EVALUATION OF THE AMELIORATIVE ROLES OF SELECTED VITAMINS ON LEAD-INDUCED TOXICITY IN *CLARIAS GARIEPINUS* (BURCHELL, 1822) FINGERLINGS

BY

SAMUEL, Patrick Ozovehe

PhD/SLS/2018/9261

DEPARTMENT OF ANIMAL BIOLOGY, SCHOOL OF LIFE SCIENCES,

FEDERAL UNIVERSITY OF TECHNOLOGY MINNA, NIGERIA

SEPTEMBER, 2023.

ABSTʳ

Heavy metals are known to be very deleterious to living organisms especially in aquatic medium. The roles of vitamins in mitigating the effects of lead nitrate ($PbNO_3)_2$) toxicant in terms of morphological differentiations, haematology, production levels of reduced glutathione (GSH), malondialdehyde (MDA), aspartate amino transferase (AST), alanine amino transferase (ALT) and histopathology of selected organs of *Clarias gariepinus* were investigated for a period of 12 weeks. A total number of 2500 samples of *C. gariepinus* fingerlings (initial weight, 3-11g; standard length, 7.9 9.4cm and total length, 8.9-10.9cm) were subjected to acute and chronic toxicity phases. The sub-lethal phase consisted of 00, 26 mg/L, 44 mg/L, 61 mg/L and 79 mg/L as T1-T4, respectively. The various treatment groups include Pb (Pb only), PbVA (Pb+vitamin A), PbVC (Pb+vitamin C) and PbVE (Pb+vitamin E).Twenty-six mg/L of the vitamins was administered across all in each case. The morphometric parameters measured on weekly basis for a period of 12 weeks were standard lengths (SL), total lengths (TL) and weight from 2 randomly selected samples from each treatment. The weight performances taken as specific growth rate (SGR), weight gain (WG) and % weight gain (% WG) were also calculated. Three samples of the fish were randomly selected and sacrificed every 4^{th} week. The blood collected were analyzed for White Blood Cell count (WBC), Red Blood Cells (RBC), Haemoglobin Concentration (HGB), Pack Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelet Count (PLT). Three samples of the fish were also randomly selected, sacrificed every 2 weeks; gills, kidneys and livers were excised, homogenized in sodium phosphate buffer; and assayed for GSH, MDA, AST and ALT productions levels in each case. The data generated in each case were subjected to one way ANOVA and considered significant at $P \leq 0.05$. Two randomly selected samples from the Pb only group and PbVE were also sacrificed; liver, kidneys and gills excised from them were analysed for tissue alterations. From the results; Pb toxicant elicited physical and behavioural responses such as lassitude, emaciation, frequent gulping and gasping for air, lacerations, exuding of blood from sides of the opercular, bulging of the abdominal region of the body before their eventual death. The 96 hrs LC_{50} of Pb was 174.71mg/L. The highest weight gain of the samples was recorded in 26 mg/L samples at the 12^{th} week with the value of 83.26 g. This percentage WG was 429 %. Samples exposed to PbVE treatments displayed higher values of WBC, MCV, MCH, MCHC, PLT at various stages of the sampling and concentrations of the toxicant. The varying alterations in the liver, kidney and gills of the fish were ameliorated to certain extent similar to control in some treatments in the PbVE treatment group. The vitamins supplemented treatments displayed varying levels of ameliorations far better than the Pb only group. The kidneys of the PbVE group exhibited the highest level of GSH production (83.51±0.07 µg/ml) in comparison to other organs. The MDA production levels were significantly high in Pb only group (80.28±0.06 nM/mg) and PbVA (80.30±0.05 nM/mg). The high levels of production of AST and ALT (153.12±0.19 nM/mg and 87.20±0.15 nM/mg, respectively) indicate physiological perturbations and as such, suggest that they are good biomarkers of liver damage elicited by the presence of the toxicant. The kidneys and livers of *C. gariepinus* were fully engaged in mitigating the effects of the toxicant in the presence of the vitamins. There should be routine check on this important staple food especially those obtained from the wild to ensure that the level of contamination is within permissive limit by relevant authority as well as creating public awareness on the

dangers (such as vomiting, birth defects, malaise, lassitude and headache) of exposure to Pb toxicant.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table
Page

LIST OF FIGURES

Figure
Page

LIST OF PLATES

weeks
127

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Fish is a rich source of animal protein throughout the world. Due to its nutritional value (Tingman *et al*., 2010), the demand for fish food has been on the increase with increasing human population (FAO 2010, 2012). African catfish, *Clarias gariepinus* is an important commercial fish due to its high growth rate, high consumer acceptability, and ability to withstand poor water quality, and oxygen depletion (Adewolu *et al*., 2008; Karami *et al*., 2010).

The presence of pollutants in the environment of an aquatic organism such as fish can lead to the production of reactive oxygen species and consequently, oxidative stress. Heavy metals are known to elicit oxidative stress in organisms when the threshold is exceeded. Heavy metals are also known to promote oxidative damage by increasing the cellular concentration of reactive oxygen species (ROS) in fish, consequently, a response of antioxidative defences (Monteiro *et al*., 2010). Oxidative stress is a mechanism of toxicity which leads to cell death and disruption of the physiological processes in fish (Banaee, 2013). Oxidative stress is a consequence of the imbalance between the antioxidant enzyme activities and ROS production; also it is when the antioxidant system is incapacitated in eliminating or neutralizing the excess ROS (Nishida, 2011; Hamed, 2015).

In this respect, fishes are endowed with antioxidants that ensure their survival provided the threshold is not exceeded. Vitamins A, C and E are known to play ameliorative roles in the attenuation of the effects of pollutants on organisms. Fishes survive oxidative stress by mobilizing enzymatic as well as non-enzymatic antioxidant

defences (Ahmad *et al*., 2008; Van Der Oost *et al*., 2003). Also, Vitamins C and E supplementations have been reported to play a positive role in detoxification of mercury toxicity especially at lower concentrations (Thakur and Kanshere, 2014). For instance, Vitamin E played an important function in the elimination of mercury stress through antioxidant free radical mechanism (Agarwal *et al*., 2010). Fishes serve as early warning indicators of pollution in the aquatic systems and can be considered to be the most standard choice as test organisms because they are the best understood organism in the aquatic environment and its importance to man (and other organisms) as a source of protein (Murtala *et al*., 2012). Vitamins A, C and E and other exogenous anti-oxidants such as carotenoids and flavonoids act as antioxidant agents and can control oxidative stress. Furthermore, high levels of ascorbic acid are efficient in amelioration of toxicity, preventing disease and enhancing fish tolerance to environmental stress (Abdel-Tawwab *et al*., 2001). Osfor *et al*. (2010) demonstrated that vitamin E could improve daily food intake, body weight gain and feed efficiency ratio. It can also reduce Pb and Cu levels in serum and tissues of liver and kidney as well as reduce Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), urea and creatinine levels in Pb and Cu intoxicated male rats. In addition to this, Vitamin C is readily available, cheap and relatively non-toxic antioxidant and also known to have great impact in the reduction of toxic effects by most xenobiotics (Uchendu *et al*., 2012).

Heavy metals could be essential or non-essential. Heavy metals such as Fe, Cu, Zn, Ni, Co, Cr, and Mn are vital to human only at lower concentrations, but they become more toxic when they are taken up more than the bio-recommended limits (Shilpi *et al*., 2015). It is also known that even essential metals may be toxic on the biological

activities of organisms above certain concentrations (Merciai *et al*., 2014). The quantity of metal accumulated has been reported to be directly related to the concentration to which the organisms are exposed and the duration of exposure (Otitoloju and Don-Pedro, 2001). Several reports have indicated that Pb can cause neurological, haematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes_ all of these are related to the dose and time of exposure to Pb (Mirhashemi *et al*., 2010).

1.2 Statement of the Research Problem

Currently, all over the world and the developing countries in particular are fraught with the problems of pollutants and waste disposal both from municipal and industrial sources. The aquatic environment bears the brunt of pollution through run-offs and seepages. The aquatic biota bioaccumulate these pollutants and in most cases, biomagnify them beyond tolerant levels. The non- essential trace elements such as lead have no known beneficial biological significance in the body of an organism. Their bioaccumulative and magnification tendencies over time make them deleterious. The presence of trace elements in the environment of an organism lead to oxidative stress and oxidative stress is an inherent aspect of aerobic life. It is as a result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defences in living organisms (Nishida, 2011). Fish are particularly vulnerable and heavily exposed to pollutants due to feeding and living in aquatic ecosystems, because they cannot avoid pollutant harmful effects (Ahmed *et al*., 2020). Heavy metals enter fish by direct absorption from water through their gills and skin, or by ingestion of contaminated food (Ayyat *et al*., 2020). It is also known that in the aquatic environment there are myriads of pollutants at play at one point or the other. There is

paucity of information on the long-run interactions amongst these elements and other pollutants in the aquatic matrix and the physiological effects of such complex interactions. There is also paucity of information on how the vitamins are capable of attenuating the deleterious effects of specific toxicants.

The presence of toxicants in the environment of organisms is also known to initiate cascade of reactions bearing on the tissue architecture (Saliu and Bawa-Allah, 2012; Samuel *et al*., 2017a), the haematological parameters of the exposed organism over a long period of time. Changes in the haematological components of cat fishes have been reported from field and laboratory researches (Guedenon *et al*., 2012; Bolognesi and Cirillo, 2014; Singh *et al*., 2017) but there is paucity of information on the effects of specific toxicant such as Pb and when supplemented with vitamins.

In addition, little is known about the influence or effects of Pb as well as the supplemented treatments with vitamins on the growth parameters of *Clarias gariepinus* as morphological manifestations of the physiological changes taking place in the organism due to the presence of the toxicant and vitamins.

1.3 Aim and Objectives of the Study

The aim of this research was to evaluate the ameliorative roles of selected vitamins (A, C and E) in attenuating the toxicological effects of lead toxicant on *Clarias gariepinus* fingerlings.

The objectives of this research are to determine:

i. the 96 hours LC_{50} of lead toxicant on *Clarias gariepinus* fingerlings

ii. the growth parameters of *Clarias gariepinus* fingerlings exposed to sub-lethal concentrations of lead toxicant as well as of the supplemented treatments with vitamins A, C and E
iii. the haematological effects of sub-lethal concentrations of lead toxicant and vitamins A, C and E supplements on *Clarias gariepinus* fingerlings
iv. antioxidants levels in *Clarias gariepinus* fingerlings exposed to sub-lethal concentrations of lead toxicant as well as of the supplemented treatments with vitamins A, C and E
v. histopathology of the tissues of *Carias gariepinus* fingerlings exposed to sub-lethal concentrations of lead toxicant as well as supplemented treatment with vitamins E.

1.4 Justification for the Study

Fish is one of the chief protein sources for man that play a major role in lowering the blood cholesterol level and offers omega-3 fatty acids that minimize the danger of stroke and heart related disorders (Al- Busaidi *et al*., 2011). Heavy metal accumulation could occur in animal body tissues gradually and, overtime, can reach toxic concentration levels, much beyond permissible limits (Suruchi and Khanna, 2011). Bioaccumulation pattern of lead (Pb) in the head capsule and body muscle of *Clarias gariepinus* exposed to paint emulsion effluents demonstrated that fish can bioaccumulate these metals from a polluted environment which can culminate in the reduction or impairement of natural population size; and could be sources of these metals to man (Dahunsi *et al*., 2012) with deleterious effects over a long period of time. Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in

humans (El-Shehawi *et al*., 2013). Production of free radicals and reactive oxygen species as a result of oxidative stress usually lead to increased exploitation of non-enzymatic antioxidants such as reduced glutathione (GSH), vitamins C and E (Rajagopalan *et al*., 2010) as well as enzymatic antioxidants. For instance, Vitamin C is known to play a crucial role in the immunological and antioxidant properties of vertebrates capable of maintaining the integrity, fluidity of membranes and capable of controlling the oxidizing reactions of fatty acids, thus keeping cellular respiration and avoiding cell death (Abdel-Warith *et al*., 2011). Also, vitamin administration is known to be effective in improving the physiological and histological integrity in fishes of polluted aquatic environment (Kumar *et al*., 2014). This is why understanding the mechanisms of action of specific toxicant such as Pb in the body of vertebrates in terms of production of some antioxidants as well as nn-functional plasma enzymes that can help in reducing the effects of such pollutants and how such effects can be ameliorated by the presence of certain concentrations of vitamins would go a long way in addressing the problems of bioaccumulation and magnification of pollutants from the environment.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of *Clarias gariepinus*

The African cat fish, *Clarias gariepinus* is a tropical hardy species belonging to the Phylum Chordata, class Actinopterygii and family Clariidae. *Clarias* species is a widely distributed fish in Asia and Africa. In these areas, the fish is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and its somewhat acceptable market price (FAO, 2003). In Nigeria, *Clarias* species is an indigenous fish occurring in freshwater throughout the country. It is suspected that apart from tilapia, *Clarias* is the most abundant cultivated fish species in Nigeria (FAO, 2003). The common species found are *Clarias gariepinus*, *Clarias anguillaris*, *Clarias buthupogon* and *Clarias lazera*. The biological features of *C. gariepinus* include: Elongated body; large depressed and bony head with small eyes; narrow and angular occipital process with wide gill opening; air-breathing organ arising from gill arches. The first gill arch has about 24-110 gill rakers. The cleithrum is pointed and narrow with longitudinal ridges and sharpness. They also possess large terminal mouth with four pairs of barbells. There is presence of long dorsal and anal fins. There is no dorsal fin spine and adipose fin. They have round caudal fin and serrated pectoral spine. Colour varies widely from yellow through gray to olive or sometimes dark with dark greenish-brown markings and white belly (FAO Fish Stat, 2019).

2.2 Fish and Fish Organs

Fish organs such as kidney, gills, liver, muscle, skin are usually explored in biomarking and testing for oxidative damages due to one form of pollutant or the other.

This is majorly because these organs are in contact with the aquatic medium and play a major role in survival and protection of the fish. For instance, kidney plays an essential role in the water and electrolyte balance and in the maintenance of a stable internal body environment (Palaniappan *et al*., 2009). Depending on the physiological status of the fish, the organs are known to respond in various capacities to the presence of reactive oxygen species (ROS). For instance, increase in reduced glutathione (GSH) level in fish tissues was attributed to presence of defence system to protect the fish from the oxidative stress or could appear as an antioxidant adaptation to metal exposure (Marzouk *et al*., 2017). Also, Hermenean *et al*. (2015) observed that the liver has a higher capacity and adaptability to counteract ROS compared to kidney. Fish accumulate pollutants preferentially in their fatty tissues like liver and the effects become apparent when concentrations in such tissues attain a threshold level (Omar *et al*., 2014). Furthermore, Rajagopalan *et al*. (2010) demonstrated how constant production of free radicals resulted in increased exploitation of the antioxidants leading to their depletion in which the levels of non-enzymatic antioxidants such as reduced glutathione, vitamins C and E were significantly reduced in the alcohol and PUFA (Poly Unsaturated Fatty Acid) treated livers of rats because of their complete utilization due to the oxidative stress. This is why Doherty *et al*. (2010) noted that fish species are suitable candidates for the assessment of biomarkers of oxidative stress induced by pollutants because they play a dual role of being on top of the aquatic chain as vertebrates and respond strongly to stress conditions. In like manner, Yildirin *et al*. (2011) reported that stressful conditions lead to the formation of excessive free radicals which are major internal threat to cellular homeostasis of aerobic organisms.

2.3 Lead and its Effects on Aquatic Organisms

Heavy metals such as lead, mercury, and cadmium naturally occur in the deep layers of the earth and are present in the soils, rocks and sediments with high concentrations (Waheed *et al*., 2020). The ability of heavy metals to bioaccumulate and biomagnifying and difficult to be eliminated from the body by the ordinary metabolic activities make them one of the most dangerous sources of chemical water pollution to fish, causing big losses to fish and effects on the fish consumers (Mirghaed *et al*., 2018).

As a result of human activities, such as fossil fuel burning, mining, and manufacturing, lead (Pb) and lead compounds can be found in all parts of our environment such as air, soil, and water. Lead is used in many different ways. It is used to produce batteries, ammunition, metal products like solder and pipes, and X-ray shielding devices. Lead is a highly toxic metal and, as a result of related health concerns, its use in several products like gasoline, paints, and pipe solder, has been drastically reduced or discouraged in recent years. The most common sources of lead exposure are lead-based paints and possibly water pipes in older homes, contaminated soil, household dust, drinking water, lead crystal, lead in certain cosmetics and toys, and lead-glazed pottery.

Lead is a probable human carcinogen and can affect every organ and system in the body. Long-term exposure of adults to Pb can result in decreased performance in some tests that measure functions of the nervous system; weakness in fingers, wrists, or ankles; small increases in blood pressure; and anaemia. Exposure to high levels of Pb can severely damage the brain and kidneys and ultimately cause death. In pregnant

women, high levels of exposure to lead may cause miscarriage. Also, gastrointestinal disturbances in children, as well as nephrotoxicity and neurotoxicity in adults, are the common adverse effects of lead toxicosis in humans (El-Hack *et al*., 2019). Under usual circumstances, more than 90 % of the lead (Pb) reserved in the body exists in the skeleton. Moreover, during lactation and pregnancy, lead moves from mother's bones to breastfed infants and fetuses (Suruchi and Khanna, 2011).

High level exposure in men can damage the organs responsible for sperm production. Absorbed lead is rapidly taken up into blood and soft tissue, followed by a slower redistribution to bone. Bone accumulates lead during much of the human life span and may serve as an endogenous source of lead that may be released slowly over many years after the exposure stops (WHO, 1995). The presence of lead in the environment of organism can produce plethora of effects that could be debilitating and deleterious. For example, Peixoto *et al*. (2013) demonstrated that oxidative stress responses, lipid peroxidation and histology of the liver of barbells are sensitive indicators of the contaminants present in Vizela River water and are valuable biomarkers for monitoring purposes.

2.4 Growth Parameters of *Clarias gariepinus* and Toxicants

Growth is a natural phenomenon that occurs in every living organism which could be in terms of weight, length, height and proportion or dimensions. In aquatic environments there are plethoras of anthropogenic activities that either inhibit or facilitate growth of organisms. Some of these activities could arise from smelting and minning of iron ore and other metals, municipal and industrial sources. Several aquatic lives would be affected when they eventually end up in water. For instance, the effects

of Cd (100 µg/L) on the embryonic, larval or both stages of the ide (*Leuciscus idus*) showed that mortality rate, body size, various body morphometrics and deformities (vertebral curvatures and yolk sac deformities) were affected; and that the highest weight gain was found in control whereas the lowest weight gain was observed in the treatment with the highest concentration of the toxicant (Witeska *et al*., 2014). Ayegbusi *et al*. (2018) also reported growth performance decline and obtained SGR (Specific Growth Rate) value of 1.95±0.015 mg/L when *Clarias gariepinus* samples were exposed to sub-lethal concentrations of $PbCl_2$ for a period of 21 days. In like manner, there was reduced fish growth when Nile Tilapia was subjected to various concentrations of Cd (Rahman *et al*., 2018). They found the highest length (27.92 ± 3.2 cm) in control samples where as in T-VI (the highest concentration with 3 mg/L) the length gain was the lowest (16.14 ± 1.2 cm); and decrease gradually with increase in Cd concentration. Fish growth was also found to be higher in control compared to other treatments with Cd concentrations; and highest Cd accumulation was found in the liver as the major organ of detoxification. Also, exposure of *Platichthys stellatus* to varying concentrations of chromium led to decreased daily length gain, daily weight gain and condition factor after exposure to 400 ppb for 2 weeks (Ko *et al*., 2019). In like manner, growth performance was significantly reduced with increasing Zn concentrations in comparison with the control which fared better; and the optimum feed intake and feed conversion ratio in control groups (Abdel-Tawwab *et al*., 2013). It is also known that essential elements are required in diet for optimal growth, functioning and sustenance of the internal environment (Isibor and Imoobe, 2017) but when certain thresholds have been reached, they could become deleterious. In addition to this, Oluwatosin *et al*. (2018) reported that the sub-lethal exposure of *C. gariepinus*

showed reduction in growth with an increase in $PbCl_2$ concentration and the specific growth rate in the control was higher than other treatments with $PbCl_2$. In another development, Han *et al*. (2019) found that growth and hematological parameters determined decreased with increasing arsenic concentration, while the concentration of plasma components measured increased. Also, fish exposed to 10, 50 and 100 µgHg/L showed a significant decrease in growth rates as from days 14 to 35 (Pratap, 2016). This was attributed to utilization of energy to overcome physiological stress induced by the toxicant, thus affecting fish growth. Furthermore, Puvaneswari and Karuppasamy (2007) posited that the length of time of exposure affected the sensitivity of fish larvae and influenced the weight gain in *Heteropneutes fossilis* exposed to Cd. Growth reduction under metal contamination increased the energy costs due to increased metabolism (Sherwood *et al*., 2000); and growth inhibition being a prominent effect of metal accumulation following chronic exposure (Zebral *et al*., 2018).

Fishes that live in unfavourable environmental conditions face all manners of challenges. In order to ensure survival and subsequent reproduction of its kinds they mobilize various antioxidants (enzymatic and non-enzymatic) to counteract the effects of the pollutants. The use of supplements in both natural and artificial ponds and, or set-ups have been known to have significant positive effects on fish. Previous study has shown that vitamin E can improve daily food intake, body weight gain and feed efficiency ratio (Osfor *et al*., 2010). Kadry *et al*. (2012) showed that, fish fed diet supplemented with Vitamin E exhibited protective effects by minimizing the atrazine induced toxicity on female *Clarias gariepinus*, to the extent that the values obtained in other treatment were more or less similar to control group fish. Furthermore, the

administration of vitamin E to lead exposed fish has been reported to prevent the bioaccumulation of lead in tissues, and enhance the growth factor of fish (Abdalla, 2009).

2.5 Haematology of Fish Exposed to Toxicants

The presence of toxicants in the environment of organisms has myriads of effects on fish physiology. The haematological profile of a species is a good indicator of the levels of environmental stress and, consequently, changes in blood parameters have been used to assess effects of environmental pollution (Vinodhini and Narayanan, 2009). Some of the parameters that have shown sensitivity to contaminants are Red Blood Cells count (RBC) and White Blood Cells count (WBC) levels, clotting times, pack cell volume (PCV) and haemoglobin content. In line with this, Priyadharshani *et al*. (2011a) reported how immunological parameters such as total white blood cell (WBC) counts, differential WBC counts, spleen weigh per body weight ratio and neutrophil per lymphocyte ratio were affected by heavy metals. In a similar manner, a laboratory study reported heavy metals induced immunomodulation of phagocytes of *E. hexadactyla* where all the phagocytes showed immune suppression (reduces the activation or efficacy of the immune system) with the increased concentrations of heavy metals, except the blood leukocytes (Priyadharshani *et al*., 2011b).

The blood parameters are usually affected by the presence of xenobiotics. For instance, Hounpaktin *et al*. (2012) demonstrated a significant decrease in white and red blood cell count, reduced hemoglobin and mean corpuscular concentrations when high concentrations of mercury and the combination of high concentrations of cadmium and mercury were administered. However, co-administration of mercury, cadmium and mercury and vitamin C had a protective effect on the harmful metals. The values of the

haematological parameters were also increased due to treatment with vitamin C. Similarly, Ognjanović *et al.* (2003) has shown that pretreatment with vitamin E and C had a protective role on the toxic effects of cadmium on haematological values and lipid peroxide. According to Siess *et al.* (2000) vitamin C acts by preventing the binding of free radicals on DNA by activation of detoxification; and protection of the capillary walls as reported by Kawabata *et al*. (1990). Awodele *et al*. (2010) also reported that the administration of vitamin C (8 mg/kg) corrected some of the potential harmful rifampicin of the deoxyribonucleic acid (DNA) in mice.

2.6 Antioxidants and its Variance

Antioxidants comprises of enzymatic and non-enzymatic types. The enzymatic antioxidants are commonly represented by Super Oxide Dismustase (SOD), Catalase (CAT), Glutathione transferase (GST), Acetyl cholinesterase, Lipid peroxidase (LPO). Non-enzymatic antioxidants are commonly represented by reduced glutathione (GSH), metallothioneine (MT), uric acid, polyphenols, vitamins A, C and E. These antioxidants are known to exhibit varying roles and functions in counteracting the effects of oxidative stress in the environment of organisms. Antioxidant enzymes are crucial in their effort to decrease oxidative stress produced by exposure to toxicants (Saglam *et al*., 2014). It has also been reported that antioxidant may ameliorate, protect and remove the oxidative damage to a target organ or molecule (El-Shenawy and Al-Ghamdi, 2014). Non-enzymatic antioxidants such as vitamins C and E can also act to overcome oxidative stress, being a part of the total antioxidant system. They prevent the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues. Antioxidants (both enzymatic and non enzymatic) provide protection against deleterious metal-mediated

free radical attacks. The assessment of alterations in key enzymatic activities of organisms following exposure to polluted waters has been one of the major uses of biomarkers in environmental studies (Almedia *et al*., 2009). Cellular biomarkers represent early diagnostic tools since they can identify changes at sub-organismal level (that is, cellular and molecular) before becoming evident at higher levels of biological organisation (Doherty *et al*., 2010).

