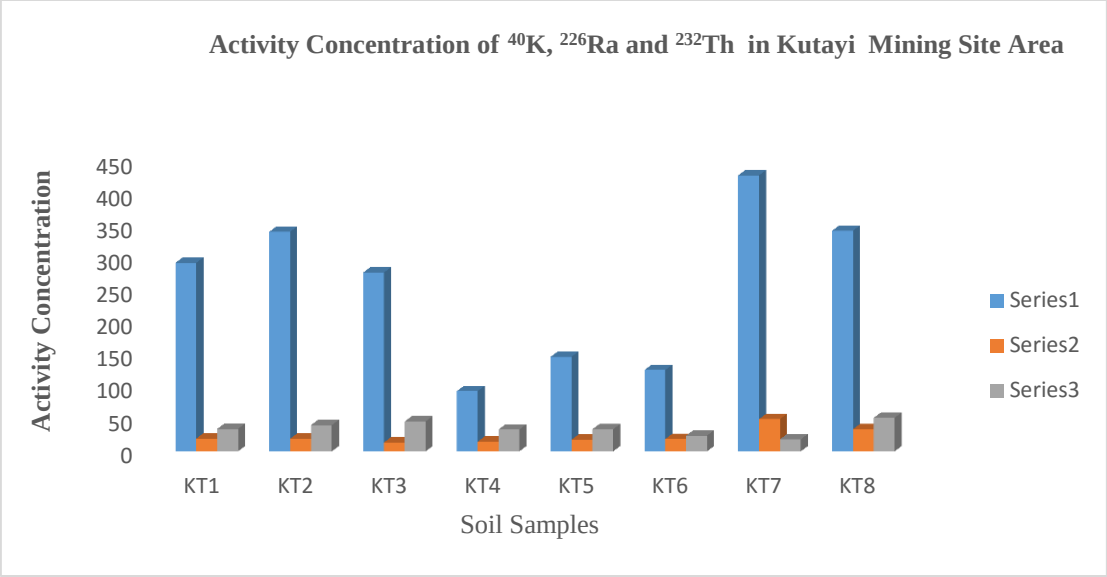
NOTE: With further provision that the effective dose must not exceed 50mSv in any single year (provided the 100 mSv (max) dose averaged over 5 years is maintained). Recommended tissue weighting factors are listed in the Radiation Safety guidelines to deter

RESULTS

All the raw data obtained from the detector were converted to conventional units using calibration factors to determine the activity concentrations of ^{40}K, ^{226}R and ^{232}Th respectively. Using equation (3), the activity concentrations were calculated and the results obtained are presented in Table (3) and Figure (2).

Table 3: Activity Concentration of ^{40}K, ^{226}Ra and ^{232}Th in Kutayi Mining Sites.

Soil Sample ID	Activity Concentration of ^{40}K in $Bqkg^{-1}$	Activity Concentration of ^{226}Ra in $Bqkg^{-1}$	Activity Concentration of ^{232}Th in $Bqkg^{-1}$	Total Activity Concentration in $Bqkg^{-1}$
KT1	293.0016	20.0464	34.8917	347.9397
KT2	341.2131	19.6987	40.8210	401.7328
KT3	277.7605	13.7891	46.5222	338.0718
KT4	93.6236	15.0637	33.9795	142.6668
KT5	146.9673	18.0765	34.3216	199.3654
KT6	126.4386	19.2352	24.5154	170.1892
KT7	428.9269	50.1738	18.7457	497.8464
KT8	342.7683	34.4148	52.1095	429.2926
Total	2045.6999	190.4982	285.9066	2527.1047
Mean	255.7125	23.8123	35.7383	315.8881



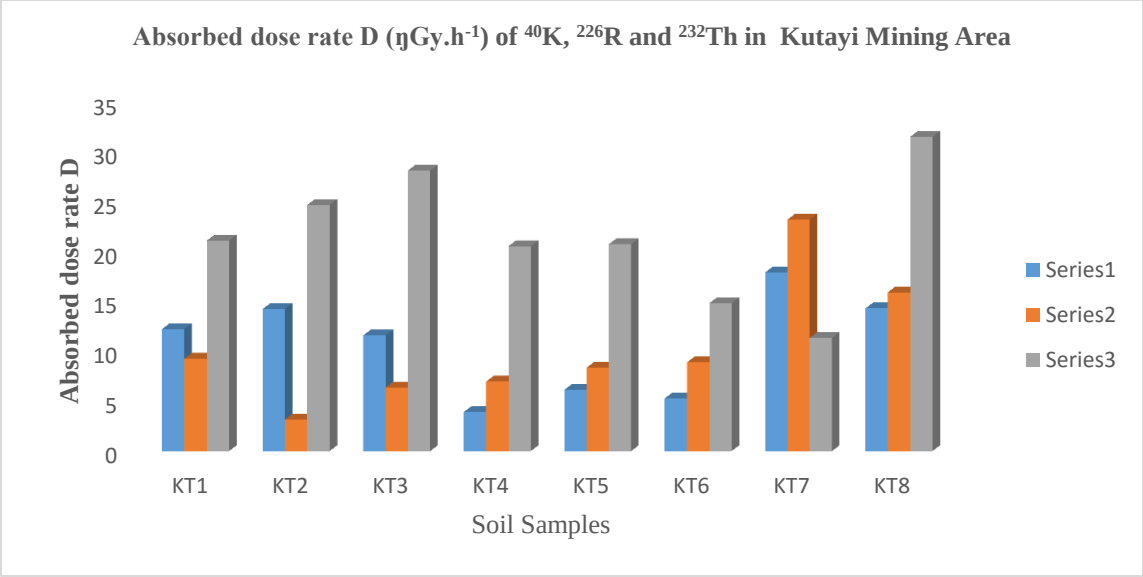
KEY: Series 1= ^{40}K ; Series 2 = ^{226}Ra and Series = ^{232}Th

Figure 2: Activity Concentration of ^{40}K, ^{226}Ra and ^{232}Th in Kutayi Mining Sites.

The external absorbed dose rate D (ŋGy.h^{-1}) due to gamma radiation in air at 1 meter height above the ground level due to activity concentrations of ^{40}K, ^{226}R and ^{232}Th for the 7 soil samples were evaluated based on international standard guidelines using equation (4) (UNSCEAR, 2000). Table (4) and figure (3) present the results of the external Absorbed Dose rate D (ŋGy.h^{-1}) in air at 1m above the ground level due to activity concentrations of ^{40}K,

Table 4: Absorbed Dose Rate D (ŋGy.h^{-1}) of ^{40}K, ^{226}R and ^{232}Th in Kutayi mining Sites

Serial	Sample ID	^{40}K (ŋGy.h^{-1})	^{226}Ra (ŋGy.h^{-1})	^{232}Th (ŋGy.h^{-1})	Total D (ŋGy.h^{-1})
1	KT1	12.2182	9.2614	21.0746	42.5542
2	KT2	14.2286	3.1614	24.6559	42.0459
3	KT3	11.5826	6.3706	28.0994	46.0526
4	KT4	3.9041	6.9594	20.5236	31.3871
5	KT5	6.1285	8.3513	20.7302	35.2100
6	KT6	5.2725	8.8867	14.8073	28.9665
7	KT7	17.8863	23.1803	11.3224	52.3890
8	KT8	14.2934	15.8996	31.4741	61.6671
9	Total	85..5142	82.0707	172.6875	340.2724
10	Mean	10.6893	10.2588	21.5859	42.5341



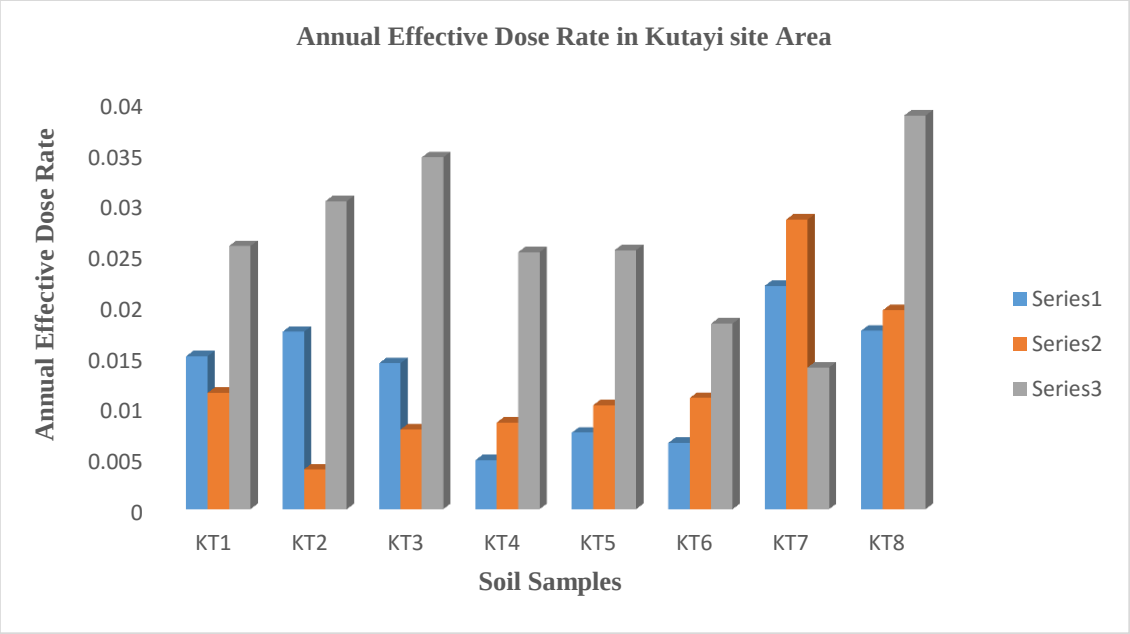
KEY: Series 1= ^{40}K ; Series 2 = ^{226}Ra and Series = ^{232}Th

Figure 3: Absorbed Dose Rate D (ηGy.h^{-1}) of ^{40}K, ^{226}R and ^{232}Th in Kutayimining Sites.

The annual effective dose rates (mSv.yr^{-1}) was calculated using equation 6 and the results shown in Table 5 and Figure 4:

Table 5: Annual Effective Dose Rates $E_d(\mathbf{mSv.y^{-1}})$ for Kutayi mining Sites

Serial	Sample ID	^{40}K $(mSv.y^{-1})$	^{226}R $(mSv.y^{-1})$	^{232}Th $(mSv.y^{-1})$	Total D $(mSv.y^{-1})$
1	KT1	0.0150	0.0114	0.0258	0.0522
2	KT2	0.0174	0.0039	0.0302	0.0516
3	KT3	0.0143	0.0078	0.0345	0.0565
4	KT4	0.0048	0.0085	0.0252	0.0385
5	KT5	0.0075	0.0102	0.0254	0.0432
6	KT6	0.0065	0.0109	0.0182	0.0355
7	KT7	0.0219	0.0284	0.0139	0.0642
8	KT8	0.0175	0.0195	0.0386	0.0756
9	Total	0.1049	0.1006	0.2118	0.4173
10	Mean	0.0131	0.0126	0.0265	0.0522



KEY: Series 1= ^{40}K; Series 2 = ^{226}Ra and Series = ^{232}Th

Figure 4: Annual Effective Dose Rate in Kutayi Mining Sites.

The results obtained for Ra_{eq} using equation 7 are presented in Table (6) and figure (5). The results show that, the mean radium equivalents ranged from 82.7770 $BqKg^{-1}$ (ER4) to 171.9653 $BqKg^{-1}$(ER2).

The results of the External Hazard Index obtained using equation 8 are shown in Table 7 and Figure 6**.** The mean external hazard index ranged from 0.2236 $Bqkg^{-1}$ (ER4) to 0.5003 $Bqkg^{-1}$ (ER6).

Table 6: Radium Equivalent in the Kutayi Mining Site.

Soil Sample ID	Radium Equivalent Ra_{eq} of the Soil Samples ($Bqkg^{-1}$)
KT1	92.5026
KT2	104.3461
KT3	101.7035
KT4	70.8634
KT5	78.4728
KT6	64.0280
KT7	110.0076
KT8	135.3245

Table 7: External Hazard index in Kutayi Mining Site of the Soil Samples

Soil Sample ID	External Hazard index of the Soil Samples
KT1	0.2498
KT2	0.2817
KT3	0.2746
KT4	0.1914
KT5	0.2120
KT6	0.1730
KT7	0.2972
KT8	0.3438

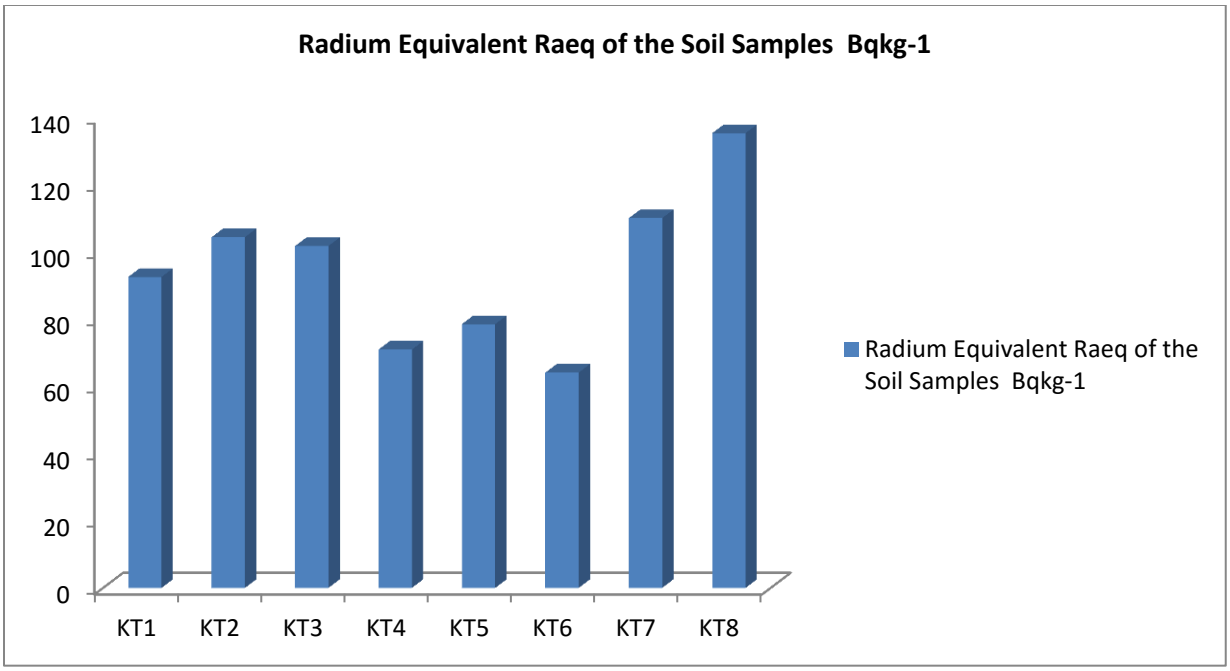


Figure 5: Radium Equivalent of the soil sample in Kutayi Mining Site Area

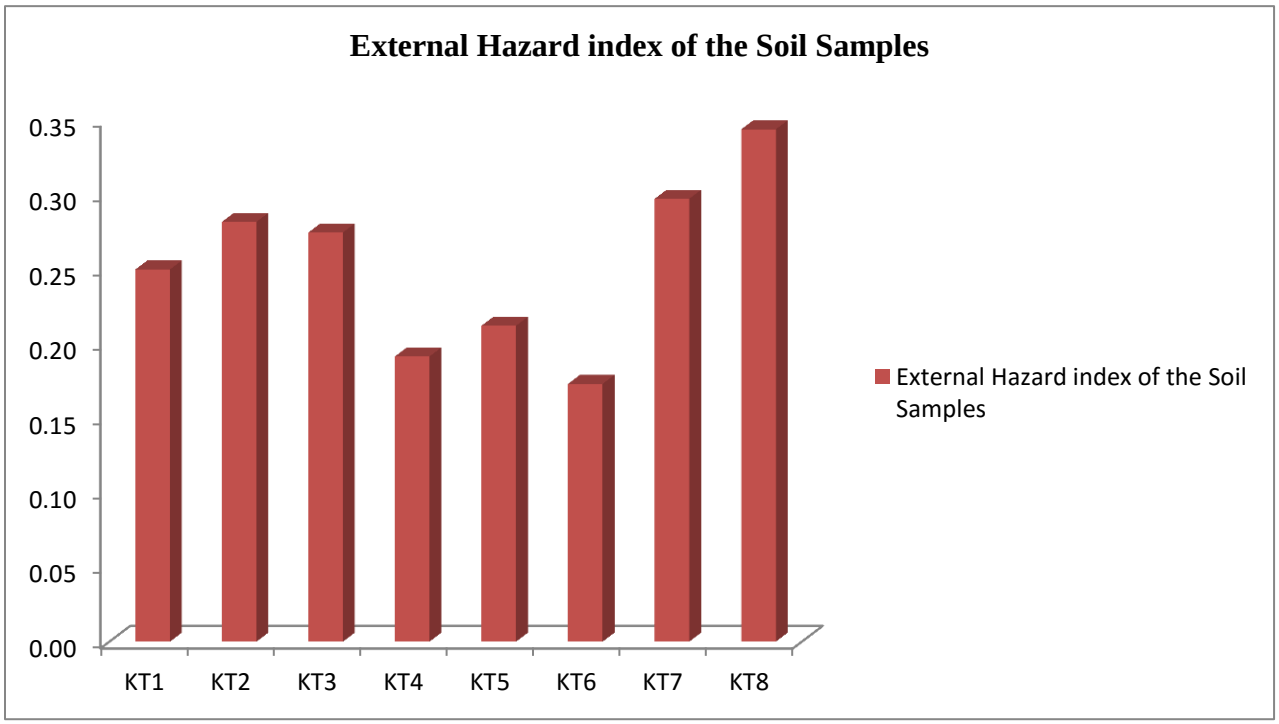


Figure 6: External Hazard Index of the soil sample in Kutayi Mining Site Area

The results of the internal hazard index obtained from equation 9 are shown in Table 8 and Figure 7.

Table 8: Internal Hazard Index of the Soil Samples

Soil Sample ID	Internal Hazard Index of the Soil Samples
KT1	0.3040
KT2	0.5425
KT3	0.3118
KT4	0.2321
KT5	0.2608
KT6	0.2250
KT7	0.4328
KT8	0.4585

The mean value of excess alpha radiation (I_α) calculated from equation 10ranged from 0.1165 $Bq.Kg^{-1}$ to 0.3766 $Bq.Kg^{-1}$, These results are shown in Table 9 and Figure 8**.**

Table 9: Excess Alpha Radiation of the Soil Samples in Kutayi Mining Site.

