PHYTOEXTRACTION OF HEAVY METALS IN INDUSTRIAL WASTE WATER USING BERMUDA GRASS (*Cynodon dactylon)*

BY

YAKUBU JOEL

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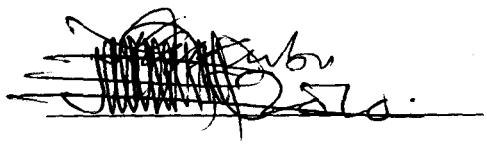
DECLARATION

I hereby declare that this project work is a record of a research work that was undertaken and written by me. It has been presented before for any degree or diploma or certificate at any university or institution. Information derived from personal communications, published and unpublished work were duly referenced in the text.



16/12/2020.

Yakubu Joel

Date

CERTIFICATION

This is to certify that the project entitled "Phytoextraction of Heavy Metals in Industrial Wastewater using Bermuda grass (*Cynodon dactylon*)" by Yakubu Joel meets the regulations governing the award of the degree of Bachelor of Engineering (B.ENG.) of the Federal University of Technology, Minna, and it is approved for its contribution to scientific knowledge and literary presentation.

Engr. (Mrs.) H.I Mustapha	13.12.10
Supervisor	Date
Engr. Dr. A.A. Balami	15/12/2010
Head of Department	Date
External Examiner	8/12/2010
	Date

DEDICATION

I dedicate this project work to my parent, Mr. and Mrs. Yakubu Ofiwayindalo for seeing me throughout the process of this project and the completion of the piece of work.

ACKNOWLEDGEMENTS

I acknowledge God almighty for seeing me through the process of this project work and the completion of this piece of work and for also seeing me throughout my stay in this campus.

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ABSTRACT

The project work discusses phytoextraction technologies applied in the remediation of industrial wastewater. This topic is of relevance to the manufacturing industries and the nation at large since they are the highest generator of wastewater. This work will help them in using natural adsorbent to treat their wastewater and will be available and cheaper. The extraction of the heavy metals from the industrial wastewater was done using Bermuda grass *(cynodon dactylon)*. The heavy metals such as Aluminium and Manganese were taking into consideration; Nitrogen and Phosphorus were also taken into consideration. The level of contamination of the industrial wastewater before planting were thus; aluminium, Al (0.509mg/l), manganese, Mn (0.138mg/l) also nitrogen, N was (18.8mg/l) and phosphorus, P was (0.26mg/l) and after planting the level of heavy metals drastically reduce and the results were thus; aluminium, Al (0.043mg/l), manganese, Mn (0.027mg/l), and also nitrogen, N and phosphorus, P were (5.902mg/l) and (0.0016mg/l) respectively which is insignificant and could be said that the heavy metal were drastically reduce. Though the quantity of water, duration of treatment and plant selection is been recommended and it is believe if taken into consideration heavy metals can be extracted and the wastewater will be safe for disposal.

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Abbreviations

IBBI	International Breweries and Beverages Industries.
COD	Chemical Oxygen Demand.
EPA	Environmental Protection Agency.
PAHs	Polyaromatic Hydrocarbons
BOD	Biochemical Oxygen Demand.
CGP	Consolidated Growers and Processors.
USA	United State of America.
CCA	Chromium, Copper and Arsenic.
SVE	Soil Vapour Extraction.
pH	Hydrogen Concentration.
BDL	Below Determination Level.
SS	Suspended Solid.
DO	Dissolve Oxygen.
AAS	Atomic Absorption Spectrophotometer.
MSRTUM	Multi Study and Research Track University of Maryland, Baltimore.

CHAPTER ONE

1. INTRODUCTION

1.1 Background to the Study

Pollution is the introduction of contaminants into an environment that causes instability, disorder, harm or discomfort to the ecosystem, i.e. physical system or living organisms (Gari, 2002). Pollution can take the form of chemical substances or energy, such as noise, heat or light (David, 1994). Pollutants, the elements of pollution, can be foreign substances or energies or naturally occurring; when naturally occurring, they are considered contaminants when they exceed natural levels (Gari, 2002). There are basically different types of environmental pollution, and each one has detrimental effects on wildlife, human habitation, and the quality of life in the affected area. The followings are the types of pollution known; Air pollution, water pollution, and Soil or land pollution, noise pollution, radioactive pollution, thermal pollution, light pollution, visual pollution, and Personal pollution (Sungmin, et. al., 1996).

Land pollution is the contamination of the land surface of the earth through dumping urban waste matter indiscriminately, dumping of industrial waste, mineral exploitation, and misusing the soil by harmful agricultural practices. Land pollution includes visible litter and waste along with the soil itself being polluted. The soil gets polluted by the chemicals in pesticides and herbicides used for agricultural purposes along with waste matter being littered in urban areas such as road, parks and streets. Land pollution comprises of solid waste and soil pollution. Solid waste and semisolid or solid matter that are created by human or animal activities, and which are disposed because they are hazardous or useless. Most of the solid wastes, like paper, plastic containers, bottles, cans, nylons, polythenes, and even used cars and electronic goods are not biodegradable, which means they do not get broken down through inorganic or organic processes. Thus, when they accumulate they pose a detrimental effect both on soil and human

being. The decaying waste also attracts household pests and result in urban areas becoming unhealthy and dirt to reside in. Moreover, it also causes damage to terrestrial organisms, while also reducing the use of the land for other, more useful purposes (Anonymous, 2010).

Some of the sources of solid waste that cause land pollution include; waste from agriculture, mining, industries, solid from sewage treatment, ashes, and garbage. They contain increasing amounts of paper, cardboards, plastics, glass, nylons, polythenes, old construction material, packaging material and toxic or otherwise hazardous substances. The portion of solid waste that is hazardous such as garbage dump sites (Anonymous, 2010). Industrial waste water contain some toxic and hazardous substances that when release to the environment causes damage to the terrestrial organism , human ,and agricultural soil , thereby reducing soil fertility for agricultural and other purposes (Schnoor, 1997).

One of the burning problems of our industrial society is the high consumption of water and the high demand for clean drinking water and agricultural purposes. Numerous approaches have been taken to reduce water consumption, but in the long run it seems only possible to recycle waste water into high quality water. It seems timely to discuss alternative water remediation technologies that are fit for industrial as well as developing countries like (Nigeria) to ensure a high quality of drinking water throughout the country (Anonymous, 2010).

Garbage comprises of the waste matter from food that are decomposable and other waste matter that are not decomposable such as glass, metal, cloth, plastics, wood, paper, and so on. The presence of this decomposable and non-decomposable material causes detrimental effect to the soil (Gari, 2002).

Phytoremediation is the use of plant to degrade, remove, and metabolize potential toxic compounds from the environment; phytoremediation can also be defined as the use of vegetation for in situ treatment of contaminated soil, sediment, and water (Mwegoha, 2008).

The phytoremediation process work sequentially or simultaneously, depending on the type of contaminants and treatment goal (Schnoor, 2002). Different processes may act on different contaminants or at different exposure concentrations (McCutcheon and Schnoor, 2003). Hence, phytoremediation is best applied at site with shallow contamination of organic, nutrients, or metal pollutant that are amendable by the following process; phytostimulation, phytoaccumulation, Phytovolatilization, phytodegradation, Phytostabilization, phytoextraction (Aken and Schnoor, 2002, McCutcheon and Schnoor, 2003).

1.2 Statement of the Problem

The contamination of the environment with industrial waste water has been a concern in many states in the country (Nigeria) where industries are sited and their effect to the soil, terrestrial organism, and human. The lost of micro-organism in the soil, aquatic animal in our water, and contacting of diseases through the ground water which is our main sources of portable water caused by indiscriminate disposal of unsafe water to the environment which pose the question on how to remove the contaminants in the industrial waste water before disposal to the environment in such away that it will not cause detrimental effect to the environment.

1.3 Objectives of the Study

1. To determine the type of contaminants present in industrial waste water.
2. To remove the contaminants in the industrial waste water using Bermuda grass.
3. To analyze the effect of the contaminants on the environment.

1.4 Justification of the Study

Determination of the type of contaminants present in industrial waste water will help in identifying the type of plant and method to be used, which will reduce many hazards caused by the waste water.

Removal of contaminants from the waste water will help in reducing hazards like, groundwater contamination through the infiltration of the waste water to water table, destruction of soil, terrestrial organism, aquatic animal and contacting of disease by human.

Analyzing the effect of contaminants on the environment will help in creating more awareness about the technology and improving the performance of existing waste water treatment.

1.5 Scope of the Study

The scope of this project work will be based on the industrial waste water from International Breweries and Beverages Industries (IBBI), Kaduna and it going to concentrate on the removal of contaminants from the waste water before disposing to the environment, and know the potentials of phytoremediation in removing contaminants from industrial waste water using method of Phytoextraction and Bermuda grass for the treatment.

CHAPTER TWO

2. LITERATURE REVIEW

Population explosion, haphazard rapid urbanization, industrial and technological expansion, energy utilization and waste generation from domestic and industrial sources have rendered many water unwholesome and hazardous to men and other living resources. There are little or no stringent laws guiding environmental pollution in Nigeria. Hence, many industries discharge untreated or inadequately treated waste water into water ways thereby polluting the environment (Amuda and Ibrahim, 2006).

A number of technologies have been developed over years to remove organic matters (expressed as C.O.D) from industrial waste water. The most important technologies include coagulation and flocculation process, membrane filtration, oxidation process (Amuda et. al., 2006). These methods are generally expensive, complicated, time consuming and required skilled personnel. The high cost of coal-based activated carbon has stimulated the search for cheaper alternative. Low cost and non-conventional adsorbents includes agricultural by-product such as nut shells, wood, bone, peat, processed into activated carbon (Tam et. al.,1999) and biomass such as aspergillus tereus and Rhizopusarrhizus have been reported to be important adsorbent for the removal of metal and organic matter from industrial waste water. Waste water from industries includes employees' sanitary waste, process waste from manufacturing, wash waters, and relatively uncontaminated water from heating and cooling operations. The waste waters from processing are the major concern. They vary widely with the type of industry (Glynn and Gary, 1996).

The extent of solid waste disposal in the world has been of alarming rate (Mattina et. al., 2006). The municipal wastes are dumped in an uncontrolled manner without any provision to deal with the detrimental effect it causes to the soil and environment in general. Inadequate or

no waste disposal facilities in the urban areas has often resulted into pollution transport to various portion of the land causing or jeopardizing the fertility of the soil for various agricultural useful purposes. Contaminants of most concern are metals, polyaromatic hydrocarbons, garbage and mineral oil (Mwegoha, 2008). The current practice of remediating heavy metal contamination elsewhere relies heavily on dig-and-dump or encapsulation, neither of which addresses the issue of decontamination of the soil (Baker et. al., 1991). Immobilization or extraction by physiochemical techniques can be expensive and applicable only for small area where rapid, complete decontamination is required (Chaudhuri et. al., 2002). This practice may not be feasible for a developing country like ours.