2.6.1 Glutathione as biomarker of oxidative stress

Glutathione (GSH) is tripeptide, which has many biological roles including protection against reactive oxygen and nitrogen species. As a carrier of an active thiol group in the form of a cysteine residue, it acts as an antioxidant either directly by interacting with reactive oxygen or nitrogen species (ROS and RNS, respectively) and electrophiles or by operating as a co-factor for various enzymes. The chemical structure of GSH determines its potential functions and its broad distribution among all living organisms reflects its important biological role. GSH has been found in all mammalian cells. Probably most importantly, GSH is responsible for protection against ROS and RNS, and detoxification of endogenous and exogenous toxins of an electrophilic nature. Other functions include (i) maintaining the essential thiol status of proteins and other molecules; (ii) storage of cysteine reserves both in the cell and for inter-organ transfer; (iii) involvement in the metabolism of estrogens, leukotrienes, and prostaglandins; (iv) participation in the reduction of ribonucleotides to deoxyribonucleotides; (v) participation in the maturation of iron-sulfur clusters in proteins; (vi) copper and iron transfer; (vii) signal transduction from the environment to cellular transcription machinery. GSH is a low molecular weight thiol. It can react directly with ROS species, thereby detoxifying them. In addition, GSH is used as a

conjugating molecule by GST (glutathione S- transferase) to ease excretion of xenobiotics. GSH is also used as a reducing equivalent in the metabolism of reactive intermediates, several studies have shown that antioxidants that are affected by reactive oxygen species show adaptive responses to xenobiotics that produce oxyradicals (Di Giulio *et al*., 1995) and are potential biomarkers for oxidative stress in fish (Van der Oost *et al*., 2003). Glutathione sulfhydryl is tripeptide protein made of three amino acids namely cystein, glutamate or glutamic acid and glycine. It was first discovered by J. deRey-Pailhade from human eyeball in 1888. The enzymes derived from GSH are glutathione peroxidase (GPx) and glutathione S-transferase (GST). When the GSH level rises the enzymes levels also rise. Glutathione and other antioxidants can be produced from the oxidative stress generated from their activities. For instance, Eissa *et al*. (2014) observed that enzyme activities act as biomarkers for oxidative stress induced by metacercarial infections in cultured *Oreochromis niloticus* and *O. aureus*. Glutathione modulates cell proliferation and plays a key role in protecting cells against oxidants (Kumaraguruparan *et al*., 2005).

The presence of the toxicant can either lead to increased production or utilization of the antioxidant. For instance, Saliu and Bawa-Allah (2012) reported that the levels of GST-GSH, SOD, CAT and MDA were all reduced in fishes (*Clarias gariepinus*) exposed to $Pb(NO_3)_2$ in comparison with the control indicating the effect of the pollutant on the fish while the reverse was the case in those fish exposed to $ZnCl_2$ with increase in antioxidants except MDA. The levels of GSH concentrations were increased by 44.8, 35.3 and 32.7 (%) in the kidney, heart and liver, respectively but were decreased by 33.6 (%) in the gill in the fish samples collected from Wadi Hanefah Reservoir (Mahboob *et al*., 2014). Similarly, Osioma *et al*. (2013) observed

that blood reduced glutathione was significantly lower in other sites in comparison with the control site (Ethiope River). Sharma and Ansari (2013) also demonstrated that GSH level in both the tissues (brain and muscles) were decreased for all exposure periods. The toxicity was time as well as concentration dependent. Glutathione has been found in all mammalian cells, and probably the most important aspect is the fact that GSH is responsible for protection against ROS and RNS, detoxification of endogenous and exogenous toxins of electrophilic nature (Lushchak, 2011). Alkallak (2013) demonstrated a significant difference in the accumulation concentration of cadmium and lead in the liver, kidneys, intestine, gills and muscles of the infected and un-infected fishes (*Silurus glanis*). Oxidative stress increased with an increase in the duration of physiological stress, while acute exposure mostly resulted in up-regulation of the antioxidant response (Costantini *et al*., 2011). Also, Sisein *et al*. (2014) attributed the significantly lower GSH value in the liver of *Clarias gariepinus* from Gbarantoru swamp in comparison with Niger Delta University Agricultural farm (control) partly to the increased accumulation of heavy metals which led to more utilization of GSH to detoxify metals and ROS. In the same vein, Ayoola *et al*. (2014) recorded significant differences in GSH, MDA, SOD and total protein in the gills of *Hemichromis fasciatus* and *Chrysichthys nigrodigitatus* collected from polluted Lagos lagoon.

2.6.2 Vitamins A, C and E as biomarkers of oxidative stress

Two distinct kinds of vitamins exist: fat-soluble and water-soluble. Fat-soluble vitamins consist of vitamins A, E, D and K, while water-soluble vitamins include the B vitamins and vitamin C. Vitamin A helps maintain good eyesight and support normal growth of cells. It plays a vital role in the development of the foetus and embryo.

Maintaining adequate intakes of vitamin A keeps teeth, bones, skin and mucus membranes in good health. This fat-soluble vitamin plays an important role in wound healing, immune system function, reproduction, and bone formation and growth. Excellent sources of vitamin A include orange fruits and vegetables, dark green leafy vegetables, liver and milk fortified with vitamin A.

On the other hand, vitamin C protects the body against the effects of free radicals, unstable molecules that damage DNA and may enhance the aging process and the development of health issues such as arthritis, cancer and heart disease. Vitamin C is responsible for the growth and repair of body tissues. The body needs vitamin C for repairing and maintaining bones and teeth and for healing wounds. The vitamin helps in the production of collagen, an important protein which is the key structural component of tendons, cartilage, blood vessels, skin and ligaments. Foods such as orange, cantaloupe, strawberries, tomatoes, cabbage, cauliflower, green peppers, watermelon, papaya (pawpaw) and Brussels sprouts contain considerable amounts of this important vitamin.

Also vitamin E protects the body from free radical damage. Vitamin E boosts immune system, enabling it to fight bacterial and viral infections. It also facilitates the usage of vitamin K in blood-clotting. This fat-soluble vitamin also contributes to the formation of red blood cells. Every cell in the body needs vitamin A. This vitamin regulates the growth and division of cells and it helps the body produce white blood cells. It plays a role in remodeling bone and it keeps the cells that line the body's interior surfaces healthy. Ascorbate (vitamin C) is an important water-soluble antioxidant, and may additionally serve as a co-factor for enzymes involved in collagen biosynthesis or neuro-transmitter conversions (Stegeman *et al*., 1992; LopezTorres *et al*., 1993).

Tocopherol (vitamin E) is a useful indicator of exposure to metals and organic contaminants that generate oxidative stress (Palace *et al*., 2005). Vitamin E is linked to regulation of various diseases like cancer, atherosclerosis, hypertension and male infertility. Antioxidants such as metallothionein, selenium, α- tocopherol, reduced glutathione (GSH), ascorbic acid and carotenoids share a common property that manifest protective influences by scavenging free radicals. There are a number of molecules that function as scavengers of free radicals. These include the well known dietary antioxidant molecules ascorbate (vitamin C), retinoic acid (vitamin A) or carotenoids and tocopherol (vitamin E) (Dalton *et al*., 1999). Layachi and Kechrid (2012) demonstrated how oral exposure to Cd caused reduction in lipid peroxidase (LPO) and antioxidant enzyme activities in rat's liver, and vitamin C or vitamin E may have partial ameliorative effects on these disturbances, while combination of vitamins C and E ensured a more efficient protection of the organ against the noticed oxidative stress. In like manner, Thakur and Kanshere (2014) demonstrated how Vitamin C and Vitamin E supplementation played a positive role in detoxification of mercury toxicity especially the low dose. Ascorbic acid is well known for its antioxidant activity, acting as a reducing agent to reverse oxidation in liquids (McGregor and Biesalski, 2006). Vitamin E has also been reported as a strong inhibitor of apoptosis and a stabilizer of biological membranes (Gago-Dominquez and Castelao, 2006).

The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathway (Pratt *et al*., 2010). Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular damage or disruption (Yolanda and Maria, 2012). Vitamin E (α-tocopherol) is a fat soluble antioxidant that inhibits the

production of reactive oxygen species formed when fat undergoes oxidation. Moreover, it was reported that high levels of ascorbic acid are efficient in reduction of toxicity, preventing disease and enhancing fish tolerance to environmental stress (Abdel-Tawwab *et al*., 2001). Similarly, Abdel-Tawwab *et al*. (2001) also found that a high level of ascorbic acid enhanced the weight gain, specific growth rate and survival rate in Nile tilapia exposed to sub-lethal dose of mercury.

2.6.3 Aspartate amino transferase (AST) and alanine amino transferase (ALT) as toxicity markers

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) belong to the plasma non-functional enzymes which are normally localized within the cells of liver, heart, gills, kidneys, muscle and other organs. These enzymes are liberated into the blood in pathological situations and therefore are of clinical importance. AST and ALT are highly conservative indicators in liver, and are commonly located in hepatic cytoplasm and would release into the circulation when hepatocytes necrotize (Arenas *et al*., 2017). In the environmental studies also, blood and tissues level of AST and ALT have been measured to assess the toxic impact of aflatoxicosis and ochratoxicosis (Ellakany and Gaafar, 2002). The presence of pollutant can trigger the utilization or increased production of AST and ALT. For instance, cadmium in plasma of goldfish significantly increased the activities of plasma glutamic acid oxaloacetic acid-transaminase (GOT/AST) and glutamic acid-pyruvic acid transaminase (GPT/ALT) (Zikic *et al*., 2001).

Ellakany and Gaafar (2002) reported that in *Oreochromis niloticus*, there was a marked reduction in AST in liver and muscle in response to the lower or higher level of ochratoxin. They attributed the reduced levels of aminotransfersase in various organs

to tissue damage and consequently the reduction of enzyme biosynthesis for reasons related to the presence of ochratoxin. On the other hand, the ALT activities in liver and muscle were found to increase during the time course of endogenous cortisol elevation induced by ochratoxin intoxication. The results obtained also indicated that the tissue injury in toxicated fish recovered when they were fed dietary ascorbic acid because the AST and ALT activities in fish exposed to different doses of combined ochratoxin and vitamin C became similar to what were obtained in control fish. Likewise, activities of the hepatic enzymes lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were found to be significantly elevated, particularly in summer (Yancheva *et al*., 2014). Serum ALT and AST are the most used markers of hepatocellular necrosis and are considered sensitive indicators of hepatic injury (Friedman *et al*., 1996) and cell membrane damage and leakage (Kaplan, 1993). The ameliorative role of vitamins was evident when Vitamin E and metallothionein treatments protected against Cd-induced damage of liver in grass carp by decreasing AST and ALT content, repairing organelles, and maintained the antioxidant system by elevating CAT, SOD, and GSH-Px activity and regulating related mRNA transcript expression (Feng *et al*., 2018). ALT and AST are liver specific enzymes and provide a more sensitive measure of hepato-toxicity and histopathological changes that can be assessed within a short time (Balint *et al*., 1997). Oluah (1999) noted that increases or decreases in the values of ALT and AST indicate tissue damage in liver, kidney, muscle and gill. Also, Atamanalp *et al*. (2002) found a significant decrease in the activities of ALP, LDH, AST and creatinine kinase (CK) and lactate in blood plasma. Gabriel *et al*. (2012) also recorded a decline in the activities of enzymes in all the organs (gill, kidney and liver) of *C. gariepinus* with

ALP mostly affected when exposed to varying concentrations of cypermethrin. ALT activity reflects a change in endoplasmic reticulum mass; it is also known to occur in the cell membrane and may be involved in metabolic activities (De Silva *et al*., 2002). Furthermore, increased activities of AST, ALT and ALP in Indian major carps exposed to nitrite toxicity have been recorded (Das *et al*., 2004). Changes or alterations in responses to toxicants in most cases are concentration dependent. However, changes in enzyme responses were not concentration-dependent and lowest activity was recorded for ALT (4.00±0.00 IU/L) in the plasma of *C. gariepinus* as reported by Akani and Gabriel (2016).

2.6.4 Malondialdehyde (MDA) as biomarkers of oxidative stress

MDA is an end product of lipoperoxydation, and is considered a biomarker of oxidative stress and cellular damage (Kim *et al*., 2000; Dotan *et al*., 2004). MDA production is a well-known oxidation product of polyunsaturated fatty acids, influencing cell membrane fluidity as well as the integrity of bio-membranes (Ercal *et al*., 2001; Almroth *et al*., 2005), and can be used as an indicator of lipid peroxidation. It is usually produced in large quantity when elicited by the presence of toxicants. For instance, Sissein *et al*. (2014) showed that the polluted Gbarantoru swamp in Niger Delta, Nigeria contain higher levels of heavy metals, high levels of MDA in liver cells of *C. gariepinus* and low levels of vitamins and glutathione as compared to the levels in the *C. gariepinus* harvested from Niger Delta University Agricultural Farm (Control). Likewise, Karadag *et al*. (2014) demonstrated that the activity of catalase and level of malondialdehyde increased while activity of superoxide dismutase and glutathione level decreased in fish from Sitilce site when compared to Samsat site (Control). Also, a significant increase ($p < 0.001$) in CAT, GSH and MDA with

concomitant decrease in SOD concentrations were observed in the treated mice with contaminated well water and electronic waste leachate (Bakare *et al*., 2012). Furthermore, malondialdehyde (MDA) and advanced oxidation protein products (AOPP) increased significantly in the liver and kidney of fish from downstream site compared to upstream one, whereas reduced glutathione (GSH) decreased; the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) increased significantly in livers, whereas SOD increased in kidney (Hermenean *et al*., 2015). This study also revealed that liver has a higher capacity and adaptability to counteract ROS compared to kidney. In like manner, Peixoto *et al*. (2013) reported high levels of lipid peroxidation, measured as MDA due to the presence of contaminated water.

Lipid peroxidation is one of the major mechanisms involved in oxidative cell injury and an increase in malondialdehyde (MDA) level is frequently observed during oxidative stress and has generally been used as a marker of oxidative damage (Yildirin *et al*., 2011). This is usually because high levels of MDA and low activity of SOD suggest a marked effect of possible fish species exposure to environmental stress (Ahamefula *et al*., 2014). Also, MDA levels were significantly elevated in the kidney of exposed fish, while the gills and liver showed no significant increase across all exposure concentrations (Adeogun *et al*., 2012). The significant increase in lipid oxidation (MDA) may indicate the susceptibility of lipid molecules to reactive oxygen species and the extent of oxidative damage imposed on these molecules. Adeogun *et al*. (2012) also observed significant increase in MDA activity in the kidney and attributed it to high antioxidant (CAT, SOD and GSH) activities recorded in the study.

2.7 Vitamins and their Ameliorative Roles

Fishes and other aquatic organisms are sensitive to stress caused by metals and other toxicants. They also possess natural molecules like vitamins and other antioxidants that are involved in scavenging these pollutants, but when the toxicants concentrations outweigh the aquatic organisms' immune ability, it results to stress, injury and low or diminishing levels of these antioxidants (Ozden and Mustafa, 2010; Yildirin *et al*., 2011). Administration of vitamins can ameliorate or at best attenuate the effects of toxicants and the ROS generated from them in the environment of organisms. For instance, Sajitha *et al.* (2010) reported that administration of vitamin E decreased the histopathological and biochemical alterations induced by Pb intoxication in female Sprague-Dawley albino rats. Likewise, Bharrhan *et al*. (2010) studied the effect of vitamin E supplementation on lipopolysaccharide (LPS)-induced liver damage in rats. They reported that the challenge with LPS resulted in a significant increase in the activities of serum ALT, AST and ALP along with histological alterations in the liver. They also reported that these responses were associated with elevated levels of malondialdehyde (MDA) and reduced levels of GSH, SOD and catalase as well as increased levels of tumor necrosis factor alpha (TNF-a) in the liver homogenates. Supplementation with vitamin E decreased the incidence and severity of LPS-related histological changes of liver. In like manner, administration of vitamin E alleviated the liver biochemical disturbances and histopathological alterations from the oxidative stress produced by exposure to heavy metals (AlAttar, 2011). Also, Vitamins C and E either alone, or in combination (as antioxidants) have been shown to ameliorate the hepato-renal and testicular toxicity of abamectin, but were not completely protective, especially in liver tissue (Magdy *et al*., 2016). Among antioxidants, ascorbic acid

(vitamin C) and tocopherol (vitamin E) used as a nutritional supplements, are the essential elements in almost all biological systems. Vitamin C is readily available, cheap and relatively non-toxic antioxidant and possesses great benefit in the amelioration of toxic effects by most xenobiotics (Uchendu *et al*., 2012). Also, it has been shown that $CdCl_2$ increases TLC (Total leukocytes count) and decreases Hb content as compared to control; and the exposure of heavy metal with ascorbic acid led to decrease in the TLC and increase in Hb contents as compared to those of heavy metal intoxicated fishes (Borane, 2013). Ascorbic acid also reduced the levels of lead in blood, liver and kidney in rats. The cellular respiration in vertebrates depends on the availability of iron associated with Hb. The change in hemoglobin and leukocytes indicate the impact of lead and ascorbic acid (Borane, 2010) on the fish. In addition, Vitamin C has potent antioxidant activity against cadmium and mercury sensitive haematological parameters (Hounkpatin *et al*., 2012). Mekkawy *et al*. (2011, 2012) also reported that tomato paste and vitamin E expressed high protective potentials against cadmium-induced biochemical changes especially liver transaminases and liver histopathological alterations. A study has also shown how vitamin E and metallothionein treatments protected against Cd-induced damage of liver in grass carp by decreasing AST and ALT content, repairing organelles, and maintained the antioxidant system by elevating CAT, SOD, and GSH-Px activity and regulating related mRNA transcript expression (Feng *et al*., 2018).

2.8 Heavy Metals and Tissue Histopathology

Heavy metals can be taken up into fish either from ingestion of contaminated food via the alimentary tract or through the gills and skin (Sfakianakis *et al*., 2015). Heavy metal accumulates in kidneys (and other organs of fish), damaging filtering

mechanisms and affecting structure and ultra-structure, depending on the exposure time and dose (Costa *et al*., 2013). In natural environment there are myriads of pollutants at play that can affect or culminate in the generation of reactive oxygen species and consequently, oxidative stress on the biota. For example, Radic *et al*. (2013) observed that tissue histopathology, oxidative damage to biomolecules and modulation of antioxidant enzyme activity in carp and plant tissues exposed to River Ilova is the result of synergistic toxic effects of micro-pollutants present in the surface water. In like manner, Chavan and Muley (2014) reported that the major histopathological changes in liver included loss of cellular architecture, necrosis in hepatocytes and accumulation of fat in parenchymal cells. They also observed congestion of blood vessels. Annabi *et al*. (2013) demonstrated slightly similar results in mosquito fish's (*Gambusia affinis)* kidney and liver.

However, under laboratory conditions known toxicants are deployed; and the consequent effects can be attributed to them. For instance, Bijoy and Nimila (2011) reported vacuolar degeneration and focal necrosis in hepatocytes of *Etroplus maculatus* exposed to lindane. In addition to this, Makinde *et al*. (2015) reported an advancing hepatic necrosis in the liver of *Clarias gariepinus* exposed to 2, 4-D amine. Likewise, Paul and Sengupta (2013) demonstrated how sub-lethal concentration of lead acetate has the capacity to bio-accumulate, thereby altering the normal functional activities of freshwater fish *C. punctata*. As an important detoxifying organ in fish, liver is generally considered as the richest accumulation position of heavy metals (Al-Balawi *et al*., 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Samples/Materials Collection and Acclimatization

A total number of two thousand five hundred (2500) fingerlings of *Clarias gariepinus* (15.50.±00 mg) were purchased from a commercial fish farmer in Ilorin, Kwara State, and transported in 50 L containers filled with water to the Old Farm Research Unit of the Department of Water, Aquaculture and Fisheries Technology, Bosso Campus, Federal University of Technology, Minna, Nigeria. The fishes were placed in fish ponds with water for acclimatization. The fishes were fed twice daily (morning and evening, 8.00 hr and 17.00 hr) with vital feed (3 mm) for 14 days (2 weeks) for the acclimatization. The holding water was changed every three days during the period.

The vitamins A, C and E granules were purchased from commercial chemical stores. 1.5 kg units of the granules in each case were used as the supplements in percentages corresponding to the sub-lethal concentrations of the treatments. The toxicant Pb (3 pieces of 500 g) analytical grades were purchased from commercial chemical stores and stored in a cool dry condition throughout the period of the experiment. This toxicant was administered according to the concentrations and the sub-lethal concentrations corresponding to the sub-lethal concentrations of the treatments during the acute and chronic phases of the exposure.

3.2 Experimental Set-up

The acute toxicity test for Pb was carried-out to determine the LC_{50}. Five treatments including control with two replicates in each treatment were set-up for the Pb, Vitamins A, C and E; and the sub-lethal exposures were run for a period of twelve (12)

weeks. The first group of treatments was tagged Pb (Pb only with T1-T4 and replicates), second PbVA (Pb+vitamin A with T1-T4 and replicates), third PbVC ((Pb+vitamin C with T1-T4 and replicates) and fourth PbVE (Pb+vitamin E with T1-T4 and replicates).

Sampling was made from each trough randomly as from the 14th day of exposure_that is, fortnightly for the twelve weeks. Fish tissues such as gills, kidneys and liver were excised for AST, ALT, MDA and GSH analyses. Growth parameters such as standard length, total length and weight were determined on weekly basis for the twelve weeks exposure period. The physico-chemical parameters such as Dissolved Oxygen (mg/L) which was determined with Portable Dissolved Oxygen Analyser (Model JPB-607), Electrical conductivity (µS/cm), Total Dissolved Solids (mg/L) and Total Alkalinity (mg/L) and pH of the test media were determined with Multiple Parameters Probe Metre (HANA..H19813_GROCHEK). The probe was immersed in the water medium for 5 minutes until a stable reading was read and recorded in each case for each parameter, respectively. Liver, kidneys and gills were excised from the Pb only treatment group and the PbVE treatment group, as well as the control for the histopathological analysis of the tissue for possible alterations and amelioration.

3.3 Acute Toxicity Test of Pb

Experimental set-up included 20 L plastic aquarium (43.8 cm by 30.8 cm) containing 10 fingerlings (15.50.±00 mg) in each trough. The test was made up of six treatments with two replicate in each case with the following nominal Pb concentrations: 0 (control), 220, 240, 260, 280, 300 and 320 mg/L carefully measured out using weighing balance. Detailed physical and behavioural changes within the first 2-4 hrs

and then, 12 hrs later were noted. Further observations were made every 24 hrs subsequently for the 96 hrs exposure period. In each tank, the dead specimens were removed as soon as possible. The 96 hrs LC_{50} value were determined using Probit analysis (Finney, 1971).

3.4 Chronic Toxicity Test of Pb

In order to assess long term effects of lead nitrate ($Pb(NO_3)_2$, the fishes were exposed to five sub-lethal treatments of lead nitrate concentrations corresponding to 0 % (control), 15 %, 25 %, 35 % and 45 % of the LC_{50} which translated into 26 mg/L, 44 mg/L, 61 mg/L and 79 mg/L. Each treatment was in two replicates containing 32 fish in 20 L plastic aquarium for the Pb, Vitamins A, C and E supplemented exposures. The minimum concentration of the toxicant serves the same concentration of the vitamins. The water was changed and fresh toxicants with the same set of concentrations were added at every 72 hours according to Organization for Economic Co-operation and Development (OECD, 2007) standards. Four fish samples were picked at random and sacrificed from each trough on every 14^{th} day for the twelve weeks exposure period. The liver, gills and kidney were excised, homogenized in sodium phosphate buffer solution using ceramic mortar and pestle; and stored in sample tubes, then refrigerated until needed for analyses such as GSH, MDA, ALT and AST.

3.5 Preparation of Sodium Phosphate Buffer

Sodium phosphate buffer solution (0.2 M) was prepared from the mixture of sodium dihydrogen orthophosphate with 0.1 M and disodium hydrogen orthophosphate with 0.1 M. The pH was adjusted to 8.0.

3.6 Determination of Growth Parameters of *Clarias gariepinus* Exposed to Sub-lethal Concentrations of Lead

3.6.1 Standard length

At every sampling day 2 randomly selected specimens were taken from each of the sub-lethal concentration exposure and replicate including the control (samples not exposed to the toxicant) for the determination of the standard length in centimeters. The standard length was measured from the tip of the snout (face bone) to the tail lobe (caudal peduncle) using a metre rule graduated in centimetres.

3.6.2 Total length

The total length of two randomly selected fish samples from each treatment and replicate including the control were taken at each sampling day of the chronic exposure. The total length was measured from the tip of snout to the end of the tail fin using metre rule graduated in centimetres.