Soil Sample ID	Excess Alpha Radiation of the Soil Samples
KT1	0.1002
KT2	0.0985
KT3	0.0690
KT4	0.0753
KT5	0.0904
KT6	0.0962
KT7	0.2509
KT8	0.1721

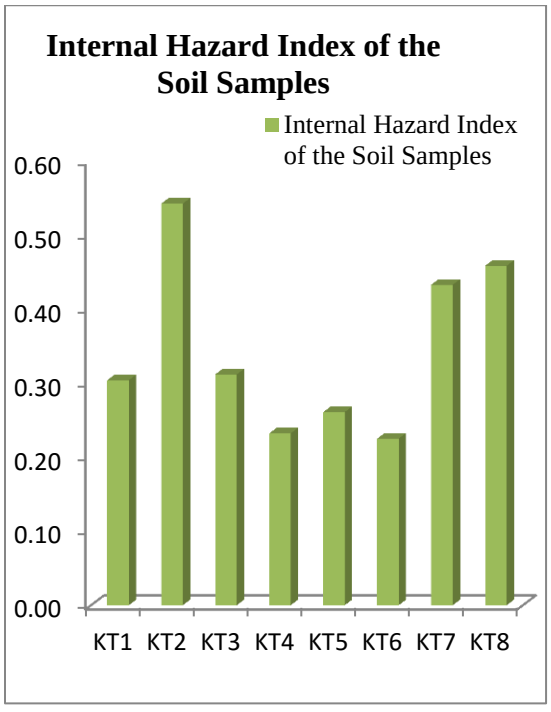


Figure 7: Internal Hazard Index of the soil sample in Kutayi Mining Site Area

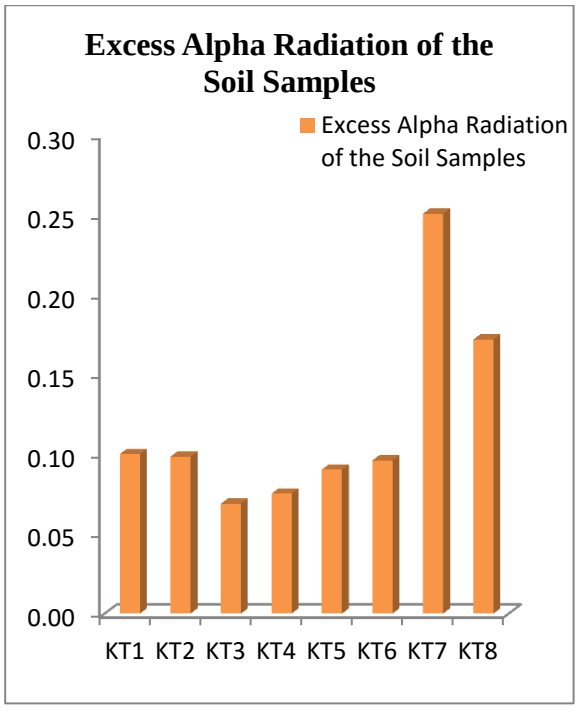


Figure 8: Excess Alpha Radiaton of the soil sample in Kutayi Mining Site Area

DISCUSSION

The method of gamma spectrometry was used to measure the radioactivity concentration of soil samples collected from the mining sites of Kutayi in Muya Local Government area of Niger State, North Central Nigeria. The result shows that, the highest radioactivity concentration of ^{40}K was found in soil sample KT7 with 428.9269BqKg^{-1} this high value could be due to the presence of abundant radioactive minerals such as Kaolinite and Feldspars in the sample. The radioactivity concentration order was followed by soil sample KT8 with 342.7683Bqkg^{-1}. The least radioactivity concentration of ^{40}K was found in soil samples KT4with 93.6236Bqkg^{-1}.

The highest radioactive concentration of ^{226}Ra was found in soil sample KT7with 50.1738Bqkg^{-1}. This high value could be due to high presence of Uranium minerals such as Uraninite, Zircon, and Monazite. The radioactivity concentrations order of ^{226}Ra were followed by soil samples KT8 and KT1 with 34.4148 and 20.0464Bqkg^{-1} respectively. Soil sample KT34 had the lowest radioactivity concentration of 13.7891 Bqkg^{-1}.Also, the highest radioactive concentration of ^{232}Th was found in soil sample KT8with 52.1095 Bqkg^{-1}.This could be due to presence of abundant radioactive Thorium minerals such as Monazite, Zircon and Thorianite(Okeyodeand Akanni., 2009).The least radioactivity concentration of ^{232}Th was also found in soil sample KT7with 18.7457 Bqkg^{-1}.The result also shows that the total concentration of ^{266}Ra is 190.4982BqKg^{-1} whichis less than that of ^{232}Th which has a total concentration of 285.9066 BqKg^{-1}, while ^{40}K leads the table of radioactivity concentrations with total value of 2045.6999 BqKg^{-1}.

From Table 4, and Figure 3, the absorbed dose rate due to the three radionuclides is highest for soil sample KT8 with absorbed dose rate of 61.6671 ŋGy.h^{-1}, this might be due to accumulation of mineral sands from different mining sites. The average absorbed dose rate of the soil samples is 42.5341 ŋGy.h^{-1}.According to Table 4,^{232}Th had the highest value of total absorbed dose rate of 172.6875ŋGy.h^{-1} among the three radionuclides detected in the soil samples collected, thus it had the highest dose level in the study areas follow by ^{40}K which has the total absorbed dose rate of 85.5142ŋGy.h^{-1}, while^{226}R had the least total absorbed dose rate of 82.0707 ŋGy.h^{-1}.

From Table 5 and Figure 4, the annual effective dose rate in air at the study area ranged from 0.0355- 0.0756 mSvy^{-1} (i.e. 36 – 76 µSv.y^{-1})and the average annual effective dose rate in air at the study area was 0.0522 mSv.y^{-1} which is slightly less than the maximum recommended world average outdoors exposure to external terrestrial radiation. (UNSCEAR, 2000). Thus, the exposure level for the members of general public is still within the recommended value of 1 mSv.y^{-1}IAEA, (1999) & UNSCEAR (2000;).

Therefore, this is an indication that the mining activities in the study areas do not appear to have any impact on the radiation burden of the environment.

The Ra-equivalent concentration (Ra_{eq}) should be less than 370 Bqkg^{-1} so as to keep the annual radiation dose below 1.5 mGy y^{-1}(UNSCEAR, 2000). The results obtained for Ra_{eq} as presented in Table 6, shows that, the mean radium equivalents ranged from 64.0280 BqKg^{-1} (KT6) to 135.3245 BqKg^{-1} (KT8) . This result shows that the recommended radium equivalent concentration of $\leq$ 370 BqKg^{-1}for

soil materials to be used for dwellings by OECD (Organization for Economic Cooperation Development) Ahmad Hussein, 1998) is applicable to the soils collected around the mine sites. This behavior of radium equivalent activity is similar to that of radiation dose rate i.e. if the value of absorbed dose rate is high, the value of radium equivalent activity is also high and vice-versa.

The external hazard index (H_{ext}) is also a criterion used for evaluation of external exposure to gamma radiation in the air.This has served as a safety criterion in many countries of the world. It was proposed by Krisiuk *et al.* (1971) and supported by Stranden (1976) and was used by Berektka and Mathew (1985).In order to limit the external gamma radiation dose from the soil materials to 1.5 mGy y^{-1} this index should be equal to or less than unity ($H_{ext} \leq = 1$). The maximum allowed value (H_{ext}= 1) corresponds to upper limit of Ra_{eq} (370BqKg^{-1}) (Beretka and Matthew, 1985). The results obtained are shown in Table 7 and Figure 6, which show that the, mean external hazard index (H_{ext}) ranged from 0.2250 Bqkg^{-1} (KT6) to 0.5425 Bqkg^{-1} (KT2).

The use of soils from and around these mining sites may pose external radiation and internal hazard as a result of inhalation of radon and its decay products, which are predominantly alpha emitters to dwellers and miners. The mean internal hazard index (H_{ext}) ranged from0.2250 Bqkg^{-1} (KT6) to 0.5425 Bqkg^{-1} (KT2) as shown in Table 8 and Figure 7.

The mean value of Excess Alpha Radiation (I_α) ranged from 0.0690 Bq.Kg^{-1} (KT3) to 0.2509 Bq.Kg^{-1} (KT7) and this is presented in Table 9 and Figure 8. All these values for I_α are below the maximum permissible value which is I_α=1 which corresponds to 200 Bq.Kg^{-1}.It can therefore be said that no radiological hazard is envisaged to dwellers and miners in the of study areas.

It can therefore be said that no radiological hazard is envisaged to dwellers of Kutayi mining **Sites**and the miners working on these sites.

CONCLUSION

This study presents results of Activity Concentrations, Absorbed dose rate and the Annual Effective dose rates of naturally occurring radionuclides (^{40}K, ^{232}Th and ^{226}Ra) absorbed in 8 soil samples collected from different areas within the Kutayimining sites in Niger State, North Central Nigeria.

A laboratory γ-ray spectrometry NaI (Tl) at the Centre for Energy Research and Training (CERT), Ahmadu Bello University Zaria, was used to carry out the analysis of the soil samples. The values of Activity Concentration for ^{40}K ranged from 428.9269 ± 1.4308 to 93.6236 ± 3.7325BqKg^{-1}; for ^{226}Ra it ranged from 50.1738 ± 8.2271 to 13.7891 ± 3.8238BqKg^{-1} and for ^{232}Th the ranged is from 52.1095 ± 1.8244 to 18.7457 ± 3.9909 Bq.Kg^{-1}.

The Absorbed Dose for ^{40}K ranged from 17.8863 ± 0.0597 to 3.9041 ± 0.1556 ŋGy.h^{-1}, for ^{226}Ra the range is from 23.1803 ± 3.8009 to 3.1614 ± 1.2152 ŋGy.h^{-1} and for ^{232}Th range from 31.4741 ± 1.1019 to 11.3224 ± 2.4105 ŋGy.h^{-1}. The total average Absorbed Dose rate of the 8 soil samples was 42.5341 ŋGy.h^{-1} and the estimated Annual Effective Dose for the sampled areas range from 0.0355- 0.0756mSvy^{-1} (i.e. 36 – 76 µSv.y^{-1}), with an average Annual Effective Dose of 0.0522 mSv.y^{-1} (i.e. 52.2 µSv.y^{-1}).

These results showthat the radiation exposure level reaching members of the public in the study areas is lower than the recommended limit value of 1 $mSv.y^{-1}$ (UNSCEAR, 2000).

Also the mean Radium Equivalents obtained ranged from 64.0280 $BqKg^{-1}$ (KT6) to 135.3245 $BqKg^{-1}$ (KT8). These results show that the recommended Radium Equivalent Concentration is $\leq$ 370 $BqKg^{-1}$ which is the requirement for soil materials to be used for dwellings; this implies that the soil from this site is suitable use for residential buildings.

The mean External Hazard Index (H_{ext}) ranged from 0.1730 $Bqkg^{-1}$ (KT6) to 0.3438 $Bqkg^{-1}$ (KT8). While the maximum allowed value of (H_{ext}= 1) corresponds to the upper limit of Ra_{eq} (370 $BqKg^{-1}$) in order to limit the external gamma radiation dose from the soil materials to 1.5 mGy y^{-1}. That is, this index should be equal to or less than unity ($H_{ext} \leq$ = 1). Furthermore, the mean Internal Hazard Index (H_{ext}) ranged from 0.2250 $Bqkg^{-1}$ (KT6) to 0.5425 $Bqkg^{-1}$ (KT2) .

Finally, the mean value of the Excess Alpha Radiation (I_α) ranged from 0.0690 $Bq.Kg^{-1}$ (KT3) to 0.2509 $Bq.Kg^{-1}$ (KT7). All these values for I_α are below the maximum permissible value of I_α=1 which corresponds to 200 $Bq.Kg^{-1}$.

It can therefore be said that no radiological hazard is envisaged to dwellers of the study areas and the miners working on those sites.

AUTHORS' CONTRIBUTIONS

This study was carried out by the two authors. Author IKS collected and prepared the field samples, participated in the laboratory procedures, performed the statistical analysis and wrote the draft of the manuscript and also designed the study and contributed to the statistical analysis. Author AM contributed in the analysis of the study. The authors read and approved the final manuscript.

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COMPETING INTEREST

There is no competing interest whatsoever that could have influenced the results of this study in any manner

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Analysis of Boron Content of Aqueous Environmental Samples

Ibrahim A.A.

Department of Chemistry, Ibrahim Badamasi Babangida University Lapai, Nigeria

*Correspondence: Email address: alituraki@gmail.com; Phone number: +2348039719683

ABSTRACT

Boron exists naturally in aqueous environment as boric acid or tetrahydroxyborate(1-) ion. Analysis of boron content in aqueous environmental samples was carried out to determine if tide levels affect boron concentration. The analysis was also undertaken to investigate the impact of anthropogenic activities in the sampled locations. A spectrophotometric analytical method based on solvent extraction of boron complex with azomethine-H reagent was used for the analysis of the water samples. Statistical analysis was carried out to test if the results obtained were significantly different. A study to determine the effects of dilution on boron extraction was also carried out. The boron concentrations found for seawater range from 3.65 (Conwy) to 6.57 ppm (Rhos on Sea) at high tide and 1.30 (Llanfairfechan) to 6.64 ppm (Rhos on Sea) at low tide. These data were consistent with the reported literature data. The results obtained tend to suggest that anthropogenic activities are insignificant and do not affect the boron concentrations around north Wales' coastal waters environments. It also shows that tide level does not affect boron concentration in seawater.

Keywords: Boron, azomethine-h, tide, boric acid

INTRODUCTION

Boron, like most metals, exists in associated form in the environment as borates, borosilicates and boric acid (Bryjak, Wolska, & Kabay, 2008). Environmental concentration of boron is variable, determined by the geochemistry of the soil, freshwater, seawater, rocks and anthropogenic pressure of the local environment. The range of environmental boron concentration is given as 0.0001 to 10 ppm (Lee & Aronoff, 1967). Boron concentration in crustal rock is about 9 ppm, 5 ppm in basalts and as high as 100 ppm in shales (Schubert, 2003). In aqueous environment boron exists as boric acid or tetrahydroxyborate(1-) ion. The concentration of boron in water varies for different waters. Seawater concentration ranges from 0.5 to 9.6 ppm with a global average of 4.5 ppm (Schubert, 2003). Lee *et al* (2010) in their studies observed that boron concentration varies with salinity and chlorinity. Boron concentration in freshwaters ranges from less than 0.01 to 1.5 ppm (WHO, 1998) depending on factors such as the proximity to marine coastal regions, the geochemistry of the drainage area and the presence of boron rich

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deposits and local anthropogenic pressures. Higher levels of about 360 ppm have been reported in areas of high boron reserves in some parts of Turkey. The major boron inputs into freshwater are precipitation and soil and underground water drainage (Kabay *et al*., 2015; William, 1994).

Over the years, there has been concern about the increasing level of environmental boron (Kabay*et al*., 2015, William, 1994; Matthews, 1974). Natural and anthropogenic activities have contributed to increased levels of boron in water. Weathering of clay rich sedimentary rocks containing boron has led to the release of boron into the world's water bodies. Oceans and geothermal streams are natural sources that have persistently increased boron concentration in water. Anthropogenic sources of boron include the discharge of industrial effluents, synthetic herbicides and fertilizers from agricultural activities, combustion of fossil fuels and the glass and ceramic manufacturing industries. Domestic use of detergents, soaps, cleaners, cosmetics and sewage discharge into the environment have led to a slight increase in the level of boron (John and Keith, 1998). Discharge of sewage and sewage sludge into water has resulted in increased levels of boron in water, which is not removed by conventional water treatment (William, 1994).

Fate of environmental boron

The survival of waterborne boron (boric acid) in the environment is dependent on the rate and frequency of the adsorption-desorption reactions occurring. These reactions result in the adsorption of boric acid by soils and sediments and these reactions are dependent on the pH and the concentration of boron in solution with a pH of 7.5 to 9.0 optimum for boron adsorption. Weathering slowly releases boron into the environment from sediments of borosilicate and borate reserves (WHO, 2009; Nasef, *et al* 2014). Fyodor (2015), in his review of boron in the environment, reported that the concentration of boron in the environment is governed by the three major cycles of the atmosphere/ocean/land cycle, the marine ecosystem and the soil-plant terrestrial ecosystem. The marine and soil-plant terrestrial ecosystem is controlled by biological activities and plant primary production and utilization.

Behaviour of boric acid in water

Boric acid, being a very weak acid, does not dissociate in water as a Bronsted acid instead it accepts a hydroxyl ion and acts as a Lewis acid forming a tetrahydroxyborate(1-) ion. The equation in Figure **1** gives the behaviour of boric acid in water in continuous interchange with tetrahydroxyborate(1-) ion.

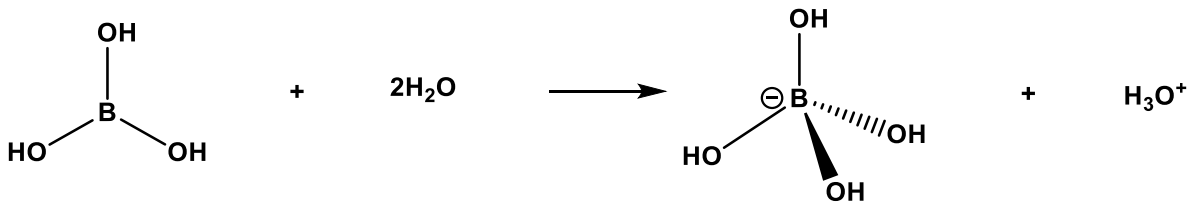

Figure **1:** Illustration of the aqueous of chemistry of boric acid (Gluer *et al*., 2015).

Applications of boron and its compounds

Zinc borate, $Zn[B_3O_4(OH)_3]$, is a boron derivative and is used as an additive in polymers to give them special properties such as fire retardance (Schubert, 2003). Ulexite, a borate mineral is used in the production of fiberglass and micronutrient fertilizers in boron deficient soil. Hydroboracite is used mainly for the production of ceramic glass (Schubert, 2003). Kernite is another important industrial mineral used in the manufacture of refined borax, boric acid and borates (Schubert, 2003; William, 1994).

Borates are used in the preservation of wood against attack by wood boring insects and in the paper industry, they are used as carriers of alkanility in the kraft pulping process (Schubert, 2003). Peroxoborates are widely used in consumer laundry detergents in oxidizing and non-oxidizing bleach detergents(Schubert, 2003, William, 1994).Boron, is widely used in different sectors (such as the food industry, furniture, health, agriculture, detergent and paper industries) its waste and the chemistry of its by-products in the environment should be of great concern. Most importantly, boron is an active migrant and is highly soluble in water. Owing to this, much research is carried out to monitor boron in natural water sources.