The method used for remediating the solid waste dumpsites and wastewaters are phytoremediation. Phytoremediation describes the treatment of environmental problem through the use of plant which mitigate the environmental problem without the need to excavate the contaminant material and dispose of it elsewhere. The word's etymology comes from the Greek word 'phyto' which means plant, and Latin 'remedium' meaning restoring balance, or remediation. Phytoremediation consists in mitigating pollutant concentrations in contaminated soils, with plant able to contain, degrade or eliminate metals and its derivatives, and various other contaminants, from the media that contain them (Greger and Landberg, 1999). Phytoremediation is applied wherever the and water has become polluted. Example is the solid waste dumpsite like garbage dumpsites and industrial waste water.

Phytoremediation is considered to the natural ability of certain plants called hyperaccumulators to bioaccumulate degrade or render harmless contaminants in soils and water (Meagher, 2000).Contaminants such as metals and polythenes have been mitigated in phytoremediation projects. Many plants such as mustard plants, beans, penny cress, pigweed, and maize have proven to be successful at hyperacculating contaminants at toxic waste sites.

This process is considered a clean, cost-effective and non-environmentally disruptive technology, as opposed to mechanical cleanup methods such as soil excavation (Hannink, and Rosser, 2001).

Natural or planted vegetation on polluted sites such as land fills; mines area and dumpsites play an important role in controlling erosion and removing contaminants such as leachate, besides imparting aesthetic value (Nagendran et al., 2006). Nevertheless, despite the availability of literature on the potentials of phytoremediation in achieving the original treatment of a wide range contaminant found in municipal solid waste dumpsite and mining areas, there is little to no awareness about this technology in Nigeria.

2.1 Advantages of Phytoremediation

As with most new technologies phytoremediation has many problems and controls when compared to other more traditional methods of environmental remediation (Nagendran et. al., 2006). It becomes clearer what the detailed advantages are:

- More economically viable using the same tools and supplies as agriculture (Schnoor, 1997).
- It is it is less disruptive to the environment and does not involve waiting from new plant communities to recolonise the site.
- Disposal sites are not needed.
- It is more likely to be accepted by the public as it is more aesthetically pleasing than traditional methods (Morikawa and Erkin, 2003).
- It has the potential to treat sites polluted with more than one type of pollutant (Schnoor, 2002).
- The plants can be easily monitored.

- It is potentially the least harmful method because it uses naturally occurring organisms and preserves the environment in more natural state (McCutchen and Schnoor, 2003).

2.2 Disadvantages of Phytoremediation

According to Erakhrumen and Agbontalor (2007), the following are the disadvantages of phytoremediation

- It is dependent on the growing conditions required by the plants (i.e. climate, geology, altitude, temperature, etc.)
- Large scale operation requires access to agricultural equipment and knowledge.
- Success is dependent on the tolerance of the plant to the pollutant.
- Time taken to remediate sites far exceeds that of other technologies.
- Contaminants solubility may be increased leading to greater soil or land damage.
- It is limited to the surface area and depth occupied by the roots.
- The survival of the plants is affected by the toxicity of the contaminated land and the general condition of the soil (Schwitzguebel, 2000).

It has been noted that around the country and the world at large, there is an increasing trend in areas of land affected by contamination from solid waste such as garbage dumps, industrial and agricultural activities either due to ignorance, lack of vision, or carelessness (Mwegoha, 2008). The build of toxic pollutants not only affects natural resources but also causes a major stain on ecosystems. Remediation of contaminated sites using conventional practices such as 'dig-and-dump' techniques, is often expensive, has limited potential, and is usually only applicable to small areas. Additionally, these conventional approaches to remediation often make the soil infertile and unsuitable for agriculture and other uses by destroying the micro environment. Hence there is the need to develop and apply alternative, environmentally sound technologies,

taking into account the probable end use of the site once it has been remediated (Baker et. al., 1994).

2.3 Effects of Industrial Waste Water on the Environment

Waste water given off by various industries and factories are often considered to be one of the prime factors contributing to water and soil pollution. According to the Environmental Protection Agency (EPA), it has been estimated that industrial waste water is responsible for almost 50 percent of the pollution present in the country. There are various wide-ranging effects, as well as serious consequences, of industrial pollution on the ecological balance of the atmosphere (Anonymous, 2010).

2.3.1 Water Pollution

Pollution emitted from the industries is also one of the major factors contributing towards water pollution. Dumping of various industrial waste products into water sources, and improper contamination of industrial wastes, often result in polluting the water. Such water pollution disturbs the balance of the ecosystem inside, resulting in the death of various animal and plant species present in the water (Anonymous, 2010).

2.3.2 Soil Pollution

Soil pollution is defined as a phenomenon is which the soil loses its structure and fertility due to various natural and artificial reasons. Dumping of industrial wastes is one of the prime factors contributing towards soil pollution. Industrial wastes contain large amounts of various chemicals which get accumulated on the top layer of the soil, resulting in loss of fertility of the soil. Such loss of fertility ultimately results in changes in the ecological balances of the environment due to reduction in plant growth (Anonymous, 2010).

.3.3 Other Common Effect

Certain other common effects of industrial pollution include damaging buildings and structures, increasing the risk of various occupational hazards such as asbestosis, pneumoconiosis, among others (Anonymous, 2010).

2.4 Working Principle of Phytoremediation

The uptake of contaminants in plants occurs primarily through the root system, in which the principal mechanisms for preventing contaminant toxicity are found. The root system provides an enormous surface area that absorbs and accumulates the nutrient essential for growth, as well as other non-essential contaminants. Researchers are finding that the use of trees (rather than smaller plants) is effective in treating deeper contamination because tree roots penetrate more deeply into the ground (Mwegoha, 2006). Plant roots also cause changes at the soil root interface as they release inorganic and organic compounds (root exudates) in the rhizosphere. These root exudates affect the number and activity of the microorganisms, and stability of the soil particles around the root, and the availability of the contaminants. Root exudates, by themselves can increase (mobilize) directly or indirectly the availability of the contaminants in the root zone (rhizosphere) of the plants through changes in soil characteristics, release of organic substances, change in chemical composition, and/or increase in plant-assisted microbial activity (Mwegoha et. al., 2007).

Phytoremediation is an alternative or complimentary technology that can be used along with or, in some cases in place of mechanical conventional cleanup technologies that often require high capital inputs and are labour and energy intensive. Phytoremediation is an original remediation technology that utilizes the inherent abilities of living plants. It is also an

ecologically friendly, solar energy driven clean-up technology, based on the concept of using nature to cleanse nature (Baker, et. al., 1994).

2.5 Site where Phytoremediation can Work

Phytoremediation technologies can be used to clean up metals, pesticides, solvents, explosive, polyaromatic hydro-carbons, present in industrial waste water and landfills. Phyto-remediation can be used in combination with other cleanup approaches as a 'finishing' or 'polishing' step. Some phytoremediation applications are slower than mechanical and chemical methods and are limited to the depths that are within the reach of plants roots (Ghosh and Singh, 2005).

Generally, the use of phytoremediation is limited to sites waste water with low to medium contaminant concentrations and contaminations in shallow soils where phytotoxicity does not occur and the roots of plants can easily access the contaminant. Plants can also be used to clean up contaminants in streams and groundwater (Schnoor, 2002).

2.6 Phytoremediation Process

Depending on the underlying processes, applicability and type of contaminant, phytoremediation can be broadly categorized as:

2.6.1 The Use of Phytoremediation to Treat Organic Contaminants

Organic contaminants are common environmental pollutants. There are several ways that plants can be used for the phytoremediation of these contaminants: phytodegradation, rhiz-odegradation, and phytovolatilisation (Mwegoha, 2006).

2.6.1.1 Phytodegradation

Phytodegradation, also called phyto-transformation, is the breakdown of contaminants taken up by plants through metabolic processes within the plant, or the breakdown of contaminants surrounding the plant through the effect of compounds produced by the plants. Complex organic pollutants are degraded into simpler molecules and are incorporated into the plant tissues to help the growth faster. Plants contain enzymes that catalyse and accelerate chemical reactions. Some enzymes breakdown and convert ammunition wastes, other degrade chlorinated solvents such as trichloroethylene and other degrade herbicides (Baker and Brooks, 1989).

2.6.1.2 Phytovolatilization

This is the process whereby plants uptake contaminants which are water soluble and release them into the atmosphere as they transpire the water. The contaminants may become modified along the way, as the water travels along the plant's vascular system from the roots to the leaves, whereby the contaminants evaporate or volatilize into the air surrounding the plant, there are varying degrees of success with plants as phytovolatilize with one study showing poplar trees to volatilize up to 90% of the trichloroethylene they absorb (Baker, and Brooks, 1989).

2.6.1.3 Rhizodegradation

This is also known as biodegradation, phytostimulation. This is the breaking down of organic contaminants in the soil by soil dwelling microbes which are enhanced by the rhizospheres presence. Micro-organisms consume and digest organic substances for nutrition and energy. Certain micro organism can digest organic substances such as fuel or solvents that are hazardous to human and break them down into harmless products in a process called biodegradation. Natural substances released by the plant root-sugars, alcohols and acid – contain

organic carbon that provides food for soil microorganisms and the additional nutrients enhance their activity (Greger and Landberg, 1999).

Biodegradation is also aided by the way plants loosen the soil and transport water to the area.

2.6.2 The Use of Phytoremediation to Treat Metal Contaminants

At sites and waste water contaminated with metals, plants can be used to either stabilize or remove the metals from the soil through three mechanisms: Phytoextraction, Rhizofiltration and Phytostabilization.

2.6.2.1 Photoextraction

This is the name given to the process where plant root uptake metal contaminants from the soil and translocate them to their above soil tissues. As different plant have different abilities to uptake and withstand high levels of pollutants many different plants may be used. Certain plants called hyperaccumulators, absorbs unusually large amounts of metals in comparison to other plants. Once the plants have grown and absorbed the metal pollutants they are harvested and disposed off safely (McCutcheon and Schnoor, 2003). This process is repeated several times to reduce contamination to acceptable level. If plants are incinerated, the ash must be disposed off in a hazardous waste land fill, but the volume of ash will be less than 10% of the volume that would be created if the contaminated soil itself were dug up for treatment. Metals such as nickel, zinc, and copper are the best candidates for removal by phytoextraction because the majority of the approximately 400 known plants that absorb unusually large amounts of metals have a high affinity for accumulating these metals (Meagher, 2000).

2.6.2.2 Rhizofiltration

'Rhizo' means 'root' is the absorption or precipitation onto plant root (or absorption into the roots) of contaminants that are in solution surrounding the root zone. Rhizofiltration is

similar to phytoextraction, but the plants are used to cleanup contaminated groundwater rather than soil. The plants to be used for cleanup are raised in green houses with their roots in water. Contaminated water is either collected from a waste site or brought to the plants or the plants are planted in the contaminated area, where the roots then take up the water and the contaminated dissolved in it. As the roots become saturated with contaminants they are harvested (Meagher, 2000).