3.6.3 Weight

The weight of the fish was recorded using the electronic battery powered weighing balance (Digital Pocket Scale, 2*PCS, 3V AAA batteries). The fish was weighed in gram by placing them inside a basket whose weight has been taped to zero in order to get the actual weight of the fishes. The weight of the fish sample was determined from two randomly selected specimens from each treatment and replicate including the control. The specimen was weighed separately and the average taken to represent each treatment and replicate at each sampling day. From the measurements the following parameters were determined:

3.6.3.1 Weight gain (g)

The fish weight gain (WG) was calculated as the difference between the final weight of fish at the end of the experiment and the initial weight in grams (Ahmed, 2012).

3.6.3.2 Percentage weight gain (%)

The Percentage weight gain was calculated as described by Ahmed (2012) thus:

$$\text{Weight gain (\%)} = \frac{\text{Final body weight} - \text{initial body weight}}{\text{Initial body weight}} \times 100$$

3.6.3.3 Specific growth rate (g/day)

The specific Growth Rate (SGR) was calculated using the formula;

$$\text{SGR(g/day)} = \frac{(\ln W2 - \ln W1)}{(T2 - T1)} \times 100$$

Where W1 = initial weight

W2 = Final weight

T_2-T_1 = Number of days of exposure

3.7 Determination of Haematological Parameters of *C. gariepinus* Exposed to Sub-lethal Concentrations of Lead

Blood samples were collected every 4th week of the exposure (once every month) for a duration of the exposure from each treatment and replicate. The blood samples were collected with 1ml heparinized sterile syringe into EDTA test tubes containing little quantity of EDTA anti-coagulant (Abdulkareem *et al*., 2017; Usman *et al*., 2019). The method of collection involved the insertion of the syringe in between the opercula end

and the pectoral fin on the ventral surface of the fish. The syringe was held perpendicularly and blood drawn out with suction pressure. Larger quantity of blood was drawn with this method in comparison to the little quantity available from the caudal end of the fish.White Blood Cells count (WBC), Red Blood Cells (RBC), Haemoglobin Concentration (HGB), Haematocrit (HCT), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelet Count (PLT) of the blood collected from the samples of each treatment and replicate were determined in the Medical Laboratory Services of Minna General Hospital, Niger State**.** These parameters were determined using Mindray (BC-5300) Auto Haematology Analyzer for full blood count. This works on the principle of laser scatter, flow cytometry and chemical dye to provide reliable and accurate 5-part differentiation on blood cells. The erythrocyte indices such as MCHC, MCH and MCV were calculated using the following formular:

MCV (Fl) = PCV (%) × 10/ RBC in millions/mm^3

MCH (Pg) = Hb (g/dl) × 10/RBC in millions/mm^3

MCHC (g/dl) = Hb (g/dl) × 100/PCV (%)

3.8 Determination of Anti-oxidants Production Levels in *C. gariepinus* Exposed to Sub-lethal Concentrations of Lead

3.8.1 Reduced glutathione bioassay

The GSH (reduced glutathione) produced in each organ of the fish from each treatment and replicate were determined from their homogenates in Africa Centre for Excellence Laboratories (Step B), Bosso, Niger State. The fish organs (gill, liver and Kidney) was homogenized using ceramic mortar and pestle with sodium phosphate buffer. The

following reagents were used for the analysis: 0.2 M phosphate buffer (8.40 g of NaH_2PO_4 and 9.94 g of Na_2HPO_4 was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH8.0); 10 % Trichloroacetic acid (10 g of TCA was dissolved in distilled water and made up to 100 ml in the volumetric flask); and Ellman' reagent (19.8 mg of 5,5'-Dithiobis Nitro Benzoic acid (DTNB) in 100 ml of 0.1 % sodium nitrate).

To 150 µL of the tissue homogenate (in phosphate-saline pH 7.4), 1.5 ml of 10 % TCA was added, and centrifuged at 1500 g for 5 mins. One (1.0) ml of the supernatant was treated with 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer (2.0 m pH 8.0). The absorbance was read at 412 nm. Estimation of Reduced Glutathione was determined by the method of Ellman (1959) as described by Rajagopalan *et al*. (2004). The amount of glutathione was calculated using a GSH standard curve and expressed as micron grams of GSH formed/mg protein in each case.

3.8.2 Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) determination

Fish tissues' AST and ALT were determined as described by Reitman and Frankel (1957) from all the treatments and replicates in Africa Centre for Excellence Laboratories (Step B), Bosso, Minna, Niger State. Spectrophotometric method was used for the assay of aspartate and alanine aminotransferase. The homogenates were prepared in the laboratories as follow: 100 µl (0.1 ml) of the tissue homogenate was added into test tubes with 500 µl (0.5 ml) of reagent 1(buffer). The mixture was incubated for 30 minutes at 37 ^{o} C in samples of *C. gariepinus* analyzed for ALT. On the other hand, the mixture was incubated for 60 minutes at 37 ^{o} C in samples of *C. gariepinus* analyzed for AST. Subsequently, 500 µl (0.5 ml) of reagent 2 (2, 4-

dinitrophenylhydrazine) was added and kept for 20 minutes at 25 ^{0} C. The reaction was terminated with the addition of 5000 µl (5.0 ml) of 0.4 Mol/L NaOH to the mixture. The blank was prepared with 500 µl (0.5 ml) of reagent1 and 0.1 µl (100 µl) of distilled water. The absorbance in each case was read at 546 nm.

3.8.3 Malondialdehyde (MDA) determination

Malondialdehyde (MDA), as an in vitro marker of lipid peroxidation, was determined according to the fluorimetric method of DelRio *et al*. (2003) in Africa Centre for Excellence Laboratories (Step B), Bosso, Minna, Niger State. A volume of 700 µL of 0.1 M HCl and 200 µL of sample was incubated for 20 minutes at room temperature. Subsequently, 900 µL of 0.025 M thiobarbituric acid was added, and the mixture was incubated for 65 minutes at 37 ° C. Finally, 400 µL of Tris– EDTA protein extraction buffer was added. MDA fluorescence was recorded using A Jasco FP750 spectrofluorometer (Tokyo, Japan) with A 520/549 (excitation/emission) filter. A calibration curve with MDA in the range of 0.05– 5 µM was used to calculate the MDA concentration. The results were expressed as nmoles of MDA/Mg protein.

3.9 Determination of the Histopathology of the Tissues of *Clarias gariepinus* Exposed to Sub-lethal Concentration of Lead Nitrate

The histopathology of the gills, kidneys and liver of *Clarias gariepinus* from the Pb only treatment group and the PbVE treatment group and replicate in each case were carried-out in comparison with the control samples. These organs were excised from the fishes and preserved in 10 % neutral buffered formalin until required for analysis. The histopathological analyses were carried-out in the histopathology unit of the University of Ilorin Teaching Hospital, Kwara State, Nigeria. Gills, kidneys and liver

of the fish were fixed in Bouin's fluid for 24 hours; it was then dehydrated in graded ethanol concentrations and embedded in paraffin wax. Sagittal sections of 3-5 µm thickness were cut and mounted on glass slides. The sections were de-paraffinized in xylene, hydrated in ethanol and stained with hematoxylin-eosin (HE). The possible changes that took place as observed in the gills, liver and kidneys were indicated from the selected photomicrograph prepared and observed under light microscope at ×400 magnification.

3.10 Data Analyses

Probit analysis was used to analyse the ranges of values from LC_{10}-LC_{99} from the acute exposure of the samples. The antioxidants levels and the haematological parameters in samples exposed to sub-lethal concentrations of the toxicant as well as those treatments supplemented with vitamins were analysed using One Way Analysis of Variance followed by Duncan Multiple Range Test to separate the means where significant at $P< 0.05$ level of significance using SPSS Statistical Package (version 20.0 for Windows).

Length-Weight Relationships were determined using regression analysis and correlation coefficient of the length and weight measurements of the samples in all the treatments.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Range finding test for Pb: physical and behavioural changes

When *Clarias gariepinus* samples (5 fish samples) were exposed to 200 mg/L of Pb toxicant there was no visible effect within 24 hours. However, on exposure to 300 mg/L concentration there was frequent gasping for air in about 2-4 hours which became normalized afterwards with 1 death within 24 hours. Vigorous and frequent gasping for air characterized the exposure to 450 mg/L within an hour; lethargic swimming then followed. Furthermore, gasping for air, hanging at the surface for a long period of time before returning to the bottom characterized the samples exposed to 550 mg/L concentration of the toxicant. There was one death within 4 hours of exposure with blood oozing out of the either sides of the opercula. Two more died within 24 hours.

4.1.2 Definitive tests

All samples of *C. gariepinus* exposed to Pb toxicant within the range of 220-320 mg/L displayed initial agitation in about 2-4 hours of exposure especially in the higher concentrations, and afterwards swarm normally in the 12 hours of exposure. Mortalities were recorded as from 15-17 hours of exposure. After the initial mortalities in the higher concentrations (2 each in 300 and 320 mg/L) there were no further deaths but displayed severe lassitude. (Table 4.1). Majority displayed weakness, slow movement and emaciation. There was an unusual inverse relationship between concentration and mortality and the LC_{50} was 174.71 mg/L. (Table 4.2).

Table 4.1: Mortality Rates of Samples of *C. gariepinus* Exposed to Lethal Concentrations of Pb.

Concentration (mg/L)	No. of individuals per trough	No. of respondents per trough	% mortality
220	10	3	30
240	10	4	40
260	10	4	40
280	10	4	40
300	10	2	20
320	10	2	20
00	10	0	0

Table 4.2: Probit Range of Values (LC_{10}-LC_{99}) of *C. gariepinus* Exposed to Lethal Concentrations of Pb.

Lethal Con.	Values (mg/L)
LC_{10}	543.54
LC_{20}	368.15
LC_{30}	277.98
LC_{40}	218.66
LC_{50}	174.71
LC_{60}	139.59
LC_{70}	109.81
LC_{80}	82.91
LC_{90}	56.16

LC_{99}	22.26

4.1.3 Growth parameters of *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with vitamins A, C and E

4.1.3.1 Standard Length of C. gariepinus exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with Vitamins A, C and E.

The standard length of the control samples ranged from 8.90 cm to 13.40 cm. The highest standard length in the Pb treatments was recorded in samples treated with 26 mg/L giving 14.90 cm at the 12th week of exposure. On the other hand, the lowest value was obtained in specimen treated with 44 mg/L which had SL of 7.50 cm after the 1st week of exposure to the toxicant. There were slight gradual increases in length from week 1 to 12 in 26 mg/L. (Figure 4.1). The highest standard length in PbVA (Pb treatments supplemented with vitamin A) was obtained in the highest concentration of 79 mg/L with 12.60 cm while the lowest was recorded in treatments with 44 mg/L at week 1 with 7.0 cm. However, no particular trend was established as there were decreases or increases at one point or the other. (Figure 4.2).

The maximum standard length obtained in PbVC (Pb treatments supplemented with vitamin C) was 12.30 cm in set-ups containing 26 mg/L concentration of the toxicant at the 10th week of exposure while the minimum was 7.50 cm recorded in treatments with 79 mg/L at the 7th week of exposure. (Figure 4.3). Unlike in other treatments above, there were marked increases in the standard length with improved growth throughout the PbVE (Pb treatments supplemented with vitamin E) when compared to the control samples. The marked improvements were recorded in the treatments with 26 mg/L with the lowest concentration of the toxicant. The highest standard length was

recorded in sample treated with 26 mg/L which was 19.40 cm while the lowest was recorded in specimens exposed to 61 mg/L with 7.20 cm at the 12th week. There were general improvements in the standard length in all the treatments at the 9th week; while the same improvements were also recorded at week 12 in treatments with 26 mg/L through 61 mg/L. These improvements were significantly higher in specimens treated with 26 mg/L than in others. (Figure 4.4).

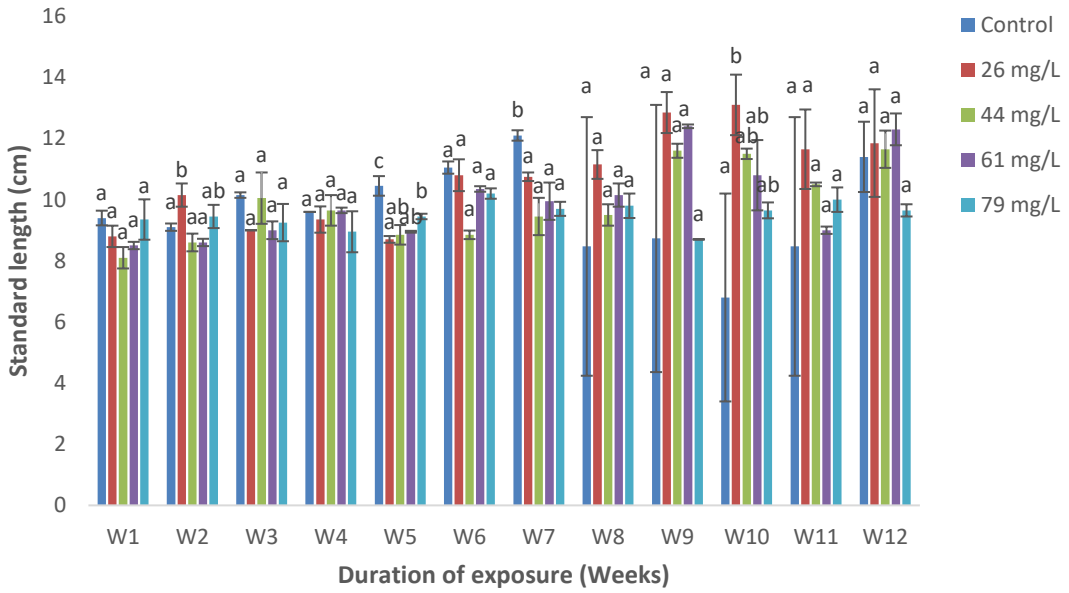

Figure 4.1 Mean values of standard lengths of *C. gariepinus* exposed to sub-lethal Concentrations of $Pb(NO_3)_2$ only for a period of 12 weeks.

Bars with different superscript differ significantly (P<0.05) from each other. Bars are mean±SEM of 2 individual observations.

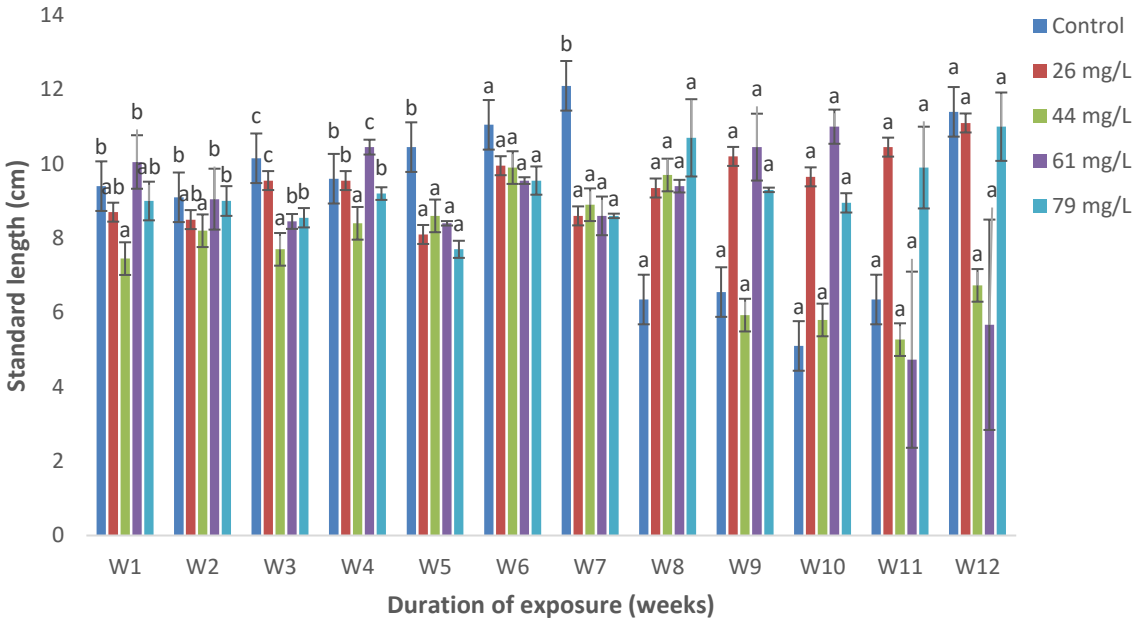

Figure 4.2 Mean values of standard lengths of *Clarias gariepinus* subjected to sub-lethal concentrations of Lead nitrate and treated with vitamin A for a duration of 12 weeks.

Bars with same superscripts are not significantly different from each other (P>0.05). Bars are mean±SEM of 2 individual observations.

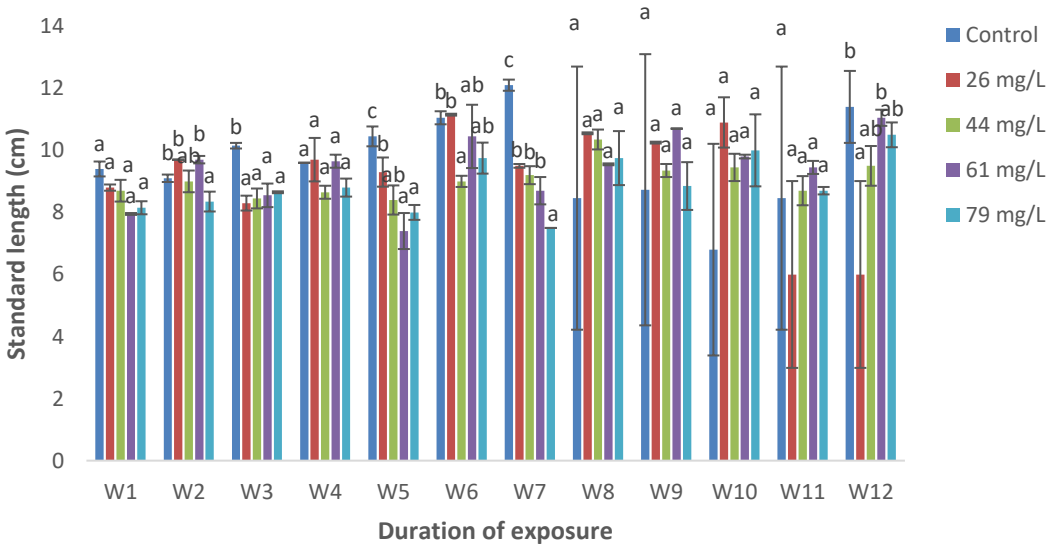

Figure 4.3 Mean values of standard lengths of *Clarias gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C for a ength of

time of 12 weeks.

Bar charts are mean±SEM of 2 specimens. Bars with different superscripts are significantly different (P<0.05) from each other.

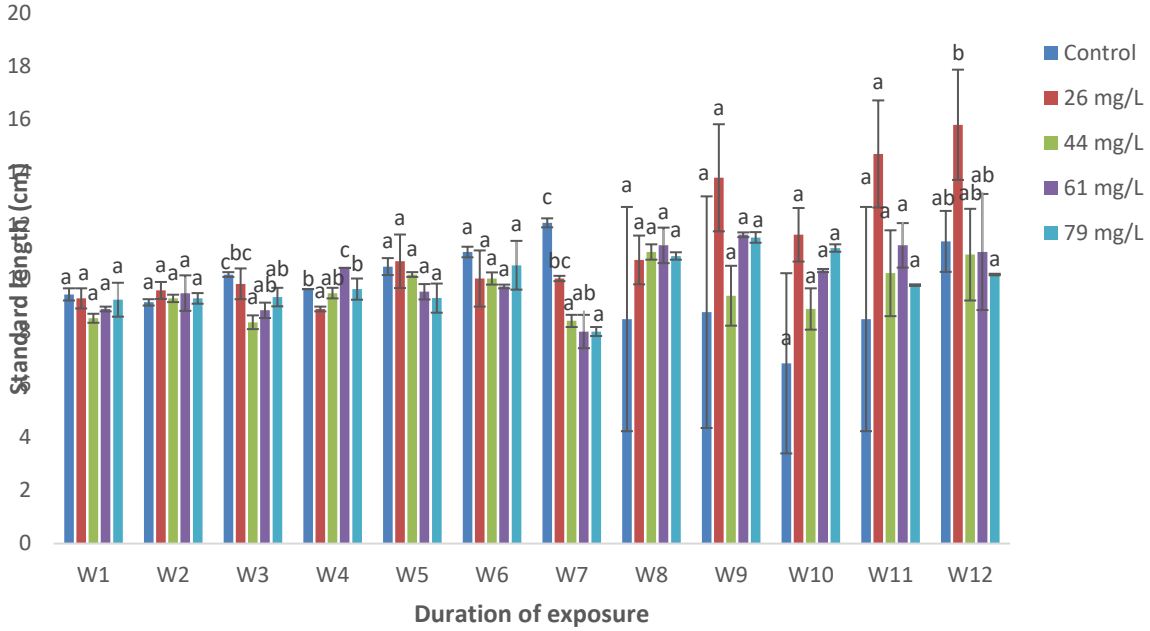

Figure 4.4 Mean values of standard lengths of *Clarias gariepinus* treated with sub-lethal concentrations of Lead nitrate for a period of 12 weeks.

Bars with different superscripts are significantly (P<0.05) different from each other. Bars are mean±SEM from 2 individual observations.

4.1.3.2 Total length of C. gariepinus exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with vitamins A, C and E

The control samples have 14.90 cm as the highest total length at the 9th week while the lowest was recorded in the 1st week after exposure with 9.80 cm. There were general increases in total length when compared to the control. The highest total length of 17.60 cm was recorded in samples treated with 26 mg/L at the 12th week; while the lowest was recorded in specimens subjected to 61 mg/L with 8.30 cm at the 1st week after exposure. (Figure 4.5).

The highest total length obtained in PbVA was 14.10 cm in the maximum concentration of 79 mg/L at the 12th week of exposure; while the lowest was recorded in the set-up having 61 mg/L at the 1st week after exposure with 6.90 cm. There was gradual increase in the total length from week 1 to week 12 in samples with 26 mg/L. (Figure 4.6).

The lowest value recorded in PbVC treatments was 8.20 cm in treatment with 61 mg/L at the 5th week; while the highest total length was 13.90 cm in the lowest treatment of 26 mg/L at the 10th week after exposure to the toxicant and the supplement. (Figure 4.7).

There were general improvements in all the treatments exposed to PbVE treatments. There was increase in the total length from week 1 to 12 in treatments with 26 mg/L to 61 mg/L. The highest total length was recorded in the set-up with26 mg/L at the 12th week of exposure with 22.30 cm which was significantly higher than other treatments; while the lowest was recorded in subjects exposed to 61 mg/L at the 12th week with 8.40 cm. (Figure 4.8).

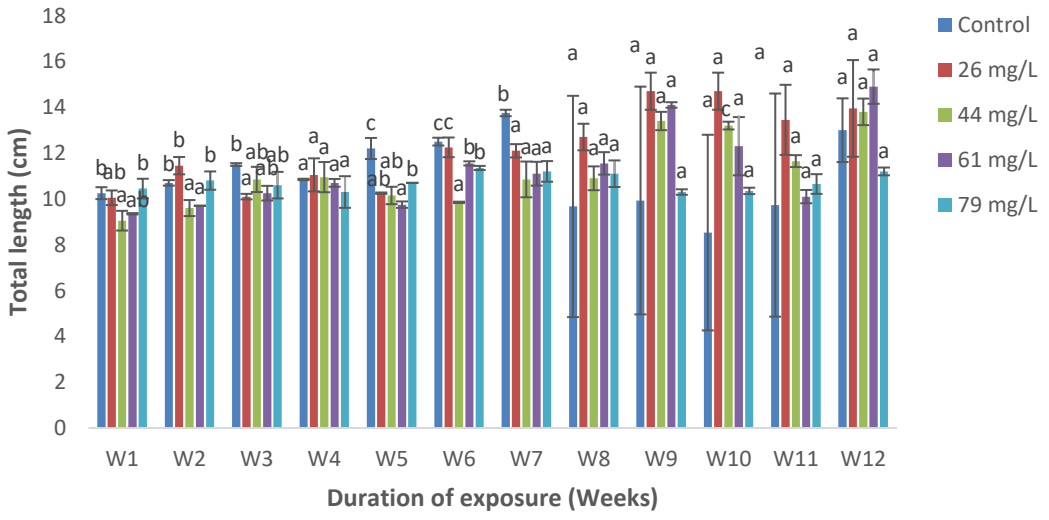

Figure 4.5 Mean values of total lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb for a period of 12 weeks.

Bars are mean±SEM from 2 individual observations. Bar chart with different superscripts are significantly different (P<0.05) from each other.