Importance of Boron

Boron is an essential micronutrient required for plant nutrition. Findings have confirmed that boron is essential for a variety of algae and bacteria strains (Goldbach *et al*., 2007). Güler *et al*. (2015) reported that boron is known to stimulate the growth of yeasts (*Saccharomyces cerevisiae*). Addition of boron as boric acid to phytoplankton also has a significant effect on primary production and assimilation of carbon. Plants absorb boron through their roots from the soil in the form of undissociated and uncharged boric acid(H_3BO_3) (John and Keith, 1998). Soil solutions on average contain 10 ppm of boric acid. In addition, Noguchi *et al* (2003) reported that the cell wall of *Arabidopsis* showed a lower degree of cross linking under limited boron supply which resulted in a low boron level in shoots, suggesting a role for boron in maintaining cell wall integrity. Studies have shown the roles of boron in the functioning of enzymes and other proteins of the plasma membrane, transport processes across the membrane and membrane integrity. There is an indication that boron deficiency affects leaf photosynthesis by causing photo inhibition of the chloroplast (Blevins, 1998) O' Neill *et al.* (2001) observed that boron deficiency affects cell differentiation in plant xylem and embryogenesis cells during the period of plant growth. Boron is an essential and integral nutrient in animal nutrition with roles in the enhancement of bone growth and maintenance (Blevins, 1998). Boron improves the functions of the central nervous system and reduces cancer risk. Evidence suggests that a boron rich diet may reduce the risk of diabetes and heart diseases (Yuan *et al*, 2014). A daily intake greater than 1.0 mg enhances the health benefits which is achieved by eating food rich in boron, such as fruits, nuts, vegetables and pulses, which are recommended to enhance healthy living (Nielsen, 2000).Nielsen reported decreased activities in several membrane bound hormones due to a shortage of boron in humans (Nielsen, 2000 & Nielsen, 2016). The World Health Organisation Committee on drinking water quality reviewed the recommended boron concentration in drinking water from 1.0 to 2.4 ppm, due to the beneficial effects of boron (Yuan *et al*, 2014; WHO, 2011).

Boron toxicity

At higher levels boron becomes toxic to animals and plants. The World Health Organisation gave 0.5 ppm as the threshold concentration of boron in irrigation water (WHO, 1998; Yuan *et al*, 2014). Plants show a wide range of responses to toxicity of boron exhibiting physical features such as slow growth of shoots and roots, decreased division of root cells, a gross decrease in photosynthetic capacity, reduced chlorophyll in leaves and poor productivity in plants (Nielsen, 2016). Abnormalities in colouration of leaf edges, increased decay and plant death are also attributed to boron toxicity (Nielsen., 2016).

In humans and animals short term effects of boron toxicity include irritation of the throat, nose and eye. Ingestion of boric acid also causes negative health effects, for instance, vomiting, headache, diarrhoea, nausea, dizziness, weakness of the body and may result in kidney damage. The fatal dose of orally ingested boric acid is about 5 – 6 g for infants and more than 20 g for adult(Fyodor, 2015).Contact through broken skin causes poisoning (Sah & Brown 1997; WHO, 1998).There is evidence that malfunctioning of the cardiovascular, digestive, nervous and sexual systems of humans and animals can be attributed to the long-term consumption of water and food with increased boron content. (WHO, 2009)

Review of methods used for boron analysis

Over the years, several analytical methods have been employed in the analysis of boron and these include colorimetric methods, potentiometric methods, and neutron sources (Sah & Brown 1997). Plasma and chromatographic technique such as high-performance liquid chromatography and ion chromatography have also been reported. In this work colorimetric method was used.

Colorimetric methods employed for the determination of boron in water are based on the colorimetric reaction of the oxy-boron complex with different reagents such as ferroin, carmic acid, curcumin and azomethine-H (Gluer *et al*., 2015; Potter & Lovelace, 1984). The optical density of the solution is measured at a particular wavelength and this is usually a function of the boron in solution. The ferroin method is based on the extraction of boron as ferroin borodisalicylate salt, an orange coloured complex which is usually insoluble in water but soluble in organic solvents. This method suffers from interference from chloride ions above 1000 ppm and phenol of 10 ppm which are usually present in natural water. The azomethine-H method has been widely used for the spectrophotometric determination of boron in different matrix such as plants, siliceous material and water (Sah & Brown 1997; Zaijun *et al*., 1999; Lopez and Gimenez, 1992).This method is very suitable for routine boron analysis because it can be applied in a dilute acidic solution unlike most reagents usually employed for boron analysis, which involves hazardous experimentation due to the use of concentrated acids, and it is also less time consuming (Mohammed *et al,* 2014; Zaijun *et al*., 1999; Oxspring *et al*., 1995). Evidence from NMR studies from literature suggests that the complex formed is a 1:1 bischelate complex with tetrahedral boron, through the full processes illustrated in Figure2 (Lopez & Gimenez, 1992; Potter & Lovelace, 1984)′ In this work, boron concentration was determined azomethine-H method.

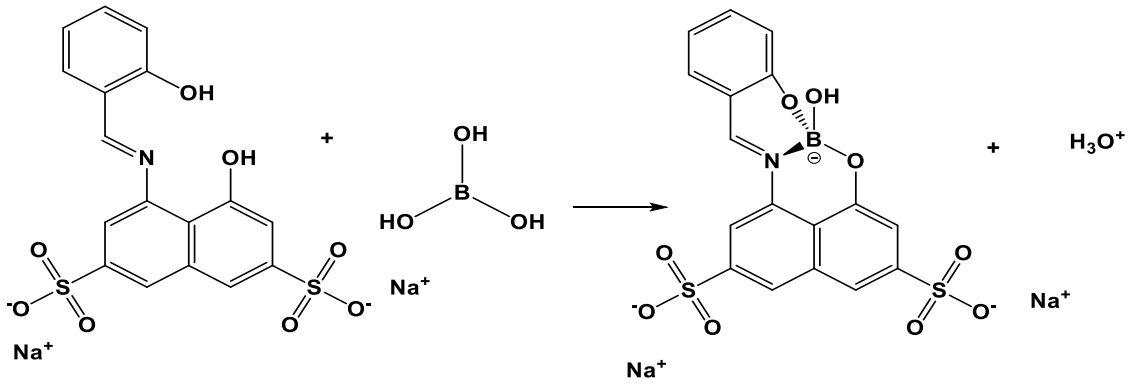

Figure 2**:** Interaction of boric acid with azomethine-H to form a coloured compound (Matsuo *et al.*, 2004).

Recently ICP has been coupled to different types of mass spectrometers to increase sensitivity and lower detection limits. ICP atomic emission spectrometry (AES) and ICP mass spectrometry (MS) have quantification limits of 5 ppb and 0.15 ppb (Kochkodan *et al*., 2015). However, ICP is often limited to freshwater with low chloride content and is not suitable for seawater that usually contains a high chloride ion concentration that may cause blocking of the nebuliser.

MATERIALS AND METHODS

A Perkin Elmer Lambda 35 ultraviolet visible spectrophotometer (Perkin Elmer, Inc. 940 Winter Street Waltham, MA 02451 USA) was used for the determination of boron concentrations in seawater collected at low and high tide. A Hanna Instrument (HI 2211 pH/ORP) pH meter was used for measurement pH and an analytical balance with a measurement accuracy of 0.0001 g was used for weighing chemicals. Borosilicate glassware were strictly avoided throughout this work.

Reagents and Standards

The reagents and all chemicals used were of analytical reagent grade. Deionised water prepared by USF Elga Ultrapure water system (Fisher Scientific UK Ltd, Bishop Meadow Road Loughborough) was used for the preparation of all standard solutions. Masking solutions was prepared using 250 g of ammonium acetate (Sigma Aldrich), 6.7 g EDTA disodium salt (Fisher scientific), 125 mL ≥ 99.7 % acetic acid and 60 mL of ≥ 98 % thioglycolic acid (Supplied both by Sigma Aldrich) in 500 mL volumetric flask made up to mark with deionised water. Azomethine-H Extraction solution was prepared by dissolving 0.9 g azomethine-H monosodium salt hydrate (Sigma Aldrich) and 2 g of L (+) ascorbic acid (Acros organic) in a 100 mL volumetric flask.5.7160 g of 99+ % (Acros organic) boric acid was dissolved in a 1000 mL volumetric flask to obtain a stock concentration of 0.092 M $B(OH)_3$ (equivalent to 1000 ppm of B). Working solution was prepared by diluting appropriate volumes of the stock solution (0.09 M B $(OH)_3$ equivalent to1000 ppm B) to 100 mL in a to give solutions of 1, 2, 3, 4 and 5 ppm, respectively.

Sample collection and sampling site

Twenty-three (23) water samples were taken from 11 sample locations of natural water sources of north Wales at low and high tides except Holyhead where sampling was done at

only high tide. The samples were collected around coastal sites at low (1.21 - 2.45 m) and high (3.56 – 7.61 m) tides. The local sea sample locations (with ordnance survey grid references) are Bangor Port (232133), Bangor Pier (237130), Menai Bridge (219942), The Swellies (220261), and Llanfairpwll PG (217231) in the County of Gwynedd. Samples taken in Conwy County were from Conwy Dock (280624), Llanfairfechan (259683), Penmaenmawr (267835), Llandudno (322527) and Rhos on Sea (316195). Samples were also taken from Holyhead (3138463). Boric acid solution prepared from dissolving analytical grade boric acid in deionised was used as control.

pH Measurement

A Hanna Instrument (HI 2211 pH/ORP) pH meter used for measuring the pH and adjusting the pH of solutions was calibrated with NIST Organics buffer solutions of pH 4 (phthalate), pH 7 (phosphate) and pH 11 (borate). pH Value and were carried out in the laboratory.

The azomethine-H method Procedure

A 5 mL water sample was measured into a 15 mL polypropylene centrifuge tube and 4 mL buffer masking solution and 2 mL of the azomethine-H solution were then added. The tube was placed on vortex mixer SAB (BioCote) for about 10 seconds at 1800 rpm for the solution to mix thoroughly. The solution was allowed to stand for about 60 to 120 minutes in fume cupboard before analysis. Calibration standard used for 0, 1, 2, 3, 4 and 5 ppm boron concentrations.

RESULT AND DISCUSSION

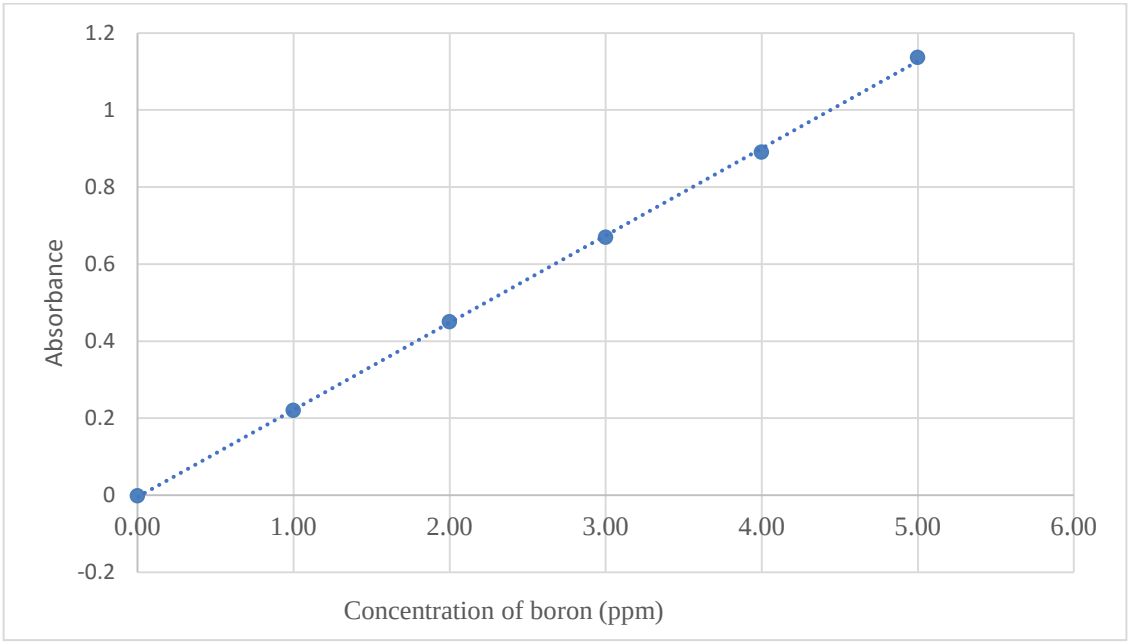

Figure 3**:** Linear plot of absorbance against concentration of boron (ppm) standards of the azomethine-H method.

Limits of detection and quantification

The limit of detection ($\bar{x}$ + 3σ) of boron found was 0.04 ppm while 0.08 ppm was obtained as limits of quantification ($\bar{x}$ + 10σ).

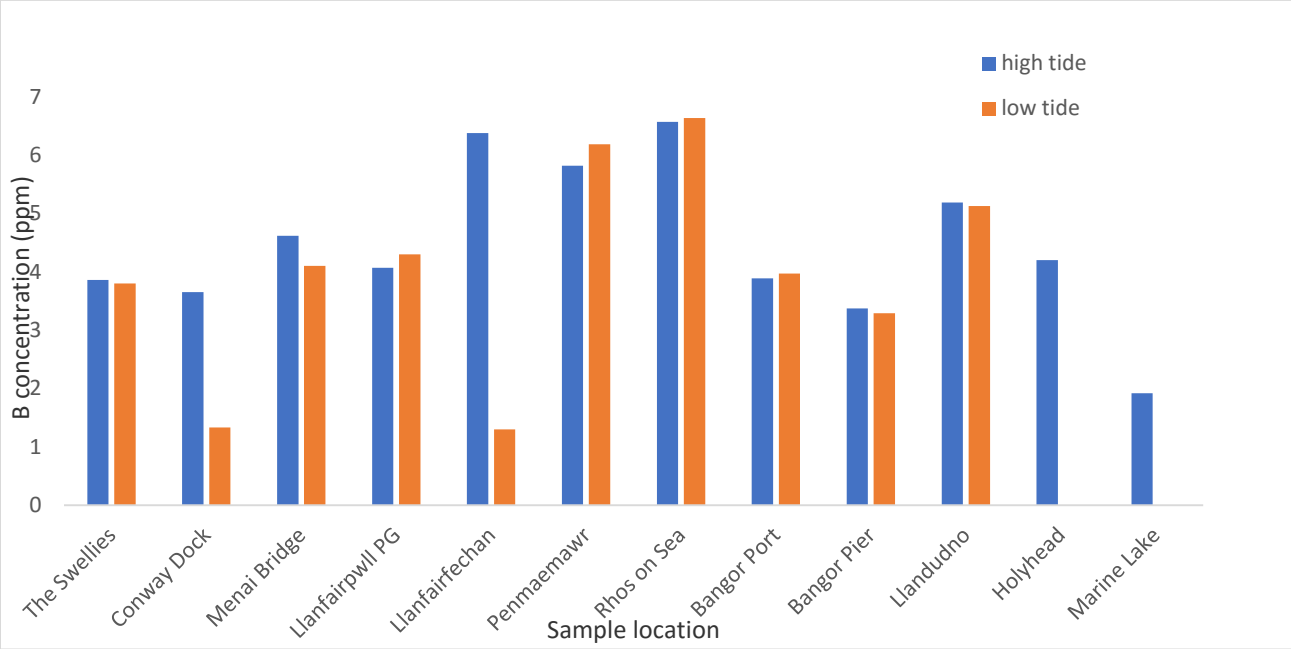

Figure 4**:** Results of boron concentrations in seawater at high and low tide.

Seawaters

The concentrations of boron determined from samples collected at coastal sites at high tide with the azomethine-H method obtained range from 3.65 (Conwy) to 6.57 ppm (Rhos on Sea) at high tide and 1.30 (Llanfairfechan) to 6.64 ppm (Rhos on Sea) at low tide (Figure4). The values obtained agreed with literature data for global boron concentrations in seawater of 0.5 to 9.5 ppm and are similar to those reported by Matthews (1974) of 4 to 5 ppm in seawater around Britain using the ferroin method. The results also agree with finding of Lopez *et al.* (1992) of about 5 ppm in Spanish seawater using the azomethine-H method and values found by Lee *et al.* (2010).

Variation in boron concentration by sample location

Clearly, sample location, freshwater influence and other local factors will affect the observed boron concentrations. The differences in boron concentrations (Figure 4) at various sample locations is possibly due to adsorption/desorption onto and from sea sediment (Nasef *et al;* 2014). This is because sea sediment is heterogeneous and it composition is usually not the same from one location to another. A number of reports have also suggested the adsorption of boron by natural materials (Bryjak, Wolska & Kabay, 2008). The variation in boron concentration is likely due to the adsorption of boron by natural material sorbents. These materials are classified into natural mineral and plant material sorbents

formed from the weathering of rocks and the decaying of dead plants. The natural mineral sorbents include sepiolite, red mud, cristobalite, alunite, zeolite, kaolinite and calcite. Examples of plant material sorbents include plants seed, residues of wheat, rice and walnut. Biopolymers such as calcium alginate and chitosan have also been demonstrated to have good adsorption capacity for boron. Generally, plants materials sorbent has been reported to have higher adsorption capacity than mineral sorbents. It is suggested that there is a chemical interaction between boron and these natural materials (Gazi & Shahmohammadi, 2012)

Another factor is tide height and possible freshwater contamination. The height of tides at low tide varied from 1.21 to 2.45 m while at high tide the range was from 3.56 to 7.61 m. The low values obtained for some samples collected at low tide (Figure**4**) is because the seawater around these sampling point becomes contaminated with freshwater from surface runoff and river flow which is slightly acidic, at low tide this dilutes seawater around coastal regions when the volume of seawater becomes low. This results in lower pH values obtained for some samples collected at low tide such as Conwy and Llanfairfechan than their corresponding high tide (Figure 4) and consequently suggests a possible cause of lower boron concentrations, due to a decrease in salinity, as a result of freshwater flowing from Llanfairfechan and Gyffin rivers, diluting the seawater around the sample location (Lee *et al*., 2010) In general, the results obtained showed a relatively constant concentration of boron around the coastal sites sampled, since the variation in boron concentration observed is not statistically significantly different from one location to another. This suggests that the anthropogenic input of boron in seawater around north Wales is negligible.

t-Test between samples collected at both tide levels

A significance test was conducted to check the effect of freshwater contamination on boron concentrations at varying tide levels. The result showed that boron concentration at high and low tide (Figure 4) are statistically significantly different ($p = 0.024$). Since the probability value is less than 0.05, the result is significant at 5 % level. This implies that boron concentration does vary with tidal level. One possible explanation is that this occurs as a result freshwater contamination, close to the sample location. Therefore, inflow of river water dilutes the boron concentration at low tide, for example Conwy and Llanafairfechan. However, when the test was repeated at locations without freshwater contamination a p-value of 0.95 was obtained which implies that the difference in boron results can be attributed only to freshwater contamination close to the sample location, and not with tide height.

Study of the effect of dilution on boron extraction

A study of the effect of dilution on boron extraction and to determine the dynamic range for which the method is linear for analysis of boron in natural water (seawater) was carried out. The azomethine-H method was used as the preferred method for this study. The results obtained for both approaches (5-fold dilution and without dilution) showed that linearity of the seawater is within a boron concentration of 1 to 3 ppm and both approaches did not show much variation in boron concentration obtained within this range. Variation was observed for samples with boron concentrations greater than 3 ppm with 5-fold dilution having higher values.