2.6.2.3 Phytostabilisation

Phytostabilisation is the use of certain plant species to immobilize contaminants in the soil through and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants (rhizosphere). This process reduces the mobility of the contaminant and prevents migration to the ground water or air, and also reduces bioavailability for entry into the food chain. This technique can be used to re-establish a vegetative cover at sites where natural vegetation is lacking due to high metal concentration in surface soil or physical disturbances to surficial materials. Metal tolerance species can be used to restore vegetation to the sites, thereby decreasing the potential migration of contamination through wind erosion and transport of exposed surface soils and leaching of soil contaminants to groundwater. Once a community of tolerant species has been established, the potential for wind erosion and thus spread of the pollutants is reduced and leaching of the soil contaminants is also reduced (Meagher, 2000).

Table 2.1 **Application of Phytoremediation**

Application	*Description*	*Contaminants*	*Type of plants*
Phytotransformation	Sorption, uptake and transformation of contaminants	Organic, including Nitroaromatics and chlorinated aliphatics	Tree and grasses, beans.
Rhizosphere biodegradation	Microbial degradation in the rhizosphere stimulated by plants	Organics such as PAHs, petroleum hydrocarbons, TNT, pesticides	Grasses, alfalfa, many other species including trees.
Phytostabilization	Stabilization of contaminants by binding, holding soils and/or decreased leaching.	Metals, organics.	Various plants with deep or fibrous roots system.
Phytoextraction	Uptake of contaminants from soil into roots or harvestable shoots	Metal, inorganics, radionuclide	Variety of natural and selected hyper accumulators e.g. alyssum, Brassica or Thelaspi.

Table 2.1. Cont'd

Rhizofiltration	Sorption of contaminants from aqueous solutions onto or into roots	Metals, radionuclides, Hydrophobic organics	Aquatic plants e.g. pennywort; also *Brassica* and sunflower.
Hydraulic control/plume capture/phytotrans	Removal of large volumes of water from groundwater and/or aquifers by trees	Inorganics, nutrients, chlorinated solvents	Poplar, willow trees.
Phytovolatilization	Uptake and volatilization from soil water and groundwater, conversion of Se and Hg to volatile species	Volatile organic compounds, Se and Hg.	Trees for VOCs in groundwater, brassica, grasses, wetland plants for Se and Hg in Soil/ sediments.
Vegetative caps	Use of plants to retard leaching of hazardous compounds from landfills	Organics, inorganics, wastewater, landfill Leachate	Trees such as poplar, plants such as alfalfa, and grasses.

Sources: Schnoor (2002)

2.7 Bermuda grass as Industrial Waste Water Remediator

Bermuda grass *(Cynodon dactylon),* a warm-season grass native to southeast Africa, is widely grown in the southeastern United States and is gaining popularity in southern Missouri. It is a deep-rooted, sod-forming grass that spreads by means of stolons and rhizomes and grows to a height of 15 to 24 inches. Perhaps its greatest advantage is that it is productive during the months of June, July and August when the quantity and quality of cool-season grasses such as tall fescue *(Festuca arundinacea)* and orchardgrass *(Dactylis glomerata)* are poor. Another advantage of bermudagrass is that herbage production is distributed more evenly throughout late spring and summer than that of other warm-season grasses. Bermuda grass produces an extensive root system that provides some drought tolerance. It responds well to nitrogen fertilization and produces a large quantity of dry matter for either grazing or hay production when soil moisture is not limiting. Although both seeded and sprigged varieties of bermudagrass are available, sprigged varieties generally have a yield advantage over seeded varieties. Hybrid Bermuda grass are popular for hay production because they are responsive to nitrogen fertilizer, have a high yield potential and are relatively fast drying. Bermuda grass makes good use of animal manures and, if well fertilized, gives high animal weight gains per acre. Bermuda grass is adapted to a wide range of soil conditions but is best suited to a well-drained site. Hulled seed of common Bermuda grass or other seed-propagated varieties should be planted in spring. Hybrid varieties can be planted in April or May as sprigs. Because hybrid varieties produce little or no viable seed, they must be vegetatively propagated (sprigged). In the southern United States, annual clovers, small grains and annual ryegrass are often overseeded in the fall to provide winter-spring production (Anonymous, 2010). Bermudagrass is used by soil and water conservation engineers for remediation of soil polluted sites and waste water, both domestic and industrial. It

best use in wastewater that is contaminated with the following contaminants: Heavy metals ch as Aluminium and manganese, suspended solid, phosphorus, nitrate in form of nitrogen, OD, COD, etc (Anonymous, 2010).

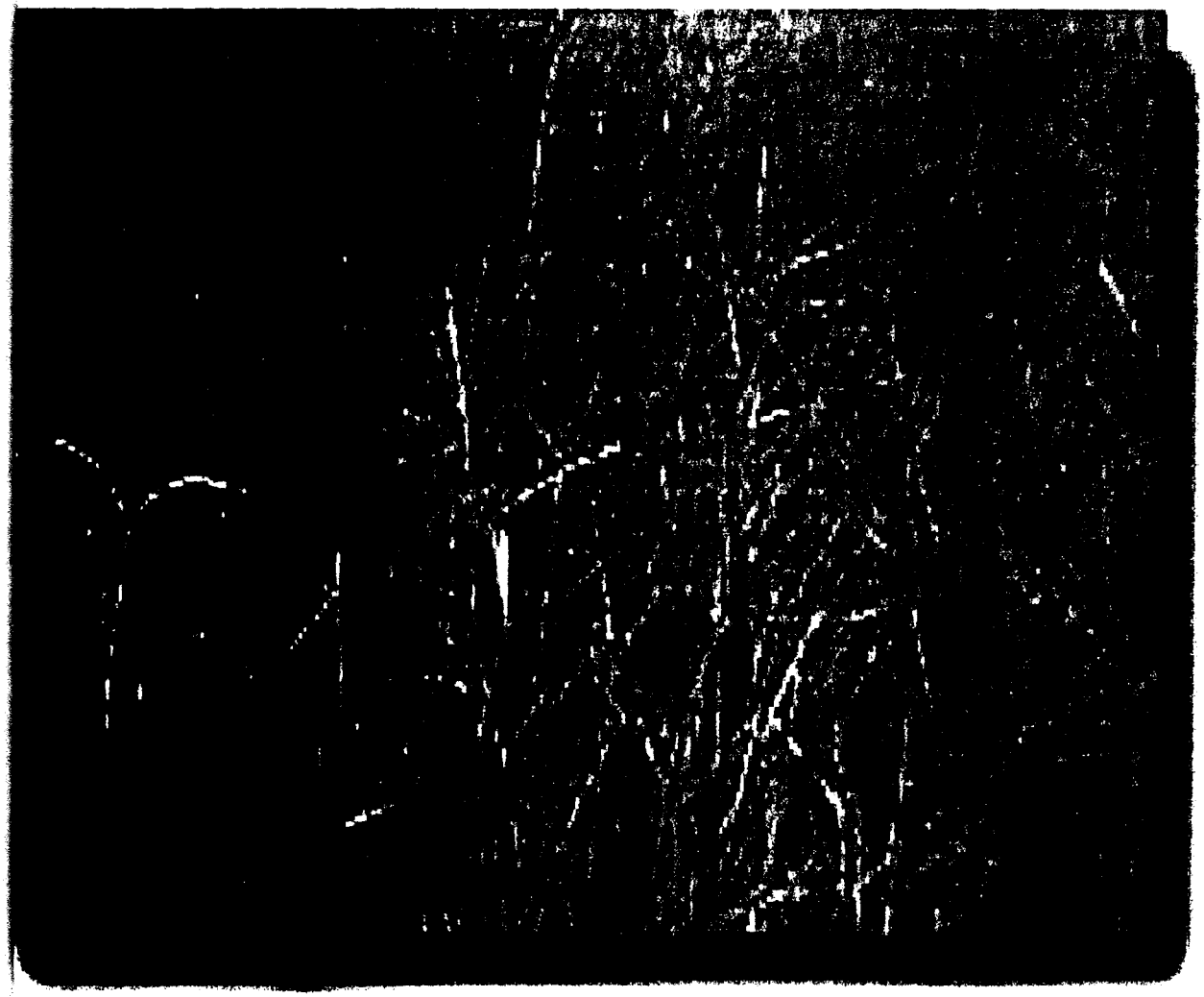


Plate1: Bermuda grass

Plate2: Bermuda

2.7.1 Other plant used in Phytoremediation order than Bermuda grass

The following are some of the plants that can be used in phytoremediation of polluted soil, stewater (industrial, domestic and municipal). Plant such as maize, beans, alfalfa, sunflower . can be used in phytoremediation, but due to their economic important to the society they :n restricted for use.

The economic importance of some of the plant is as follow:

2.7.1.2 Sunflower

This is a very good phytoremediator of contaminated soil and water by heavy metals and other contaminants but mostly not use due to its economic importance. Several species of sunflowers are of economic importance. Below is a list of species that have a widespread use in society. Many more species of Asteraceae of narrow distribution, especially in tropical regions, are used locally for various medicinal and food purposes. The economic importance of many species of sunflowers is yet to be fully explored. According to Dempewolf et al. (2008) give the following as the importance of sunflower;

2.7.1.2.1 Oils

- Niger seed oil, Guizotia abyssinica (L. f.) Cass, Asteroideae: Millerieae, northeast tropical Africa.
- Safflower oil, Carthamus tinctorius L. Carduoideae: Cynareae, central Asia.
- Sunflower oil, Helianthus annuus L. Asteroideae: Heliantheae, North America.

2.7.1.2.2 Food

- Artichoke, Cynara cardunculus L., Carduoideae: Cynareae and Eurasia.
- Endive, Cichorium endivia L., Cichorioideae: Cichorieae and Europe.
- Jerusalem artichoke, Helianthus tuberosus L., Asteroideae: Heliantheae and North America.
- Lettuce, Lactuca sativa L., Cichorioidea: Cichorieae, cultivar of Asian species.
- Mexican tarragon, Tagetes lucida Cav., Asteroideae: Tageteae and Mexico.
- Radicchio, Cichorium intybus L., Cichorioideae: Cichorieae and Europe.
- Salsifi, Tragopogon porrifolius L., Cichorioideae: Cichorieae and Europe.

- Sunflower seeds, Helianthus annuus L., Asteroideae: Heliantheae and North America.
- Tarragon, Artemisia dracunculus L., Asteroideae: Anthemideae and Eurasia.

2.7.1.2.3 Ornamentals

- Black-Eyed Susans, Rudbeckia hirta L., Asteroideae: Heliantheae, USA, Canada.
- Chrysanthemums, Chrysanthemum several species, Asteroideae: Anthemideae, Asia.
- Dahlias, Dahlia coccinea Cav., Dahlia pinnata Cav., Asteroideae: Coreopsideae, Mexico.
- Echinaceas, Echinacea purpurea (L.) Moench, Echinacea paradoxa, (Norton) Britton, Asteroideae: Heliantheae, North America.
- Marigolds, Tagetes erecta L., Asteroideae: Tageteae, Mexico, Central America.
- Santolinas, Santolina, several species Asteroideae: Anthemideae, Europe.
- Zinnias, Zinnia angustifolia Kunth, Zinnia peruviana (L.) L., Zinnia violacea Cav., Asteroideae: Heliantheae, Mexico, South America.