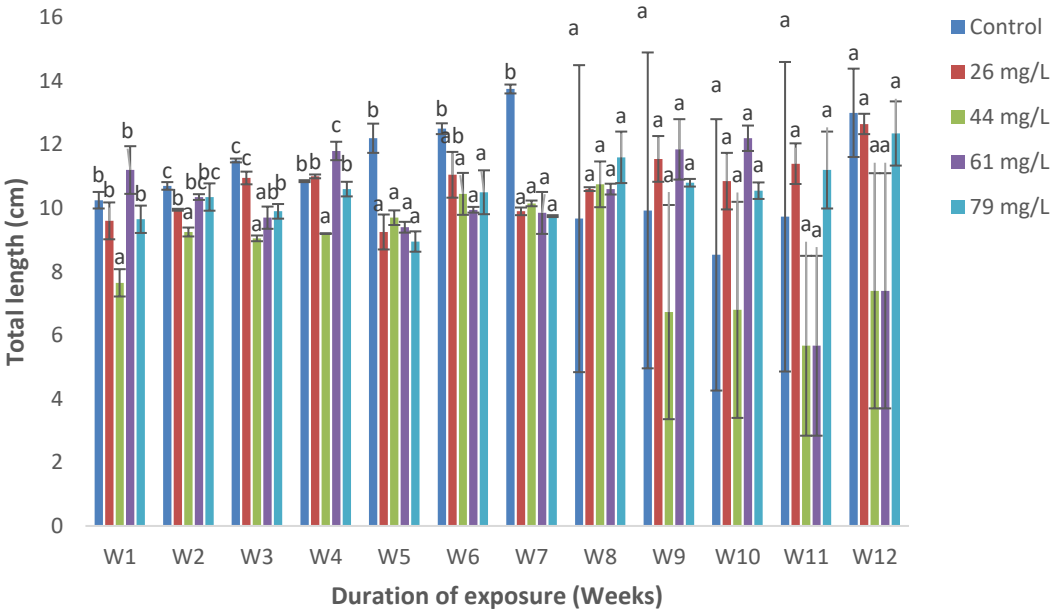

Figure 4.6 Mean values of total lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin A for a period of 12 weeks.

Bars with different superscripts are significantly different (P<0.05) from each other. Charts are mean±SEM from 2 individual observations.

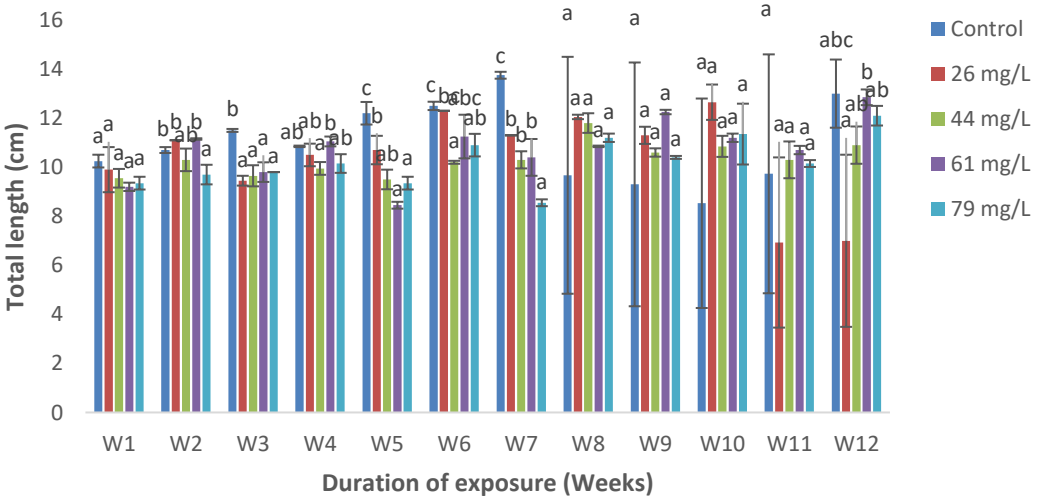

Figure 4.7 Mean values of total lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin C for a period of 12 weeks.

Bars with same alphabeths as superscripts are not significantly different (P>0.05) from each other. Bars are mean±SEM from 2 individual observations.

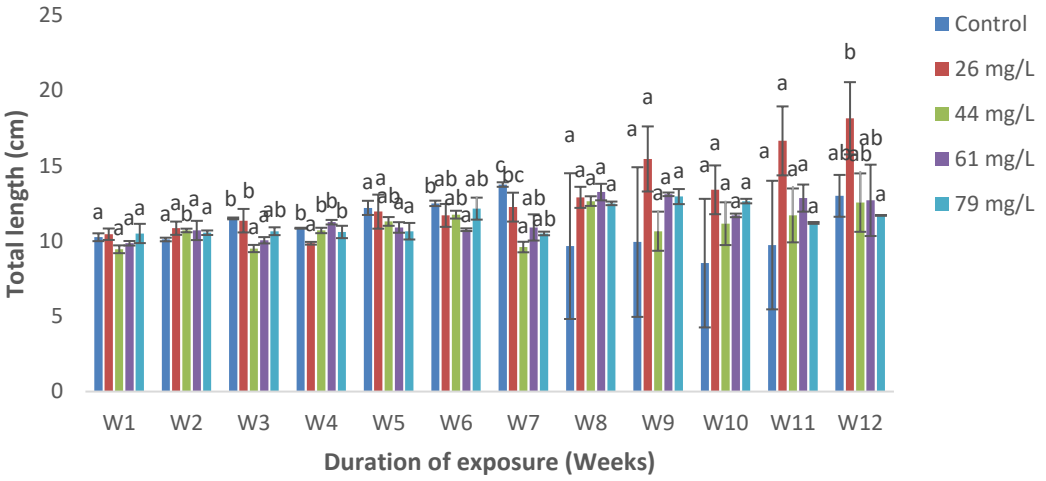

Figure 4.8 Mean values of total lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin E for a period of 12 weeks.

Bars with different alphabeths as superscripts are significantly different (P<0.05) from each other. Bars are mean±SEM from 2 individual observations.

4.1.3.3 Weight parameters of C. gariepinus exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with vitamins A, C and E

The weight of the control samples ranged from 8.22 g to 41.72 g. There was general slight increase in weight values in samples treated with 26 mg/L of Pb from weeks 1 to 12. The highest weight value was 36.09 g and recorded at week 12 in specimens exposed to 26 mg/L while the lowest was 5.32 g after the 1st week of exposure in those subjected to 44 mg/L. Specimens exposed to 26 mg/L and 61 mg/L had the highest percentage weight gain and specific growth rate in comparison with the control. (Tables 4.3 and Figure 4.9).

The highest weight value in samples exposed to PbVA treatments was obtained in the highest concentration (79 mg/L) with 21.64 g at the 8th week of exposure; while the lowest weight of 3.91 g was recorded in treatments with 44 mg/L after the 1st week of exposure. Contrary to what was obtained in Pb treatments, there was negative percentage weight gain (% WG) and low specific growth rate (SGR) (Table 4.4 and Figure 4.10).

The maximum weight obtained in samples exposed to PbVC treatments was 23.48 g in samples exposed to 26 mg/L at the 10th week; while the lowest was recorded in 44 mg/L set-up after the 1st week of exposure with 3.84 g. The highest % WG and SGR were recorded in samples with 61 mg/L treatments. (Table 4.5 and Figure 4.11).

In the samples exposed to PbVE there was general improvement in weight values in all treatments with marked growth in specimens subjected to 26 mg/L to 44 mg/L with exceptional performance in fishes subjected to 26 mg/L. The highest weight gain of the samples was recorded in 26 mg/L treatments (with 40.07 g) at the 12th week with the value of 83.26 g; while the lowest was recorded in the set-up with 44 mg/L at the 12th week with 5.02 g. There were also increased % WG and SGR in 26 mg/L and 61 mg/L set-ups, respectively. (Table 4.6 and Figure 4.12).

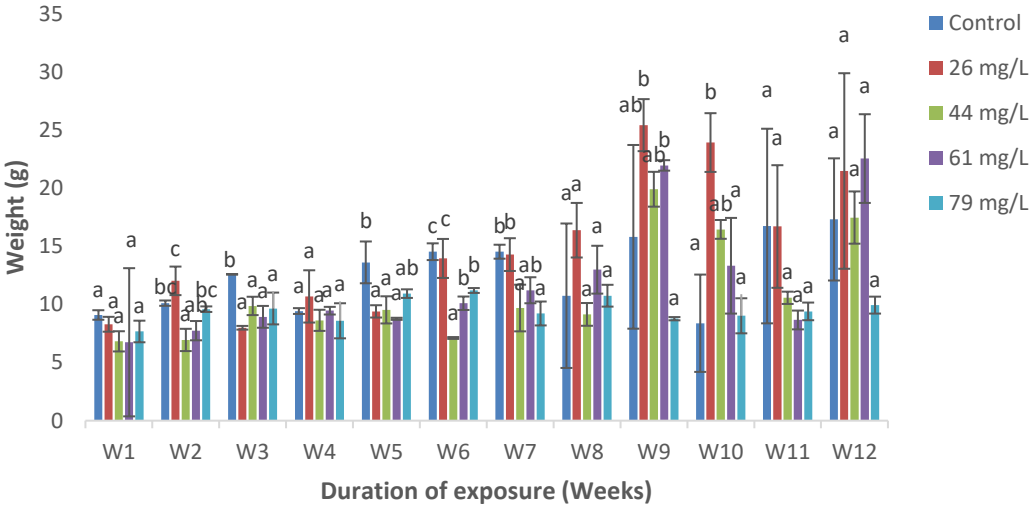

Figure 4.9 Mean values of weight of *C. gariepinus* exposed to sub-lethal concentrations of Pb for a period of 12 weeks.

Bars with different alphabeths as superscripts are significantly different ($P<0.05$) from each other. Bars are mean±SEM from 2 individual observations.

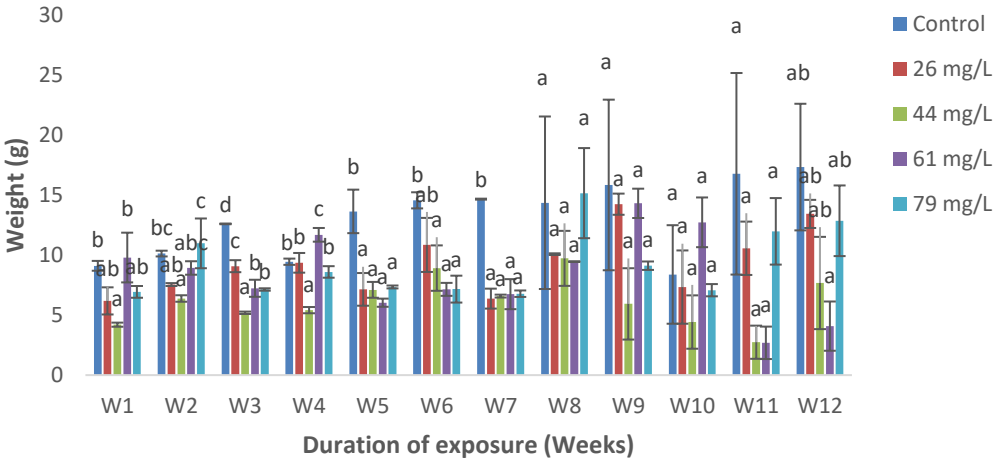

Figure 4.10 Mean values of weight of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin A for a period of 12 weeks.

Bars with same alphabeths as superscripts are not significantly different ($P>0.05$) from each other. Bars are mean±SEM of 2 specimens.

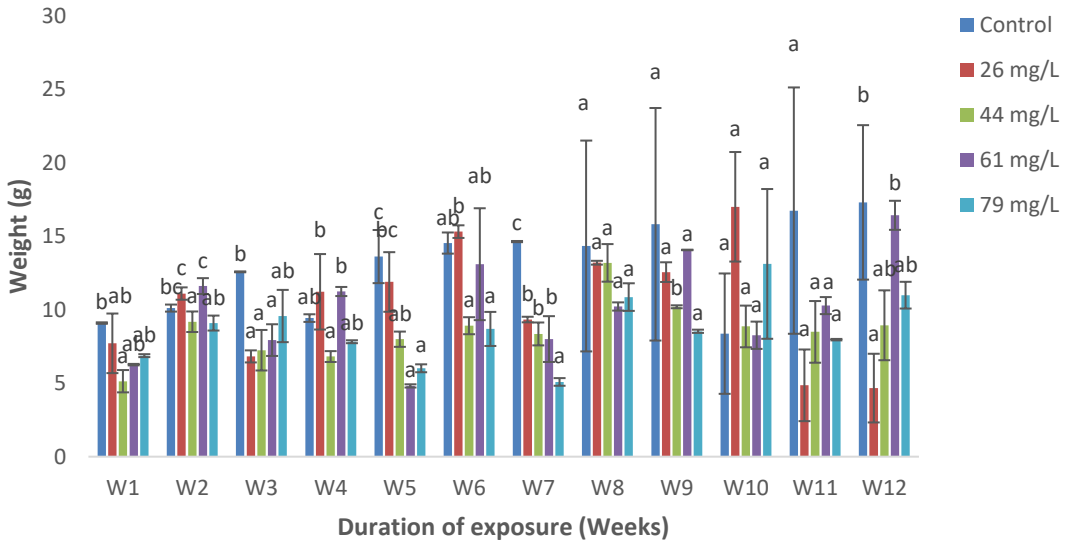

Figure 4.11 Mean values of weight of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin C for a period of 12 weeks.

Bars with different alphabeths as superscripts are significantly different (P<0.05) from each other. Bars are mean±SEM for 2 individual observations.

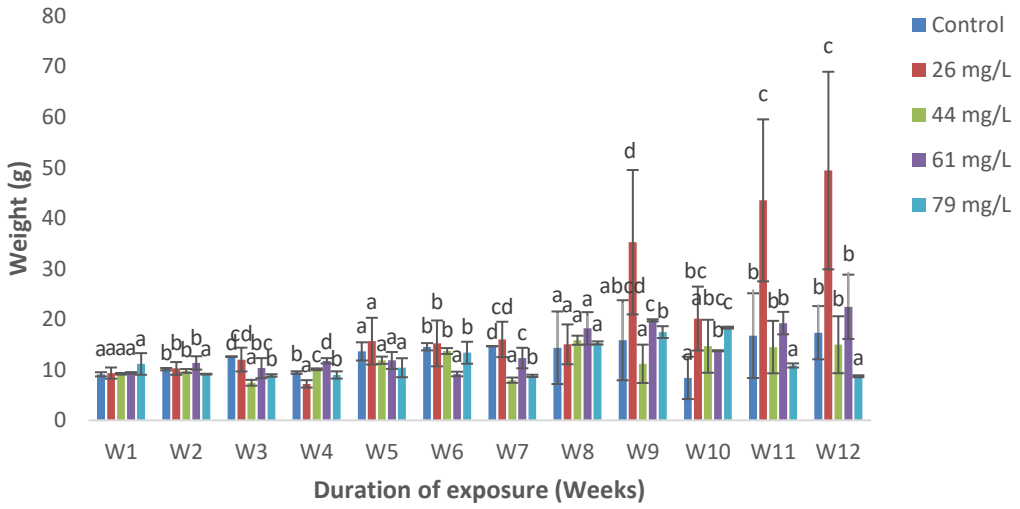

Figure 4.12 Mean values of weight of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin E for a period of 12 weeks.

Bar charts with different alphabeths as superscripts are significantly different (P<0.05) from one another. Bars are mean±SEM from 2 individual observations.

Table 4.3: Growth Performances of *C. gariepinus* Exposed to Sub-lethal Concentrations of Lead Nitrate for a Period of 12 Weeks

Treatments mg/L	W_1 (g)	W_0 (g)	WG (g)	% WG	SGR(g/day)
Control	17.33	9.10	8.23	90	3.23
26	21.51	8.30	13.21	159	3.53
44	17.49	6.84	10.65	156	3.29
61	22.57	6.76	15.81	234	3.60
79	9.95	7.68	2.27	30	2.46

W_1 is the final weight of the fish, W_0 dpicts the initial weight of the specimen, WG stands for the weight gain, % WG is the percentage weight gain and SGR indicates the specific growth rate of the fish sampled.

Table 4.4: Growth Performances of *C. gariepinus* Exposed to Sub-lethal Concentrations of Lead Nitrate Supplemented with Vitamin A for a Period of 12 Weeks

Treatments mg/L	W_1 (g)	W_0 (g)	WG (g)	% WG	SGR(g/day)
Control	17.33	9.10	8.23	90	3.23
26	13.43	6.18	7.25	85	2.92
44	11.51	4.20	7.31	57	2.75
61	6.13	9.79	-3.66	-37	1.60
79	12.85	6.90	5.95	86	2.85

W_1 stands for the final weight of the fish, W_0 dpicts the initial weight of the specimen, WG shows the weight gain, % WG is the percentage weight gain and SGR indicates the specific growth rate of the fish sampled.

Table 4.5: Growth Performances of *C. gariepinus* Exposed to Sub-lethal Concentrations of Lead Nitrate Supplemented with Vitamin C for a Period of 12 Weeks

Treatments mg/L	W_1 (g)	W_0 (g)	WG (g)	% WG	SGR(g/day)
Control	17.33	9.10	8.23	90	3.23
26	7.01	7.72	-0.71	-09	1.91
44	8.96	5.51	3.81	74	2.37
61	16.44	6.27	10.17	162	3.19
79	11.01	6.88	4.13	60	2.63

W_1 stands for the final weight of the fish, W_0 dpicts the initial weight of the specimen, WG represnts the weight gain, % WG is the percentage weight gain and SGR is the specific growth rate of the fish sampled.

Table 4.6 Growth Performances of *C. gariepinus* Exposed to Sub-lethal Concentrations of Lead Nitrate Supplemented with Vitamin E for a Period of 12 Weeks

Treatments mg/L	W_1 (g)	W_0 (g)	WG (g)	% WG	SGR(g/day)
Control	17.33	9.10	8.23	90	3.23
26	49.41	9.34	40.07	429	4.59
44	14.95	9.20	5.75	62	3.02
61	22.46	9.39	13.07	139	3.58
79	8.72	11.15	-2.44	-22	2.19

W_1 is the final weight of the fish, W_0 stands for the initial weight of the specimen, WG represnts the weight gain, % WG is the percentage weight gain and SGR stands for the specific growth rate of the fish sampled.

4.1.3.4 Length-Weight Relationships of C. gariepinus exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments for a period of 12 weeks

The relationships between length and weight of *C. gariepinus* were linear throughout the period of exposure in all Pb treatments. Samples subjected to 44 mg/L concentration in all Pb treatments had the highest R^2 values (0.6796). (Figures 4.25-4.32).

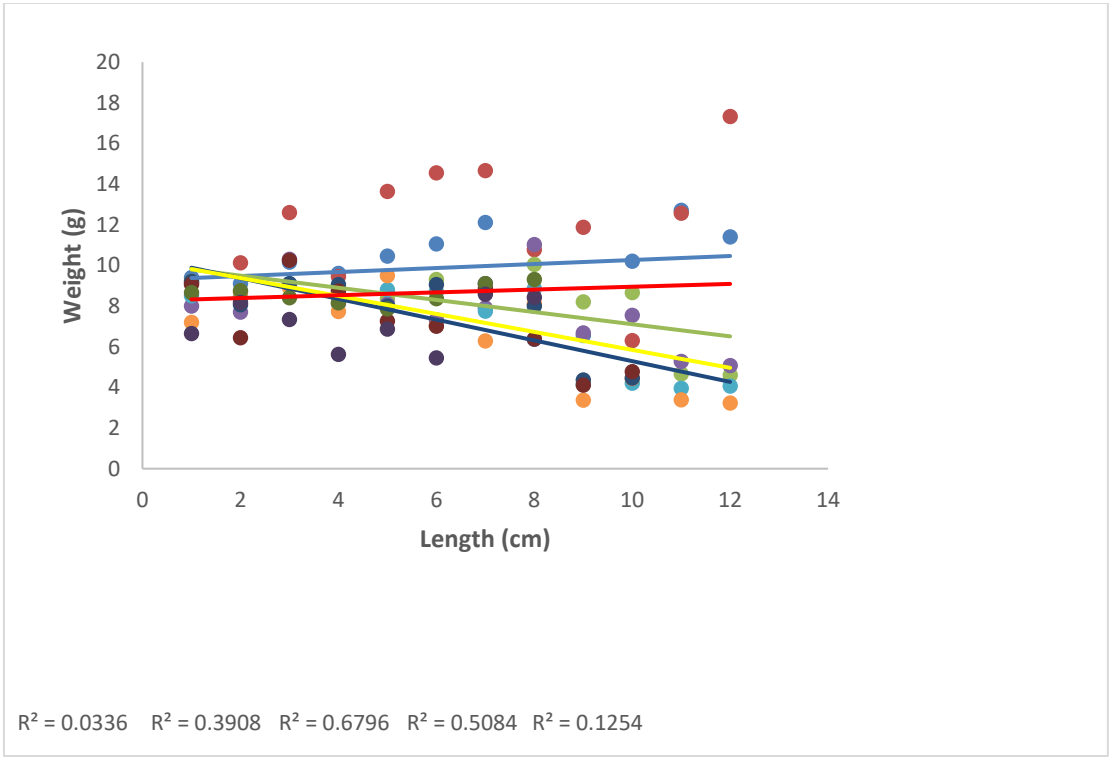

Figure 4.13 Length-Weight regression analysis of *C. gariepinus* exposed to sub-lethal concentration of lead for a period of 12 weeks.

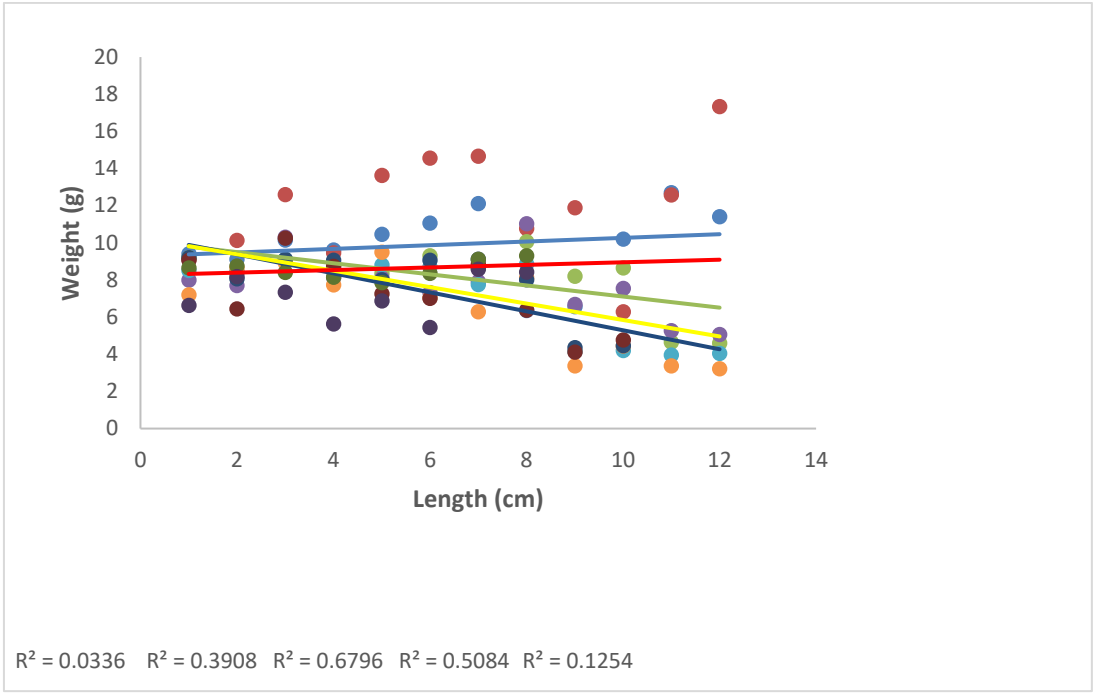

Figure 4.14 Length-Weight regression analysis of *C. gariepinus* exposed to sub-lethal concentration of lead supplemented with vitamin A for a period of 12 weeks.