When a significance test was carried out, the results from the 5-fold dilution compared to those without dilution are significantly different when using a one tailed t-test ($p = 0.038$), however, they are only significantly different ($p = 0.076$) at the 10 % level for a two tailed test. This suggest that applying the azomethine-H method without diluting the samples gives slightly different results and suggests that the range of boron concentrations for which the method can be used is narrow**.**

CONCLUSION

The analysis of boron of aqueous environmental samples (seawater) in north Wales was successfully carried out through solvent extraction with azomethine-H reagent. The values obtained for boron concentrations were within the global range and average for seawater of 0.5 to 9.5 ppm and 4.5 ppm respectively (Schubert, 2003) The values obtained also agrees with the values reported by Matthews (1974) of 4 to 5 ppm in seawater around Britain using the ferroin method. The results also agree with finding of Lopez *et al* (1992) of about 5 ppm in Spanish seawater using the azomethine-H method. This agreement suggests that there was reproducibility with the methods. A t-test suggests that boron concentration is constant at varying tide height except for influence of freshwater. Furthermore, application of the test was used to suggest that dilution of seawater improves boron extraction. The results of the boron concentrations of seawater determined around the sampled locations indicate that there is no boron rich deposit around the north Wales coastal sites. It also tends to suggest that the variation in boron concentrations at different sample locations is due to adsorption/desorption onto and from sea sediments. The finding of this work also tends to suggest that freshwater contamination from river flow and surface runoff affects the boron concentration at low tide close to the sampling points at coastal sites. Currently, anthropogenic activities such as biomass burning, discharge of sewage, and the agricultural use of boron containing fertilizer are insignificant, and do not cause a variation of boron concentration in seawater. However, constant monitoring is required in view of growing anthropogenic activities.

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Estimation of Entrance Surface Dose During Diagnostic X-Ray Procedure at Minna General Hospital

[*]Olaide K., Kolo M.T., Ahmadu U., & Olarinoye, I.O.

[1]Department of Physics, Federal University of Technology Minna, Nigeria

*Correspondence: Email: kehindeolaide24@gmail.com Phone number: +2348133388954

ABSTRACT

The risk of occurrence of cancer in developed and developing nations of the earth has become a major concern in the scientific and medical circle. Ionizing radiation which plays a significant role in x-ray diagnostic procedure has been identified as one of the factors responsible for the risk of cancer. This have therefore called for imperative measures to determine how much of radiation these medical imaging delivers to the patient. The aim of the study is to determine the entrance surface dose for patient undergoing diagnostic x-ray procedure at Minna general hospital. A total of 125 patient participated in the study with different examination such as the lumber (LAT & AP), chest (PA &LAT) and pelvis (AP). Patient parameter collected after all necessary approved consent include the age, sex, height, weight, and the exposure factors which are the kVp, mAs and the focus-to-skin distance. Using the geometric and technical parameters, the ESD were calculated. The obtained ESD ranged from 0.3-1.5mGy for chest (PA), 0.3-1.0mGy for chest (LAT), 1.33-5.67mGy for lumber (AP),1.5-5.4mGy for lumber (LAT), 1.6-5.21.5.2mGy for pelvis (AP). These values when compared with IAEA(1996) and NPRB(2002) values were found to be low which indicates an improvement in optimization and does not pose any health risk to the patient.

Keywords: ESD, cancer, radiology, diagnostic X-ray.

INTRODUCTION

The risk of occurrence of cancer in developed and developing countries has been of major concern. Developed countries has done significantly well in terms of early detection and radiotheraphy but the death rate in developing countries shows that not much has been done either in early detection or radiotheraphy (Ahmedin *et al.,* 2011). An essential energy, ionizing radiation used during diagnostic x-ray procedure is one of the factors responsible for the risk of occurrences of cancer. This has therefore called for an imperative measure to determine how much of radiation these medical Imaging delivers to patients by determining the entrance surface dose. The entrance surface dose which is a deterministic effect is the

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maximum amount of X-radiation dose absorbed by the entrance skin of a living tissue at the central point of the irradiated area (Sharifat & Olarinoye, 2009). Due to the risk of exposure during diagnostic x-ray procedure, minimum amount of x-radiation should be recommended and the entrance surface dose should be measured and monitored (Alatta et al., 2017).

Akinlade (2011) carried out a research on assessment of detrimental health effect of radiation associated with diagnostic x-ray examinations at four centres in Nigeria. ESD and effective dose(ED) was estimated using PCXMC programand obtained results were compared with similar examinations in published studies. From estimated ED in some of the selected facilities, the risk of fatal cancer was higher than the ICRP recommended limit. Abubakar *et al*.(2017) investigated ESD for patient in five different projections in Sebbha city of Libya. The mean ESD values were found to be higher than the mean ESD reference values which indicate the necessity to reduce the patient dose to the acceptable levels recommended by ICRP. Worldwide interest in dose measurement and guidelines includes National Radiological Protection Board (NRPB), International Atomic Energy Agency (IAEA).

The aim of this study is to determine the patient dose arising from diagnostic X-ray procedure in Minna general hospital and the knowledge from this investigation will help to determine if the patient dose in this facility is as low as reasonably achievable.

MATERIALS AND METHOD

Data Collection

General hospital Minna, a government owned health care institution in the capital city of Niger state, is a source of medical aid to more than half of the 300,000 population. It was considered due to the high number of patients that visit the facility for x- ray examination and the number of trained personnel. The facility has a record of two radiographers, one radiologist and three x-ray technician.

The available machine used for x-ray examination in Minna General hospital is the Nortek rotating anode x-ray tube with 1.00 mmAl/75 kv filtration. It is a three-phase x-ray machine, installed and manufactured in the year 2014 which makes use of Agfa digital processor for the X-ray photographic film.

125 adult patients (male and female, between the ages of 18-90 years) were considered in this investigation. The human parameters considered in this study includes sex, age and body mass index (BMI) of every patient under investigation. The body weight and height of each patient from which their respective BMI were calculated, were obtained directly using a weighing balance and tape rule respectively. The five projections of interest to this research in the daily X-ray examination of patients conducted between January and March, 2019 (3 months), are chest (PA & LAT), lumber (AP & LAT) and pelvis (AP). The kVp, and mAs values for each examination was read directly from the

control panel of the x-ray machine. The focus-to-skin distance for each patient was also recorded.

Data Analysis

The entrance surface dose was calculated using the equation (Sharifat & Oyeleke, 2009).The data inputto get the ESD are the kVp, mAs and the focus-to-skin distance.

$$\mathrm{ESD} = \mathrm{Y(d)} \times \left(\frac{\mathrm{kV}}{80}\right)^2 \times \left(\frac{100}{\mathrm{FSD}}\right)^2 \times \mathrm{mAs} \times \mathrm{BSF} \quad (1)$$

where;

ESD is the entrance surface dose

Y(d) is the radiation output $\frac{mGy}{mAs}$, And multiplied by 0.00877/mAs to convert from milliroentgen to air kerma in mGy/mAs

kV is the tube kilovoltage

FSD is the focus-to-skin distance,

mAs is the current-time product and

BSF the backscattering factor which is 1.35 obtained from literature (Suliman *et al*. 2006, Sherifat & Olarinoye, 2009).

The obtained data was analysed using Excel

RESULT AND DISCUSSIONS

Five examinations were considered in this study, with chest x-ray having the largest number of patients (67.2%) and pelvis (8.8%) with the least number. The projections considered are the anteror-posterior (AP), posterior-anteror (PA) for chest and lateral (LAT) projection. The patient geometric data is shown in Table 1 with the mean age as 38years, 66kg as the mean weight, 161.4cm as the mean height and the mean BMI derived from weight/(height)^{2}as 25.5.

Table 1: Patient's geometric information, mean values and ranges indicated.

RADIOGRAPH	PROJECTION	AGE	WEIGHT (KG)	HEIGHT (CM)	BMI (Range)
CHEST	LAT	48.2(33-64)	70.8 (50-98)	164.6 (150-178)	28.7(19.5-38)
CHEST	PA	40.4(18-71)	67 (42-120)	159.5 (148-172)	26.4 (18-53)
LUMBER	AP	46(31-58)	62 (48-76)	158.8(145-177)	30.3(21.3-50)
LUMBER	LAT	36(23-58)	76.5(63-110)	158.5 (140-174)	23.5(20-25.7)
PELVIS	AP	36(29-48)	73.2 (50-110)	164 (146-190)	27.1(22-30.5)

The exposure parameters are presented in Table 2, revealing the range of tube loading (58kVp-78kVp), mAs (8-33) and the focus-to-skin distance (57-106) cm. The use of low mAs and high kVp in this facility is known to substantially reduce patient dose and it is been recommended by many other previous study.

Table 2: exposure factors and ESD, mean values (ranges) indicated.

RADIOGRAPH	PROJECTION	kVp	mAs	FSD(cm)	ESD (mGy)
CHEST	LAT	71.2(70-73)	20.4(12-33)	106	0.42
CHEST	PA	66.1(56-72)	11.3(8-18)	103	0.77
LUMBER	AP	74.1(70-78)	23.4(20-25)	67.4(57-100)	2.24
LUMBER	LAT	74.3(70-78)	25.7(12.5-32)	73.6(57-100)	2.14
PELVIS	AP	71.3(68-75)	20.8(16-25)	63.7(58-75)	2.75

A comparison of patience dose between this study and the study carried out in this facility by Sherifat and Oyeleke (2009) and international established values, IAEA (1996) and NPRB (2002) is presented in Table 3.

Table 3: estimated ESD compared with other published and established values.

RADIOGRAPH	PROJECTION	THIS STUDY	SHERIFAT & OLARINOYE (2009)	IAEA (1996)	NRPB (2002)
CHEST	LAT	0.42	NA	1.5	1.0
CHEST	PA	0.77	4.4	0.4	0.2
LUMBER	AP	2.24	NA	5	6
LUMBER	LAT	2.14	NA	15	14
PELVIS	AP	2.75	NA	10	4

NA- Not Applicable.

A remarkable level of increase in optimization over the years can be deduced from the result obtained and shown in Table 3 after comparing the mean ESD of this study with that of Sherifat & Oyeleke (2009), this is a reflection of reduction in the exposure factors which contribute immensely to the reduction of ESD from 4.4mGy to 0.77mGy(86% decrease). The minimum ESD in this study is 0.42mGy which is the case of chest (LAT) and the maximum dose being 2.75mGy as with the case of pelvis. In all cases of examination, low doses are obtained when compared with international standards. The inverse square law is established as the

intensity of the X-ray beam is a function of distance from the target, hence maximizing distance is the best protection measure from ionizing radiation (Olarinoye & Igwe, 2010). The relationship between higher X-ray tube filtration and low dose is well established (Saeed, 2015), as the absorbing material used for the purpose of an added filtration is to harden the beam and thus eliminate the soft photons that could be detrimental to health when absorbed. The use of inherent filtration 1.0mmAl only for a machine that is barely five years as at the time of this study could be another reason for the increase in patient dose. Diagnostic reference level are not to be exceeded when good and normal procedure regards diagnostic and technical performance is applied, as a result of this comparison, it therefore shows that there is a need for increase in the focus-to-skin distance.

ESDs calculated for pelvis and lumbar spine, 2.75mGy and 2.19mGy respectively were found to be within the corresponding DRLs recommended by NRPB (2002) and IAEA (1996).

CONCLUSION

The entrance surface dose of patients in Minna general hospital is presented in this study, this was to ascertain the level of improvement in the optimization of X-ray procedure. Five common projections were carried out on a total of 125 patients. Comparison were made between the present measurement and a previous study alongside international standards. The mean dose for lumber (AP), lumber (LAT) and pelvis are 2.24mGy, 2.14mGy and 2.75mGy respectively.

The mean entrance surface dose was found to be generally low. However, though variations are observed, the facility employed safe and optimal use of high kVp and low mAs as a means of dose reduction. Although this indicate a level of improvement in the system but a reasonable low dose is still achievable by setting guidelines for hospitals to always compare their dose for better optimization.

RECOMMENDATION

These results showed that there is the need for continual optimized procedure in Minna general hospital especially for chest examination which is the commonest examination. Optimum choice of the technical factors especially the FSD and filtration which can reduce the dose to patient should be put in check.

ACKNOWLEDGEMENT

The approval of the consent to use the facility is hereby acknowledged, and so also are the entire staff of the X-ray unit appreciated for their timely assistance and cooperation.

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Refining Shear Butter Using Activated Bone Char Obtained from Various Activation Methods

Maikarfi A.A. & *Muhammad H.I.

Department of Chemistry, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria.

*Correspondence: Email address: hauwalarai2010@gmail.com; Phone Number: 08067986163

ABSTRACT

Shea butter has a wide range of home and industrial usage. This present study was able to portray the possible enrichment obtainable for Shea butter when passed through chemical analysis. Pulverized cow bone sample was activated using 20% H_2So_4 , KOH, Na_2Co_3 and steam for 24hours, The dried mass was subjected to carbonization process at 400°C, then powdered well and finally activated at a temperature of 700 °C for a period of 60 minuteswith the sample labelled A, B, C and D respectively. The activated bone sample was characterized and crude Shea butter was degummed and refined using sample A, B, C and D. physic-chemical parameters were also determined. Acidified sample (A), showed a higher percentage charcoal yield (91.48) compared to others with: 72.55, 85.67 and 61.69 for samples B, C and D respectively. An FT-IR analysis of the samples revealed the presence of carbonyl (-C= O), hydroxyl (-OH) Amine ($-NH_2$), and methyl (-CH) groups. The SEM carried out on the samples showed a good porosity for the activated cow bone sample. The refining of crude Shea butter using chemical methods has proven an improved outcome of the shea butter in use in the locality of research.

Keywords: Shea Butter, Chemical Analysis, Acid Activation, FT-IR and SEM.

INTRODUCTION

Shea tree is an indigenous oil producing plant that grows wild in Africa (Honfo *et al*., 2012). Shea butter is a vegetable fat extracted from the kernel of the fruit of the Shea tree (Vitellaria paradoxa), a tree belonging to the family of sapotaceae. The shea tree although takes much longer time to mature (like 45 years) in comparison to other trees grown as plantation but continuously produce Shea nuts for up to 200 years in commercial quantities (Alander, 2004).

Tocopherol content in shea gives the natural anti-oxidant properties it possesses. Shea nut contains 37-55 % of fats; it is composed mainly of two fatty acids, stearic and oleic, which together account for 85-90 % of the total fatty acids (Ferris *et al*., 2001; Maranz *et al*., 2004). Soft Shea butter has high oleic content (Badifu, 1989). Honfo *et al*. (2012) reported Shea butter oil to contain 2-6 % palmitic acid (C16), 15-25 % stearic acid (C18), 60-70 % oleic acid (C18) and less than 1 % linoleic acid (C18) and 5-15 % linolenic acid.

Variations in the Physico-chemical composition of vegetable oils which depends on environmental factors such as availability of

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rain- fall, soil fertility, maturity period, agronomic practices and genetic substitution (Asuquo *et al*., 2010) gives a need to refine shea butter.

METHODOLOGY

Sample collection

Cow bone used for research work was obtained from Lapai abattoir in Niger State. Crude Shea butter for this work was obtained from Mayaki, a town in Lapai, Niger State.

Sample Carbonization and Activation

Acid Activation:

Acid activation was carried out as described by Toa and Xlaoqin (2008) using 20% H_2SO_4 solution for 24 hrs period. The cow bone were powdered and activated in a muffle furnace kept at 700 °C for a period of 60 minutes. After activation, the carbon of obtained were washed sufficiently with large volume of water to remove free acid, Then, the obtained material was washed with plenty of water to remove excess of acid, dried then to desired particle size.The final products obtained were stored in vacuum desiccators for further experiment. The resulting carbon labelled A.

Carbonization:

Samples were carbonized using three solution methods (Toa and Xlaoqin, 2008).

a. Carbonization with Potassium-hydroxide

The portion to be carbonized was soaked in 20% solutions of Potassium hydroxide for 24 hrs. After impregnation, the insoluble solution portion was decanted off and then dried.The dried mass was subjected to carbonization process at 400 °C, powdered well and finally thermally activated at 700 °C for a period of 60 minutes. The resulting carbon sample was labelled B.

b. Carbonization with Carbonate Salts

The portion to be carbonized with a salt was soaked in 20% Sodium-carbonate solution for 24 hrs. After impregnation, the insoluble solution portion was decanted off and then dried. The dried mass was subjected to carbonization process at 400 °C, powdered well and finally thermally activated at 700 °C for a period of 60 minutes. The resulting carbon sample was labelled C.

c. Carbonization with Steam

The sample was placed at the Muffle Furnace at 400^{o}C for 60 minutes, pulverized and finally activated at 700^{o}C for a period of 60 minutes. The resulting carbon sample was labelled D.

Characterization of the Activated Carbon

Ash Content, Moisture Content, Pore Volume, Bulk (apparent) Density Determinations were carried out using standard methods (Akpan et al., 2005).

Characterization using Spectroscopic Techniques

Activated cow bone charcoal prepared in this research work was also characterized by FT-IR spectroscopy and SEM techniques.

Degumming of Crude Shea butter

180g of crude Shea butter was put into separating funnel and 80g of boiling distilled water added to the oil and it was shaken vigorously for about 7 mins. Two layers were obtained, since the water and gum are denser

than oil, the oil settles above the water. The water and gum were decanted off and the de-gummed Shea butter was obtained. Akpan *et al*. (2005).

Refining of the Shea butter:

Refining of the shea butter was carried out according to methods used by Akpan *et al*., (2005). Also, moisture content, acid value, Free Fatty Acid, Saponification value, Peroxide value determinations were carried out following the same methods of Akpan *et al*., (2005).

RESULTS

Table 1: Physico-Chemical Characterization of Bone Char

Parameters	Sample A	Sample B	Sample C	Sample D
Ash content (%)	10.75	19.00	23.45	25.75
Moisture content (%)	3.9	4.5	5.4	6.7
Pore volume (g/cm^3)	0.32	0.40	0.50	0.46
Bulk density	2.54	2.26	2.30	2.32
Yield charcoal (%)	91.48	72.55	85.67	61.69

KEY= A≡ bone char produced using H_2SO_4, B≡ bone char produced using KOH, C≡ bone char produced using Na_2CO_3, D≡ bone char produced using Steam.