2.7.1.2.4 Medicinal

- Anti-malarial, Artemisia annua L., Asteroideae: Anthemideae, eastern Asia.
- Chamomile tea, Matricaria recutita L., Asteroideae: Anthemideae, Europe.
- Echinacea tea, Echinacea purpurea (L.) Moench, Asteroideae: Heliantheae, North America.

2.7.1.2.5 Industrial

- Absinthe, alcoholic beverage flavoring, Artemisia absinthium L., Asteroideae: Anthemideae, Europe.

- Insecticides (pyrethrins and cinerins), Tanacetum spp. Asteroideae: Anthemideae, Eurasia.
- Orange dye, Tagetes erecta L., Tagetes patula L., Asteroideae: Tageteae, Mexico.
- Orange dye, Carthamus tinctorius L., Cichorioideae: Cichorieae, central Asia.
- Rubber, Guayule, Parthenium argentatum A. Gray, Asteroideae: Heliantheae, North America.
- Sweetener, Stevia rebaudiana (Bertoni) Bertoni, Asteroideae: Eupatorieae, South America.

Today, many researchers, institutes, and companies are funding scientific efforts to test different plants' effectiveness at removing a wide range of contaminants. Raskin favors Brassica juncea and Brassica carinata, two members of the mustard family, for phytoremediation. In laboratory tests with metals loaded onto artificial soil (a mix of sand and vermiculite), these plants appeared to be the best at removing large quantities of chromium, lead, copper, and nickel. Several members of this family are edible and yield additional products such as birdseed, mustard oil, and erucic acid, which is used in margarine and cooking oil. Researchers at the DuPont Company have found that corn, Zea mays, can take up incredibly high levels of lead. Z. mays, a monocot in the Poaceae or grass family, is the most important cultivated cereal next to wheat and rice, yielding such products as corn meal, corn flour, cornflakes, cooking oil, beer, and animal feed. Phytokinetics, a company in Logan, Utah, is testing plants for their ability to remove organic contaminants such as gasoline from soil and water. Applied Natural Sciences in Hamilton, Ohio, is taking a slightly different route by using trees to clean up deeper soils, a process they call "treemediation." University researchers from the UK reported in the May 1999 issue of Nature Biotechnology that transgenic tobacco plants

can play a role in cleaning up explosives. In February 1996, Phytotech, Inc., a Princeton, NJ-based company, reported that it had developed transgenic strains of sunflowers, Helianthus sp., that could remove as much as 95% of toxic contaminants in as little as 24 hours. Subsequently, Helianthus was planted on a styrofoam raft at one end of a contaminated pond near Chernobyl, and in twelve days the cesium concentrations within its roots were reportedly 8,000 times that of the water, while the strontium concentrations were 2,000 times that of the water. Helianthus is in the composite, or Asteraceae, family and has edible seeds. It also produces oil that is used for cooking, in margarine, and as a paint additive. H. tuberosus was used by Native Americans as a carbohydrate source for diabetics. In 1998, Phytotech, along with Consolidated Growers and Processors (CGP) and the Ukraine's Institute of Bast Crops, planted industrial hemp, Cannabis sp., for the purpose of removing contaminants near the Chernobyl site. Cannabis is in the Cannabidaceae family and is valuable for its fiber, which is used in ropes and other products. This industrial variety of hemp, incidentally, has only trace amounts of THC, the chemical that produces the "high" in a plant of the same genus commonly known as marijuana. (EPA, 1996).

2.8 Risk Assessment of Phytoremediation

The use of phytoremediation in the field and waste water is subject to many environmental concerns, especially in the light of the recent public hysteria about the release of genetic modification crop into the environment (Erakhrumen and Agbontalor, 2007). Even if non genetic modification strains of plants are used there are still many concerns:

- Do volatilized contaminants remain 'safe' levels in the atmosphere?
- Exposure of the ecosystem to contaminants is prolonged as phytoremediation is a relatively slow process (Pivetz, 2001 and Schnoor, 2002).

However, there are other issues that affect the risk assessment for the use of transgenic organisms as phytoremediators. Not only do such organisms have the same risk as with the type of remediators but they also have the same risks as releasing any genetic mobilization organism into the field. The issues are:

- The potential genetic pollution of native species.
- Potential for the gene to recombine with other genes possibly leading to the hyper accumulation of non-contaminant compounds.
- The genetic mobilization plants may revert to a wild type genotype (Mwegoha, 2008).

Optimal performance is of course a key to phytoremediation being able to improve its market penetration. With the possible exception of some systems that are already widely studied and understood (e.g. the use of deep rooted poplars for soil or land control), all of phytoremediation major applications require further basic and applied research in order to optimize in-field performance which brings about the research work carried out for the project work. This need can be summarized in three areas (Schnoor, 2002):

- Mechanisms of uptake, transport and accumulation: Better understand and utilize physiological, biochemical, and genetic processes in plants that underlie the passive and sorption, active uptake, translocation, accumulation, tolerance and inactivation of pollutants.
- Genetic evaluation of hyperaccumuators: Collect and screen plants growing in soils containing elevated levels of metals or other pollutants for traits useful in phytoremediation.
- Rhizosphere interactions: Better understand and interactive roles among plant roots, microbes, and other biota that make up the rhizosphere and utilize their integrative

capacity in contaminant accumulation, contaminant degradation and mineralization (Schnoor, 2002).

All of these need primarily to be directed towards basic research, aimed at understanding the mechanisms that underlie the biological processes central to phytoremediation. The reason for undergoing this research work is to achieve the means to manipulate or control these processes to improve commercial performance, whether simply through selection and use of optimal plants for given waste scenario, or through more advanced techniques (Mwegoha, 2008).

2.9 Prospects for Use and Regulation of Transgenic Plants in Phytoremediation

The plant species being developed for phytoremediation seem capable of effective bioaccumulation of targeted contaminants, but efficiency might be improved through the use of transgenic (genetically engineered) plants. The transgenic research has been taking place in countries like U. S. A., Canada and Europe under reasonable regulatory regimes but yet to be adopted in developing countries like Nigeria. Many specific transgenic varieties have been exempted from regulation based upon a record of safe research use, and many novel or varieties are being sold and used commercially. It should be possible to routinely obtain government approval for field testing and ultimate commercial use of transgenic plants in phytoremediation (David, 1997).

2.9.1 Prospects for Genetic Engineering

All commercial and research activity to date in phytoremediation has used naturally occurring plants species which is also going to be the one we will use in the course of this project work. However, many of these are species that can be genetically engineered, including *Brassica juncea*, which is being investigated for phytoremediation of heavy metals from soils (Dushenkov et. al., 1995), sunflower, Helian thus annus, being tested for rhizofiltration of uranium and poplar trees (populous deltoids origia), being investigated for the accumulation of

with the chemicals or just concentrate them and stick them to the roots of the plants to

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Description of the Study Area

The project study area, International Breweries and Beverages Industries (IBBI), Kaduna is located in industrial layout few kilometers from Sabo in Kaduna, Kaduna State of Nigeria. Kaduna State is located in Northwest of the Federal Republic of Nigeria. It lies between the savanna zone of the tropics between latitude (4°N and 14°S) and longitude (3°W and 15°E). Its climate is influenced by rain and dry dusty or harmattan. These are mainly of the rainy and dry seasons. The rainy season begins in March and ends in October and the dry season starts in November and ends in February. Thus this study was undertaken during the rainy season. They generates a waste of about 300litres in every production and production is made in three section (i.e. the morning, afternoon and night section) from the process waste from manufacturing, sanitary wastes, wash waters, and relatively uncontaminated water from heating and cooling operations. This waste is collected in a collection chamber and treated with normal conventional method of waste water treatment i.e. the use of chemicals for treatment. They produce product such as beer (Cronebour, Goldberg, Star lager, Gulder etc) and beverages (Maltina, Malta, Moltonic etc). Treated industrial wastewater from International Breweries and Beverages Industries, IBBI, Bermuda grass (*Cynodon dactylon*) and plastic containers were part of the materials used for this research.

3.2 Methodology

The method of Phytoextraction was used in the removal of heavy metal from industrial waste water using Bermuda grass. This is the name given to the process where plant root uptake metal contaminants from soil and waste water and translocate them to their tissues. As different plant have different abilities to uptake and withstand high levels of pollutants many different plants may be used. Certain plants called hyperaccumulators, absorbs unusually large amounts of metals in comparison to other plants. Once the plants have grown and absorbed the metal pollutants they are harvested and disposed off safely (McCutcheon and Schnoor, 2003). In this experimental work the method of Phytoextraction were followed as described in the procedure for removing heavy metal and other contaminants in the industrial waste water below.

3.2.1 Laboratory analysis

In the laboratory analysis of industrial waste water from International Breweries and Beverages Industries, IBBI, Kaduna, Colometric method was used in determining the type of contaminants present in the industrial waste water.

3.2.1.1 Materials used

The materials that were used during the analysis were industrial waste water from International Breweries and Beverages Industries (IBBI), Kaduna.

3.2.1.2 Equipments/ Apparatus used

The following equipments/ apparatus were used in determining the type of contaminants present in the industrial waste water; Spectrometer, with infrared phototube for use at 880nm, Acidic-washed glassware, JENWAY 4510 Conductivity Meter, JENWAY 3505 pH Meter, JENWAY

(Phosphorus), Burette, Pipette, Beaker, Phenolphthalein indicator, Retort stand and DR/ 890 Calorimeter machine (Suspended Solid).

3.2.1.3 Pre – analysis

The industrial wastewater was first of all collected from the International Breweries and Beverages Industries, IBBI, Kaduna and the water sample of about 2litre was collected from the large sample collected from the industry and taken to the laboratory for post test before planting of the Bermuda grass. So also the Bermuda grass was also collected at the age of four week of germination from the soil and a large quantity was collected and small quantity weigh 2kg was dried and taking to the laboratory for test for heavy metals and from the same source another 2kg was taking and planted in the wastewater.

3.2.1.4 Test Procedure

The procedure used in the determination of the type of contaminants present in the industrial waste water was followed step by step as shown in Appendix A.

3.2.1.5 Test Parameters

The following parameters were measured and tested for from the industrial waste water during the analysis; Heavy metals (Aluminium and Manganese), Biochemical Oxygen Demand (B.O.D), Chemical Oxygen Demand (C.O.D), Total Phosphorus, Total Nitrogen, Acidity, Suspended Solid, Dissolved Solid, pH and Conductivity.

3.3 Procedure for the removal of heavy metals and other contaminants from industrial waste water after the waste water analysis

The following procedures were under gone while removing the heavy metals and other contaminants from the industrial waste water;

Five numbers plastic containers measured 1litre each were filled with the industrial waste water of about 0.5litre each. The Bermuda grass were dried and taken to the laboratory for test of heavy metal been uptake during the four weeks of germination in the soil. The Bermuda grass was planted into the industrial waste water at the age of four weeks after germination; the plant is suitable for the removal of heavy metals and other contaminants in the industrial waste water.