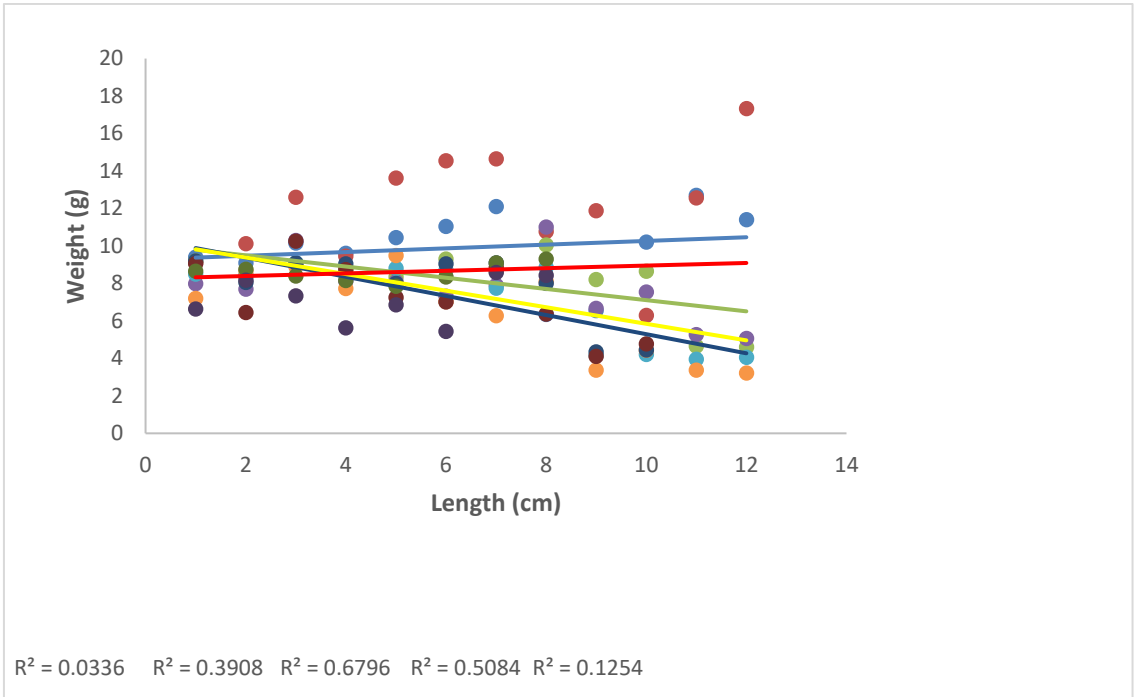

Figure 4.15 Length-Weight regression analysis of *C. gariepinus* exposed to sub-lethal concentration of lead supplemented with vitamin C for a period of 12 weeks.

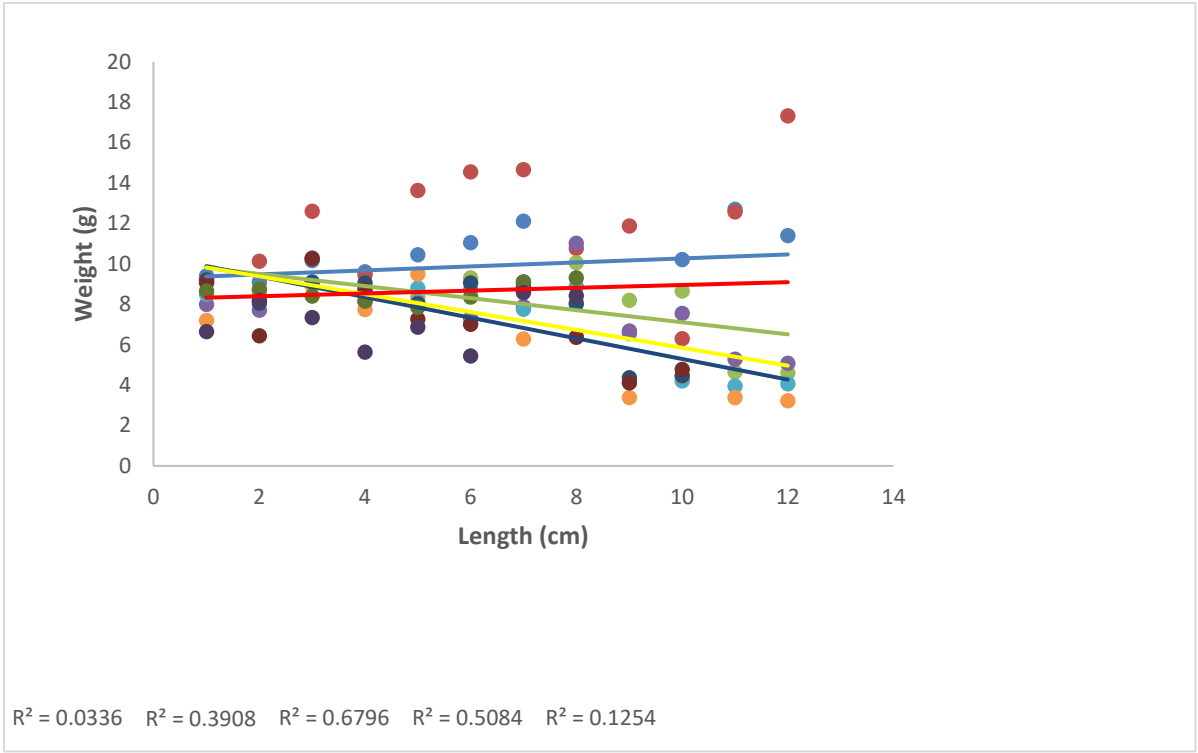

Figure 4.16 Length-Weight regression analysis of *C. gariepinus* exposed to sub-lethal concentration of lead supplemented with vitamin E for a period of 12 weeks.

4.1.4 Physico-chemical parameters of the test media of *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with Vitamins A, C and E

In the media containing samples exposed to Pb treatments there were slight variations in Temperture (T), Dissolved Oxygen (DO), pH and Electrical Conductivity (EC). The Total Dissolved Solids (TDS) varied widely from that of the control. (Table 4.7). Similar trends were also exhibited by the media of the samples exposed to PbVA treatments. (Table 4.8).

The physico-chemical parameters of the media exposed to PbVC showed slight variations in T, DO and pH. High EC and TDS values were obtained in comparison to the low values in the control. (Table 4.9). There were also slight variations in the values of T, DO, pH; and relatively higher values of EC and TDS when compared to the control in the PbV E treatment group. (Table 4.10).

Table 4.7: Physico-chemical Parameters of Test Media for Pb Treatments

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
T (°C)	23.70±0.12^{c}	23.00±0.17^{b}	22.95±0.09^{b}	23.30±0.06^{b}	22.20±0.12^{a}
DO (mg/L)	5.90±0.01^{d}	2.70±0.06^{b}	2.65±0.03^{a}	2.60±0.00^{a}	2.80±0.00^{c}
pH	7.50±0.06^{b}	7.25±0.03^{b}	7.25±0.03^{b}	7.15±0.03^{b}	6.65±0.23^{a}
EC (µS/cm)	0.28±0.02^{a}	4.10±0.00^{c}	0.39±0.01^{b}	0.41±0.00^{b}	6.38±0.01^{d}
TDS (mg/L)	202.00±0.58^{a}	288.50±1.44^{c}	275.00±5.20^{b}	293.00±1.15^{d}	288.50±1.44^{c}

Values are presented as mean±SEM (Standard error of mean). Values with different alphabets as superscripts in a row are significantly different at $p < 0.05$.

Table 4.8: Physico-chemical Parameters of Test Media for PbVA Treatments

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
T (°C)	23.70±0.24^{b}	22.70±0.06^{a}	22.40±0.29^{a}	22.60±0.06^{a}	22.60±0.03^{a}
DO (mg/L)	5.90±0.18^{b}	2.70±0.00^{a}	2.70±0.00^{a}	2.70±0.00^{a}	2.70±0.00^{a}
pH	7.50±0.18^{b}	7.05±0.03^{a}	7.05±0.03^{a}	7.05±0.03^{a}	7.15±0.03^{a}
EC (µS/cm)	0.28±0.03^{a}	0.42±0.00^{b}	0.39±0.00^{b}	0.40±0.00^{b}	0.38±0.02^{b}
TDS (mg/L)	202.00±1.74^{a}	297.00±0.58^{e}	281.50±2.03^{c}	288.50±2.60^{d}	272.10±9.49^{b}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at $p < 0.05$.

Table 4.9: Physico-chemical parameters of Test Media for PbVC Treatments

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
T (°C)	23.70 ± 0.40^{b}	22.70 ± 0.00^{a}	22.95 ± 0.03^{a}	23.00 ± 0.06^{a}	23.35 ± 0.14^{ab}
DO (mg/L)	5.90 ± 0.35^{b}	2.65 ± 0.03^{a}	2.60 ± 0.00^{a}	2.65 ± 0.03^{a}	2.55 ± 0.03^{a}
pH	7.50 ± 0.06^{c}	7.25 ± 0.03^{a}	7.20 ± 0.00^{a}	7.40 ± 0.00^{b}	7.35 ± 0.03^{b}
EC (µS/cm)	0.25 ± 0.02^{a}	0.43 ± 0.14^{b}	0.41 ± 0.00^{b}	0.42 ± 0.01^{b}	0.44 ± 0.01^{b}
TDS (mg/L)	202.00 ± 2.31^{a}	305.00 ± 6.9^{c}	292.00 ± 0.29^{b}	293.50 ± 0.29^{bc}	310.00 ± 7.50^{d}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at $p < 0.05$.

Table 4.10: Physico-chemical Parameters of Test Media for PbVE Treatments

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
T (°C)	23.70 ± 1.73^{c}	23.55 ± 0.09^{c}	23.15 ± 0.03^{b}	22.55 ± 0.09^{a}	22.60 ± 0.12^{a}
DO (mg/L)	5.90 ± 0.23^{b}	2.70 ± 0.06^{a}	2.70 ± 0.00^{a}	2.90 ± 0.12^{b}	2.85 ± 0.03^{b}
pH	7.50 ± 0.23^{c}	7.10 ± 0.06^{ab}	6.95 ± 0.03^{ab}	6.90 ± 0.00^{a}	7.30 ± 0.00^{bc}
EC (µS/cm)	0.28 ± 0.02^{a}	0.42 ± 0.01^{b}	0.40 ± 0.00^{b}	0.40 ± 0.00^{b}	0.40 ± 0.00^{b}
TDS (mg/L)	202.00 ± 0.58^{a}	299.50 ± 8.95^{e}	278.00 ± 4.04^{c}	254.00 ± 2.31^{b}	282.50 ± 1.44^{d}

Values are presented as mean±SEM. Values with different alphabets as superscripts in a row are significantly different at $P < 0.05$.

4.1.5 Haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with Vitamins A, C and E for a duration of four, eight and twelve weeks

In samples exposed to Pb treatments for a period of four weeks, there were increased values of production of WBC in all treatments when compared to the control. Also, there were significant ($P < 0.05$) decreases in the production values of PLT in all treatments when compared to control samples. Similarly, after the eight week of exposure, there were significant ($P < 0.05$) values of WBC in all treatments when compared to the control. There were also decreased values of RBC, Hb and PCV in all treatments when compared to the control group. Apart from 26 mg/L and 44 mg/L concentrations, there were drastic reductions in values of PLT in all treatments when compared to the control. Furthermore, there were increased values of WBC after twelve weeks of exposure in all treatments. Decreased values of RBC, Hb, MCV and PCV were also obtained in all treatments. Decreased PLT values were also recorded in all treatments when compared to the control. From the statistical analysis after the 4^{th} week of exposure, the mean values of WBC in lowest and highest concentrations were significantly higher than 44 mg/L and 61 mg/L conentrations. RBC mean values in the control and treatment with 61 mg/L were significantly higher than treatments with 44 mg/L, 79 mg/L and 26 mg/L concentrations, respectively. Hb and PCV mean values in the control were significantly higher than treatments with 26 mg/L all through to 79 mg/L concentrations. MCV mean values in samples treated with 44 mg/L were significantly higher than Control, 26 mg/L, 44 mg/L and 79 mg/L concentrations. Likewise, MCH mean values in subjects treated with 44 mg/L were significantly higher than those subjected to 26 mg/L, Control, 61 mg/L and 79 mg/L. MCHC mean

values in the control were significantly higher than specimens treated with 26 mg/L to 79 mg/L. All the mean values of PLT were significantly different with the control significantly higher. After the 8th week, the mean values of WBC of samples in 44 mg/L were significantly higher than treatments with 61 mg/L, 79 mg/L and 26 mg/L. The RBC, Hb, PCV, MCHC and PLT mean values in the control were significantly higher than fishes treated with26 mg/L to 79 mg/L. Furthermore, at the end of the 12th week, the mean values of WBC in the peak concentration were significantly higher than 26 mg/L to 61 mg/L and the Control. The RBC, Hb, PCV, MCV, MCH, MCHC and PLT in the control were significantly higher than those of samples treated with 26 mg/L to 79 mg/L concentrations. (Table 4.11- 4.13).

Table 4.11: Haematological Parameters of *C. gariepinus* Exposed to Sub-lethal Concentrations of Pb for a Period of Four Weeks

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	9.80 ± 0.12^{a}	19.50 ± 0.87^{d}	16.00 ± 0.58^{b}	19.50 ± 0.87^{d}	19.00 ± 1.15^{c}
RBC (Mil/mm^3)	3.60 ± 0.06^{d}	2.15 ± 0.20^{a}	1.95 ± 0.09^{a}	2.60 ± 0.12^{b}	2.80 ± 0.00^{c}
Hb (g/dl)	10.05 ± 0.14^{d}	6.05 ± 0.20^{a}	7.00 ± 0.35^{c}	6.65 ± 0.55^{b}	6.25 ± 0.32^{a}
PCV (%)	29.50 ± 0.29^{c}	18.00 ± 0.58^{a}	20.05 ± 0.87^{b}	20.00 ± 1.73^{b}	18.50 ± 0.87^{a}
MCV (Fl)	81.50 ± 2.02^{b}	86.50 ± 10.68^{c}	104.50 ± 0.29^{d}	76.00 ± 3.46^{a}	75.50 ± 2.60^{a}
MCH (Pg)	27.00 ± 0.58^{c}	29.00 ± 3.46^{d}	35.50 ± 0.29^{e}	25.00 ± 1.15^{b}	22.00 ± 1.15^{a}
MCHC (g/dl)	33.50 ± 0.29^{b}	31.50 ± 1.44^{ab}	31.00 ± 1.73^{ab}	29.50 ± 0.29^{a}	29.00 ± 0.00^{a}
PLT (Cmm)	206.00 ± 1.15^{e}	103.00 ± 1.15^{a}	107.00 ± 0.00^{b}	121.50 ± 2.60^{d}	110.50 ± 2.02^{c}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. (n=3).

Table 4.12: Haematological Parameters of *C. gariepinus* Exposed to Sub-lethal Concentrations of Pb for a Period of Eight Weeks

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	9.40±0.23^{a}	16.50±0.29^{b}	18.50±0.29^{c}	17.50±0.29^{bc}	17.00±0.58^{b}
RBC (Mil/mm^3)	3.60±0.12^{d}	2.90±0.06^{c}	2.20±0.06^{a}	2.60±0.17^{b}	2.45±0.14^{a}
Hb (g/dl)	10.45±0.03^{d}	8.05±0.14^{c}	6.05±0.20^{a}	6.25±0.38^{ab}	6.80±0.06^{b}
PCV (%)	31.00±0.00^{d}	23.50±0.29^{c}	18.00±0.58^{a}	18.50±0.87^{ab}	20.00±0.00^{b}
MCV (Fl)	86.50±2.60^{c}	81.00±2.31^{b}	82.00±4.62^{b}	78.50±12.41^{a}	82.00±4.62^{b}
MCH (Pg)	28.50±1.44^{c}	27.50±0.87^{b}	28.50±2.02^{c}	24.50±3.18^{a}	27.50±2.02^{b}
MCHC (g/dl)	32.00±1.73^{b}	29.00±0.00^{a}	29.00±0.00^{a}	29.50±0.29^{ab}	29.50±0.29^{ab}
PLT (Cmm)	227.50±7.79^{e}	179.00±41.57^{d}	169.50±28.00^{c}	120.00±6.35^{b}	114.00±4.04^{a}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. (n=3).

Table 4.13: Haematological Parameters of *C. gariepinus* Exposed to Sub-lethal Concentration of Pb for a Period of Twelve Weeks

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	8.00±0.00^{a}	17.00±5.78^{b}	18.00±0.00^{b}	18.50±2.89^{b}	20.50±0.87^{c}
RBC (Mil/mm^3)	3.45±0.14^{b}	2.55±0.03^{a}	2.55±0.09^{a}	2.50±0.00^{a}	2.60±0.06^{a}
Hb (g/dl)	11.50±0.29^{b}	7.00±0.17^{a}	6.40±0.35^{a}	7.00±0.17^{a}	6.60±0.12^{a}
PCV (%)	34.50±0.87^{b}	20.50±0.29^{a}	19.00±1.15^{a}	20.50±0.29^{a}	19.00±0.58^{a}
MCV (Fl)	100.50±6.64^{c}	81.00±1.73^{b}	74.00±2.31^{a}	82.00±1.15^{b}	73.00±0.58^{a}
MCH (Pg)	34.50±3.18^{c}	27.50±0.87^{b}	25.50±0.58^{a}	28.00±0.58^{b}	25.00±0.00^{a}
MCHC (g/dl)	30.00±0.00^{c}	27.00±0.58^{a}	29.50±0.29^{bc}	29.50±0.29^{bc}	29.50±0.29^{b}
PLT (Cmm)	247.00±5.20^{e}	110.50±0.87^{a}	163.00±34.06^{d}	115.50±0.87^{b}	129.00±1.15^{c}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. (n=3).

In another development, PbVA treatments in the first four weeks of exposure indicated increased production values of WBC in all treatments. Similarly, increased values of PLT were obtained in all treatments. Reduced values of PCV in all treatments were also recorded. On the other hand, samples exposed for eight weeks indicated that there were increased production values of WBC. However, more or less the same values of MCV, MCH and MCHC and PLT (nearly at par with PLT values of the control) were obtained. Moreover, samples exposed for a period of twelve weeks also displayed increased values of WBC. From the statistical analysis, after the 4^{th} week of exposure, the mean values of WBC in samples exposed to 44 mg/L were significantly higher than subjects treated with 26 mg/L, 79 mg/L, 44 mg/L and Control. The MCV mean values in specimens treated with 61 mg/L were significantly higher than fishes in 44 mg/L, Control, 79 mg/L and 26 mg/L treatments. Moreso, MCH mean values in samples treated with 44 mg/L were significantly higher than those subjected to 61 mg/L, 26 mg/L, Control and 79 mg/L treatments. Likewise, mean values of PLT in samples treated with 79 mg/L were significantly higher than fishes exposed to 61 mg/L, 44 mg/L, 26 mg/L and the Control. After the 8^{th} week of exposure, mean values of WBC in the highest concentration samples were significantly higher than 44 mg/L, 61 mg/L, 26 mg/L concentrations. Mean values of samples treated with 61 mg/L were significantly higher than those subjected to 26 mg/L, 44 mg/L and 79 mg/L concentrations. The PLT mean values in fishes subjected to 44 mg/L were significantly higher than those exposed to 26 mg/L, 61 mg/L and 79 mg/L treatments. After the 12^{th} week on the other hand, the mean values of WBC in samples exposed to 79 mg/L and 26 mg/L concentrations are significantly higher than those treated with 44 mg/L and 61 mg/L. The RBC, Hb and PCV mean values in specimens treated with 79 mg/L were

significantly higher than 26 mg/L to 61 mg/L. The PLT mean values in fishes subjected to 79 mg/L were significantly higher than those exposed to26 mg/L to 61 mg/L. (Tables 4.14- 4.16).

Table 4.14: Haematological Parameters of *C. gariepinus* Exposed to Sub-lethal Concentration of Pb Supplemented with Vitamin A for Length of Time of Four Weeks

Parameters	**Treatments (mg/L)**				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	9.80 ± 0.12^{a}	15.50 ± 0.29^{cd}	13.50 ± 0.29^{b}	16.00 ± 0.58^{d}	15.00 ± 1.15^{c}
RBC (Mil/mm^3)	3.60 ± 0.06^{c}	3.10 ± 0.17^{b}	2.80 ± 0.17^{a}	2.65 ± 0.14^{a}	3.00 ± 0.17^{b}
Hb (g/dl)	10.50 ± 0.14^{b}	7.85 ± 0.55^{a}	8.10 ± 0.17^{a}	7.45 ± 0.32^{a}	7.20 ± 0.23^{a}
PCV (%)	29.50 ± 0.29^{c}	23.00 ± 1.73^{b}	24.00 ± 0.58^{b}	22.00 ± 1.15^{a}	21.50 ± 0.87^{a}
MCV (Fl)	81.50 ± 2.02^{b}	73.50 ± 1.44^{a}	86.00 ± 3.46^{c}	94.50 ± 3.18^{d}	81.50 ± 4.33^{b}
MCH (Pg)	27.00 ± 0.58^{bc}	24.50 ± 0.29^{ab}	29.00 ± 1.15^{c}	28.50 ± 2.60^{c}	24.00 ± 0.58^{a}
MCHC (g/dl)	33.50 ± 0.29^{c}	31.50 ± 1.44^{b}	29.00 ± 0.00^{a}	29.50 ± 0.29^{ab}	29.50 ± 0.29^{ab}
PLT (Cmm)	206.00 ± 1.15^{a}	211.50 ± 0.29^{ab}	211.50 ± 6.06^{ab}	212.50 ± 0.87^{b}	217.00 ± 2.31^{c}

Values are presented as mean±SEM (Standard error of mean). Values with different alphabets as superscripts in a row are significantly different at P <0.05. (n=3).

Table 4.15: Haematological Parameters of *C. gariepinus* Treated with Sub-lethal Concentrations of Pb Supplemented with Vitamin A for a Duration of Eight Weeks

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	9.40 ± 0.23^{a}	13.50 ± 0.87^{bc}	12.50 ± 0.29^{b}	14.00 ± 0.00^{bc}	15.00 ± 1.15^{c}
RBC (Mil/mm^3)	3.60 ± 0.12^{c}	3.30 ± 0.17^{bc}	2.90 ± 0.06^{a}	3.15 ± 0.20^{bc}	3.05 ± 0.09^{a}
Hb (g/dl)	10.45 ± 0.03^{c}	9.50 ± 0.17^{b}	9.00 ± 0.17^{a}	9.65 ± 0.03^{b}	9.30 ± 0.00^{ab}
PCV (%)	31.00 ± 0.00^{d}	28.00 ± 0.58^{b}	26.50 ± 0.29^{a}	29.00 ± 0.00^{c}	27.00 ± 0.00^{a}
MCV (Fl)	86.50 ± 2.60^{a}	85.50 ± 6.06^{a}	91.00 ± 2.89^{c}	93.00 ± 5.78^{d}	88.50 ± 2.60^{b}
MCH (Pg)	29.50 ± 1.44^{a}	28.50 ± 2.02^{a}	31.00 ± 1.15^{b}	30.50 ± 2.02^{b}	31.50 ± 0.29^{b}
MCHC (g/dl)	32.00 ± 1.73^{b}	29.00 ± 0.00^{a}	29.00 ± 0.00^{a}	29.50 ± 0.29^{ab}	29.50 ± 0.29^{ab}
PLT (Cmm)	227.50 ± 7.79^{c}	221.50 ± 2.02^{b}	242.00 ± 4.62^{d}	219.00 ± 1.15^{a}	219.50 ± 0.87^{a}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. (n=3).

Table 4.16: Haematological Parameters of *C. gariepinus* Subjected to Sub-lethal Concentrations of Pb Supplemented with Vitamin A for a Period of Twelve Weeks

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	8.00 ± 0.00^{b}	13.50 ± 0.87^{c}	7.33 ± 3.67^{b}	4.00 ± 4.00^{a}	14.50 ± 0.29^{cd}
RBC (Mil/mm^3)	3.45 ± 0.14^{d}	2.90 ± 0.06^{c}	1.87 ± 0.93^{b}	0.93 ± 0.93^{a}	3.25 ± 0.14^{d}
Hb (g/dl)	11.50 ± 0.29^{e}	8.45 ± 0.09^{c}	6.53 ± 3.27^{b}	2.93 ± 2.93^{a}	9.65 ± 0.09^{d}
PCV (%)	34.50 ± 0.87^{e}	24.50 ± 0.29^{c}	18.67 ± 9.33^{b}	8.67 ± 8.67^{a}	28.50 ± 0.29^{d}
MCV (Fl)	100.50 ± 6.64^{e}	84.50 ± 2.60^{c}	66.67 ± 33.33^{b}	30.67 ± 30.67^{a}	88.00 ± 2.89^{d}
MCH (Pg)	34.50 ± 3.18^{e}	29.00 ± 1.15^{c}	23.33 ± 11.67^{b}	10.33 ± 10.33^{a}	30.00 ± 1.15^{d}
MCHC (g/dl)	30.00 ± 0.00^{d}	28.00 ± 0.58^{c}	19.33 ± 9.67^{b}	9.67 ± 9.67^{a}	29.50 ± 0.29^{d}
PLT (Cmm)	247.00 ± 5.20^{d}	219.50 ± 0.87^{c}	162.00 ± 81.00^{b}	78.00 ± 78.00^{a}	259.50 ± 13.00^{e}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at $P < 0.05$. (n=3).