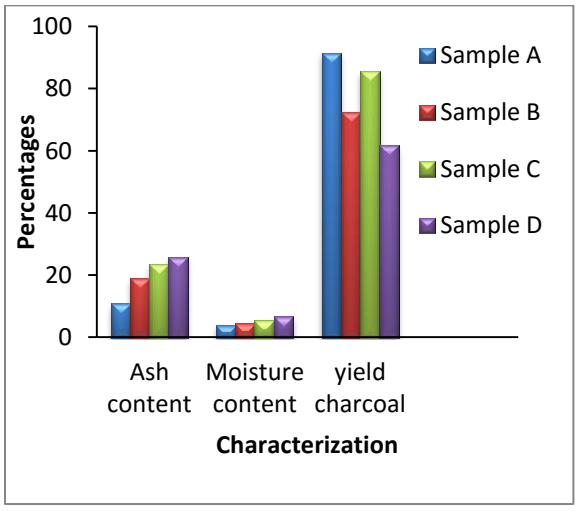


Fig 1: Physico-chemical characterization of the bone char

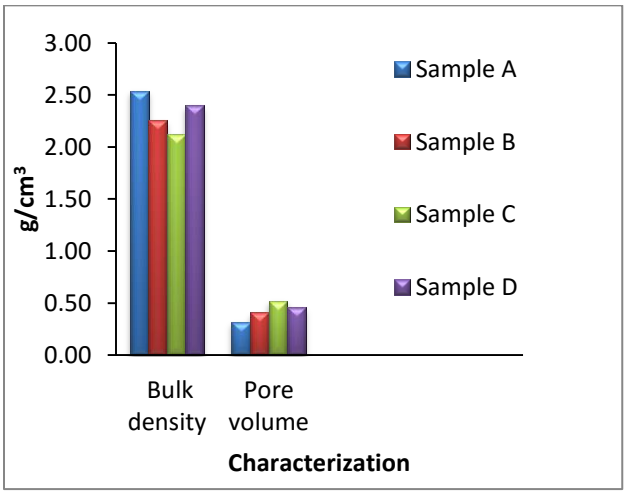
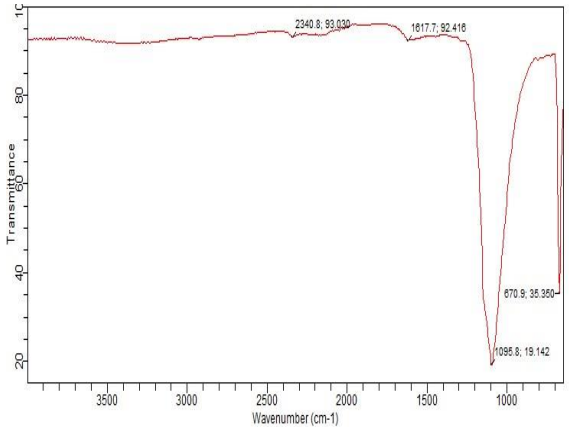


Fig 2: Bulk density and pore volume of sample A, B, C and D

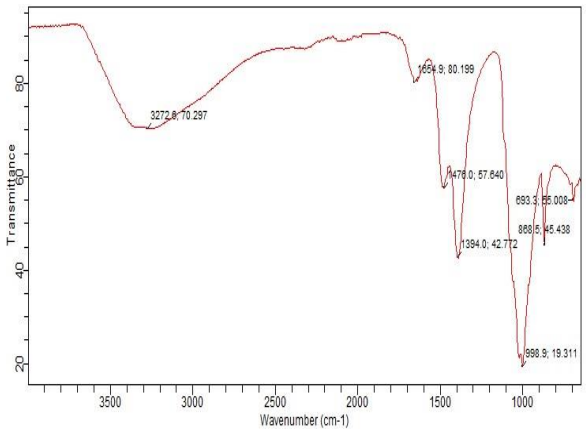
Table 2: Characterization of unrefined and refined Shea butters

Characteristics	Required	Crude oil	Refine oil using A	Refine oil using B	Refine oil using C	Refine oil using D
Saponification value (mgKOH/g)	180 – 360	389.89	162.56	165.72	167.71	169.70
pH	3.18±0.83	4.38	3.38	3.62	3.78	3.45
Free Fatty Acid (%)	1.1 – 3.0	4.21	1.68	1.79	1.89	1.96
Acid Value (mg KOH/g)	2.30 ± 1.6	8.42	3.36	3.57	3.78	3.92
Peroxide Value (Meq/kg)	-------	15	10.40	10.62	10.78	10.84
Moisture content (%)	0.06 – 0.2	2.29	0.12	0.14	0.15	0.17

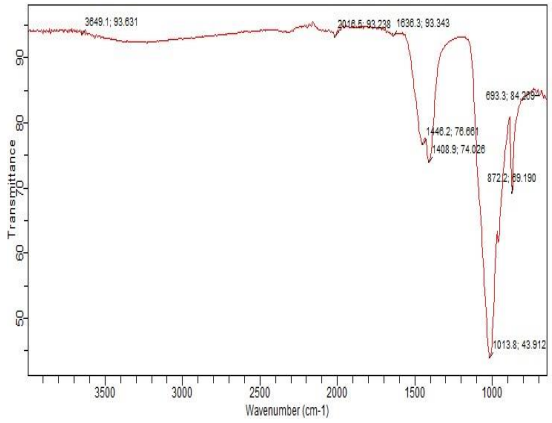
KEY= A≡ bone char produced using H_2SO_4, B≡ bone char produced using KOH, C≡ bone char produced using Na_2CO_3, D≡ bone char produced using Steam



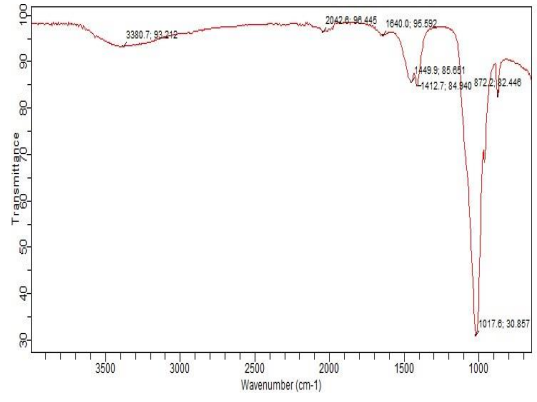
Sample A



Sample C

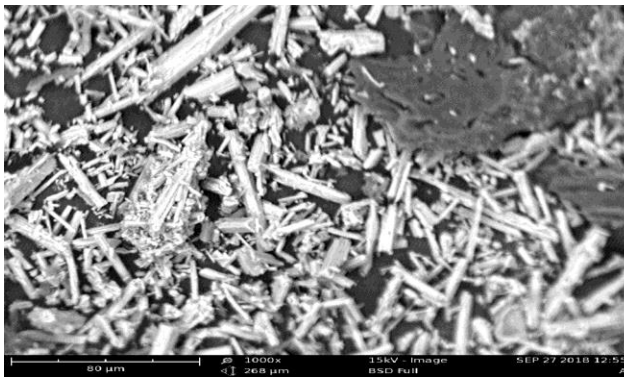


Sample B

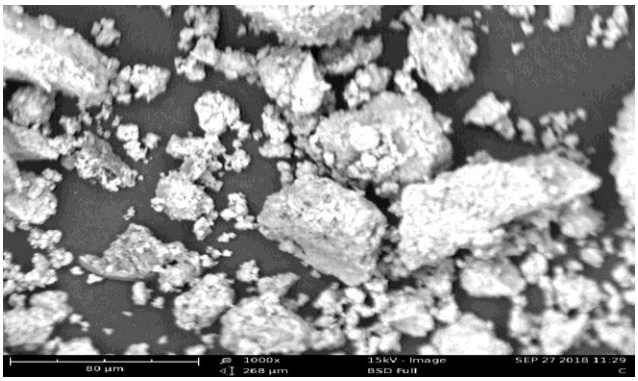


Sample D

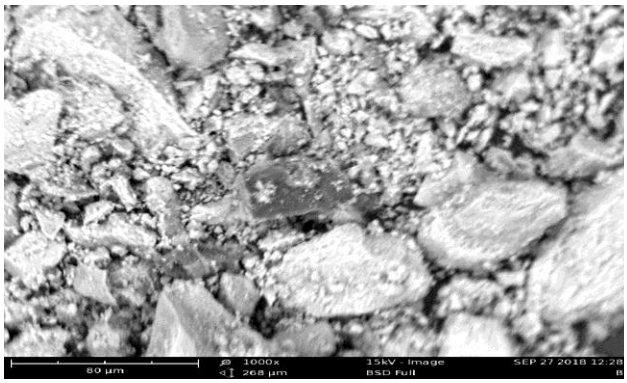
Fig 3: FT-IR Result of Samples A, B, C and D.



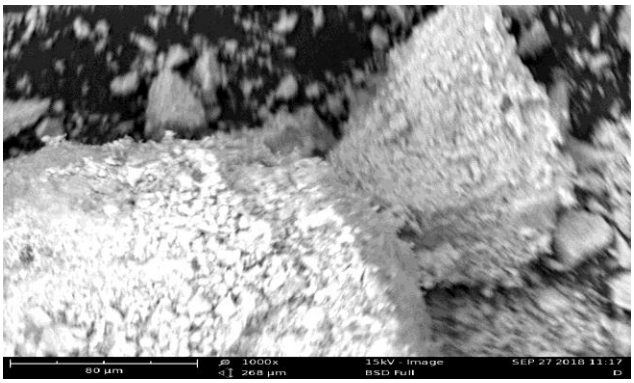
Sample A



Sample C

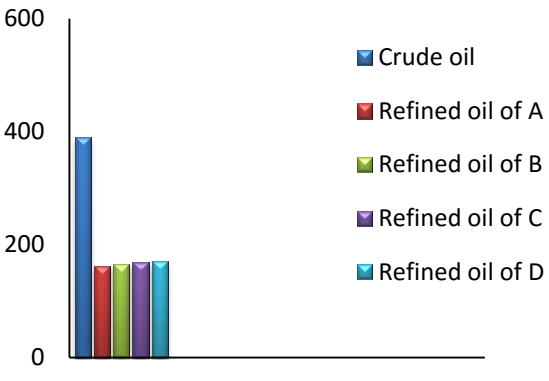


Sample B



Sample D

Fig 4: SEM Images of Samples A, B, C and D.



Saponification value (mgKOH/g)

Fig 5: Saponification value of refined shea butter using sample A, B, C and D

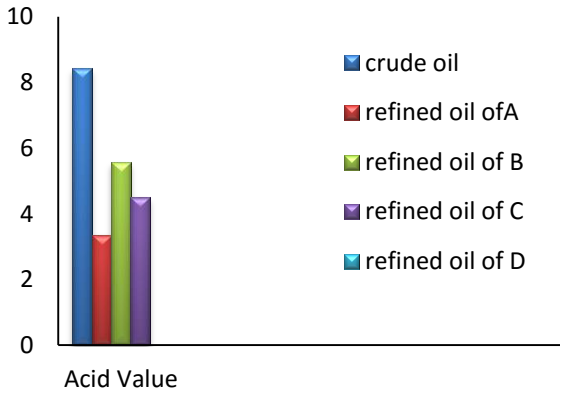


Fig 6: Acid value of sample A, B, C and D.

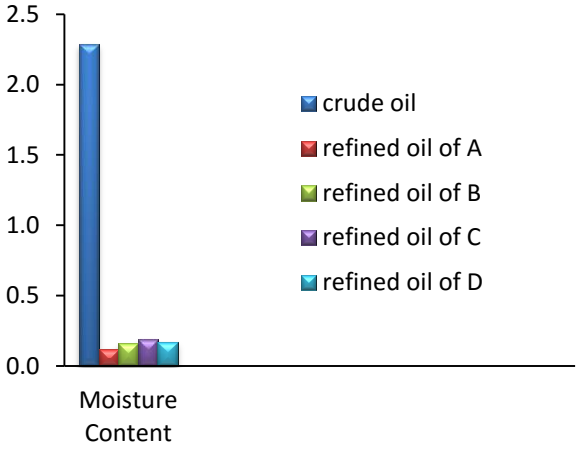


Fig 7: moisture content of the refined oils

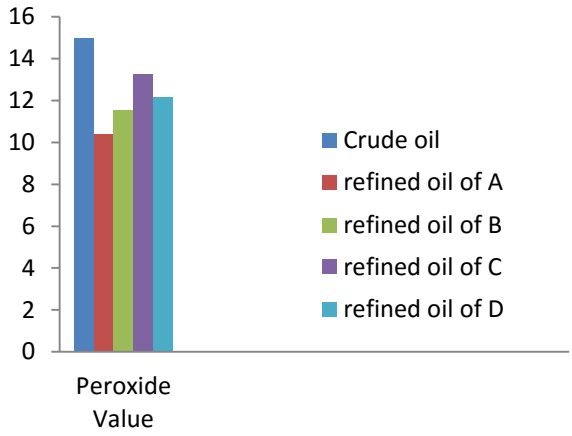


Fig 8: Peroxide Value of the Refined Oils

DISCUSSION

Physico-chemical characterization of bone char

The higher the surface area and carbon content, the more effective the activated carbon produced.

This result have exhibited the physico-chemical characterization of sample A to have its ash content at 10.75 %, moisture content 3.9 %, pore volume 0.32 and yield charcoal 91.48 %.

This result hence shows sample A to be the most effective adsorbent with low properties of ash content, moisture content and pore volume.

Characterization of Activated Cow Bone Charcoal by Infrared spectroscopy

FT-IR Analysis of Sample A:

The FT-IR analysis of Sample A revealed an intense/sharp -OH stretching of carboxyl, phenol and alcohol vibration around 1100 cm^{-1} and aliphatic -CH stretching absorption around 2340 cm^{-1}. A saturated aliphatic ether showing a strong band in the region 1108.69 cm^{-1} is said to be attributed to carbonyl groups, and a broad band in the region 1500 to 1900 cm^{-1} could be due to C=0 stretching. Hence, the broad band observed in the region of 1000 to 1250 cm^{-1} could be attributed to a characteristic absorption of -OH group. These results are in good agreement with literature (Ttreybal,1981).

FT-IR Analysis of Sample B:

The FT-IR spectrum of sample B as observed at 3272.6cm^{-1} which indicates the presence of $-NH_2$ of the amine group. Peaks around broad band region of 1408.9 and 1446.2cm^{-1} depicts the presence of aromatic groups. This makes this result in term with the literature reported by viboon, 2008. The band 1013.8cm^{-1} is attributed to calcium phosphate. Region 450-750 cm^{-1} shows two bands in the 480 and 485 cm^{-1} which are associated with the in plane and out-of-plane aromatic ring deformation vibrations (Socrates 1994).

FT-IR Analysis of sample C:

The bone char Sample C shows peaks at 598-680 cm^{-1} which could be due to the out-of-plane C-H bending mode of the sample. These spectra were also suggested to be due to alkaline groups of cyclic ketones and their derivatives added during activation (Park *et al.*, 1997, Guo & Lua, 1999). The IR absorption spectrum of sample C adsorbent shows also the bands

located between 1476 and 1654.9 cm^{-1}. The methylene group is detected by Yi–CH stretching at a wave number of 2927-3272.6 cm^{-1}, this is in a good conformation with the literature, reported by Viboon (2008). Aromatic groups are shown by a peak around 1396 and 1457 cm^{-1}. The spectrum at 3279 cm^{-1} indicated also the presence of $–NH_2$ group of amine.

FT-IR Analysis of sample D:

The IR absorption spectrum of sample D shows the bands between 1473 and 1629 cm^{-1}. The methylene group was detected by –CH stretching at a wave number of 2042.6 -2927 cm^{-1}, this confirms with Viboon, 2008. Aromatic groups are shown by a peak around 1376 and 1457 cm^{1}. The band 1017.6 cm^{-1} belongs to the calcium phosphate group. The spectrum at 33890.70 cm^{-1} indicated also the presence of $–NH_2$ group of amine. The broad band observed in the region of 1000 to 1250 cm^{-1} was assigned due to a characteristic absorption of -OH group. These results are in good agreement with the findings of Ttreybal (1981). Saturated aliphatic ethers show a strong band in the region 1095.80 -1108.69 cm^{-1} which attributes to carbonyl groups, and a broad band in the region of 1500 to 1900 cm^{-1} to C=0 stretching.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscope (SEM) was utilised to study the Surface structures of cow bone charcoal after activation at 1000 X magnifications. Sample A, B, C and D preview the SEM images of bone char respectively after activation. SEM images show the surface topography of bone charcoal. A fluffy structure was developed after activation of the bone char. Good porosity was observed in the activated cow bone charcoal that aided in refining in this study.

Saponification Value

From the results above, crude Shea butter has the highest saponification value and the refine oil with low saponification values, this clearly indicate the action of the adsorbent on the Shea butter. It shows that some pigment which are responsible for the high value of the saponification have been removed or adsorbed by the adsorbent used. The high saponification value of 389.89, 162.26, 165.41, 167.50 and 169.75 mg KOH/g for crude and refined Shea butter samples A, B, C and D respectively is also an indication that the Shea butter is a normal triglyceride molecule. The higher the saponification value of the oil, the higher is the lauric acid content of that oil (Asuquo *et al*., 2010).

Acid Value

The acid value of the Shea butter oil was observed to be; 8.42, 3.36, 3.57, 3.78 and 3.92 mg KOH/g for crude and refined oils using sample A, B, C and D respectively and a corresponding free fatty acid value of 4.21, 1.67, 1.79, 1.89 and 1.96. This is in close agreement with the work of Enweremadu and Alamu, (2009) who obtained a value of 3.62 mg KOH/g as acid value for shea.

Moisture Content

The research results showed the moisture contents of Shea butter oil to be 2.29 and 0.12, 0.14, 0.15, 0.17 % for crude and refined oils respectively. The value of the moistures in refined oils shows appreciable decrease with respect to the standard of 0.06 – 0.2 % as reported by Okullo et al. (2000). However, a lower value of 0.1 % was reported by Asuquo et al. (2010), though the value was higher than 0.037 % obtained by Enweremadu and Alamu,

(2010) while the crude has relatively high moisture content when compared to the refined. The difference observed could be due to the age of the oil or the method of extraction employed. Based on the classification of Shea butter, the moisture content of the crude oil falls above the Grade 3 and the refined within the Grade 2 grades of shea butter. Therefore, refining the crude oil improves its quality attributes.

Peroxide Value

The oil under study has a peroxide value of 15,10.4,11.52,13.23 and 12.84 Meq/kg for crude, refined oil using sample A, B, C and D respectively. This low peroxide value showed that the oil cannot easily go rancid. It is important to add that the value obtained from this study for refined oil is lower than 14.2 and 12.5 reported by Asuquo et al., (2010) and Eweremadu and Alamu, (2009) respectively but shows a close proximity to the value obtained for crude.

CONCLUSION

Though shea butter is widely used for various purposes in Nigeria, but the awareness of obtaining a much higher quality by refining using chemical methods is yet to be passed to the population. This research has successfully obtained a higher grade level of shea butter after refining the crude. It revealed the use of acid activation to have the highest refining power over other methods by obtaining the best yield of shear butter from the research. Hence, a better industrial outcome of products is expected from these new and improved version of shea butter if put to use.

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Morphological Characterization of Acid Modified Clays Obtained Locally From Niger State

Onyema C.F, *Muhammad H.I, Agwuncha S.C. & Abdulkareem A.