The growing processes of the plant were observed and records were taken every day for four weeks. The plants were harvested after four weeks and the Biomass of the plant was taken. The plants were tested in the laboratory to ensure that the heavy metals are been extracted and safely disposed. The treated water was return to the laboratory to ensure that it is free from the heavy metals and other contaminants.

3.4 Plant Sampling and Methods

Plant sampling has been widely used as an aid in the determination of the status in crops and the sources in which they grow i.e. soil or water. Plant analysis can also be used to detect or confirm nutrient deficiencies or toxicity in plant i.e. type of heavy metals and nitrogen, phosphorus, BOD, COD, SS, DS and pH in the waste water (Ojukwu, 2010). It is an important method of monitoring the uptake of contaminants by plant when used in conjunction with water analysis.

The samples were collected at four week stage of the Bermuda (*Cynodon dactylon)* grass. Taking a minimum of 25-30 Bermuda grass sample from the waste water. The collected samples were prepared by drying, transferred into nylon and taking to the laboratory for analysis.

The samples (Bermuda grass) were analyzed for heavy metals like Aluminium, Manganese, Zinc, Manganese, Arsenic, iron and Lead.

3.5 Procedure for plant test

The procedure for the plant test to ensure the uptake of heavy metals and nitrogen, phosphorus, BOD, COD, SS, DS, and pH in the industrial waste water were followed as shown in Appendix B.

3.6 Plant selection

The plant selection of this experimental work was based on the type of contaminants present in the industrial waste water after the test analysis was carried out. The selection of Bermuda grass was based on the presence of the heavy metals and nitrogen, phosphorus, BOD, COD, SS, DS, pH and it's suitability for the remediation of the industrial waste water (Schnoor, 2002).

3.7 Test procedure after the removal of contaminants

The procedures after the removal of contaminants from the waste water through the method of Phytoextraction were as shown in Appendix A.

3.8 Statistical Analysis

The statistical analysis of the wastewater laboratory analysis results before and after planting and also that of the plant tested before and after planting were carried out for ANOVA, Correlation and Regression and the bar chart representation of the comparison between tested values of both the industrial wastewater and plant before and after planting and the result is shown in Appendix C.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Presentation of Results

Industrial waste water contains a variety of organic and inorganic contaminants. The results of the waste water analysis are presented below;

Table 4.1: The Laboratory analysis Result of the industrial waste water before planting

Parameters	Units	Measured Value
Aluminum	mg/L	0.509
Manganese	mg/L	0.138
BOD_5	mg/L	8.08
COD	mg/L	483
Phosphorus	mg/L	0.26
NO_3-nitrogen	mg/L	18.8
Acidity	mg/L	0.02
Suspended Solid	mg/L	107

)issolve Solid	mg/L	1.72

Table 4.1 Continued

·H	-	4.75
Conductivity	µS/cm	3.29

Table 4.2: The Laboratory analysis Result of the industrial waste water after planting

Parameters	Units	Measured Value
Aluminum	mg/L	0.043
Manganese	mg/L	0.027
BOD_5	mg/L	4.30
COD	mg/L	36
Phosphorus	mg/L	0.0016
NO_3-nitrogen	mg/L	59.02
Acidity	mg/L	0.0012
Suspended Solid	mg/L	34

Table 4.2 continued

Dissolve Solid	mg/L	185.59
pH	-	7.17
Conductivity	µS/cm	277

Table 4.3 plant Analysis Result before Planting

Metals	Unit	Tested Value
Copper (Cu)	µg/l	6.88
Manganese (Mn)	µg/l	0.61
Zinc (Zn)	µg/l	14.2
Molybdenum (Mo)	µg/l	0.40
Magnesium (Mg)	µg/l	1.55
Iron (Fe)	µg/l	10.22
Arsenic (As)	µg/l	BDL
Lead (Pb)	µg/l	BDL

BDL—Below Determination Level

Table 4.4 plant Analysis Result after Planting

Metals	Unit	Tested Value
Copper (Cu)	µg/l	6.88
Manganese (Mn)	µg/l	0.70
Zinc (Zn)	µg/l	14.2
Molybdenum (Mo)	µg/l	0.55
Magnesium (Mg)	µg/l	1.55
Aluminium (Al)	µg/l	0.47
Iron (Fe)	µg/l	10.22
Arsenic (As)	µg/l	BDL
Lead (Pb)	µg/l	BDL

BDL—Below Determination Level

4.2 Discussion of Results

Table 4.1, 4.2, 4.3 and 4.4 shows the first and second physico-chemical analysis of waste water and plant test for heavy metals before and after planting respectively as shown above.

4.2.1 Physico-Chemical Analysis

I. Chemical Oxygen Demand (COD):- This is the measure of the total amount of organic material which may eventually be oxidized by microbes, the COD test (a rapid oxidation by action of chemicals) also measure materials such as larger pieces of cellulose only slowly degraded by micro-organisms (Samgodaya and Mson, 1997). Chemical Oxygen Demand (COD) is the equivalent amount oxidizing chemical required to act on behalf of the bacterial. The essence of this analysis is to determine the amount of biodegradable organic matter in waste water. The amounts of biodegradable organic matter in the industrial wastewater before planting was determined to be (483mg/l) and after planting it was determine to be (36mg/l) which shows the effectiveness of phytoextraction as wastewater remediators.

II. Biological Oxygen Demand (BOD):- It is a measure of the organic material in the slurry which can be easily metabolized by aerobic bacteria and which can cause oxygen depletion and pollution in water. It is usually measured by allowing a sample of water at 20°C for five days and calculating the amount of oxygen used up during the oxidation of the organic matter by bacteria (Ojukwu, 2010). The measured value from the analysis before after planting was (8.08mg/l) and (4.30mg/l) respectively.

III. Suspended Solid (SS):- This is the material organic or inorganic in suspension but not in solution in slurry. The organic and inorganic solution in slurry in the industrial wastewater wa

(107mg/l) before planting and (34mg/l) after planting. This result has shown that there was 70% uptake of suspended solid by the Bermuda grass.

IV. Dissolved Solid (DO): - This is a measure of the impurities in a water sample. It can also be referred to as the total salt concentration of water sample. It is one of the most important agricultural water quality parameters (Ojukwu, 2010). It was present in the industrial wastewater to the following level before and after planting (1.75mg/l) and (185.59mg/l) respectively which signified that the solid contaminants were properly dissolved.

V. pH: - this is hydrogen ion concentration in a substance. This is a measure of the acidity or alkalinity of a solution. A pH of 7 is neutral, a lower pH acid and higher pH alkaline. Most natural biological system runs at a pH between 6 and 7.5 (Samgodaya and Mson, 1997). The main use of pH in water analysis is for detecting abnormal water. The normal range of pH in a save water is between 6.5 to 8.5 (Motsara and Roy, 2008). The pH of the industrial waste water from the analysis is 4.75 which indicate the stability of pH in the waste water.

VI. Aluminium (Al), Manganese (Mn) and Phosphorus:-These are heavy metals that are detrimental to the environment. The wastewater that contains these metals at threshold value can cause soil toxicity resulting to destruction of the terrestrial organism when disposed. The value of aluminium (0.509mg/l) and manganese (0.138mg/l) before planting was at threshold value which may cause destruction to both soil and aquatic life.

VII. Nitrogen: - Nitrate represents the final product of the biochemical oxidation of ammonia. In water, the present of nitrate is probable due to the presence of nitrogen organic matters. From the industrial waste water analysis, the level of nitrate in nitrogen content is high (18.8mg/L) which pose great threat on the environment. The total nitrogen includes all forms of inorganic

nitrogen such as NH_4, NO_3 and NH_2 (Urea), and organic nitrogen compounds such as amino acid and other derivatives.

4.2.2 **Plant Analysis**

The plant analysis was carried out to identify the level of heavy metal in industrial waste water before and after planting of Bermuda grass which was extracted during the experiment and the result presented as shown in table 4.3 and 4.4 above. But in this study, the heavy metals analyzed are listed above.

For better examination and evaluation, Plate 3-7 shows the observable physical growth of the plant and growing condition from planting to harvesting.