In the case of samples exposed to PbVC for a period of four weeks, there were increases in WBC and PLT values. After eight weeks of exposure, there were increased productions in values of WBC with higher values obtained in samples subjected to 26 mg/L. There were increased values of Hb and MCV when the concentrations increased from 44 mg/L to 79 mg/L. Furthermore, after twelve weeks of exposure, there were increased values of WBC. From the statistical analysis, after 4 weeks of exposure, the WBC mean values in samples exposed to 26 mg/L were significantly higher than those subjected to 61 mg/L, 79 mg/L and 44 mg/L concentrations. The RBC mean values in fishes treated with 61 mg/L were significantly higher than samples exposed to 26 mg/L, 44 mg/L and 79 mg/L. The mean values of MCV in 44 mg/L were significantly higher than fishes subjected to 26 mg/L, 61 mg/L and 79 mg/L treatments. Likewise, the MCH mean values in samples subjected to 44 mg/L were significantly higher than samples exposed to 26 mg/L, 79 mg/L and 61 mg/L concentrations. The MCHC mean values in fishes treated with 26 mg/L were significantly higher than those subjected to 44 mg/L to 79 mg/L. After the 8^{th} week of exposure, the mean values of RBC in 26 mg/L samples were significantly higher than those exposed to 44 mg/L to 79 mg/L cncentrations. Furthermore, at the end of the 12^{th} week of exposure, only the mean values of samples treated with 44 mg/L in all the parameters and treatments were significantly lower. (Tables 4.17-4.19).

Table 4.17: Haematological Parameters of *C. gariepinus* Treated with Sub-lethal Concentration of Pb Supplemented with Vitamin C After Four Weeks

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	9.80±0.12^{a}	12.00±0.00^{c}	10.50±0.29^{ab}	11.00±0.58^{b}	11.00±0.00^{b}
RBC (Mil/mm^3)	3.60±0.06^{b}	3.35±0.03^{a}	3.25±0.03^{a}	3.75±0.03^{c}	3.30±0.06^{a}
Hb (g/dl)	10.05±0.14^{c}	8.95±0.03^{ab}	9.45±0.26^{bc}	9.05±0.20^{ab}	8.60±0.35^{a}
PCV (%)	29.50±0.29^{c}	27.00±0.00^{ab}	28.00±0.58^{bc}	27.00±0.58^{ab}	25.50±0.87^{a}
MCV (Fl)	81.50±2.02^{ab}	80.00±0.58^{ab}	86.00±2.31^{b}	82.00±3.46^{ab}	77.00±1.15^{a}
MCH (Pg)	27.00±0.58^{b}	26.50±0.29^{b}	31.00±0.58^{c}	23.50±0.87^{a}	26.00±0.58^{b}
MCHC (g/dl)	33.50±0.29^{c}	31.50±0.87^{b}	29.50±0.29^{a}	29.00±0.00^{a}	30.00±0.00^{a}
PLT (Cmm)	206.00±1.15^{a}	226.50±4.33^{b}	225.00±4.61^{b}	234.00±5.20^{c}	226.00±2.31^{b}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at $P \leq 0.05$. (n=3).

Table 4.18: Haematological Parameters of *C. gariepinus* Exposed to Sub-lethal Concentration of Pb Supplemented with Vitamin C for a Period of Eight Weeks

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	9.40 ± 0.23^{b}	11.00 ± 0.58^{d}	10.00 ± 0.00^{c}	10.00 ± 0.00^{c}	6.67 ± 3.33^{a}
RBC (Mil/mm^3)	3.60 ± 0.12^{b}	3.85 ± 0.09^{c}	3.15 ± 0.03^{ab}	3.15 ± 0.14^{ab}	2.00 ± 1.00^{a}
Hb (g/dl)	10.45 ± 0.03^{c}	10.10 ± 0.17^{c}	10.80 ± 0.17^{b}	10.80 ± 0.75^{b}	7.13 ± 3.57^{a}
PCV (%)	31.00 ± 0.00^{b}	30.00 ± 0.58^{b}	32.00 ± 0.58^{b}	32.00 ± 2.31^{b}	20.67 ± 10.33^{a}
MCV (Fl)	86.50 ± 2.60^{c}	78.00 ± 0.00^{b}	103.00 ± 0.00^{e}	100.50 ± 2.60^{d}	68.67 ± 34.33^{a}
MCH (Pg)	28.05 ± 1.44^{c}	26.00 ± 0.00^{b}	35.50 ± 0.29^{d}	36.00 ± 2.31^{d}	23.33 ± 11.67^{a}
MCHC (g/dl)	32.00 ± 1.73^{cd}	30.50 ± 0.29^{c}	32.50 ± 1.44^{d}	29.50 ± 0.29^{b}	19.33 ± 9.27^{a}
PLT (Cmm)	227.50 ± 7.79^{d}	214.50 ± 1.44^{b}	223.50 ± 7.80^{c}	227.50 ± 7.22^{d}	144.60 ± 72.33^{a}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at P < 0.05. (n=3).

Table 4.19: Haematological Parameters of *C. gariepinus* Exposed to Sub-lethal Concentrations of Pb Supplemented with Vitamin C for a Duration of Twelve Weeks

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	8.00 ± 0.00^{b}	9.50 ± 0.29^{c}	3.00 ± 3.00^{a}	9.50 ± 2.89^{c}	10.00 ± 0.00^{c}
RBC (Mil/mm^3)	3.45 ± 0.14^{b}	3.40 ± 0.17^{b}	1.23 ± 1.23^{a}	3.75 ± 0.03^{d}	3.60 ± 0.12^{c}
Hb (g/dl)	11.50 ± 0.29^{c}	10.05 ± 0.14^{b}	3.40 ± 3.40^{a}	10.85 ± 0.20^{b}	11.60 ± 0.23^{c}
PCV (%)	34.50 ± 0.87^{d}	29.50 ± 0.29^{b}	10.00 ± 10.00^{a}	32.00 ± 0.58^{c}	34.50 ± 0.87^{d}
MCV (Fl)	100.50 ± 6.64^{e}	89.50 ± 6.35^{c}	27.00 ± 27.00^{a}	86.00 ± 1.78^{b}	95.50 ± 0.87^{d}
MCH (Pg)	34.50 ± 3.17^{d}	30.00 ± 2.31^{cb}	9.00 ± 9.00^{a}	28.50 ± 0.29^{b}	32.00 ± 0.00^{c}
MCHC (g/dl)	30.00 ± 0.00^{b}	33.00 ± 1.73^{c}	10.00 ± 10.00^{a}	29.50 ± 0.29^{b}	30.00 ± 0.00^{b}
PLT (Cmm)	247.00 ± 5.20^{e}	224.00 ± 10.97^{b}	85.33 ± 85.33^{a}	239.00 ± 1.15^{d}	226.50 ± 8.37^{c}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at P < 0.05. (n=3).

In addition, samples exposed to PbVE treatments after four week, displayed higher values of WBC. MCV, MCH and MCHC recorded in fishes subjected to 44 mg/L had higher values than other treatments. There were increases in the production values of blood PLT in all treatment. After eight weeks of exposure, there were increased values of WBC. The increased values of MCV were recorded in 44 mg/L to 79 mg/L samples. Moreover, after the twelve weeks, there were also increased values of WBC, slightly decreased values of RBC, Hb, PCV and MCV in all treatments. From the statistical analysis, after the 4th week of exposure, the mean values of WBC in specimens exposed to 26 mg/L and 61 mg/L were significantly higher than thos exposed to 44 mg/L and 79 mg/L. The MCV mean values in 44 mg/L samples were significantly higher than fishes subjected to 26 mg/L, 61 mg/L and 79mg/L. Also, the mean values of PLT in treatments 44 mg/L to 79 mg/L were significantly higher than those subjected to 26 mg/L. There were no significance differences after the 8^{th} week of exposure. At the end of the 12^{th} week of exposure however, the mean values of WBC in the specimens exposed to highest concentration were significantly higher than 61 mg/L, 44 mg/L and 26 mg/L specimens. The MCHC mean values in fishes exposed to 26 mg/L were significantly higher than samples subjected to 44 mg/L to 79 mg/L. (Tables 4.20-4.22).

Table 4.20: Haematological Parameters of *C. gariepinus* Exposed to Sub-lethal Concentration of Pb Supplemented with Vitamin E for a Period of Four Weeks

Parameters	Treatments (mg/L)				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	9.80 ± 0.12^{a}	15.00 ± 0.58^{c}	12.50 ± 0.87^{b}	14.50 ± 0.87^{c}	12.50 ± 1.44^{b}
RBC (Mil/mm^3)	3.60 ± 0.06^{b}	3.00 ± 0.00^{a}	2.95 ± 0.09^{a}	3.15 ± 0.03^{a}	3.15 ± 0.14^{a}
Hb (g/dl)	10.05 ± 0.14^{c}	7.85 ± 0.26^{a}	9.00 ± 0.06^{b}	7.50±0.17a	8.50 ± 0.23^{b}
PCV (%)	29.95 ± 0.29^{c}	23.00 ± 0.58^{a}	26.50 ± 0.29^{b}	22.50 ± 0.58^{a}	25.00 ± 0.58^{b}
MCV (Fl)	81.50 ± 2.02^{c}	76.50 ± 2.02^{a}	89.50 ± 3.75^{d}	79.50 ± 4.33^{bc}	79.00 ± 1.73^{b}
MCH (Pg)	27.00 ± 0.58^{c}	25.50 ± 0.87^{b}	30.00 ± 1.15^{d}	23.50 ± 0.29^{a}	26.50 ± 0.29^{c}
MCHC (g/dl)	33.50 ± 0.29^{b}	32.00 ± 1.73^{ab}	29.50 ± 0.29^{a}	29.50 ± 0.29^{a}	29.50 ± 0.29^{a}
PLT (Cmm)	206.00 ± 1.15^{a}	208.00 ± 2.31^{b}	223.50 ± 4.33^{d}	225.00 ± 3.18^{e}	219.50 ± 1.44^{c}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at P < 0.05. (n=3).

Table 4.21: Haematological Parameters of *C. gariepinus* Treated with Sub-lethal Concentration of Pb Supplemented with Vitamin E for a Duration of Eight Weeks

Parameters	Treatments				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	9.40 ± 0.23^{b}	7.33 ± 3.67^{a}	7.33 ± 3.67^{a}	7.33 ± 3.67^{a}	9.33 ± 4.67^{b}
RBC (Mil/mm^3)	3.60 ± 0.12^{b}	2.13 ± 1.07^{a}	1.87 ± 0.93^{a}	2.00 ± 1.00^{a}	2.00 ± 1.00^{a}
Hb (g/dl)	10.45 ± 0.29^{c}	5.80 ± 2.90^{a}	6.53 ± 3.27^{b}	6.27 ± 3.13^{a}	6.53 ± 3.27^{b}
PCV (%)	31.00 ± 0.00^{c}	17.33 ± 8.67^{a}	19.33 ± 9.67^{b}	18.00 ± 9.00^{a}	19.3 ± 19.66^{b}
MCV (Fl)	86.50 ± 2.60^{e}	54.00 ± 27.00^{a}	68.67 ± 34.33^{d}	60.00 ± 30.00^{b}	64.00 ± 32.00^{c}
MCH (Pg)	28.50 ± 1.44^{e}	18.00 ± 9.00^{a}	23.33 ± 11.67^{d}	20.67 ± 10.33^{b}	21.33 ± 10.67^{c}
MCHC (g/dl)	32.00 ± 1.73^{c}	19.33 ± 9.67^{a}	19.33 ± 9.67^{a}	20.00 ± 10.00^{b}	20.00 ± 10.00^{b}
PLT (Cmm)	227.50 ± 7.79^{d}	148.00 ± 74.00^{c}	144.67 ± 72.33^{b}	147.33 ± 73.67^{bc}	142.67 ± 71.33^{a}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at P < 0.05. (n=3).

Table 4.22: Haematological Parameters of *C. gariepinus* Exposed to Sub-lethal Concentration of Pb Supplemented with Vitamin E for a Period of Twelve Weeks

Parameters	Treatments				
	Control	**26**	**44**	**61**	**79**
WBC (10^9cells/L)	8.00 ± 0.00^{a}	10.50 ± 0.29^{b}	13.50 ± 0.29^{c}	14.00 ± 0.58^{c}	16.50 ± 0.29^{d}
RBC (Mil/mm^3)	3.45 ± 0.15^{c}	2.90 ± 0.06^{a}	3.00 ± 0.00^{a}	3.15 ± 0.58^{b}	2.90 ± 0.58^{a}
Hb (g/dl)	11.50 ± 0.29^{c}	9.45 ± 0.03^{b}	8.80 ± 0.35^{a}	9.25 ± 0.29^{b}	8.80 ± 0.06^{a}
PCV (%)	34.50 ± 0.87^{c}	27.50 ± 0.29^{b}	25.50 ± 0.87^{a}	27.00 ± 0.00^{ab}	26.00 ± 0.00^{ab}
MCV (Fl)	100.50 ± 6.64^{d}	95.00 ± 2.89^{c}	85.00 ± 2.89^{a}	86.00 ± 2.31^{a}	89.50 ± 1.45^{b}
MCH (Pg)	34.50 ± 3.18^{c}	32.50 ± 0.87^{b}	29.00 ± 0.58^{a}	29.50 ± 0.29^{a}	29.50 ± 0.29^{a}
MCHC (g/dl)	30.00 ± 0.00^{a}	32.00 ± 1.16^{b}	29.00 ± 0.58^{a}	29.50 ± 0.29^{a}	29.50 ± 0.29^{a}
PLT (Cmm)	247.00 ± 5.20^{e}	219.00 ± 4.62^{b}	229.00 ± 4.61^{c}	216.50 ± 2.80^{a}	231.00 ± 5.78^{d}

Values are presented as mean±SEM (Standard error of mean).Values with different alphabets as superscripts in a row are significantly different at P <0.05. (n=3).

4.1.6 Antioxidants levels of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ toxicant and the respective supplemented treatments with vitamins A, C and E for a period of twelve weeks and sampled fortnightly

4.1.6.1 GSH production levels in Liver, Kidneys and gills of C. gariepinus exposed to sub-lethal concentrations of $Pb(NO_3)_2$ toxicant and the respective supplemented treatments with Vitamins A, C and E for a period of twelve weeks and sampled fortnightly

From the statistical analysis of the resuts of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$, the mean values of the glutathione (GSH) produced in the liver of the control samples were significanctly higher than other treatments, with samples exposed to 61 mg/L significantly higher than other treatments in the first two weeks of exposure to the toxicant. However, the mean values of fishes subjected to 79 mg/L concentration in the 4^{th} week of exposure were significantly higher than the other treatments including the control. Likewise, 44 mg/L and 26 mg/L samples' mean values were significantly higher in the 6^{th} and 8^{th} week of exposure, respectively than other treatments. On the 10^{th} week, mean values of specimens exposed to 61 mg/L were significantly higher than other treatments. At the end of the 12^{th} week, mean values of fishes subjected to 26 mg/L were significantly higher than other treatments with the control following suit. The highest mean value of 82.04±0.13 µg/ml in the liver of the fish was also obtained in treatments with 26 mg/L. (Table 4.23).

Meanwhile, in the kidneys of the samples exposed to the sub-lethal concentration of the toxicant, specimens treated with 79 mg/L and 26 mg/L concentrations had mean

values significantly higher than other treatments including the control in the first 2 weeks of exposure. These however, became significantly lower in the 4th week of exposure in comparison with the control mean values which were significantly higher than other treatments. The control mean values in the 6th week were also significantly higher than other treatments. The 61 mg/L samples mean values (of 30.84±0.10 µg/ml) in the kidney of the fish exposed to the sub-lethal concentration of the toxicant was the highest, which were also significantly higher than other treatments at the end of 8th week. The GSH mean values in the 10th week of exposure in the treatments were significantly lower than the control. In the 12th week however, the mean values of samples exposed to 26 mg/L were significantly higher than other treatments including the control. (Table 4.24).

Furthermore, in the gills of the samples exposed to sub-lethal concentration of the toxicant, 61 mg/L samples' mean values were significantly higher than other treatments including the control in the first 2 weeks of exposure. On the other hand, there were general low production levels at the end of the 4th week of exposure. Treatments with 44 mg/L and 26 mg/L had mean values significantly higher than other treatments in the 6th and 8th weeks of exposure, respectively. Similarly, the average values obtained from samples subjected to 79 mg/L and 26 mg/L were significantly higher than other treatments in the 10th and 12th weeks of exposure, respectively. The highest GSH production level (mean value) in the gill of the fish exposed to the sub-lethal concentration of the toxicant was 31.30±0.10 µg/ml in treatments with 44 mg/L concentration at the 6th week of exposure. (Table 4.25).

Table 4.23: GSH Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	19.76±0.13^{e}	7.26±0.13^{b}	13.51±0.07^{d}	19.03±0.10^{d}	9.42±0.07^{a}	57.83±0.13^{d}
26	7.32±0.10^{a}	8.11±0.10^{c}	6.70±0.13^{b}	24.88±0.07^{e}	9.59±0.10^{a}	82.04±0.13^{e}
44	9.88±0.07^{c}	7.66±1.34^{b}	17.49±0.26^{e}	17.55±0.03^{c}	19.59±0.10^{b}	12.60±0.07^{b}
61	13.51±0.07^{d}	5.90±0.46^{a}	11.45±0.31^{c}	11.75±0.10^{b}	46.13±0.13^{d}	15.22±0.07^{c}
79	9.48±0.16^{b}	11.35±0.13^{d}	5.37±0.29^{a}	8.74±0.00^{a}	27.09±0.03^{c}	10.16±0.10^{a}

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.24: GSH Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	9.88±0.07^{c}	11.70±0.13^{e}	13.80±0.03^{d}	16.13±0.13^{c}	18.80±0.10^{d}	12.55±0.10^{b}
26	13.00±0.03^{d}	3.23±0.10^{a}	13.00±0.03^{c}	6.58±0.07^{a}	11.75±0.10^{b}	23.11±0.10^{e}
44	5.56±0.13^{a}	10.94±0.04^{d}	0.00±0.00	6.81±0.13^{b}	12.83±0.07^{c}	16.30±0.10^{d}
61	6.18±0.03^{b}	8.34±0.10^{c}	12.32±0.10^{b}	30.84±0.10^{d}	7.21±0.03^{a}	14.93±0.10^{c}
79	13.34±0.10^{e}	3.97±0.13^{b}	10.33±0.07^{a}	16.13±0.07^{c}	7.21±0.03^{a}	6.53±0.10^{a}

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.25: GSH Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	12.55 ± 0.16^{b}	2.72 ± 0.07^{a}	4.88 ± 0.07^{a}	13.29 ± 0.13^{c}	16.35 ± 0.07^{d}	13.68 ± 0.10^{b}
26	12.09 ± 0.16^{a}	7.04 ± 0.07^{c}	19.99 ± 0.07^{d}	21.92 ± 0.13^{e}	13.91 ± 0.03^{b}	23.06 ± 0.07^{e}
44	15.33 ± 0.07^{c}	12.32 ± 0.10^{d}	31.30 ± 0.10^{e}	9.42 ± 0.07^{b}	8.29 ± 0.07^{a}	21.92 ± 0.07^{d}
61	24.88 ± 0.13^{d}	5.62 ± 0.10^{b}	13.74 ± 0.07^{c}	14.71 ± 0.16^{d}	14.88 ± 0.13^{c}	19.48 ± 0.03^{c}
79	15.10 ± 0.13^{c}	5.28 ± 0.10^{b}	9.82 ± 0.03^{b}	6.92 ± 0.07^{a}	29.65 ± 0.13^{e}	5.33 ± 0.07^{a}

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

From the statistical analysis of the results of the fish samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin A, the T3 and T2 mean values of the liver of the fish were significantly higher than in other treatments in the 2nd and 4th week of exposure, respectively. However, only fishes subjected to 44 mg/L mean values were significantly higher than other treatment in the 8th week of exposure. Mean values of subjects treated with 26 mg/L in the 10th and 12th weeks were significantly higher than other treatments. The highest mean value of 23.57±0.10 µg/ml in the Liver of the fish subjected to sub-lethal concentrations of the toxicant and supplemented with vitamin A was obtained in samples treated with 26 mg/L at the 10th week of exposure. (Table 4.26).

On the other hand, the kidneys of the fish indicated that samples treated with 26 mg/L had mean values that were significantly higher than other treatments at the 2nd week of exposure. Treatments with 44 mg/L and 26 mg/L had samples' mean values significantly higher than other treatments in the 4th and 8th week of exposure, respectively. However, specimens subjected to 79 mg/L had mean values significantly higher than other treatments in the 10th and 12th weeks of exposure. The highest mean value of 58.74±0.07 µg/ml in the kidney was obtained fishes treated with 79 mg/L at the 12th week of exposure. (Table 4.27).

In addition to the forgoing, the gills of the samples of the fish exposed to the sub-lethal concentrations of the toxicant and supplemented with vitamin A, the samples exposed to the highest concentration had mean values significantly higher than in other treatments. There were general low GSH production levels in the gills of the fish at the 4th week of exposure. The 44 mg/L and 26 mg/L samples' mean values were significantly higher than other treatments at the 8th and 10th weeks of exposure,

respectively. Treatments with 26 mg/L had mean values significantly higher than other treatments at the 12th week of exposure. The highest mean value of 52.72±0.07 µg/ml in the gill of the fish samples was obtained in specimens treated with 26 mg/L at the end of 12 weeks of exposure. (Table 4.28).

Table 4.26: GSH Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	19.76±0.13[b]	7.26±0.13[b]	13.51±0.07	19.03±0.10[e]	9.42±0.07[a]	57.83±0.13[d]
26	10.90±0.20[b]	5.96±0.10[a]	0.00±0.00	16.47±0.13[c]	23.57±0.10[e]	17.89±0.03[c]
44	9.25±0.16[a]	13.17±0.20[d]	0.00±0.00	17.43±0.16[d]	17.09±0.10[d]	13.91±0.10[b]
61	21.01±0.07[c]	12.21±0.10[c]	0.00±0.00	3.97±0.07[a]	14.22±0.07[b]	13.00±0.03[a]
79	9.20±0.13[a]	5.90±0.07[a]	0.00±0.00	9.14±0.10[b]	16.92±0.07[c]	0.00±0.00

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.27: GSH Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	9.88 ± 0.07^{d}	11.70 ± 0.13^{c}	13.80 ± 0.03	16.13 ± 0.13^{e}	18.80 ± 0.10^{d}	12.55 ± 0.10^{a}
26	11.87 ± 0.10^{e}	7.15 ± 0.07^{b}	0.00 ± 0.00	13.34 ± 0.10^{d}	15.39 ± 0.10^{b}	17.21 ± 0.03^{c}
44	8.46 ± 0.10^{b}	34.37 ± 0.10^{d}	0.00 ± 0.00	11.53 ± 0.10^{c}	12.26 ± 0.13^{a}	14.37 ± 0.03^{b}
61	4.14 ± 0.03^{a}	0.00 ± 0.00	0.00 ± 0.00	9.03 ± 0.10^{b}	17.15 ± 0.07^{c}	26.07 ± 0.03^{d}
79	9.03 ± 0.16^{c}	3.80 ± 0.10^{a}	0.00 ± 0.00	4.54 ± 0.07^{a}	18.74 ± 0.07^{d}	58.74 ± 0.07^{e}

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.28: GSH Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	12.55 ± 0.16^{a}	2.72 ± 0.07^{a}	4.88 ± 0.07	13.29 ± 0.13^{d}	16.35 ± 0.07^{a}	13.68 ± 0.10^{b}
26	18.51 ± 0.13^{c}	8.12 ± 0.10^{c}	0.00 ± 0.00	7.21 ± 0.03^{a}	25.50 ± 0.10^{d}	52.72 ± 0.07^{e}
44	12.83 ± 0.39^{b}	8.91 ± 0.89^{d}	0.00 ± 0.00	15.90 ± 0.13^{e}	16.75 ± 0.30^{a}	43.47 ± 0.10^{d}
61	21.81 ± 0.20^{d}	6.75 ± 0.49^{b}	0.00 ± 0.00	12.66 ± 0.03^{b}	20.16 ± 0.10^{c}	11.64 ± 0.03^{a}
79	24.31 ± 0.20^{e}	8.97 ± 0.07^{d}	0.00 ± 0.00	12.15 ± 0.07^{c}	17.21 ± 0.10^{b}	23.40 ± 0.20^{c}

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

In the samples of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C, 26 mg/L samples' mean values in the liver of the fish were significantly higher than in other treatments at the end of the second week of exposure. The mean values of samples of 44 mg/L and 26 mg/L in the 4th and 8th weeks of exposure, respectively were significantly higher than other treatments. While 44 mg/L and 79 mg/L samples' mean values in the 10th and 12th weeks of exposure, respectively were significantly higher than other treatments. The highest mean value of 25.79±0.07 µg/ml in the liver of the fish in this case, was obtained in samples exposed to 79 mg/L at the 12th week of exposure. (Table 4.29).