Department of Chemistry, Ibrahim Badamasi Babangida University Lapai, Nigeria

*Correspondence: Email address: hauwalarai2010@gmail.com Phone no: 08067986163

ABSTRACT

Samples of clays collected locally from four different local government areas of Niger State were acid modified to improve their catalytic properties. The clay samples were ground and sieved with 2 mm mesh size sieve. The sieved samples were then treated with acid solutions of concentrations in the range of 1.0 - 5.0 M under reflux for 3 h using H_2SO_4. The acid treated samples were repeatedly washed with deionized water until the washed water tested neutral to litmus. They were then dried in the oven at 120 ^oC for 4 h and stored in plastic zip bags for further analysis. The modified clays were characterized using FTIR and XRD. The morphological results obtained from the XRD indicated that the four different samples contained kaolinite, illites, muscovites and dickites. Non-clay mineral such as Quartz was also identified. The FTIR spectrogram also showed that the acid treatment lead to a gradual decrease in the –OH peak as the acid concentration was increased. The results showed that the acid modifications of the locally available clays were successful as their morphology and chemical composition were observed to have been transformed. The modified clays might be used to replace imported clays for catalytic and other industrial purposes.

KEYWORDS: Clay, Acid Modification, FTIR, XRD and Niger state.

Introduction

Clay minerals are naturally occurring alumino-silicates, vastly present on earth in geological sediments, they are made primarily of fine grained materials, which shows plastic characteristics through various range of water content, which can become harden when dried. They may contain other materials which do not impart plasticity and various organic matter (Sunil *et al*.,2018). Clays are classes of hydrous aluminum silicate minerals with corpuscle size of either < 2 µm or < 5 µm in normal spherical diameter. The literal size maybe 100 µm in one dimension and much less than 1 µm in another (Regina, 2012).

Acid activation has been widely studied as a chemical treatment method for improvement of surface and catalytic properties of fibrous clay (Ajemba & Onukwuli, 2012; Petrovic *et al*., 2012). Acid treatment of clay is aimed at modifying its structure by changing the properties of surface, porosity and acidity (Panda *et al*., 2010).

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Clay minerals are of significant importance due to its adsorptive characteristics that makes them suitable for industrial applications (Amari *et al*., 2018). Acid leaching has generally been employed when clays are used for industrial activities, for example catalyst and catalyst support for various industrial and chemical processes (Javed *et al.*, 2018). Activated clay has been used in the removal of heavy metals and dyes from wastewater (Resmi *et al.*, 2012; Qingliang *et al.*, 2013); for example, it has been used to remove cobalt from wastewater (Al-shahrani, 2013). In medicine, clay is utilized as a counter-poison in heavy metal poisoning. Personal care products such as mud packs, sunburn paint, baby and face powders, and face creams may all contain clay (EUBA, 2018). Acid leached clays has also been utilized for sea water desalination (Wibowo *et al*., 2015). Clay also offers effective de-inking properties for paper reclamation, detergents production and texile fabrications (Sidorenko, *et al*., 2018; Anirudhan & Ramachandran, 2015; Amari *et al.,* 2018). Also, acid-activated clay is used as the active ingredient in the production of carbonless copy paper (EUBA, 2018). In fact the most widely used application of clay is in bleaching, purification and stabliization of vegetable oil, clarification of food additives, wine, beer and animal feed bond. It has also been utilised in food processing i.e. bleaching earth, (Amari *et al*., 2018).

Literature reveals a number of works on the systematic examination of clay by monitoring changes in physiochemical properties using XRD and FTIR techniques. Panda *et al.,* 2010 activated natural kaolin with various concentrations of sulphuric acids and characterized the acid-leached kaolin using XRD, FTIR and other spectroscopic techniques. XRD analysis of higher acid concentrations caused amorphization resulting in the formation of amorphous silica type phase. Liu *et al*., (2012) also reported a similar trend in their research on activated bentonite using sulphuric acid. The morphological properties of the clay was observed to deteriorate even with low (0.015 M) sulphuric acid solutions; higher concentrations (up to 1M) produced higher degradation. X-ray diffraction and infrared spectroscopy were used to monitor the changes in the morphological structure of the clay after activation, and it was observed that at higher acid concentrations, the crystalline sample gradually decrease yielding a more amorphous based bentonite. Amari *et al.,* 2018 in their study which focused on the structural change of activated clays; activated green clay using sulphuric acid and characterized the samples using XRD and FTIR spectroscopic techniques. They reported that characterization of the treated clay exhibited significant changes to a greater extent of acid activation. The surface area and total pore volume also increase proportionally with the level of acid treatment.

Clay has been located in large deposit in various locations in Niger state, Nigeria such as suleja, lambata; but most of its exploited, and researched clay are mined along the bida basin. Clay located along lapai and paiko has not yet been fully exploited, and so far no record of its modification (as an adsorbent).The present study is therefore carried out in order to determine the

structural characteristics of some selected clays along minna-lapai zone of Niger state Nigeria, determine their mineralogical constituents and as well as to determine changes in physiochemical properties with respect to acid activation.

Methodology

Materials

Clay samples were obtained from selected local government areas in Niger State, Nigeria. The samples were labeled according to its geographically sourced location, and its apparent color. Table 1 shows sample labeling and their sourced locations.

Table 1: sample label

Clay location	Sample color	Sample label
Paikoro	red	PR
Lapai	red	LR
Chanchanga	gold	CG
Bosso	brown	BB

Pre-treatment of Samples

The sample was purified by method reported by Mustapha et al, 2013 and modified as follows; each of the clay samples was crushed to powdered form using a mortar and pestle, the crushed sample was transferred to a beaker and then agitated repeatedly with distilled water to give a solution. The solution was then allowed to stand for 5 minutes for the impurities to settle. The slurry above was decanted leaving behind impurities such as sand, grits and stones at the base. The decanted slurry was allowed to stand for 24 hours, the surface water decanted off and the clay was air dried for several days. The clay samples were again crushed to powdered form, filtered through a 0.2 mm sieve and stored in an air tight container for characterization, activation and other analysis.

Acid Activation of Clay

The clay was activated by method described by Usman et al, 2013, and modified as follows. Approximately 7g of the pre-treated clay sample was mixed with 70 ml of 1.0 M sulphuric acid (SIGMA-ALDRICH, Riedel-de Haen) concentration and the mixture heated at 100 °C under reflux for 3 hours. The obtained samples were then cooled, washed repeatedly with distilled water until the waste water tested neutral as indicated by a litmus test. The washed samples were dried in the oven at 105 °C for 2 hours. The acid activated clay was crushed, filtered through a 0.2 mm sieve and stored in polyethylene bags. The procedure was repeated using five concentrations of sulphuric acid (SIGMA-ALDRICH, Riedel-de Haen) 1.0 M, 2.0 M, 3.0 M, 4.0 M and 5.0 M.

Physiochemical Characterization

Physiochemical parameters for both activated and unactivated clay samples were determined.

Moisture Content

Moisture content of both raw and acid activated clays was determined by drying approximately 1 g of clay at 105 °C in the oven (DHG-9202) for 4 hours. Then after cooling in a desiccator for 2 hour to obtain constant weight, and their moisture content was determined. Each sample was analyzed

in triplicate and the average percentage reported.

pH Determination

A caliberated pH meter (JENWAY) was used in determining the pH of both activated and unactivated clay samples.

Morphological Characterization

Morphological characteristics of clay samples were determined by a wide-angle X-ray diffraction (WAXD; PanAnalytical Xpert Pro Diffractometer, The Netherlands) using a Cu-Kα (λ= 0.154 nm, V= 45kV, I= 40mA), the XRD patterns were recorded in the range of 0-80^{o}. Basal spacing of crystalline phases was compared with those of standard compounds for mineral identification. The Fourier transform infrared spectroscopy was done using a PerkinElmer Spectrum 100 spectrometer (PerkinElmer, USA) within the wave number of 400 and 4000cm^{-1}.

Results

Table 2: Moisture Content of Clay Samples

Sulphuric acid concentration	0M	1M	2M	3M	4M	5M
LR	12.598	10.000	17.347	12.687	13.028	13.112
CG	10.924	10.526	12.069	11.504	16.667	13.031
BB	6.494	16.049	18.182	12.174	19.008	19.231
PR	5.770	9.666	12.0426	11.750	10.210	12.553

Table 3: pH determination of unactivated clay samples

Clay sample	LR0	BB0	PR0	CG0
pH Value	6.10	6.70	5.61	6.71

Chemical Characterization

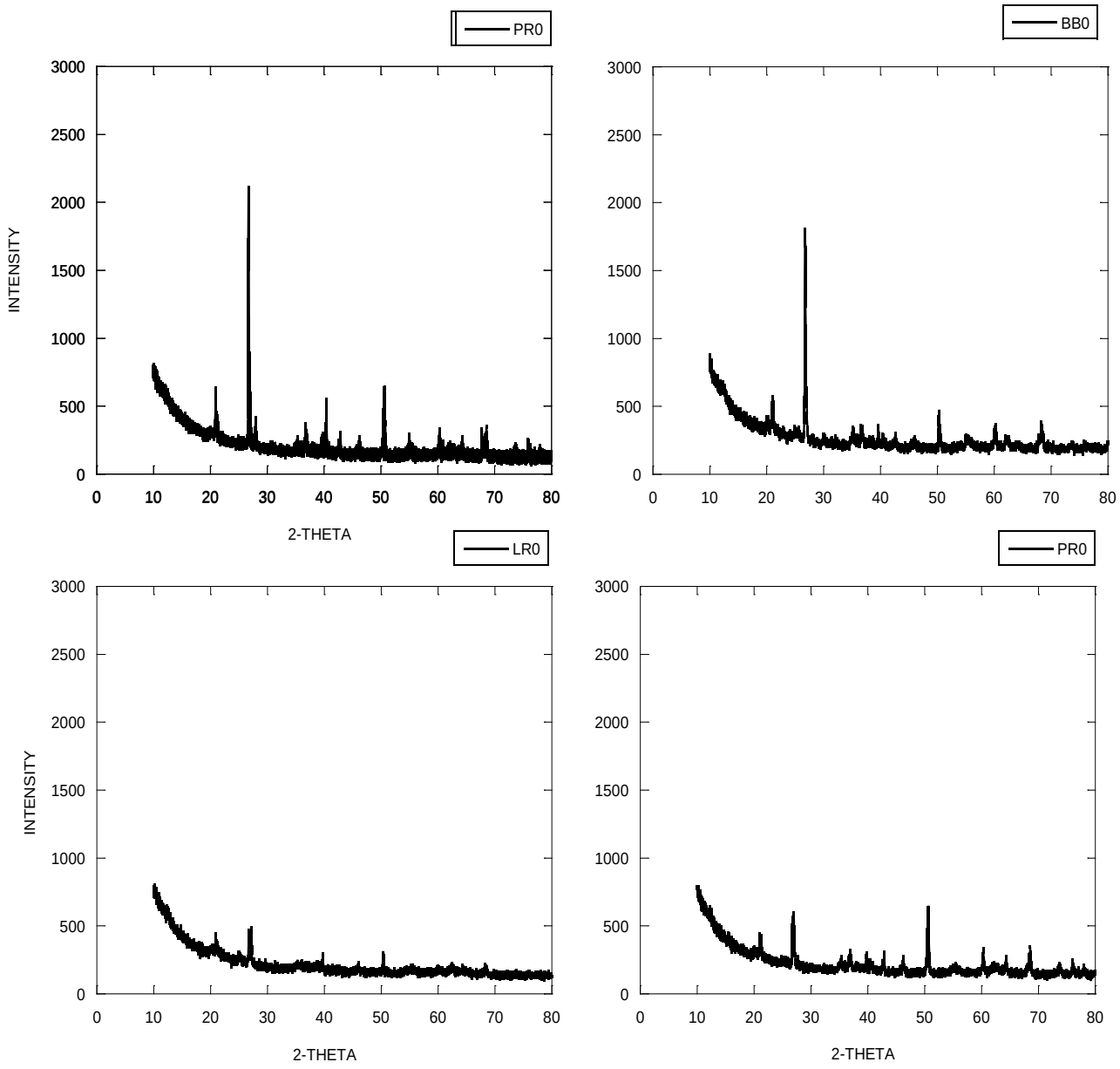

Figure 1: XRD results on LR series

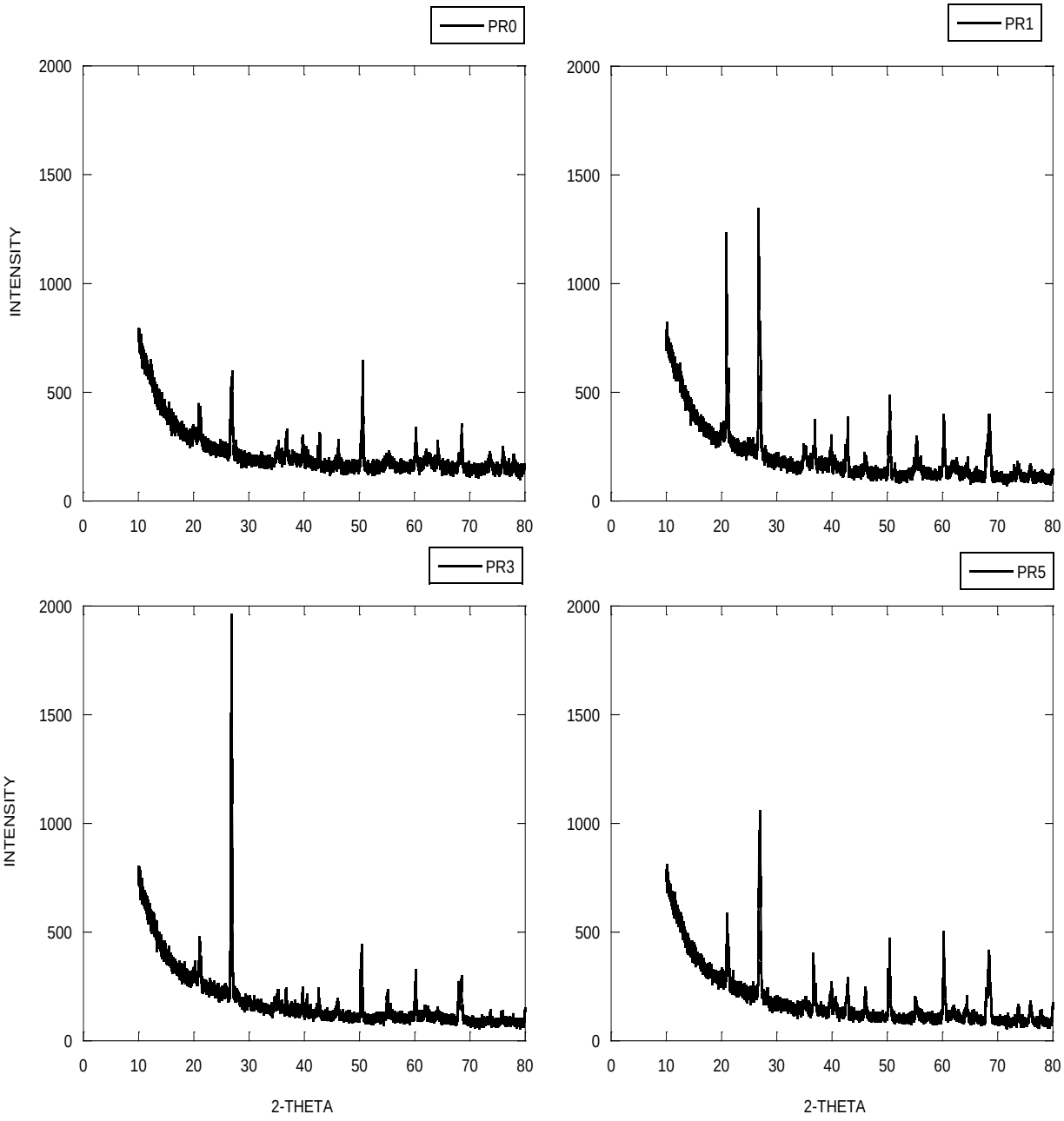

Figure 2: XRD results on PR series

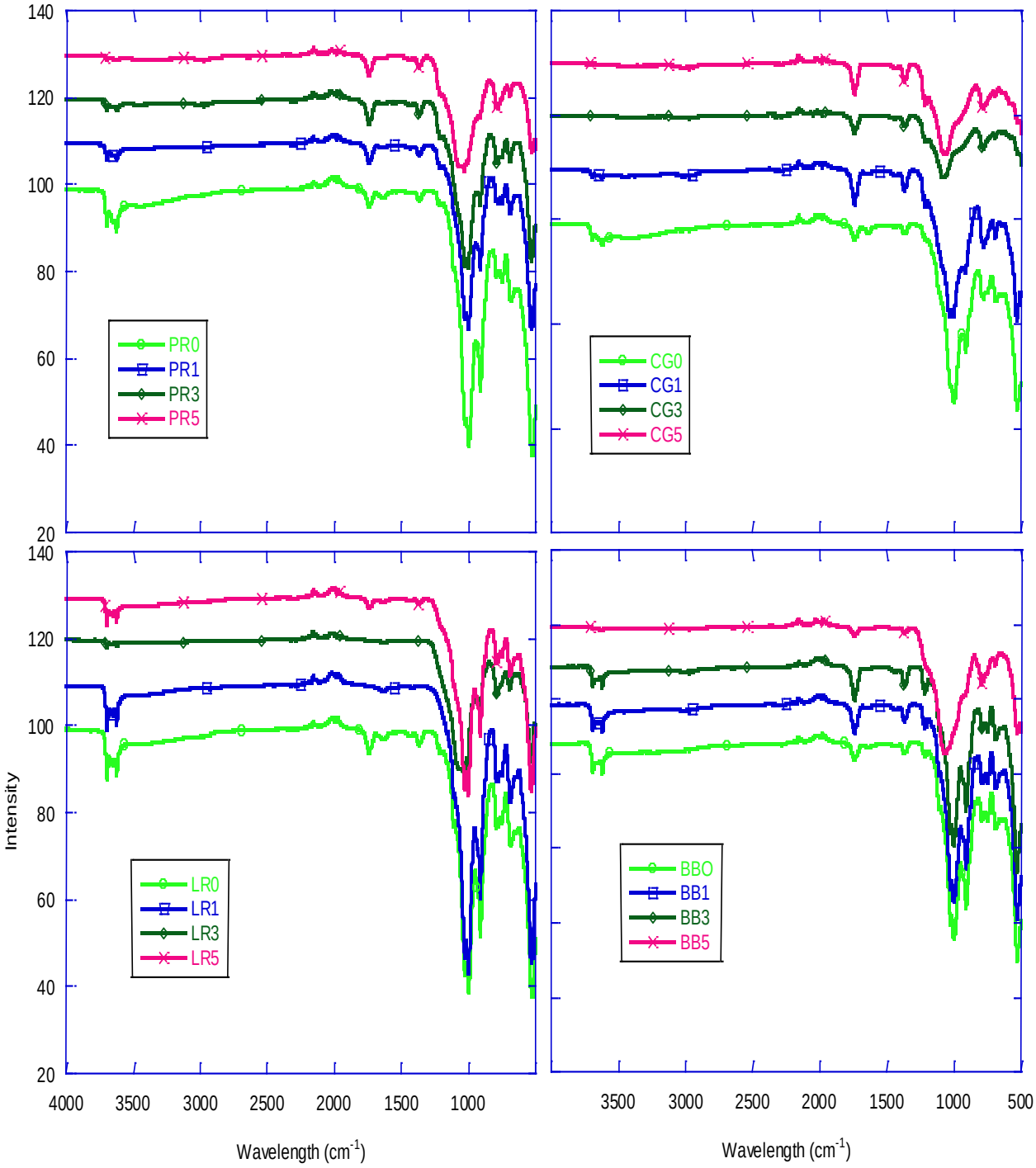

Figure 3: FTIR results on clay samples

DISCUSSION

Physiochemical Characterization

The result obtained in table 1 above revealed that acid attack at first increase the capacity of clay to retain water, a similar trend was also observed by Ujeneza (2012). Although it does not determine the bleaching properties of clay alone, it serves to explain how clay structure reacts in response to space created as metal ions are been displaced by hydrogen ions, which are of smaller volume than the metal ions, hence acid attack increases the surface area of the clay structure, hence increasing the sorbent characteristics of natural clay (Ujeneza, 2012).

pH Determination

The pH of the untreated or natural clay samples showed that all clays apart from sample PR0 are partially neutral. The sample PR0 is the only sample that is slightly acidic. The treated clay samples are all partly neutral as litmus test was employed in washing the activated sample continuously with water.