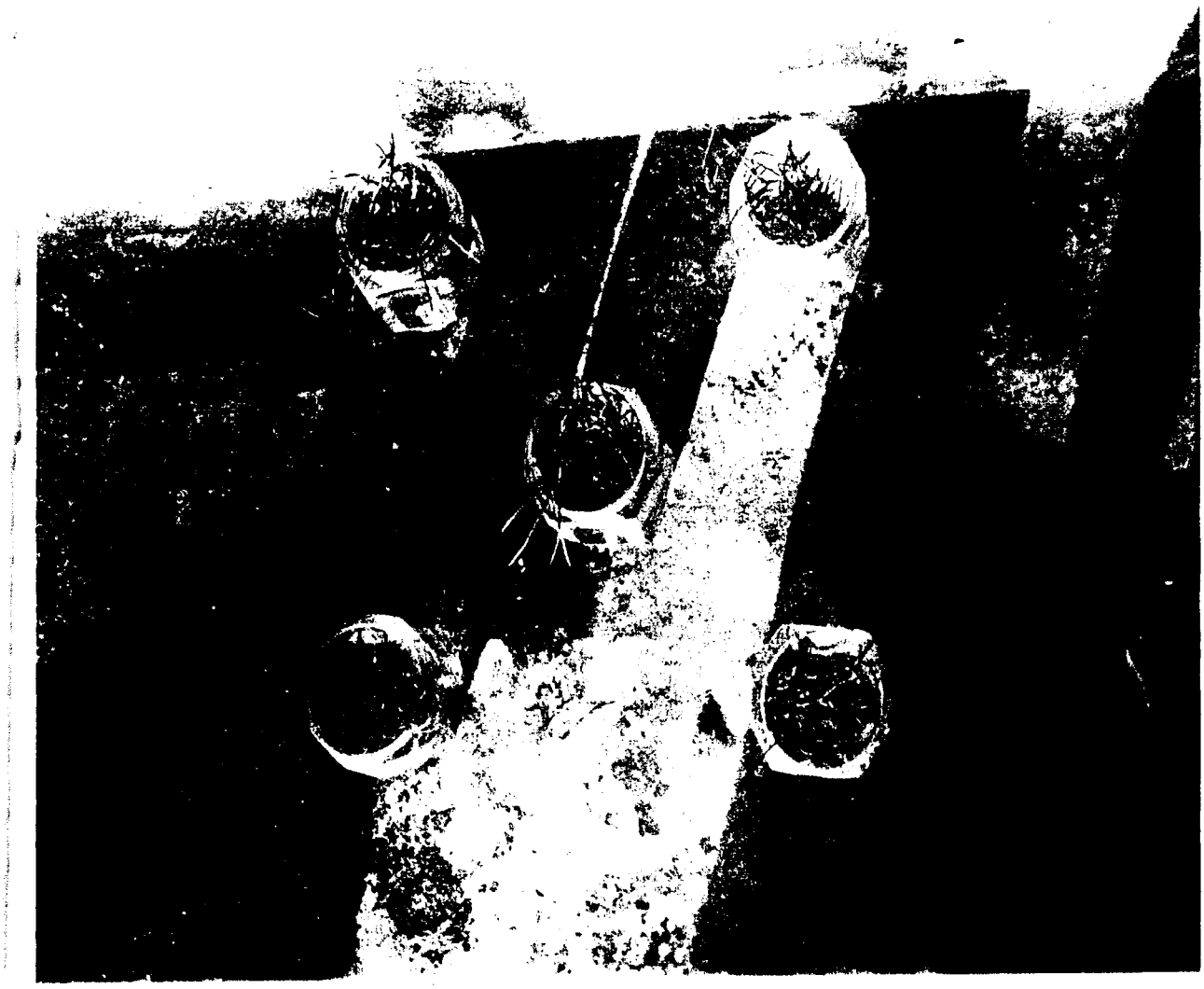


Plate3: First week of planting Bermuda grass

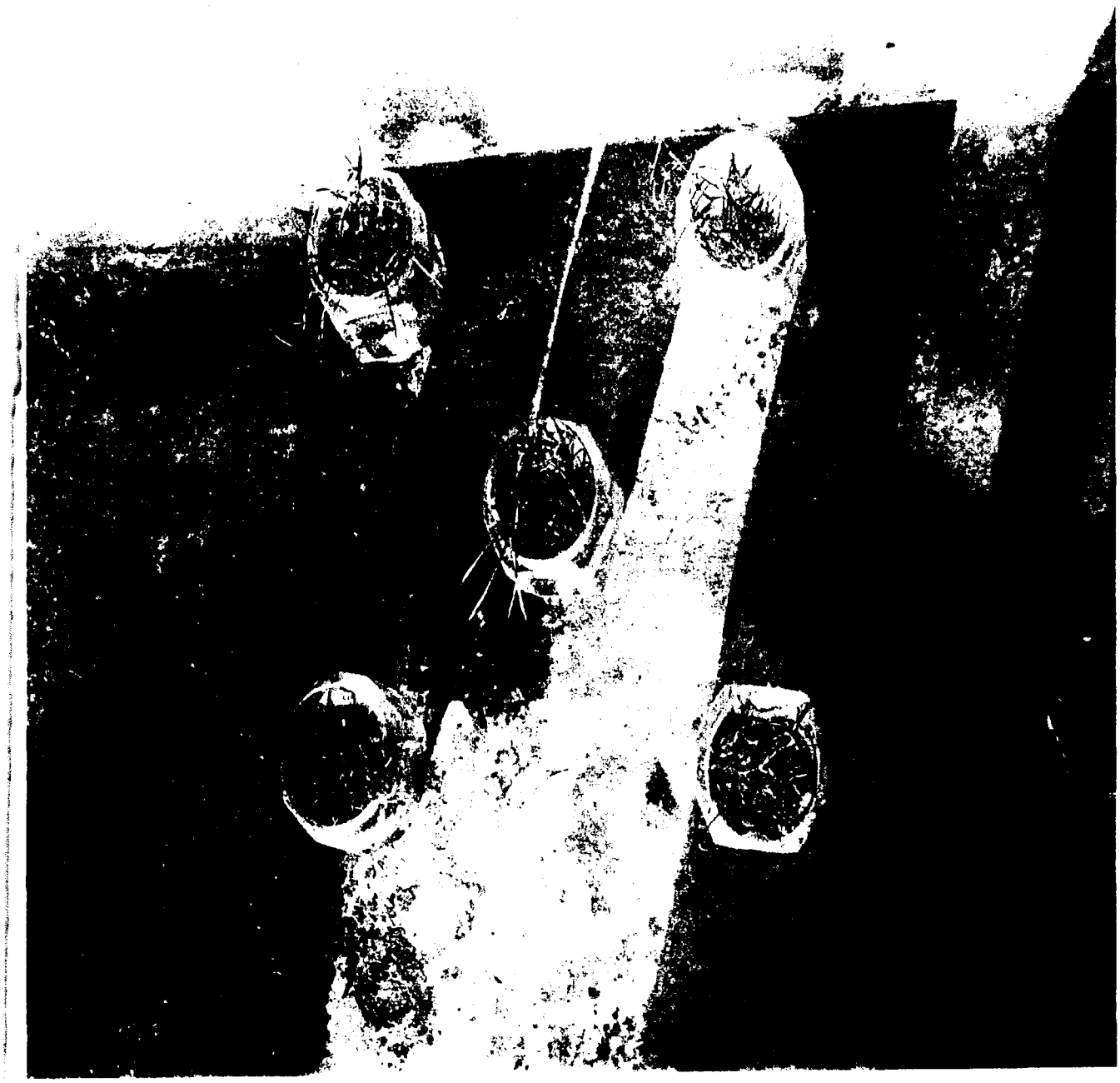


Plate 4: Third week of planting Bermuda grass

Plate 5: Eight week dried Bermuda grass after harvesting

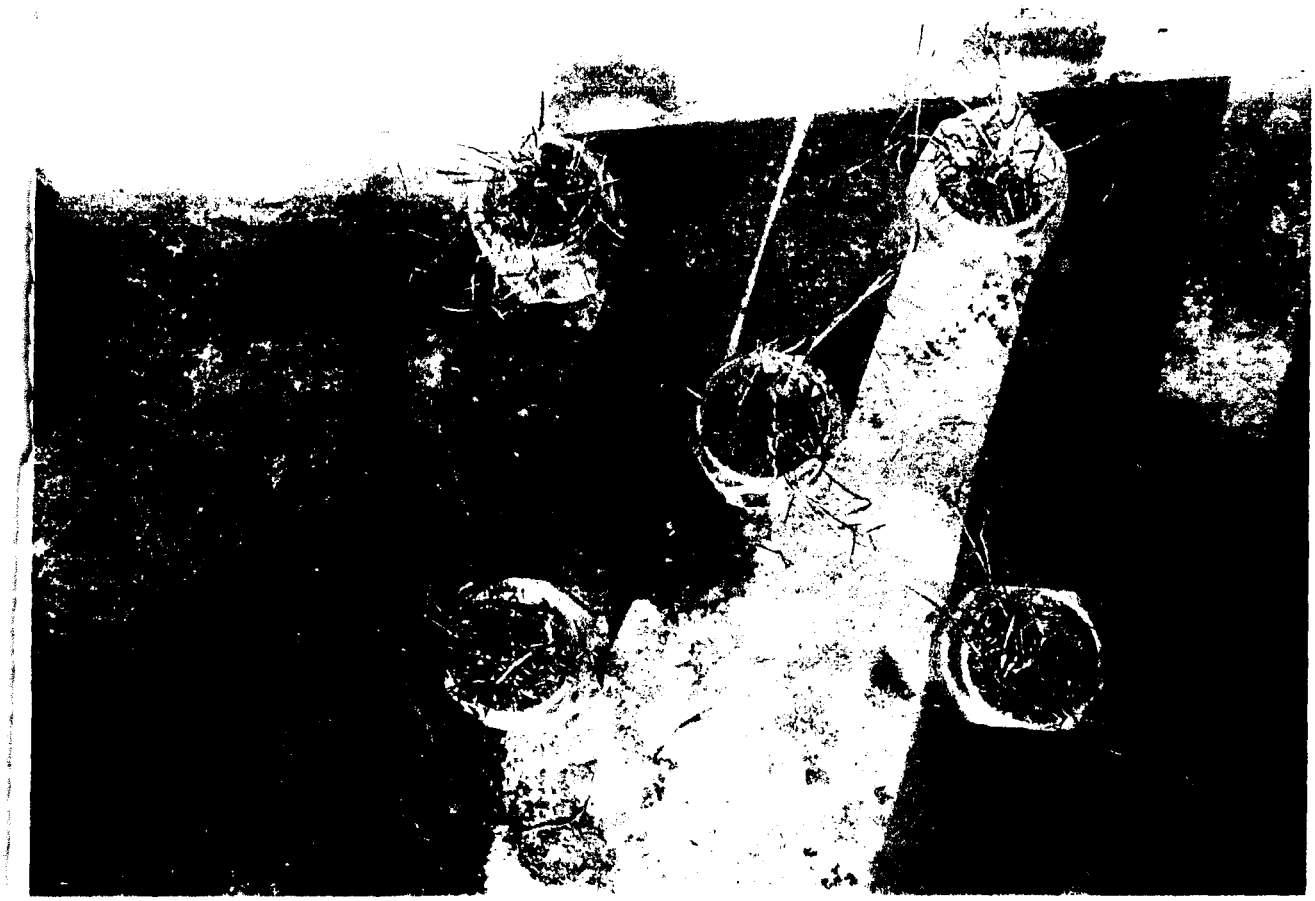


Plate 6: Fourth week of planting Bermuda grass

PLATE 7: Four weeks dried Bermuda grass before planting

Table 4.5 Observable Physical Growth of Bermuda grass in Industrial Wastewater

GROWTH STAGE	OBSERVATIONS
Planting	- At the second day of planting, there was change in plant the plant dries completely by exhibiting dying action for about five days. -The wastewater shows some kind of forming as detergent do immediately after planting.
Growing Period	- After five days there was another change in plant, the plant begin to grow back to it green leaves. -At the second week still in this stage there was change in the level of wastewater in which the plant was planted. -The colour of wastewater was brighter than before planting. -There was rapid growth of the Bermuda grass at week three.
Harvesting	-The plant grew taller than when it was planted

and green and fresh than when it was planted.

-Still in this stage the level of wastewater less than when it was started.

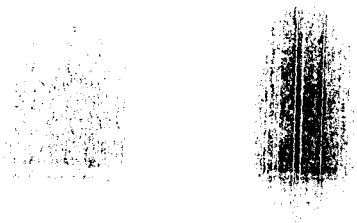
4.2.3 **Effect of selected heavy metals on the environment**

I. Aluminium Toxicity

The normal level of aluminium in water is 0.3mg/l and in soil is 0.45mg/l (MSRTUM, 2002). So the present of 0.509mg/l of aluminium in the industrial waste water is an indication of it detrimental effect it will cause on both soil and aquatic animal when dispose to the environment with out proper treatment. Though aluminium is very useful but it excessiveness will cause it deposit in the plant tissue preventing it from proper growth and destruction of aquatic life in water as poison.

II. Manganese toxicity

The normal level of manganese in both soil and water for effective use is 0.05mg/l and 0.03mg/l respectively (MSRTUM, 2002), it excess will cause harmful effect on the soil and the crop grown on the soil. The accumulation of manganese in plant tissue causes translocation in plant and causes reduction in yield.