On the other hand, mean values of samples subjected to 26 mg/L and 79 mg/L in the kidneys of the fish in the second and fouth weeks of exposure were significantly higher than other treatments. Mean values of subjects treated with 44 mg/L concentration at the 8th week of exposure were significantly higher than other treatments. Also, the fishe exposed to 79 mg/L and 44 mg/L had mean values that were significantly higher than other treatments in the 10th and 12th weeks of exposure. The highest mean value of 28.40±0.13 µg/ml was obtained in samples exposed to 44 mg/L concentration of the toxicant at the 12th week of exposure. (Table 4.30). Furthermore, the treatment with 79 mg/L had mean values in the gills of the fish exposed to the sub-lethal concentrations of the toxicant and supplemented with vitamin C that were significantly higher than other treatments in the 2nd, 4th, 8th and 10th weeks of exposure. However, those subjected to 26 mg/L had mean values in the 12th week of exposure that were significantly higher than other treatments. The highest mean value of 37.55±0.03 µg/ml in the highest concentration was obtained in the gills of the fish in this case at the 8th week of exposure. (Table 4.31).

Table 4.29: GSH Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	19.76±0.13^{c}	7.26±0.13^{a}	13.51±0.07	19.03±0.10^{c}	9.42±0.07^{b}	57.83±0.13^{e}
26	20.84±0.10^{d}	9.37±0.10^{b}	0.00±0.00	22.32±0.10^{d}	5.90±0.07^{a}	18.63±0.13^{c}
44	14.42±0.07^{b}	18.63±0.13^{c}	0.00±0.00	14.93±0.10^{b}	15.84±0.03^{d}	17.83±0.07^{b}
61	14.08±0.07^{b}	9.42±0.07^{b}	0.00±0.00	10.05±0.10^{a}	12.21±0.10^{c}	11.87±0.36^{a}
79	13.46±0.10^{a}	0.00±0.00	0.00±0.00	0.00±0.00	12.26±0.07^{c}	25.79±0.07^{d}

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.30: GSH Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	9.88±0.07^{d}	11.70±0.13^{c}	13.80±0.03	16.13±0.13^{d}	18.80±0.10^{d}	12.55±0.10^{b}
26	11.92±0.20^{e}	9.88±0.52^{b}	0.00±0.00	4.93±0.10^{a}	15.16±0.10^{c}	10.10±0.13^{a}
44	8.63±0.07^{c}	11.07±0.10^{c}	0.00±0.00	10.33±0.07^{c}	11.13±0.07^{a}	28.40±0.13^{d}
61	7.78±0.10^{b}	8.06±0.07^{a}	0.00±0.00	5.56±0.07^{b}	11.98±0.16^{b}	13.34±0.03^{c}
79	4.65±0.13^{a}	14.59±0.03^{d}	0.00±0.00	0.00±0.00	23.57±0.10^{e}	0.00±0.00

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.31: GSH Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks

Ts	Eposure Duration (Week)					
	2	**4**	**6**	**8**	**10**	**12**
C	12.55 ± 0.16^{a}	2.72 ± 0.07^{b}	4.88 ± 0.07	13.29 ± 0.13^{c}	16.35 ± 0.07^{c}	13.68 ± 0.10^{c}
26	15.05 ± 0.10^{c}	7.15 ± 0.13^{c}	0.00 ± 0.00	8.57 ± 0.50^{b}	14.82 ± 0.16^{b}	21.07 ± 0.10^{d}
44	13.12 ± 0.10^{b}	7.26 ± 0.13^{c}	0.00 ± 0.00	5.33 ± 0.13^{a}	12.38 ± 0.07^{a}	13.34 ± 0.10^{c}
61	13.17 ± 0.07^{b}	2.09 ± 0.10^{a}	0.00 ± 0.00	21.47 ± 0.13^{d}	14.82 ± 0.03^{b}	9.20 ± 0.07^{b}
79	22.38 ± 0.13^{d}	21.24 ± 0.07^{d}	0.00 ± 0.00	37.55 ± 0.03^{e}	14.82 ± 0.01^{b}	6.92 ± 0.07^{a}

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

From the results of the samples of fish exposed to sub-lethal concentrations of Pb(NO3)2 and Supplemented with vitamin E, the samples exposed to 26 mg/L and 44 mg/L had mean values significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. The samples exposed to 61 mg/L had mean values that were significantly higher than other treatments in the 6th week. Also, treatments with 44 mg/L and 61 mg/L concentrations produced mean values in the 10th and 12th weeks, respectively which were significantly higher than other treatments. The highest mean value of 57.21±0.03 µg/ml in the liver of the exposed samples of the fish was obtained in samples subjected to 61 mg/L concentration at the 12th week of exposure. (Table 4.32). The specimens subjected to 26 mg/L and 61 mg/L had mean values in the kidneys that were significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. Likewise, the treatments containing 44 mg/L and 79 mg/L generated mean values in the 6th and 8th weeks of exposure, respectively and were significantly higher than other treatments. Also, the samples exposed to 26 mg/L and 61 mg/L had mean values that were significantly higher than other treatments in the 10th and 12th weeks of exposure, respectively. The highest mean value of 83.51±0.07 µg/ml in the kidneys of the sample was obtained in the specimens subjected to 26 mg/L at the 10th week of exposure. (Table 4.33). Furthermore, treatments with 79 mg/L and 26 mg/L produced mean values in the gills which were significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. Treatments with 79 mg/L and 61 mg/L had mean values that were also significantly higher than other treatments in the 6th and 10^{th} weeks of exposure, respectively. The 61 mg/L mean value of 63.29±0.07 µg/ml obtained at the 12th week was the highest and was significantly higher than other treatments. (Table 4.34).

Table 4.32: GSH Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	19.76±0.13^{e}	7.26±0.13^{c}	13.51±0.07^{c}	19.03±0.10^{c}	9.42±0.07^{a}	57.83±0.13^{e}
26	15.84±0.10^{d}	5.84±0.16^{a}	12.21±0.23^{b}	10.39±0.10^{b}	12.21±0.10^{c}	7.49±0.07^{b}
44	9.25±0.10^{b}	7.66±0.10^{c}	17.83±0.07^{d}	0.00±0.00	22.66±0.03^{e}	3.85±0.07^{a}
61	10.67±0.13^{c}	6.47±0.13^{b}	36.75±0.10^{e}	0.00±0.00	10.67±0.07^{b}	57.21±0.03^{d}
79	8.57±0.03^{a}	6.47±0.13^{b}	10.45±0.07^{a}	9.08±0.13^{a}	13.85±0.07^{d}	39.31±0.13^{c}

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.33: GSH Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	9.88±0.07^{c}	11.70±0.13^{a}	13.80±0.03^{c}	16.13±0.13^{c}	18.80±0.10^{c}	12.55±0.10^{b}
26	11.30±0.16^{d}	12.78±0.10^{b}	0.00±0.00	2.89±0.10^{a}	83.51±0.07^{e}	22.89±0.10^{c}
44	8.34±0.10^{b}	12.66±0.16^{b}	13.63±0.07^{c}	0.00±0.00	63.80±0.10^{d}	4.93±0.10^{a}
61	7.43±0.10^{a}	39.14±0.10^{d}	8.40±0.07^{a}	0.00±0.00	9.48±0.03^{a}	56.41±0.03^{e}
79	9.71±0.23^{c}	14.03±0.16^{c}	9.03±0.16^{b}	10.28±0.16^{b}	16.24±0.07^{b}	33.40±0.13^{d}

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.34: GSH Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	12.55 ± 0.16^{a}	2.72 ± 0.07^{a}	4.88 ± 0.07^{a}	13.29 ± 0.13^{b}	16.35 ± 0.07^{c}	13.68 ± 0.10^{c}
26	19.65 ± 0.26^{b}	29.08 ± 0.13^{d}	13.12 ± 0.03^{d}	7.89 ± 0.10^{a}	13.23 ± 0.16^{b}	0.00 ± 0.00
44	23.40 ± 0.20^{c}	2.55 ± 0.10^{a}	9.37 ± 0.03^{b}	0.00 ± 0.00	12.49 ± 0.13^{a}	9.71 ± 0.10^{a}
61	25.45 ± 0.26^{d}	4.76 ± 0.13^{b}	12.32 ± 0.10^{c}	0.00 ± 0.00	18.91 ± 0.10^{d}	63.29 ± 0.07^{d}
79	25.65 ± 0.13^{d}	13.46 ± 0.10^{c}	26.30 ± 0.10^{e}	0.00 ± 0.00	12.66 ± 0.10^{a}	11.24 ± 0.07^{b}

Mean values and standard errors (µg/ml) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

4.1.6.2 MDA production levels in Liver, Kidneys and gills of C. gariepinus exposed to sub-lethal concentrations of $Pb(NO_3)_2$ toxicant and the respective supplemented treatments with Vitamins A, C and E for a period of twelve weeks and sampled fortnightly

In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, the MDA liver mean values in specimens exposed to 44 mg/L and 61 mg/L in the 2nd and 4th weeks of exposure, respectively, were significantly higher than other treatments including the control. The 44 mg/L samples' mean values in both 6th and 8th weeks of exposure were significantly higher than other treatments including the control. While the specimens subjected to 61 mg/L and 79 mg/L concentrations had mean values in the 10th and 12th weeks of exposure that were significantly higher than other treatment including the control. The highest MDA production level of the liver of the samples was 56.65±0.06 nM/mgprotein obtained in samples exposed to 61 mg/L concentration at the 10th week of exposure. (Table 4.35). In another development, the MDA produced in the kidneys of the fish indicated that mean values of sampes treated with 79 mg/L were significantly higher than other treatments including the control in the 2nd week of exposure. The mean values of the 4th, 8th and 10th weeks of exposure in the treatments were significantly lower than the control. Samples exposed to 79 mg/L concentration had mean values in both 6th and 12th weeks of exposure that were however, significantly higher than other treatments. The highest MDA production in this case was 40.33±0.06 nM/mgprotein obtained in 79 mg/L samples at the end of the 10th week of exposure. (Table 4.36). In the gills of the sample, the control mean values were significantly higher than other treatments closely followed by specimens subjected to 79 mg/L in the 2nd week of exposure. The subjects exposed to 44 mg/L

produced mean values in both 4th and 6th weeks that were significantly higher than other treatments including the control. Treatments with 61 mg/L, 44 mg/L and 79 mg/L generated mean values in the 8th, 10th and 12th weeks of exposure, respectively that were significantly higher than other treatments including the control. The highest MDA mean value of 80.28±0.06 nM/mgprotein in the gills of the samples was obtained in those treated with 44 mg/L in the 10th week of exposure. (Table 4.37).

Table 4.35: MDA Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	34.22±0.03^{d}	14.82±0.03^{c}	7.60±0.27^{a}	15.86±0.05^{c}	7.76±0.02^{b}	10.88±0.03^{b}
26	16.53±0.06^{a}	5.47±0.03^{a}	7.57±0.05^{a}	1.00±0.05^{a}	7.09±0.03^{a}	42.43±1.03^{e}
44	38.82±0.02^{e}	13.62±0.03^{b}	35.37±0.27^{d}	43.57±1.66^{d}	23.34±0.03^{c}	4.82±0.03^{a}
61	23.43±0.27^{b}	37.60±0.05^{e}	27.71±0.05^{c}	7.57±0.05^{b}	56.65±0.06^{e}	14.54±0.09^{c}
79	27.55±0.03^{c}	15.95±0.05^{d}	23.87±0.05^{b}	15.81±0.05^{c}	39.40±0.05^{d}	25.54±0.05^{d}

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.36: MDA Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	27.83 ± 0.03^{d}	39.77 ± 0.03^{e}	24.19 ± 0.02^{d}	51.49 ± 0.06^{d}	56.35 ± 0.06^{e}	0.14 ± 0.03^{a}
26	21.60 ± 0.05^{c}	16.30 ± 0.03^{d}	8.36 ± 0.05^{a}	0.70 ± 0.03^{a}	17.64 ± 0.03^{b}	13.59 ± 0.02^{c}
44	10.47 ± 0.03^{b}	8.04 ± 0.05^{a}	0.00 ± 0.00	21.49 ± 0.03^{c}	9.38 ± 0.05^{a}	19.05 ± 0.05^{d}
61	5.74 ± 0.03^{a}	11.28 ± 0.03^{b}	12.97 ± 0.03^{b}	7.20 ± 0.02^{b}	39.84 ± 0.05^{c}	4.54 ± 0.03^{b}
79	38.08 ± 0.02^{e}	14.10 ± 0.14^{c}	32.11 ± 0.05^{c}	7.09 ± 0.09^{b}	40.33 ± 0.06^{d}	34.03 ± 0.03^{e}

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.37: MDA Production Levels in the Gills of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	31.18 ± 0.05^{d}	23.38 ± 0.03^{c}	12.11 ± 0.02^{c}	10.86 ± 0.47^{d}	20.68 ± 0.05^{c}	2.11 ± 0.02^{a}
26	23.52 ± 0.03^{b}	21.49 ± 0.06^{b}	10.77 ± 0.07^{b}	1.55 ± 0.05^{a}	56.79 ± 0.05^{d}	7.11 ± 0.03^{b}
44	12.60 ± 0.06^{a}	30.84 ± 0.06^{e}	33.94 ± 0.03^{d}	2.71 ± 0.05^{b}	80.28 ± 0.06^{e}	12.11 ± 0.02^{d}
61	23.71 ± 0.06^{b}	26.18 ± 0.67^{d}	27.23 ± 0.06^{c}	42.87 ± 1.58^{e}	7.64 ± 0.03^{a}	11.30 ± 0.30^{c}
79	28.20 ± 0.03^{c}	17.23 ± 0.06^{a}	4.22 ± 0.03^{a}	8.15 ± 0.06^{c}	9.36 ± 0.06^{b}	28.55 ± 0.07^{e}

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

In another development, the samples of fish exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin A, the MDA production in the liver indicated close range of values in samples subjected to 26 mg/L to 61 mg/L with specimens treated with 44 mg/L significantly higher than other treatments in the 2nd week of exposure. At the 4th and 8th weeks of exposure, the treatment with 44 mg/L produced mean values that were also significantly higher than other treatments. Treatment with the highest concentration had mean values that were significantly higher than other treatments in the 10th and 12th weeks of exposure. The highest mean value of 40.10±0.03 nM/mgprotein at the 12th week was obtained in the peak concentration. (Table 4.38). On the other hand, the MDA produced in the kidneys indicated that the maximum concentration of the toxicant generated mean values in the 2nd, 10th and 12th weeks of exposure that were significantly higher than other treatments. The highest MDA production in this case was 58.41±0.02 nM/mgprotein obtained in the highest concentration at the 10th week. (Table 4.39). Furthermore, in the gills of the fish samples, the highest concentration produced mean values in both 2nd and 4th weeks of exposure that were significantly higher than other treatments. However, the lowest concentration of the toxicant had mean values in both 10th and 12th weeks of exposure that were significantly higher than other treatments. The highest MDA production in the gill in this case was 80.30±0.05 nM/mgprotein obtained in the lowest concntration at the 10th week of exposure. (Table 4.40).

Table 4.38: MDA Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	34.22±0.03^{b}	14.84±0.03^{b}	7.60±0.03	15.86±0.05^{c}	7.76±0.02^{b}	10.88±0.03^{b}
26	38.59±0.05^{c}	27.57±0.07^{d}	0.00±0.00	11.32±0.03^{b}	21.23±0.02^{d}	35.47±0.06^{c}
44	38.64±0.05^{c}	31.44±0.03^{e}	0.00±0.00	17.80±0.05^{d}	4.70±0.02^{a}	7.97±0.06^{a}
61	38.24±0.03^{c}	17.76±0.05^{c}	0.00±0.00	3.29±0.03^{a}	7.57±0.02^{b}	39.82±0.06^{d}
79	21.65±0.05^{a}	8.45±0.05^{a}	0.00±0.00	11.88±0.02^{b}	19.54±0.03^{c}	40.10±0.03^{e}

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.39: MDA Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	27.83±0.03^{e}	39.77±0.03^{d}	24.19±0.02	51.49±0.06^{e}	56.35±0.06^{d}	0.14±0.03^{a}
26	17.80±0.05^{c}	27.02±0.05^{c}	0.00±0.00	14.93±0.05^{d}	34.49±0.06^{c}	15.10±0.03^{c}
44	9.38±0.05^{a}	20.44±0.02^{b}	0.00±0.00	3.41±0.02^{b}	17.87±0.03^{b}	18.20±0.03^{d}
61	13.22±0.02^{b}	0.00±0.00	0.00±0.00	0.77±0.05^{a}	2.55±0.03^{a}	9.73±0.03^{b}
79	25.14±0.03^{d}	16.28±0.05^{a}	0.00±0.00	14.10±0.05^{c}	58.41±0.02^{e}	39.84±0.05^{e}

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.40: MDA Production Levels in the Gills of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	31.18±0.05^{d}	23.38±0.03^{c}	12.11±0.02	10.86±0.47^{d}	20.68±0.05^{b}	2.11±0.02^{a}
26	20.68±0.07^{b}	31.83±0.07^{d}	0.00±0.00	7.99±0.05^{c}	80.30±0.05^{e}	40.33±0.06^{e}
44	13.59±0.02^{a}	10.77±0.05^{b}	0.00±0.00	0.75±0.05^{a}	25.56±0.06^{c}	25.17±0.05^{d}
61	23.85±0.03^{c}	6.30±0.03^{a}	0.00±0.00	12.30±0.02^{e}	4.12±0.06^{a}	10.77±0.05^{c}
79	40.37±0.03^{e}	37.80±0.05^{e}	0.00±0.00	3.27±0.07^{b}	39.61±0.02^{d}	8.96±0.05^{b}

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

From the results of the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C, the sample subjected to 61 mg/L and 79 mg/L had mean values in the 2nd and 4th weeks of exposure, respectively that were significantly higher than other treatments. The specimens exposed to 44 mg/L generated mean values in both 8th and 10th weeks of exposure which were significantly higher than other treatments. The specimens treated with 61 mg/L had mean values that were significantly higher than other treatments including the control. The Highest MDA production in the liver of the fish was 46.60±0.06 nM/mgprotein obtained in samples exposed to 61 mg/L after the 2nd week of exposure. (Table 4.41). In another development, the MDA mean values in the kidneys of the samples indicated that the fishes subjected to 61 mg/L produced mean values in the 2nd and 12th week of exposure that were significantly higher than other treatments. The highest mean value in this regard was 40.21±0.02 nM/mgprotein obtained in those samples subjected to 61 mg/L at the end of 8th week of exposure. (Table 4.42). Furthermore, the mean values in control in the 2nd and 8th weeks of exposure were significantly higher than other treatments. The treatment with 61 mg/L produced mean values in both 4th and 10th weeks of exposure that were significantly higher than other treatments. The samples treated with 26 mg/L had mean values in the 12th week of exposure which were significantly higher than other treatments. The highest mean value of 45.07±0.05 nM/mgprotein was obtained in samples subjected to 61 mg/L concentration of the toxicant in the 4th week of exposure. (Table 4.43).

Table 4.41: MDA Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	34.22 ± 0.03^{b}	14.82 ± 0.03^{d}	7.60 ± 0.03	15.86 ± 0.05^{b}	7.75 ± 0.02^{a}	10.88 ± 0.03^{b}
26	40.14 ± 0.03^{d}	13.24 ± 0.03^{b}	0.00 ± 0.00	3.64 ± 0.05^{a}	9.84 ± 0.02^{c}	14.24 ± 0.05^{c}
44	23.94 ± 0.03^{a}	8.92 ± 0.05^{a}	0.00 ± 0.00	29.03 ± 0.06^{c}	16.76 ± 0.03^{d}	1.25 ± 0.03^{a}
61	46.60 ± 0.06^{e}	14.05 ± 0.12^{c}	0.00 ± 0.00	3.43 ± 0.03^{a}	9.29 ± 0.02^{b}	39.87 ± 0.08^{d}
79	39.01 ± 0.05^{c}	16.07 ± 0.03^{e}	0.00 ± 0.00	0.00 ± 0.00	21.35 ± 0.06^{e}	1.51 ± 0.02^{a}

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.42: MDA Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	27.83 ± 0.03^{e}	39.77 ± 0.03^{d}	24.19 ± 0.02	51.49 ± 0.06^{d}	56.35 ± 0.06^{e}	0.14 ± 0.03^{a}
26	17.78 ± 0.03^{b}	39.17 ± 0.06^{d}	0.00 ± 0.00	1.88 ± 0.05^{a}	23.66 ± 0.03^{d}	22.85 ± 0.05^{d}
44	14.26 ± 0.06^{a}	17.87 ± 0.03^{a}	0.00 ± 0.00	4.43 ± 0.05^{b}	18.82 ± 0.07^{c}	5.74 ± 0.02^{b}
61	24.33 ± 0.02^{d}	22.32 ± 0.03^{c}	0.00 ± 0.00	40.21 ± 0.02^{c}	13.59 ± 0.05^{b}	17.20 ± 0.05^{c}
79	19.54 ± 0.03^{c}	19.84 ± 0.02^{b}	0.00 ± 0.00	0.00 ± 0.00	10.95 ± 0.02^{a}	0.00 ± 0.00

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.43: MDA Production Levels in the Gills of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	31.85±0.05^{e}	23.38±0.03^{c}	12.11±0.02	10.86±0.46^{d}	20.68±0.05^{a}	2.11±0.02^{a}
26	22.06±0.05^{d}	39.08±0.03^{d}	0.00±0.00	9.80±0.02^{c}	25.72±0.05^{b}	32.99±0.05^{c}
44	11.88±0.05^{a}	16.81±0.03^{b}	0.00±0.00	5.61±0.03^{b}	31.90±0.03^{c}	2.83±0.03^{a}
61	21.05±0.05^{c}	45.07±0.05^{e}	0.00±0.00	5.77±0.02^{b}	35.88±0.19^{e}	13.62±0.03^{b}
79	20.47±0.03^{b}	10.35±0.05^{a}	0.00±0.00	1.95±0.06^{a}	32.50±0.03^{d}	2.92±0.06^{a}

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E, the MDA production levels in the liver of the fish indicated that the highest concentration of the toxicant produced mean values in the 2nd week of exposure that were significantly higher than other treatments. The samples subjected to 44 mg/L had mean values in both 4th and 6th weeks of exposure which were significantly higher than other treatments. The specimens subjected to 26 mg/L generated mean values in the 8th and 10th weeks of exposure that were significantly higher than other treatments. The highest mean value of MDA in the liver was 40.19±0.03 nM/mgprotein obtained in samples exposed to 26 mg/L at the 10th week of exposure. (Table 4.44). On the other hand, the fishes exposed to 26 mg/L and 61 mg/L generated mean values in the kidney of the fish which were significantly higher than other treatments in the 2nd and 6th weeks of exposure, respectively. Treatment with 26 mg/L had mean values that were significantly higher than other treatments at 12th week of exposure. The highest mean value in this regard was 40.17±0.47 nM/mgprotein obtained in samples treated with 26 mg/L at the 12th week of exposure. (Table 4.45). Furthermore, in the gills of the sample, the sample exposed to 61 mg/L had mean values in both 2nd and 4th weeks of exposure that were significantly higher than other treatments. The treatments with 79 mg/L and 26 mg/L had mean values in the 6th and the 12th weeks of exposure that were significantly higher than other treatments. The highest MDA mean value produced in the gill in this case was 77.04±0.06 nM/mgprotein obtained in fishes subjected to 26 mg/L at the end of the 12th week of exposure. (Table 4.46).