Chemical Characterization

The XRD pattern of LR clay samples is shown in figure 1, while figure 2 displays the pattern observed by sample PR. The major clay minerals identified in the study area clays are Dickite and Kaolinite, alongside with small amounts of Illite and muscovite. The non-clay mineral impurity identified is Quartz.

Clay minerals

Dickite

Dickite type of Kaolinite is a major clay mineral which was present in some of the clay samples and has been identified by the reflections at 4.25A^0 for clay sample CG0; 4.21 A^0 for sample BB0; 4.25A^0 and 3.55A^0 for clay sample LR0. The mineral was not identified in sample PR.

Kaolinite (K)

Kaolinite was present in all samples; the most distinctive peak of kaolinite appears at reflections at 2.54 A^0, 2.45A^0, 2.27 A^0 and 1.81 A^0 for clay sample BB0; 2.27 A^0, 2.25 A^0, 1.81 A^0 for sample LR0; 3.18 A^0, 2.45 A^0, 2.28 A^0, 2.23 A^0 and 1.81 A^0 for sample CG0; 4.18 A^0, 2.54 A^0, 2.43 A^0, 2.63 A^0, 1.80 A^0 and 1.66 A^0 for sample PR0.

Muscovite

Muscovite was identified by reflection at 4.07A^0 and 1.97 A^0 in sample PR and BB respectively.

Illite

Illite was identified by reflection at 2.11 A^0 for sample PR0; and 2.12 A^0 for both sample CG0 and BB0.

Non-clay minerals

Quartz

Quartz is the only non-clay mineral in all samples. Quartz was identified by distinctive reflections at 4.37 A^0, and 3.34 A^0, for sample LR0; 3.33 A^0 for sample CG0; 3.30 A^0 for sample PR0; 4.12 A^0 for sample BB0.

Effect of Acid Activation on Clay

Acid treatment was observed to have modified the structure of the clay samples. The extent of modification was also noticeable from their FTIR structure.

In figure 3 above it is noticeable that various peaks decrease on acid activation as concentration of acid increases.

In fig 3 the PR OH stretching of inner-surface hydroxyl group at 3694 and 3618 cm^{-1} gradually disappears as acid concentration increase. Hence the sample PR1 and PR3 were not fully modified in lower acid strength.

While in sample CG the OH stretching of inner-surface hydroxyl group at the range of about 3700 and 3600 cm^{-1} on each clay sample series gradually disappears on treatment. This can be attributed to the fact that the heat of the reaction displaced most molecularly bonded water in the clay structure, and the acid has broken most Si-OH, Al-OH bonds, leaving the hydroxides to be continuously removed as vapor as more protons are added via increase in concentration. Also the absorbance of about 2350 cm^{-1} to 2360 cm^{-1} in the untreated sample completely disappears on treatment; this is because the organic matter present in the clay has been destroyed completely during the digestion process. This can be clearly observed in the CG series.

The sample LR showed optimum modification at LR3 as the hydroxyl peak dropped to minimum, although the peak appeared again at LR5 which could arise probably from hydrolysis of the sample at higher concentration. The organic matter present also reduces on activation as observed in all samples.

Clay modification of sample BB was not very effective at lower acid strength. Only the organic material peak at 2358 cm^{-1} was slightly reduced, was can be as a result of temperature. The hydroxyl peaks at 3680 and 3625 cm^{-1} persists from the un-activated to the 3M acid activated sample (BB3). Only at the 5M acid activated sample was modification fully achieved.

The XRD pattern of PR series can be used to understand the effect of acid attack on the crystalline arrangement and structure of clay. The acid attack results in the dealumination of clay structure. Also a number of cations in the octahedra layer of clay, as well as metal impurities such as calcite originally present in the natural clay are removed during acid leaching with acid at elevated temperature. In addition the edges of the platelets are opened, hence a more crystalline product in yield on activation as a result of the increase in surface area and pore diameter of the clay.

From figure 2 shows the XRD diffraction patterns for the PR series in other to determine the structural modification occurring during acid leaching. The clay structure showed intense change in the diffraction profile throughout the treatment. The transformation can be observed from the first acid concentration utilized in activation, indicating that the sample has good re-activity in conditions favorable to acid treatment. Hence hydrogen ions are quickly replacing the replaceable cations present in the octahedral surface. A similar trend was also observed by Cecillia et al, 2018.

The major morphological modifications were noted in the reflections at 9-28^0 and 41-70^o 2θ. It can also be observed that there is a decrease in the intensity of peaks in the activated samples (i.e. PR1, PR3 and PR5). The peak at 4.0773 A^0 (21.800 2ɵ) which is a muscovite mineral [$(KF)_2(AL_2O_3)_3(SiO_2)_6$] was observed to decrease as acid concentration increases from 1M to 5M concentration, this due to the structural disorder that occur due to acid treatment, since there is an increase in the number of protons resulting in the displacement of more potassium ions from the mineral surface. A similar trend is also observed in other clay minerals present in the sample (I.e. kaolinite and illite) as acid activation causes a more crystalline clay sample to become more amorphous with respect to the acid strength. The increase in the peak at 27.00 θ (3.302 A^0) may be related to decrease in mean lattice strain or increase in crystallite size since the mineral present is a non-clay material (quartz). The same was observed and reported by Panda et al, 2010. A similar trend was also observed for the remaining samples on acid attack.

CONCLUSION

In this project, the effect of acid leaching on the morphological structure of activated clay from selected local government area in Niger state, Nigeria; was investigated. Chemical composition of the clay allows for detection of clay minerals such as kaolinite, dickite, illite, muscovite; and non-clay mineral such as quartz. Moreover, determination of morphological characteristics showed that sample CG responded well to acid attack more than samples BB and LR, whose physical properties did not change as much on activation. The XRD analysis showed how acid treatment affected the crystallinity of natural clay and clay minerals present in the sample. The FTIR analysis on clay treatment shows that the OH stretching of inner-surface hydroxyl group at 3694 and 3618 cm^{-1} gradually disappears as acid concentration increases to optimum. Hence optimized activation of each sample was closely monitored on acid treatment, which differs from sample to sample. Thus this method of modification can be used to produce an activated, high surface area, porous and adsorptive material which can be used as a catalyst or catalyst support as well as an adsorbent for bleaching studies.

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Synthesis and Optical Characterisation of Boron Doped Zinc Oxide Thin Film Using Electrostatic Deposition Method

[1]Umaisha R. A., [2]Ramalan A. M. & *[3]Yusuf A. S.

[1]Department of Electrical/Engineering, Nuhu Bamalli Polytechnic Zaria, Nigeria
[2]Department of Physics, University of Abuja, Abuja, Nigeria
[3]Department of Physics, Federal University of Technology, Minna, Nigeria

*Correspondence: Email: ayusuf@futminna.edu.ng Phone number: +2348063886915

ABSTRACT

ZnO thin film was doped with boron doping concentration of 5, 10, 15 and 20%, using electrostatic spray deposition method. The study on the effect on the optical properties of the thin film was carried out. The absorbance data, as-deposited, of the boron-doped ZnO (ZnO:B) thin films was obtained using UV-Visible spectroscopy at room temperature in the wavelength range of 350 – 80nm. A sharp absorption peak was observed at 370nm for undoped ZnO (0% Boron doping), the absorption peak slightly shifted to a lower wavelength when the material was doped with concentration of 5 and 10% of Boron, and significantly shifted to a much lower wavelength when doped with 15 and 20% of Boron concentration. The shift indicated the presence of Boron in ZnO thin films. The transmittance, absorption coefficient (α), optical bandgap, refractive index and extinction coefficient were evaluated from the absorbance data. The transmittance (T) of the films increased with the increase of boron concentration which was attributed to the decrease in free carrier absorption due to the elevated carrier mobility of the film. The films generally exhibit relatively poor optical transmission below 70%) which may be connected to film thickness. The optical bandgap (E_g) was found to be between 3.16 and 3.28 eV, the optical bandgap increased as the boron concentration increased from 0 to 20%, this observation can be attributed to the Burstein - Moss (B-M) effect. Refractive index (n) of ZnO:B thin films is found between 2.43 and 2.55. The extinction coefficient (k) is found between 0.4 and 0.7 which is similar to the widely reported value in its literature. It can therefore be inferred that: doping the material with boron modified/tuned atomic distribution of the ZnO thin films causes changes in the optical properties. This could be useful in many areas including optoelectronics, solar cells, photo catalysis, single or multilayer optical coatings, and dye-sensitized solar cells.

Keywords: Boron, Zinc Oxide, Electrostatic.

INTRODUCTION

Modern technology largely depends on the use of semiconductor materials due to their unique properties and functionalities. Zinc oxide (ZnO) is one of such semiconductor materials with a wide bandgap that suits several applications such as ultra violet (UV) electronics (Liu *et al*., 2000), spintronic devices, solar cell technology, sensors among others owing to its

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complementary optoelectronic properties (Jagadish and Pearton, 2006). The surging interest in ZnO is fuelled and fanned by its prospects in solar cells and optoelectronic applications including display panels, photovoltaic devices and gas sensors credited to the material direct wide bandgap (E_g ~3.3 eV at 300 K), large excited binding energy, less toxic and abundant in nature (Ozgur, 2005) (Kumar *et al*., 2015). Zinc oxide (ZnO) thin film is an inexpensive transparent conductive oxide (TCO) with excellent characteristics (Isah *et al.,* 2016)

Transparent conducting oxides (TCOs) are electrical conductive materials which combine low electrical resistance with high optical transparency in the visible range of the electromagnetic spectrum (Huiyong *et al*., 2010). For transparent conducting oxide (TCO) thin films like ZnO, it is always desired to improve the electrical conduction without affecting its excellent optical properties (Tewari and Bhattacharjee, 2011). Many studies have indicated that doping the properties of ZnO thin films have some interesting features; this markedly depends on oxygen vacancies as well as zinc interstitial defects (Yadav and Uplane, 2015). The optical-electrical properties of these films can be easily tuned by incorporating appropriate impurities of group III elements such as Indium, Gallium, Aluminium and Boron; the efficiency of dopant depends on its electrical negativity and ionic radius of the doping element (Yadav and Uplane, 2015).

MATERIALS AND METHODS

SAMPLE PREPARATION

Soda lime glass was used as substrates in the deposition of ZnO:B thin films. The glass substrates were cleaned with soap (Sodium Lauryl), rinsed with distilled water, methanol and dried under compressed hot air for 5mins. The precursors used were Zn acetate and boric acid solutions. 0.2 M of zinc acetate $Zn(CH_3COOH)_2.2H_2O$ solution was prepared using 4.39g of the zinc acetate in 100ml of methanol and stirred for 5 minutes; and also 0.2 M of H_2BO_3 solution was prepared using 1.20 g H_2BO_3 in 100ml of methanol and stirred for 5mins to obtain a homogeneous clear and transparent solution. Five ZnO:B samples were prepared, to achieve doping, the precursors were mixed using appropriate volume ratios for doping concentration according to Table 1.

Table 1: Doping concentration chart

Sample	Zinc acetate solution (ml)	Boric acid solution (ml)	% doping
ZB0	10	0	0
ZB5	9.5	0.5	5
ZB10	9	10	10
ZB15	8.5	1.5	15
ZB20	8	2	20

DEPOSITION PROCEDURE`

B-doped ZnO thin films were deposited on soda lime glass substrate using electrostatic spray deposition technique at Material Science Laboratory, Namiroch Nig. Ltd. Abuja. The precursor was loaded onto the ESD system (Plate I) with five different doping concentrations of 0, 5, 10. 15 and 20 % and constant nozzle to substrate distance of 5cm, precursor volume of 0.6 ml, Flow rate of 2400 µL/hr and voltage of 4kV and Substrates temperature of 400 ^{o}C. The deposition was carried out in ambient environment. After the deposition, the deposited films were allowed to cool down to room temperature before taken out for characterisations.

OPTICAL CHARACTERISATION

UV/V is Spectrophotometer (UV752N, Axiom) in the wavelength range 250 - 900 nm was used to determine the absorbance (A) of the ZnO:B films and transmittance, absorption coefficient (α), bandgap (E_g), and refractive index were evaluated according to the 1.0 to 1.7.

Transmittance (T)

The transmittance (T) is calculated using equation:

$$A = 2 - \log_{10} \%T \quad (1)$$

$$\%T = 10^{2-A} \quad (2)$$

Absorption Coefficient

The absorption coefficient (α) determines how far into a material light of a particular wavelength can penetrate before it is absorbed. The absorption coefficient ($\propto$) of ZnO:B thin film is calculated using the following relation (Ramalan, 2016).

$$\propto = \frac{2.303A}{d} \quad (3)$$

where d is the film thickness and A is absorbance of the film.

Energy Bandgap (Eg)

The optical bandgap (E_g) is the energy needed to move a valence electron into conduction band. The optical absorption coefficient is ($\propto$) dependent on the energy of incident photon and determined by the equation (Ramalan, 2016).

$$\propto h\nu \approx B\left(h\nu - E_g\right)^{n/2} \quad (4)$$

where B is a constant, α is the absorption coefficient, h is the plank's constant, and ν is the frequency of the incident photons, hv is the energy of the absorbance, E_g is optical energy gap and n is equal to one for direct gap material and two for an indirect gap material. Zinc Oxide is direct gap material therefore the above relation becomes (Mohite, 2015; Ramalan, 2016).

$$\propto h\nu \approx B\left(h\nu - E_g\right)^{1/2} \quad (5)$$

$$\text{Or} \qquad \left(\propto h\nu\right)^2 = h\nu - E_g \quad (6)$$

At $\left(\propto h\nu\right)^2 = 0$; $\mathrm{hv} = E_g$

The value of optical bandgap can be obtained by plotting $(\alpha h\nu)^2$ against hv known as tauc's plot. The extrapolations of the straight linear portion to the energy axis (hv) indicate the values of direct optical bandgap (Ratheesh, 2007).

Refractive Index (n)

The refractive index (n) is calculated using equation:

$$n = \left(\frac{1+R}{1-R}\right) + \sqrt{\frac{4R}{(1-R)^2} - k^2} \quad (7)$$

Where R is reflectance, n is refractive index, and k is extinction coefficient which is given as:

$$k = \frac{\alpha\lambda}{4\pi} \quad (8)$$

Where λ is the wavelength.

RESULTS AND DISCUSSIONS

THICKNESS OF THE BORON DOPED ZnO

The thickness of boron doped Zinc Oxide thin films as determined by profilometer (VEECO DEKTAK 150).

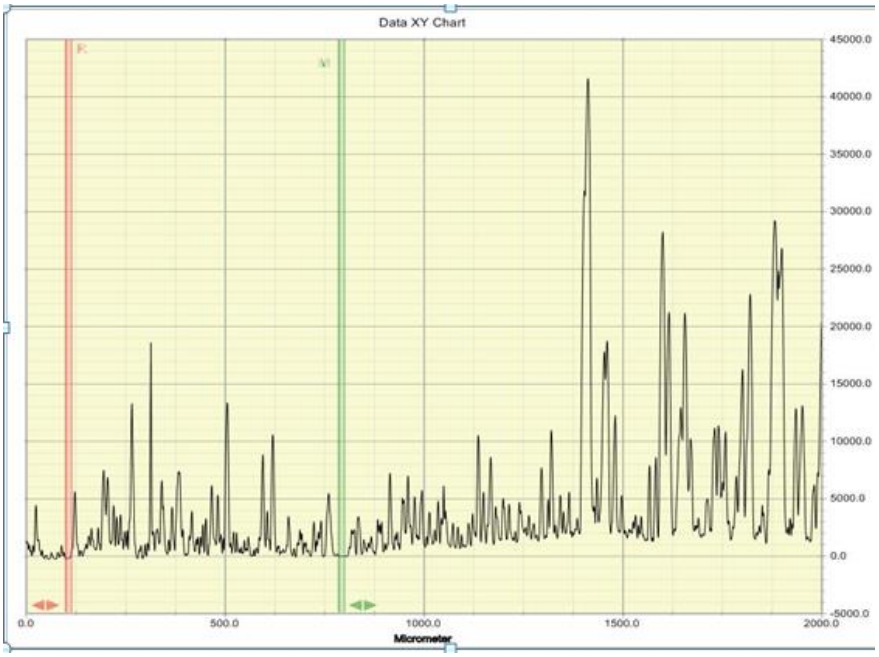

Figure 1: Thickness of boron (B) doped Zinc Oxide thin film

The thickness was determined to be 1.2 µm as shown in figure 1. The thickness is used for the determination of optical bandgap of the ZnO:B thin films.

OPTICAL ABSORPTION

The absorption spectra of undoped ZnO and B doped ZnO thin films are presented in Figure 2.