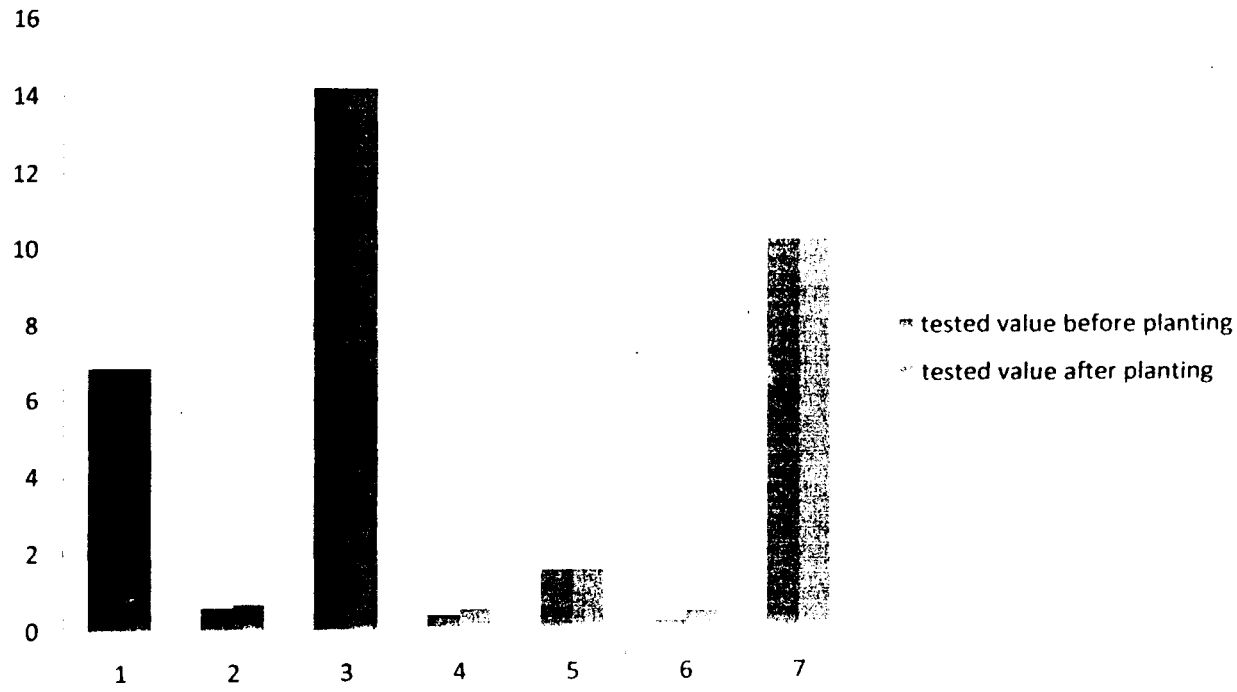


Fig4.1: Bar Chart representation of Tested Bermuda plant before and after planting

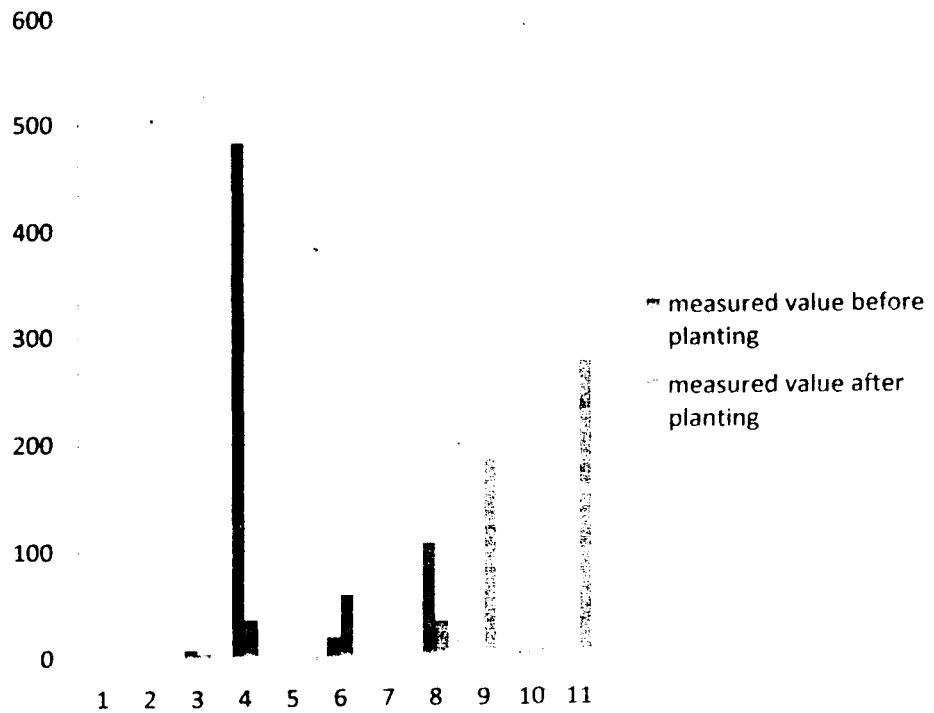


Fig4.2 Bar Chart representation of the tested wastewater before and after planting

From the above figures, the level of heavy metals in both wastewater and plant differs drastically as stated earlier, in fig4.1 it can be notice that the level of aluminium (Al) in the plant before planting (0.24µg/l) is less than that of after planting (0.47µg/l), which signify that there was an uptake of aluminium (Al) by the plant (Bermuda grass). And also the level of manganese (Mn) before planting (0.61µg/l) and after planting (1.55µg/l) was totally differ which also shows the difference in the uptake of manganese (Mn) by Bermuda grass, so also nitrogen (N), phosphorus (P), BOD, COD, pH, DO, Conductivity, SS and Acidity.

From fig4.2, the level of heavy metals in the industrial wastewater was so insignificant; therefore it did not appear on the graph though there was present of the heavy metals in the wastewater as seen in the laboratory analysis results and that of the plant.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusions

After careful analysis and testing of the industrial waste water and Bermuda grass samples, it is clearly convincing that the use of plant in treating waste water is a good and cheap technology than the conventional type of waste water treatment been adopted by most people in the treatment of both industrial, domestic and municipal waste water. The use of Bermuda grass in the extraction of heavy metal was a very successful one; it shows it ability to adapt to any condition in the treatment or uptake of heavy metals in industrial waste water.

As it relates to this study, the industrial waste water readily gives up the heavy metals and other contaminants to the plant which was subsequently extracted through the plant (Bermuda grass) through the roots. This is the reason why on analyzing the waste water after harvest of Bermuda grass has lesser percentage of heavy metals and other contaminants contents present in the waste water and more content of the contaminant in the Bermuda grass which shows the effectiveness of the methodology.

5.2 Recommendations

It is obvious that the most effective natural method of removing heavy metals and other contaminants in soil, underground water and wastewater is phytoremediation and Phytoextraction is most effective in the treatment of wastewater most especially the industrial waste water. This experiment should be recommended to all manufactured industries in other to use natural adsorbent in the treatment of heavy metal before disposal in other to reduce it ef[illegible]

on the environment. The following recommendations are enumerated to be adopted for more effective mean of removing heavy metals in industrial waste water;

5.2.1 Quantity of waste water

The quantity of waste water to be used in this experiment should be moderate as much as possible. As it relates to this study, the quantity of wastewater used in this work was a little bit less as required. Thus a further study is recommended to use 5litres of wastewater in Phytoextraction of heavy metals other than aluminium and manganese (such as arsenic, zinc, lead, mercury, chromium etc).

5.2.2 Plant selection

In the selection of plant to be used in the phytoremediation of heavy metal of industrial waste water, it will be recommended that more tolerance plant other than Bermuda grass should be used. Plants such as Alpine, Alfalfa, orchard grass etc. Also a further study should be carried out on the Phytoextraction of heavy metals using economical plant to determine the effect of the heavy metals on those plants.

5.2.3 Duration of Treatment

As it relates to this study, four weeks was used in the extraction of heavy metals present in the industrial waste water. A further study is recommend to use 8-12 weeks for the experiment so as to have more effective result.

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APPENDICES

Appendix A: Waste water Analysis

1.0 TEST PROCEDURE FOR NITRATE

- Enter the stored program number for high range nitrate nitrogen (NO3-N)- power pillows. Press: **PRGM.** The display will show: **PRGM ?**
- Press: **51 ENTER.** The display will show **mg/L, NO3-N** and the **ZERO** icon.
- Fill a sample cell with 10mLof sample.
- Add the contents of one Nitra Ver 5 Nitrate Reagent Powder Pillow to the cell (the prepared sample). Cap the sample cell.
- Press: **TIMER ENTER,** a one- minute reaction period will begin. Shake the sample cell vigorously until the timer beeps.
- After the timer beeps, the display wil show: **5:00 TIMER 2.** Press: **ENTER.** A five minute reaction period will begin.
- Fill another cell with 10mL of sample (blank). Wipe off any fingerprint or liquid.
- Place the blank into the cell holde. Tighover the sample cell with the instrument cap.
- When the timer beeps, press **ZERO**.The r will move to the right, the the display will show: **0.0mg/L NO3-I.**

- Place the prepared sample into the cell holder. Tightly cover the sam... with the instrument cap.
- Press: **READ.** The cursor will move to the right, and then the result in mg/L NO_3-N (or alternate form) will be displayed.

2.0 TEST PROCEDURE USED FOR DETERMINING CHEMICAL OXYGEN DEMAND (C.O.D).

- Enter the stored program number for chemical oxygen demand (C.O.D) low range. Press: **PRGM**. The display will show: **PRGM ?**
- Press: **16 ENTER.** The display will show **mg/L, COD** and the **ZERO** icon
- Insert the COD/TNT. Adapter into the cell holder by rotating the adapt until it drops into place. Then push down to fully insert it.
- Clean the outside of the blank with a towel.
- Place the blank in the adapter. Push straight down on the top of the via until it seat solidly into the adapter.
- Tightly cover the vial with the instrument cap.
- Press: **ZERO.** The cursor will move to the right, then the display will show **0mg/L COD.**
- Clean the outside of the sample with a towel.
- Press: **READ.** The cursor will to the right, then the result in **mg/ COD** will be display.

3.0 TEST PROCEDURE FOR ...INATION OF SUSPENDE... SOLID (SS)

- Enter the stored program number for suspended solid. Pres
  display will show **PRGM ?**
- Press: **94 ENTER.** The display will show **mg/L Suld** and the **ZERO** icon.
- Blend 500mL of sample in a blender at high speed for exactly 2 minutes.
- Pour the blended sample into a 600mL beaker.
- Fill sample cell with 25mL of tap water (the blank).
- Place the blank in the cell holder. Tightly cover the sample cell with the instrument cap.
- Press: **ZERO.** The cursor will move to the right, and then the display will show: **0mg/L Suld.**
- Stir the sample thoroughly and immediately pour 25mL of the blended sample into a sample cell (the prepared sample).
- Swirl the prepared sample cell to remove any gas bubble and uniformly suspend any residue.
- Place the prepared sample inthe cell holder. Tightly cover the sampl cell with the instrument cap.
- Press: **READ.** The cursor wve to the right, then the result in mg/ suspended solid will be disp

4.0 TEST PROCEDURE FOR D INING MANGANESE

- Enter the stored program nu ow range manganese. Press: **PRG** The display will show **PRG**
- Press: **43 ENTER.** The disp **mg/L, Mn** and the **ZERO** icon
- Fill another sample cell witl nizer water (the blank).

- Fill another sample cell with 10mL of sample (the prepared sample).
- Add the content of one ascorbic Acid Powder Pillow to each cell. Swirl to mix.
- Add 15 drop of alkaline cyanide Reagent solution to each cell. Swirl to mix.
- Press: **TIMER ENTER.** A two – minute reaction period will begin.
- After the timer beeps, place the blank into the cell holder. Tightly cover the sample cell with the instrument cap.
- Press: **ZERO.** The cursor will move to the right then the display will show **0.000mg/L Mn.**
- Place the prepared sample into the cell holder. Tightly cover the sample cell with the instrument cap.
- Press: **READ.** The cursor will move to the right, and then the result in **mg/L Mn** will be displayed.

5.0 TEST PROCEDURE FOR ALUMINIUM

- Enter the stored program number for Aluminium. Press: **PRGM.** The display will show **PRGM ?**
- Press: **1 ENTER.** The display will show **mg/L, Al** and the **ZERO** icon.
- Fill a 50-mL graduated mixing cylinder to the 50-mL mark with sample.
- Add the content of one ascorbic Acid Powder Pillow. Stopper. Invert several times to dissolve powder.
- Add the content of one Aluver 3 Aluminium Reagent Powder Pillow. Stopper.

- Press: **TIMER ENTER.** A three – minute reaction period will begin. Invert the cylinder repeatedly for the three minutes.
- Pour 25mL of mixture into a 25-mL sample cell (the prepared sample).
- Add the content of one bleaching 3 Reagent Powder Pillow to the remaining 25mL in the mixing graduated cylinder (the blank).
- The display will show: **00:30 Time 2.** Press: **ENTER.** A 30 second reaction period will begin. Vigorously shake the cylinder for the 30-second period.
- Pour the 25mL of mixture in the second 25-mL sample cell (the blank).
- The display will show: **15:00 TIMER 3.** Press: **ENTER.** A 15 minute reaction period will begin.
- Within 3-minute after the timer beeps, place the blank into the cell holder. Tightly cover the sample cell with the instrument cap.
- Press: **ZERO.** The cursor will move to the right, and then the display will show: **0.000mg/L Al.**
- Immediately place the prepared sample into the cell holder. Tightly cover the sample cell with the instrument cap.
- Press: **READ.** The cursor will move to the right, and then the result in mg/L aluminium will be displayed.

6.0 TEST PROCEDURE FOR ACIDITY

- About 30mL sample boiled on a hot plate to remove CO_2.
- The solution cooked and initial volume restored with distilled water.

- 10mL alignment of diluted sample transferred into a conical flask, add one drop of phenolphthalein, mix.
- Titrate the mixture with 0.05ml NaOH, initial pink colour first appears.

$$\text{Citric acid} = \frac{Vol. * M * 6.404}{V} \qquad (1)$$

Where Vol= Volume of NaOH used.

M= Molarity of NaOH

V= Volume of sample used (ml).

7.0 TEST PROCEDURE FOR PHOSPHORUS

- Pipette 50mL sample into a clean, dry test tube or 125mL Erlenmeyer's flask.
- Add one drop of phenolphthalein indicator. If a red colour develops, add 5ml H_2SO_4 solution drop wise to just discharge the colour.
- Add 8ml combined reagent and mix thoroughly. After at least 10 minute, but not more than 30 minute, measure absorbance of each sample at 880nm, using reagent blank as the reference solution.
- Preparations of calibration curve- prepare individual calibration curves from a series of six standards within the phosphate ranges from 0-6µg.
- Plot absorbance against phosphate concentration.

8.0 PROCEDURE FOR DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND

- Determination of initial dissolve oxygen (DO): If the sample contain material that react rapidly with DO, determine initial DO immediately after filling BOD bottle with diluted sample. If rapid initial DO uptake is insignificant, the time period between preparing dilution and measuring initial DO is not critical. Use the azide modification of the iodometric method (section 4500-O.C) or the membrane electrode method (section 4500-O.G) to determine initial DO small sample dilutions, dilution water blank and where appropriate, seed control.
- Dilution water blank: use a dilution water blank as a rough check on quality of unseeded dilution water and cleanliness of incubation bottles. Together with each batch of samples incubate a bottle of unseeded dilution water. Determine the initial and final DO as in above. The DO uptake should not be more than 0.2mg/L and preferably not more than 0.1mg/L.
- Incubation: incubate at 20°c ±1°c BOD bottles containing desired dilutions, seed control, dilution water blanks and glucose –glutamic acid checks. Water seal bottles and described in above.
- Determination of final DO: After 5-days incubation determine DO in sample dilutions, blanks and check as described above.
- When dilution water is not seeded :

$$BOD_5, mg/L = \frac{D1-D2}{P} \qquad (1.1)$$

Where;

D_1=DO of diluted sample immediately after preparation, mg/L.

D_2= DO of diluted sample after 5-days incubation at 20°c, mg/L.

P= decimal volumetric fraction of sample used.

9.0 TEST PROCEDURE FOR pH, CONDUCTIVITY AND DISSOLVE SOLID

- The sample is pour into a beaker and pH, Conductivity and Dissolve Solid Meter is deep into the sample through the electrode and the result will be displayed.

Appendix B: plant analysis

1.0 TEST PROCEDURE FOR MAGNESIUM (Mg)

Magnesium stock solution was prepared by dissolving 8.3606g of magnesium chloride 6-hydrate, $MgCl26H_2O$ in distilled water and making it up to 1litre. Magnesium standard solution was prepared by diluting 10ml stock magnesium solution into 1litre of water. Different concentrations was then prepared from the standard solution in the range of 5-40mg/l. These run through AAS with magnesium cathode lamp installed at 285.2nm. Standard calibration curve was drawn by plotting concentration of standards against absorbance. The sample was acidified with 1ml concentration. Nitric acid autoclaved at 121°C for 1hour to solubilize the particulate matter content and also run through AAS.

Magnesium is calculated using equation;

Mg (mg/l) = reading from the curve × D

Where: $D = \frac{\text{ml sample} + \text{ml water} + 1\text{ml acid}}{\text{ml of sample}}$

2.0 TEST PROCEDURE FOR COPPER (Cu)

Stock copper solution was prepared by dissolving 3.9296g copper sulphate 5- hydrate in distilled water which was made up to 1litre. Standard solution was prepared by dissolving 5ml of stock solution in 100ml of distilled water from where different concentration were then prepared in the range of 5ml-20mg/l. This was run through AAS to determine the absorbance level using copper cathode lamp at 324.7nm and calibration curve was drawn from this. The sample was run to determine copper with AAS. It was calculated using equation:

Cu (mg/l) = reading from the curve × D

Where: $D = \frac{\text{ml sample} + \text{ml water} + 1\text{ml acid}}{\text{ml of sample}}$

3.0 TEST PROCEDURE FOR ZINC (Zn)

Stock zinc solution was prepared by dissolving clean 100mg zinc metal in 1ml HCl and was made up to 1litre with distilled water. Standard zinc solution was prepared by dissolving 10ml of zinc stock solution to 1litre with distilled water. Different concentrations were then prepared in the range of 0.1-0.5mg/l. This was run through AAS to determine the absorbance level using zinc, cathode lamp at 213.8nm.The calibration curve was drawn from the results. The sample was analyzed for zinc concentration. It was calculated using equation:

Zn (mg/l) = reading from the curve x D

$$D = \frac{\text{ml sample} + \text{ml water} + 1\text{ml acid}}{\text{ml of sample}}$$

4.0 TEST PROCEDURE FOR IRON (Fe)

Stock iron solution was prepared by dissolving clean 5.0503g iron (II) ammonium sulphate, Fe $(NH_4)_2(SO_4)_2$ in 1litre with distilled water. Standard iron solution was prepared by dissolving 20ml of stock solution in 1litre with distilled water. Different concentrations were then prepared and determine for iron with AAS using iron cathode lamp at 248.3nm.The calibration curve was drawn from the results. The sample was run for iron with AAS to determine iron and the results were extrapolated from the calibration curve. It was calculated using equation:

Fe (mg/l) = reading from the curve × D

Where: $D = \frac{\text{ml sample} + \text{ml water} + 1\text{ml acid}}{\text{ml of sample}}$

5.0 TEST PROCEDURE FOR LEAD (Pb)

Stock lead solution was prepared by dissolving 1.5985g lead nitrate, Pb $(NO_3)_2$ in 1litre distilled water. Standard lead solution was prepared by dissolving 10ml lead stock solution in 1litre distilled water. The same procedure as described above.

TEST PROCEDURE FOR MANGANESE (Mn)

Stock manganese solution was prepared by dissolving 2.8766g $KMnO_4$ in distilled water and was making up of 1litre.

Standard solution was prepared by dissolving 10ml of stock solution in distilled water and make up to 1litre. Different concentrations were then prepared from standard solution and are determined for manganese using manganese cathode lamp at 279.4nm with AAS which is used to draw standard calibration curve. The samples were run to determine manganese concentration in them.

Manganese is calculated using equation:

Mn (mg/l) = reading from the curve × D

Where: $D = \frac{\text{ml sample} + \text{ml water} + 1\text{ml acid}}{\text{ml of sample}}$

APPENDIX C

ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
tested value before planting	7	34.07	4.867143	31.76299
tested value after planting	7	34.57	4.938571	31.01871

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.017857	1	0.017857	0.000569	0.981364	4.747225
Within Groups	376.6902	12	31.39085			
Total	376.7081	13				

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999904
R Square	0.999809
Adjusted R Square	0.999771
Standard Error	0.085373
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	190.5415	190.5415	26143	1.72E-10
Residual	5	0.03642	0.00728		
Total	6	190.5779			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-0.12985	0.044678	-2.90616	0.0335	-0.24479	-0.01491	-0.24479	-0.01491
tested value after planting	1.011829	0.006258	161.6874	2E-10	0.995743	1.027916	0.995743	1.027916

	tested value before planting	tested value after planting
tested value before planting	1	
tested value after planting	0.999904385	1

ANOVA: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
measured value before planting	11	627.567	57.05155	20938.98
measured value after planting	11	603.1528	54.83207	8410.579

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	27.09333	1	27.09333	0.001846	0.966153	4.3512
Within Groups	293495.5	20	14674.78			
Total	293522.6	21				

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.9999043
R Square	0.9998087
Adjusted R Square	0.9997705

Standard Error	0.0853726 02
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	190.54	190.542	26142.83	2E-10
Residual	5	0.0364	0.00729		
Total	6	190.58			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-0.1298486 2	0.0447	-2.906 2	0.0335 49	-0.245	-0.015	-0.244 7	-0.015
tested value after planting	1.0118293 41	0.0063	161.687	1.72E-10	0.9957	1.0279	0.99574	1.0279 92

CORRELATION

	measured value before planting	*measured value after planting*
measured value before planting	1	
measured value after planting	-0.082708004	1