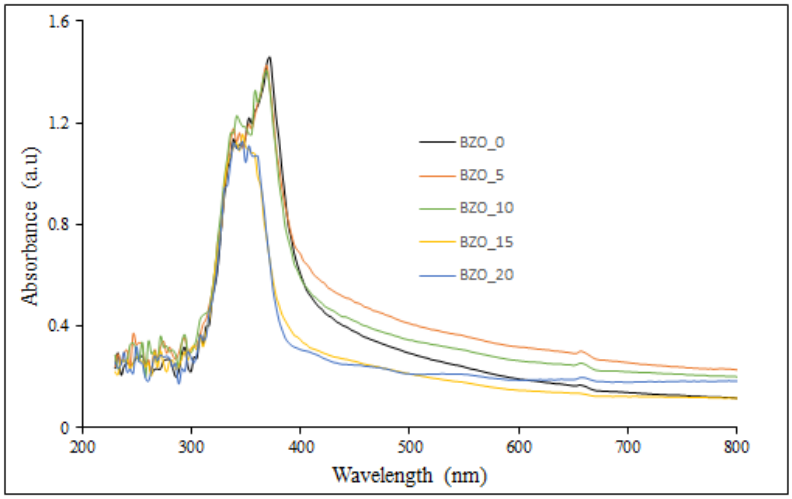

Figure 2: Absorption spectra of B doped ZnO thin films

All the thin films show low absorption in the visible region of the electromagnetic spectrum and sharp absorption peak is observed at 370 nm for undoped (0% B doping) ZnO while the peaks were observed at 369 nm, 366 nm for 5 and 10% B (i.e. BZO_5 and BZO_10) doping concentration, slight shifting of the peaks to a lower wavelength shows that the ZnO contains a foreign material (doping). It is further observed that there is a significant shift towards a lower wavelength when the B concentration was increased to 15 and 20 % (BZO_15 and

BZO_20) with absorption peak of 355 nm and 352 nm respectively. This result shows that the increase in B concentration shift the absorption peaks towards lower wavelength

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TRANSMITTANCE

Optical transmittance (T %) spectra of ZnO:B thin films deposited at various B doping concentration of 0, 5, 10, 15, and 20% are shown in Figures 3

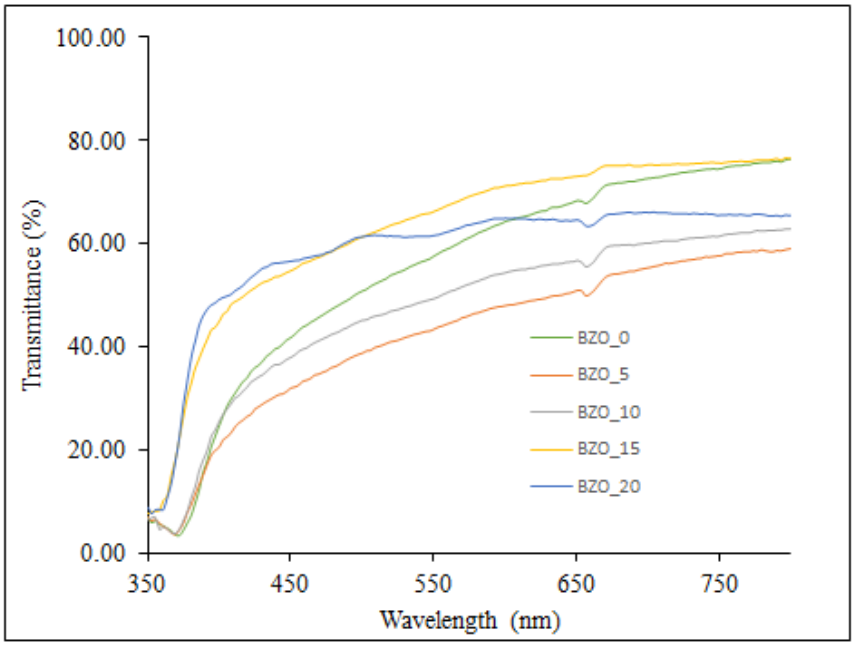

Figure 3: Transmittance spectra of ZnO:B thin films deposited at various B doping concentrations of 0, 5, 10, 15 and 20%

The transmittance of ZnO:B at 0, 5, 10, 15 and 20% B doping concentration are 55.71, 42.13, 48.09, 64.80 and 61.18% respectively. The transmittance increases with the increase of B concentration exempt that of undoped which decreased down to 48.09% at 10% B concentration before it increased to 61.18% at 20% of B doping. This increase in value of T can be attributed to the decrease in free carrier absorption due to the elevated carrier mobility of the film (Sharmin, 2015). Though the transmittance is generally poor (below 70%) which are below most of the values reported in the literature, this may be attributed to the film thickness which is significantly high than thicknesses in the literature.

Optical Bandgap

The optical bandgap is defined as the minimum energy needed to excite an electron from the valence band to the conduction band. Optical bandgap (Eg) of the ZnO and ZnO:B thin films are determined from the Tauc's plot. The optical bands for the ZnO and ZnO:B thin films as well as their comparative analysis are shown in Figure 4 (a-e) and 1.4 (f) respectively.

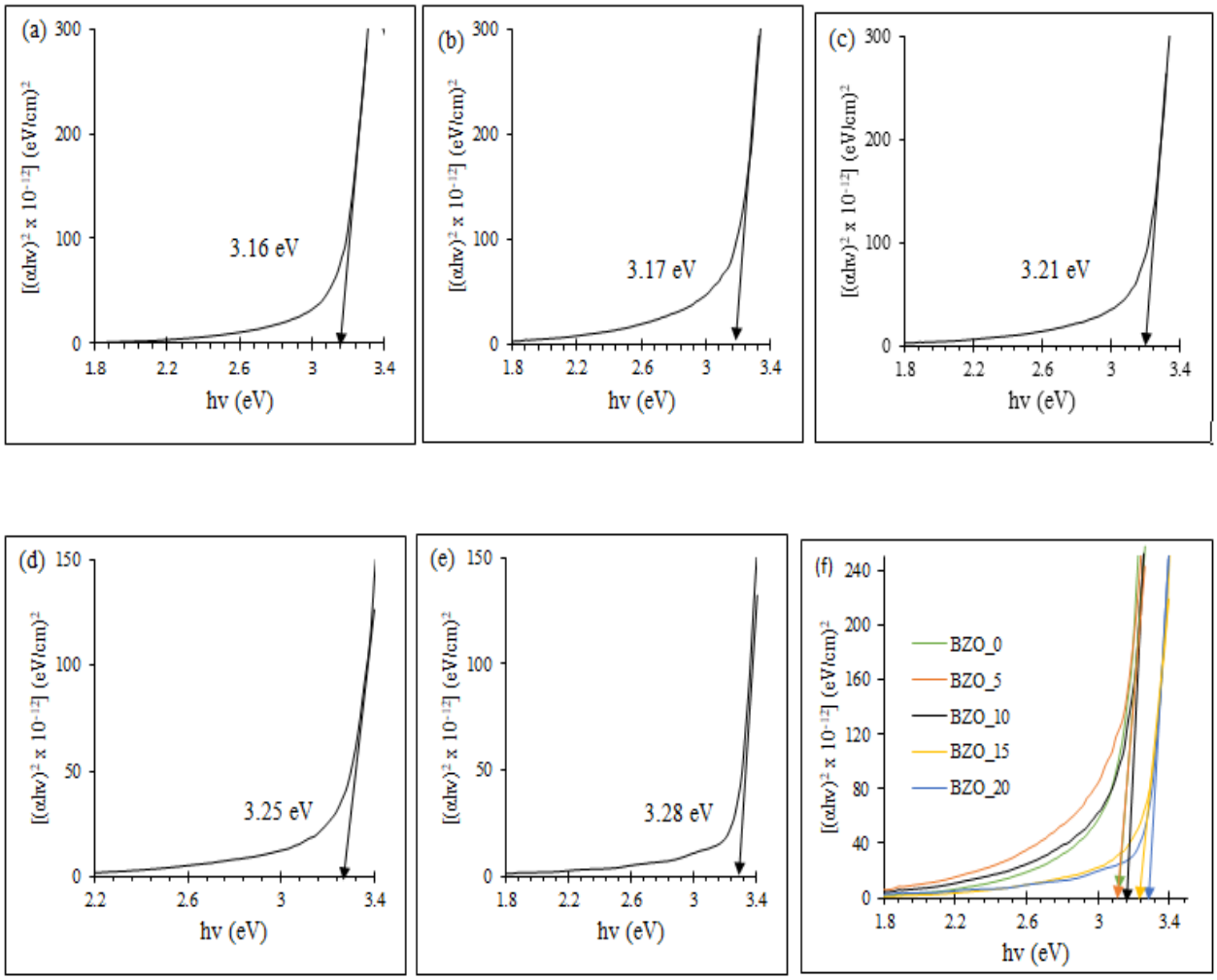

Figure 4: Tauc's plot for energies bandgaps of ZnO:B at (a) 0% (b) 5% (c) 10% (d) 15% and (e) 20% doping concentrations

According to Figure 4 (a-e), it was shown that the optical bandgap for undoped ZnO (0% doping concentration) and boron (B) doped ZnO (5, 10, 15 and 20% doping concentration) thin films exhibit 3.16, 3.17. 3.21, 3.25 and 3.28 eV respectively. It was observed that the optical bandgap increased as the doping concentration from 0 to 20%. Tauc's plots shown in Figures 4 indicate the extrapolations of the linear portion to the intercept of energy axis at $(\alpha h\nu)^2 = 0$ are used to find out the energy bandgap (E_g) of ZnO:B thin films. Bandgap values of ZnO have been reported between 3.1 and 3.3 eV for ZnO single crystal (Srikant and Clarke, 1998). The values of optical bandgap as obtained are found between 3.16 and 3.28 eV, the results obtained are in good agreement with those obtained for single crystal ZnO and ZnO:B thin films deposited onto glass substrate at by spray pyrolysis technique (Pawar *et al*., 2005) and also by Sol-gel method (Kumar *et al*., 2011).

The optical bandgap has increased slightly with B concentration and the maximum optical bandgap of 3.28 eV is obtained for 20% ZnO:B thin film. Increase of optical bandgap may be

attributed to the Burstein- Moss (B-M) effect (Thomas, 2015). B-M effect states that in a heavily doped semiconductor the donor electrons occupy energy levels at the bottom of the conduction band. Pauli principle prevents energy states from being doubly occupied and optical transitions are vertical. Thus, the valence electrons require an additional energy to be excited to the higher energy states in the conduction band to conserve linear momentum; as a result optical bandgap is shifted (Sharma and Kumar, 2014). This observation may also be attributed to many body effects like the exchange energy due to electron-electron and electron-impurity interactions which occurs when the donor density exceeds a certain value and causes narrowing (red shift) of the bandgap energy (Sharma and Kumar, 2014).

Furthermore, the difference in optical bandgap of the ZnO:B film as obtained may also be due to the existence of grain boundaries and imperfections in polycrystalline thin films as reported in case of aluminium (Al) doping (Thomas, 2015). The atomic structure at the grain boundary is different from that in the grain, which leads to larger free carrier concentrations (free electron concentration) and existence of potential barriers at the boundaries, leading to the formation of an electronic field and hence an increase of the bandgap. Similar reports suggest that the bandgap difference between the ZnO:B film and bulk ZnO is due to the grain boundary, the stress and the interaction potentials between defects and host materials. The increase in bandgap may be attributed to the partial filling of the conduction band of ZnO:B thin film resulting in a blocking of the lowest states (Sharmin, 2015; Thomas, 2015).

According to BM effect the broadening of the optical bandgap is given by the relation:

$$E_g = E_o + \Delta E_g^{BM} \quad (1.1)$$

while the Burstein-Moss (B-M) shift is related to the carrier density by the relation:

$$E_g^{BM} = E_g - E_o = \frac{h^2}{2M_\tau}\left(3\pi^2 n\right)^{\frac{2}{3}} \quad (1.2)$$

where h, E_o, M_τ and n are the planks constant, Intrinsic bandgap, reduced effectivemass and carrier concentration. E_g^{BM} is the shift of the doped semiconductor compared to undoped semiconductor. According to the equation, the bandgap increase with increasing carrier concentration ($\Delta E_g^{BM} \alpha \left(3\pi^2 n\right)^{\frac{2}{3}}$, where $\frac{h^2}{2M_\tau}$ is constant). Doping of ZnO with B will increase the carrier concentrations thereby blocking the lowest state in the conduction band owing to the filling up of low energy levels by the conduction electrons, thus increasing the bandgap. The enhancement of bandgap also ensures that boron was successfully doped in the ZnO thin films.

REFRACTIVE INDEX

Refractive index (n) of the ZnO and ZnO:B thin films was calculated and plotted against photon wavelength as shown Figures 5.

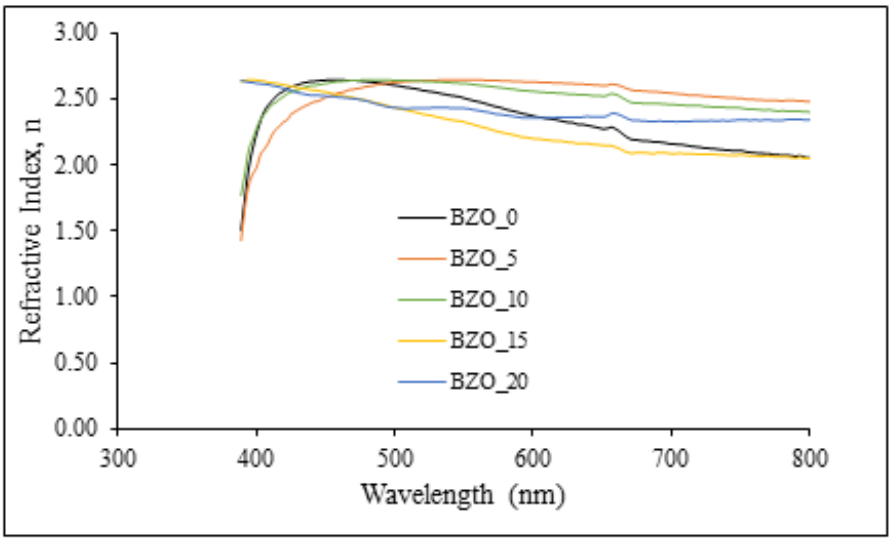

Figure 5: Variation of refractive index with wavelength for 0, 5, 10, 15 and 20 % doping concentrations of ZnO:B thin films

Table 2: Refractive index for ZnO:B thin films deposited at various doping concentrations

S/N	Sample	% Concentration	Refractive index
1	ZBO_0	0.00	2.43
2	ZBO_5	5.00	2.54
3	ZBO_10	10.00	2.55
4	ZBO_15	15.00	2.34
5	ZBO_20	20.00	2.44

The values of n become constant in the visible region of spectrum (about 390 to 700 nm). Table 2 shows average refractive indices (n) for ZnO:B thin films at the range of visible wavelength (390 to 700 nm). The n value increases with the B doping concentration. ZBO_10 (10 % B doping concentration) thin film has the highest n of 2.55 while the ZBO_0 (15 % B doping concentration) has the lowest n of 3.34. Since speed of light is inversely proportional to n, high value of n of a material indicates that light speed decreases when it passes through the material. Thus, it is found that 15% B doping shows lower n than others and it can be said that light moves faster through the films in the visible region. The values of n run between 2.43 – 2.55, these values are consistent with those obtained for ZnO and ZnO:B thin films synthesized by spray pyrolysis method under some different deposition condition (Lokhande *et al*., 2001; Kumar *et al*., 2013).

EXTINCTION COEFFICIENT

The extinction coefficient is measure of damping of the incident wave in the material (Sharma and Kumar, 2014). It also describes the attenuation of light wavelength as it goes through the deposited thin film material. Variation of extinction coefficient (k) with wavelength for ZnO:B thin films are shown in Figure 6.

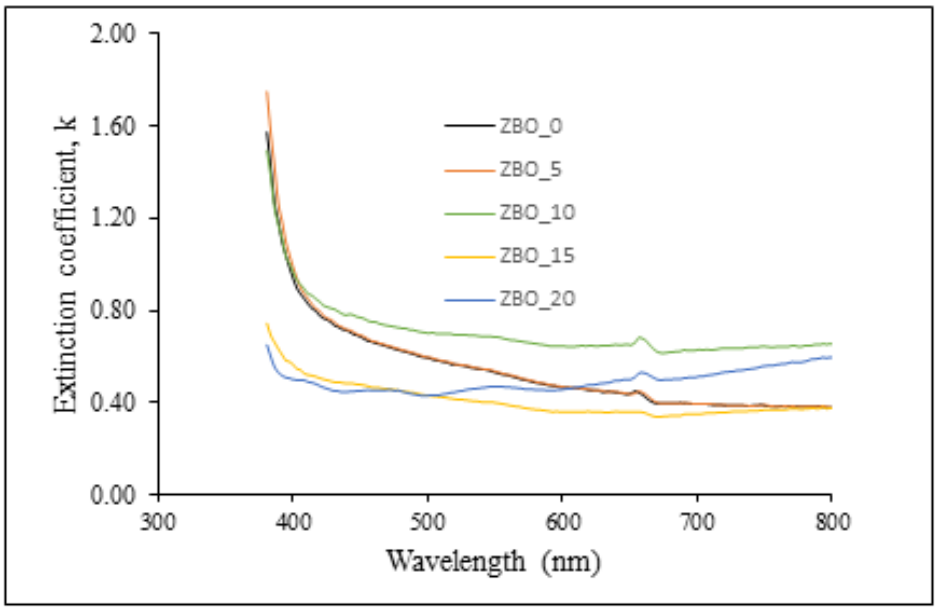

Figure 6: Variation of extinction coefficient (k) with wavelength for ZnO:B thin films deposited at 0, 5, 10, 15 and 20 % doping concentrations

The values of k is found between 0.4 and 0.7 which similar nature has been observed for ZnO:B thin films deposited by Sol-gel dip coating method (Kim *et al*., 2013; Kumar *et al*., 2011).

CONCLUSION

In this project, ZnO:B thin films were synthesized using Electrostatic Spray Deposition (ESD) technique onto glass substrates with different B doping concentrations of 0, 5, 10, 15 and 20%. The thin films have thicknesses between about 1.2 µm. The as-synthesised ZnO:B thin films were characterised using UV-Vis spectrophotometer to determine the absorbance data, the optical transmittance, bandgaps, extinction coefficients and refractive indices of theZnO:B thin films were calculated from the absorbance data.

The transmittance (T) of the films increased with the increase of B concentration; this increase in value of T can be attributed to the decrease in free carrier absorption due to the elevated carrier mobility of the film. Though the transmittance is generally poor (below 70%) which are below most of the values reported in the literature, this may be attributed to the film thickness which is significantly higher than thicknesses in the literature. The optical bandgap increased as the doping concentration from 0 to 20% of B concentration. The optical bandgap, $E_{g,}$of the prepared ZnO:B thin films are found between 3.16 and 3.28 eV, the results obtained are in good agreement with those obtained for single crystal ZnO and ZnO:B thin films deposited onto glass substrate at by spray pyrolysis technique in the literature. Increase of E_g may be attributed to the Burstein- Moss (B-M) effect.Refractive index (n) of ZnO:B thin films is found between 2.43 and 2.55; The n value increases with the B doping concentration. The values of k are found between 0.4 and 0.7.The results obtained in this project work showed good agreement with those reported in the literature for the same material.

Hence, it can therefore be inferred that doping the material with boron modified/tuned the atomic distribution of the ZnO thin films, in return, causes changes in the optical properties which could be useful in many areas including optoelectronics, solar cells, photocatalysis, single or multilayer optical coatings, and dye-sensitized solar cells.

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