Table 4.44: MDA Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	34.22±0.03^{b}	14.82±0.03^{c}	7.50±0.03^{c}	15.86±0.05^{b}	7.76±0.02^{b}	10.88±0.03^{e}
26	39.17±0.03^{d}	0.70±0.03^{a}	6.95±0.02^{b}	19.03±0.03^{c}	40.19±0.03^{e}	3.80±0.06^{b}
44	28.8±0.05^{a}	27.77±0.06^{e}	13.11±0.06^{e}	0.00±0.00	3.64±0.05^{a}	7.94±0.18^{d}
61	38.15±0.03^{c}	12.02±0.05^{b}	5.56±0.03^{a}	0.00±0.00	8.48±0.03^{c}	0.37±0.03^{a}
79	40.03±0.05^{e}	12.30±0.05^{b}	8.50±0.05^{d}	3.75±0.06^{a}	13.31±0.05^{d}	6.62±0.03^{c}

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.45: MDA Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	27.83±0.03^{d}	39.77±0.03^{e}	24.19±0.02^{d}	51.49±0.07^{c}	56.35±0.06^{e}	0.14±0.03^{a}
26	31.12±0.03^{e}	13.96±0.05^{c}	19.96±0.03^{c}	3.43±0.03^{a}	33.50±0.05^{d}	40.17±0.47^{c}
44	14.73±0.03^{c}	12.71±0.02^{b}	6.79±0.05^{a}	0.00±0.00	29.24±0.05^{c}	2.50±0.02^{b}
61	11.83±0.07^{a}	33.06±0.06^{d}	39.54±0.06^{e}	0.00±0.00	7.67±0.02^{a}	2.50±0.11^{b}
79	12.32±0.06^{b}	10.24±0.06^{a}	12.11±0.06^{b}	4.89±0.05^{b}	27.89±0.06^{b}	0.54±0.05^{a}

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.46: MDA Production Levels in the Gills of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	31.18±0.05^{d}	23.38±0.03^{d}	12.11±0.02^{b}	10.86±0.05^{a}	20.68±0.05^{d}	2.11±0.02^{a}
26	29.77±0.03^{b}	10.77±0.05^{b}	9.12±0.03^{a}	30.58±0.02^{b}	10.86±0.02^{c}	77.04±0.06^{d}
44	30.44±0.02^{c}	19.19±0.02^{c}	13.45±0.02^{c}	0.00±0.00	0.00±0.00	3.82±0.07^{c}
61	35.91±0.05^{e}	56.69±0.05^{e}	21.49±0.02^{d}	0.00±0.00	9.33±0.02^{b}	3.87±0.05^{c}
79	6.25±0.03^{a}	8.55±0.05^{a}	24.43±0.02^{e}	0.00±0.00	3.27±0.02^{a}	6.12±0.06^{b}

Mean values and standard errors (nM/mgprotein) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

4.1.6.3 AST production levels in Liver, Kidneys and gills of C. gariepinus exposed to sub-lethal concentrations of $Pb(NO_3)_2$ toxicant and the respective supplemented treatments with Vitamins A, C and E for a period of twelve weeks and sampled fortnightly

In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, the AST production levels in the liver of the fish indicated that the control mean values in the 2nd and 4th weeks of exposure were significantly higher than other treatments. The catfishes exposed to 26 mg/L concentration generated mean values in the 6th and 8th weeks of exposure that were significantly higher than other treatments including the control. The samples exposed to 26 mg/L and 44 mg/L had mean values in the 10th week of exposure that were significantly higher than other treatments including the control. The mean values of the samples of the treatment containing 61 mg/L in the 12th week of exposure were significantly higher than other treatments including the control. The highest mean value of AST in the liver was 124.68±0.19 nM/mL obtained in 26 mg/L treatment at the 6th week of exposure. (Table 4.47). On the other hand, the 61 mg/L, 79 mg/L and 26 mg/L treatments produced mean values in the kidney of the fish which were significantly higher than other treatments including the control in the 2nd, 4th and 6th weeks of exposure, respectively. However, the mean values of specimens treated with 79 mg/L were significantly higher than other treatments including the control in the 8th week of exposure. Meanwhile, the control mean values were significantly higher than other treatments at 10th week of exposure. The highest mean value in this regard was 141.40±0.10 nM/mL obtained in samples exposed to 26 mg/L at the 12th week of exposure. This value was also significantly higher than other treatments including the control. (Table 4.48). Furthermore, in the gills of the sample, the 26

mg/L had mean values in the 2nd week of exposure that were significantly higher than other treatments including the control. However, the control mean values in the 4th, 6th and 10th weeks of exposure were significantly higher than other treatments. The 44 mg/L samples' mean values in the 8th week of exposure were significantly higher than other treatments including the control. The highest AST mean value produced in the gill in this case was 124.21±0.28 nM/mL obtained in fishes exposed to 79 mg/L at the end of the 12th week of exposure. (Table 4.49).

Table 4.47: AST Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	90.62±0.18^{d}	105.00±0.19^{e}	86.90±0.06^{c}	6.56±0.37^{b}	81.25±5.78^{b}	19.21±0.28^{a}
26	87.03±0.10^{c}	11.40±0.28^{a}	124.68±0.19^{e}	21.25±0.19^{c}	119.06±0.19^{c}	104.53±0.10^{d}
44	50.93±0.37^{b}	20.00±0.19^{b}	78.50±0.12^{b}	53.59±0.28^{d}	119.68±0.19^{c}	94.53±0.28^{c}
61	98.90±0.28^{e}	39.06±0.19^{c}	23.89±0.16^{a}	3.28±0.28^{a}	5.31±0.19^{a}	115.78±0.28^{e}
79	9.53±0.46^{a}	69.84±0.01^{d}	119.68±0.19^{d}	100.31±0.19^{e}	5.78±0.28^{a}	84.84±0.28^{b}

Mean values and standard errors (nM/ nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.48: AST Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	47.03 ± 0.01^{c}	15.31 ± 0.19^{b}	70.00 ± 0.19^{c}	13.59 ± 0.82^{a}	120.31 ± 0.19^{e}	45.46 ± 0.46^{a}
26	17.50 ± 0.55^{a}	96.00 ± 0.13^{d}	103.28 ± 0.28^{e}	74.84 ± 0.28^{c}	94.53 ± 0.10^{d}	141.40 ± 0.10^{e}
44	62.65 ± 0.28^{d}	16.71 ± 0.10^{c}	34.98 ± 0.08^{b}	14.53 ± 0.10^{b}	86.40 ± 0.28^{c}	62.18 ± 0.11^{b}
61	68.28 ± 0.28^{e}	0.93 ± 0.19^{a}	97.65 ± 0.10^{d}	14.53 ± 0.10^{b}	35.78 ± 0.28^{b}	74.84 ± 0.28^{c}
79	39.68 ± 0.19^{b}	106.09 ± 0.28^{e}	20.00 ± 0.19^{a}	129.21 ± 0.28^{e}	2.49 ± 0.19^{a}	119.21 ± 0.19^{d}

Mean values and standard errors (nM/ nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.49: AST Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	41.09 ± 0.01^{b}	152.18 ± 0.19^{d}	120.15 ± 0.10^{e}	9.68 ± 0.19^{a}	100.15 ± 0.10^{e}	12.18 ± 0.19^{a}
26	118.75 ± 0.18^{e}	116.71 ± 0.10^{c}	79.37 ± 0.19^{c}	90.46 ± 0.28^{d}	94.68 ± 0.37^{d}	117.34 ± 0.46^{d}
44	13.43 ± 0.19^{a}	31.71 ± 0.28^{a}	112.50 ± 0.19^{d}	93.75 ± 0.19^{e}	13.12 ± 0.19^{c}	101.56 ± 0.19^{c}
61	117.03 ± 0.10^{d}	44.53 ± 0.10^{b}	71.56 ± 0.19^{b}	38.90 ± 0.28^{c}	3.75 ± 0.37^{a}	46.71 ± 0.18^{b}
79	104.00 ± 0.46^{c}	31.25 ± 0.37^{a}	47.03 ± 0.28^{a}	10.31 ± 0.19^{b}	9.68 ± 0.19^{b}	124.21 ± 0.28^{e}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

In another development, the samples of fish exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin A the AST production in the liver indicated that samples exposed to 26 mg/L generated mean values that were significantly higher than other treatments in the 2nd week of exposure. At the 4th and 8th weeks of exposure, respectively the treatments with 79 mg/L and 26 mg/L had mean values that were significantly higher than other treatments. Specimens subjected to 61 mg/L and 79 mg/L produced mean values in the 10th and 12th weeks of exposure which were significantly higher than other treatments. The highest mean value of 113.75±0.19 nM/mL at the 10th week was obtained from samples exposed to 61 mg/L concentration. (Table 4.50). On the other hand, the AST mean values produced in the kidneys indicated that 26 mg/L and 44 mg/L samples' mean values in the 2nd and 4th weeks of exposure were significantly higher than other treatments. However, when the fishes were treated with 44 mg/L, the mean values in both 8th and 12th week of exposure were significantly higher than other treatments. The highest AST production value in this case was 139.86±0.19 nM/mL obtained in treatment with 44 mg/L at the 12th week. (Table 4.51). Furthermore, in the gills of the fish samples, the samples subjected to 79 mg/L produced mean values in the 2nd week of exposure that were significantly higher than other treatments. The specimens subjected to 26 mg/L, 61 mg/L and 79 mg/L concentrations had mean values that were significantly higher than other treatments in the 8th, 10th and 12th weeks of exposure, respectively. The highest AST production value in the gill in this case was 115.78±0.10 nM/mL obtained in the highest concentration at the 12th week of exposure. (Table 4.52).

Table 4.50: AST Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	90.62±0.19^{c}	105.00±0.19^{e}	0.00±0.00	6.50±0.37^{a}	81.25±5.78^{c}	19.21±0.28^{a}
26	101.56±0.55^{e}	6.56±0.19^{a}	0.00±0.00	110.93±0.19^{e}	87.96±0.10^{d}	75.15±0.10^{b}
44	43.59±0.90^{b}	23.28±0.00^{c}	0.00±0.00	39.21±0.10^{d}	54.37±0.19^{b}	94.06±0.19^{c}
61	94.37±0.19^{d}	15.46±0.28^{b}	0.00±0.00	23.59±0.46^{c}	113.75±0.19^{e}	103.59±0.10^{d}
79	38.59±0.00^{a}	66.56±0.19^{d}	0.00±0.00	22.81±0.19^{b}	10.93±0.19^{a}	110.62±0.37^{e}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.51: AST Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	47.03±0.10^{c}	15.31±0.19^{a}	70.00±0.19	13.59±0.82^{a}	120.31±0.19^{e}	45.46±0.46^{a}
26	63.90±0.28^{e}	31.71±0.28^{b}	0.00±0.00	103.59±0.28^{c}	30.93±0.19^{a}	82.34±0.10^{b}
44	29.06±0.19^{b}	37.34±0.10^{d}	0.00±0.00	127.96±0.10^{e}	65.00±0.19^{b}	139.86±0.19^{e}
61	55.31±0.19^{d}	0.00±0.00	0.00±0.00	53.59±0.28^{b}	79.06±0.19^{c}	126.09±0.28^{d}
79	13.43±0.19^{a}	33.90±0.28^{c}	0.00±0.00	111.09±0.28^{d}	96.25±0.19^{d}	102.65±0.10^{c}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.52: AST Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	41.09±0.10[a]	152.18±0.19[e]	120.15±0.10	9.68±0.19[a]	100.15±0.10[b]	42.18±0.19[a]
26	93.12±0.19[d]	28.75±0.37[c]	0.00±0.00	97.96±0.10[e]	107.30±0.10[d]	99.37±1.09[d]
44	59.84±0.28[b]	10.46±0.10[a]	0.00±0.00	40.62±0.19[b]	32.96±0.28[a]	86.25±0.37[b]
61	60.46±0.10[c]	32.96±0.28[d]	0.00±0.00	69.37±0.37[c]	115.46±0.28[e]	93.43±0.19[c]
79	114.53±0.10[e]	26.87±0.19[b]	0.00±0.00	73.90±0.10[d]	106.56±0.19[c]	115.78±0.10[e]

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

From the results of the analysis of the samples of fish exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C, the control mean values of AST in the 2nd week of exposure were significantly higher than other treatments. The fishes exposed to 61 mg/L and 26 mg/L had mean values in the 4th and 8th weeks of exposure, respectively that were significantly higher than other treatments. Similarly, the specimens exposed to 26 mg/L and 44 mg/Lproduced mean values in the 10th and 12th week of exposure that were significantly higher than other treatments. The highest mean value was 129.06±0.37 nM/mL obtained in samples treated with 26 mg/L at the 10th week of exposure. (Table 4.53). In another development, the AST mean values produced in the kidneys indicated that the samples subjected to 61 mg/L generated mean values in both 2nd and 4th weeks of exposure, respectively which were significantly higher than other treatments. The specimen treated with 44 mg/L had mean values in the 8th week that were significantly higher than other treatment. The mean values produced from samples exposed to 26 mg/L and 61 mg/L in the 10th and 12th weeks of exposure were significantly higher than other treatments. The highest AST mean value in the kidneys of the fish was 112.81±0.19 nM/mL obtained in fishes treated with 26 mg/L at the 10th week of exposure. (Table 4.54). Furthermore, the gills' AST production levels indicated that specimens treated with 44 mg/L generated mean values in the 2nd and 4th weeks of exposure, respectively which were significantly higher than other treatments. Likewise, samples subjected to 79 mg/L had mean values in the 8th and 10th weeks of exposure, respectively that were significantly higher than other treatments. The specimens treated with 44 mg/L produced mean values in the 12th week of exposure that were significantly higher than other treatments. The highest

AST mean value in the gill was 125.78±0.28 nM/mL obtained in in the peak concentration at the 8th week of exposure. (Table 4.55).

Table 4.53: AST Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	90.62±0.19^{e}	105.00±0.19^{c}	0.00±0.00	6.56±0.37^{a}	81.25±5.78^{a}	19.21±0.28^{b}
26	46.71±0.28^{c}	72.96±0.28^{b}	0.00±0.00	58.43±0.37^{d}	129.06±0.37^{e}	82.50±0.19^{b}
44	37.81±0.37^{b}	23.59±0.10^{a}	0.00±0.00	8.28±0.10^{b}	97.34±0.28^{c}	122.65±0.28^{e}
61	22.65±0.10^{a}	116.71±0.10^{d}	0.00±0.00	28.59±0.28^{c}	82.34±0.28^{b}	30.31±0.19^{a}
79	71.25±0.19^{d}	0.00±0.00	0.00±0.00	0.00±0.00	112.18±0.19^{d}	84.37±0.19^{c}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.54: AST Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	47.03±0.10^{d}	15.31±0.19^{c}	70.00±0.19	13.59±0.62^{b}	120.31±0.19^{e}	45.46±0.46^{a}
26	30.46±0.10^{b}	7.34±0.28^{b}	0.00±0.00	13.12±0.19^{b}	112.81±0.19^{d}	79.53±0.28^{b}
44	32.65±0.28^{c}	7.65±0.28^{b}	0.00±0.00	25.31±0.19^{c}	102.03±0.10^{c}	97.81±0.19^{c}
61	47.96±0.10^{e}	30.93±0.19^{d}	0.00±0.00	10.78±0.10^{a}	97.81±0.19^{b}	100.00±0.19^{d}
79	29.53±0.10^{a}	3.43±0.19^{a}	0.00±0.00	0.00±0.00	44.21±0.28^{a}	0.00±0.00

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.55: AST Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	41.09±0.10^{e}	152.18±0.19^{e}	120.15±0.10	9.68±0.19^{a}	100.15±0.10^{d}	42.18±0.19^{c}
26	9.68±0.19^{a}	39.21±0.10^{b}	0.00±0.00	69.21±0.10^{c}	38.75±0.19^{a}	56.87±0.19^{d}
44	27.18±0.19^{d}	89.53±0.28^{d}	0.00±0.00	62.65±0.10^{b}	69.84±0.28^{c}	94.06±0.19^{e}
61	16.56±0.19^{b}	58.28±0.28^{c}	0.00±0.00	109.68±0.19^{d}	42.65±0.10^{b}	18.90±0.28^{a}
79	19.68±0.19^{c}	30.46±0.28^{a}	0.00±0.00	125.78±0.28^{e}	100.00±0.19^{d}	27.18±0.37^{b}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E, the AST production in the liver of the fish indicated that, fishes exposed to 44 mg/L,26 mg/L,79 mg/L, 79 mg/L, 61 mg/L and 26 mg/L produced mean values across their respective columns in the 2nd to the 12th weeks of exposure which were significantly higher than other treatments. The highest mean value in this perspective was 135.78±0.28 nM/mL obtained in samples treated with 26 mg/L at the 4th week of exposure. (Table 4.56). In addition to the forgoing, the 26 mg/L samples' mean values in the kidneys in 2nd week of exposure were significantly higher than other treatments. The specimens exposed to 44 mg/L produced mean values in both 4th and 6th weeks of exposure that were significantly higher than other treatments. Treatments with 61 mg/L and 79 mg/L generated mean values in the 10th and 12th week of exposure that were significantly higher than other treatments. The highest mean value of AST produced in the kidney was 123.90±0.10 nM/mL obtained in the maximum concentration of the toxicant at the end of the 12th week of exposure. (Table 4.57). On the other hand, the 26 mg/L, 44 mg/L and 26 mg/L samples' mean values of AST produced in the gill at the end of the 2nd, 4th and 6th weeks of exposure, respectively were significantly higher than other treatments. Also, specimens subjected to 79 mg/L and 26 mg/L had mean values in the 10th and 12th weeks of exposure which were significantly higher than other treatments. The highest mean value in this case was 153.12±0.19 nM/mL obtained in the samples exposed to 44 mg/L at the end of the 4th week. (Table 4.58).

Table 4.56: AST Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	90.62±0.19^{b}	105.00±0.19^{d}	0.00±0.00	6.56±0.37^{a}	81.25±5.78^{a}	19.21±0.28^{a}
26	84.84±0.28^{a}	135.78±0.28^{e}	23.75±0.37^{a}	37.96±0.28^{b}	91.56±0.19^{b}	122.34±0.28^{d}
44	99.53±0.10^{d}	42.81±0.19^{b}	72.34±0.28^{b}	0.00±0.00	93.75±0.19^{c}	0.00±0.00
61	94.68±0.37^{c}	45.78±0.28^{c}	110.00±0.19^{c}	0.00±0.00	128.28±0.10^{e}	82.96±0.28^{b}
79	99.06±0.19^{d}	22.81±0.19^{a}	119.53±0.19^{d}	95.00±0.19^{c}	120.31±0.19^{d}	90.93±0.19^{c}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.57: AST Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	47.03±0.10^{b}	15.31±0.19^{a}	70.00±0.19^{a}	13.59±0.82^{b}	23.75±0.19^{a}	45.46±0.46^{b}
26	96.71±0.28^{e}	45.62±0.19^{d}	106.71±0.10^{c}	44.53±0.10^{c}	53.75±0.37^{b}	73.59±0.28^{d}
44	75.15±0.10^{c}	72.50±0.37^{e}	120.15±0.10^{e}	0.00±0.00	53.75±0.37^{b}	32.81±0.19^{a}
61	94.84±0.10^{d}	29.53±0.28^{c}	109.53±0.10^{d}	0.00±0.00	82.96±0.28^{d}	68.59±0.10^{c}
79	25.31±0.19^{a}	20.46±0.10^{b}	89.06±0.19^{b}	5.62±0.19^{a}	56.87±0.37^{c}	123.90±0.10^{e}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.58: AST Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	41.09 ± 0.10^{b}	152.18 ± 0.19^{d}	120.15 ± 0.10^{e}	9.68 ± 0.19^{b}	100.15 ± 0.10^{d}	42.18 ± 0.19^{b}
26	101.40 ± 0.10^{e}	134.53 ± 0.10^{c}	113.59 ± 0.28^{d}	0.93 ± 0.19^{a}	4.84 ± 0.28^{a}	123.90 ± 0.10^{e}
44	100.00 ± 0.19^{d}	153.12 ± 0.19^{e}	91.25 ± 0.19^{c}	0.00 ± 0.00	96.09 ± 0.10^{b}	11.09 ± 0.28^{a}
61	66.09 ± 0.10^{c}	3.43 ± 0.18^{a}	48.43 ± 0.19^{a}	0.00 ± 0.00	99.84 ± 0.28^{c}	62.96 ± 0.28^{c}
79	20.31 ± 0.37^{a}	22.65 ± 0.10^{b}	82.50 ± 0.19^{b}	0.00 ± 0.00	110.62 ± 0.37^{e}	91.87 ± 0.19^{d}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

4.1.6.4 ALT production levels in Liver, Kidneys and gills of C. gariepinus exposed to sub-lethal concentrations of $Pb(NO_3)_2$ toxicant and the respective supplemented treatments with Vitamins A, C and E for a period of twelve weeks and sampled fortnightly

From the results of the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, the ALT production levels indicated that samples exposed to 26 mg/L and 79 mg/L generated mean values in the 2^{nd} and 4^{th} weeks of exposure, respectively which were significantly ($P<0.05$) higher than other treatments including the control. Also, the specimens exposed to 79 mg/L had mean values in both 6^{th} and 8^{th} weeks of exposure that were significantly higher than other treatments including the control. Similarly, the 61 mg/L samples' mean values in the 10^{th} and 12^{th} weeks of exposure, respectively were significantly higher than other treatments including the control. The highest mean value of ALT produced in the liver of the samples was 87.20±0.15 nM/mL obtained in the maximum concentration of the toxicant at the 4^{th} week of exposure. (Table 4.59). On the other hand, the samples exposed to 26 mg/L had mean values in the kidneys of the fish that were significantly higher than other treatments including the control in both 2^{nd} and 4^{th} week of exposure. The control mean values in the 6^{th}, 8^{th} and 10^{th} weeks of exposure were significantly higher than other treatments. The samples treated with 61 mg/L concentration produced mean values in the 6^{th} week of exposure that were significantly higher than other treatments. The 79 mg/L treatment had mean values in the 12^{th} week of exposure that were significantly higher than other treatments. This peak concentration of the toxicant generated mean value (65.76±0.20 nM/mL) in the 12^{th} week of exposure was also the highest ALT produced in the kidney. (Table 4.60). Furthermore, specimens subjected to 26 mg/L, 61 mg/L and 79 mg/L

concentrations of the toxicants produced mean values in the gill at the end of the 2nd, 4th and 6th weeks of exposure, respectively were significantly higher than other treatments including the control. The treatments with 26 mg/L and 61 mg/L generated mean values in samples at the 8th and 12th weeks of exposure, respectively which were significantly higher than other treatments including the control. The control mean values in the 10th week of exposure were significantly higher than other treatments. The highest ALT mean value produced in the gill was 69.92±0.05 nM/mL obtained in samples exposed to 61 mg/L at the end of the 12th week of exposure. (Table 4.61).

Table 4.59: ALT Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	48.48±0.20^{d}	8.73±0.15^{c}	66.69±0.05^{c}	65.17±0.05^{d}	48.90±0.15^{a}	23.12±0.15^{a}
26	55.43±0.20^{e}	8.90±0.05^{c}	57.71±0.15^{b}	49.32±0.10^{c}	67.46±0.20^{d}	43.48±0.15^{c}
44	27.12±0.10^{b}	4.49±0.15^{b}	26.02±0.15^{a}	2.54±0.10^{a}	63.90±0.20^{c}	26.19±0.15^{b}
61	34.66±0.05^{c}	1.01±0.10^{a}	26.19±0.05^{a}	5.76±0.10^{b}	70.26±0.05^{e}	71.27±0.05^{e}
79	26.78±0.10^{a}	87.20±0.15^{d}	68.48±0.10^{d}	72.46±0.15^{e}	62.12±0.05^{b}	49.66±0.10^{d}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.60: ALT Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	48.48±0.20^{d}	21.87±0.10^{d}	68.39±0.15^{d}	12.54±0.10^{e}	54.32±0.05^{e}	16.10±0.10^{a}
26	55.43±0.20^{e}	32.29±0.15^{e}	51.44±0.15^{b}	5.00±0.15^{b}	53.65±0.15^{d}	32.71±0.10^{b}
44	27.12±0.10^{b}	8.65±0.20^{b}	0.00±0.00	6.01±0.24^{c}	49.32±0.20^{b}	57.20±0.15^{d}
61	34.66±0.05^{c}	5.34±0.15^{a}	59.75±1.42^{c}	3.82±0.15^{a}	20.76±0.15^{a}	46.95±0.10^{c}
79	26.78±0.10^{a}	12.29±0.15^{c}	46.44±0.10^{a}	7.71±0.15^{d}	52.37±0.10^{c}	65.76±0.20^{e}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.61: ALT Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	26.61±0.20^{b}	10.68±0.20^{b}	7.80±0.10^{a}	19.32±0.10^{c}	77.37±0.05^{e}	24.49±0.05^{a}
26	63.6±0.10^{d}	2.37±0.10^{a}	37.88±0.15^{b}	24.66±0.05^{e}	53.22±0.10^{b}	57.71±0.15^{c}
44	11.01±0.10^{a}	48.56±0.05^{d}	60.68±0.10^{c}	22.04±0.10^{d}	54.24±0.10^{c}	45.00±0.05^{b}
61	26.70±0.05^{b}	61.36±0.10^{e}	60.09±0.05^{c}	4.07±0.20^{a}	30.17±0.10^{a}	69.92±0.05^{d}
79	57.03±0.15^{c}	17.54±0.05^{c}	66.87±0.15^{d}	9.41±0.05^{b}	55.17±0.15^{d}	45.76±0.10^{b}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

From the results of the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, and supplemented with vitamin A, the ALT production levels in the liver indicated that samples treated with 26 mg/L had mean values in both 2nd and 4th weeks of exposure, respectively which were significantly higher than other treatments including the control. Also, the specimens exposed to 61 mg/L, 79 mg/L and 61 mg/L generated mean values in the 8th, 10th and 12th weeks of exposure which were significantly higher than other treatments including the control. The highest mean value of ALT produced in the liver of the samples was 77.12±0.20 nM/mL obtained in samples subjected to 26 mg/L at the 4th week of exposure. (Table 4.62). On the other hand, the 26 mg/L samples' mean values in the kidneys of the fish were significantly higher than other treatments in 2nd week of exposure. The fishes treated with 44 mg/L and 61 mg/L produced mean values in the 4th and 8th weeks of exposure that were significantly higher than other treatments. The specimens exposed with 26 mg/L and 61 mg/L had mean values in the 10th and 12th weeks of exposure which were significantly higher than other treatments. The highest mean value of ALT produced in the kidney was 84.75±0.10 nM/mL obtained from samples treated with 61 mg/L at the end of the 12th week of exposure. (Table 4.63). Furthermore, the highest and lowest concentrations of the toxicant generated mean values in the gill of the samples at the 2nd and 4th weeks of exposure, respectively which were significantly higher than other treatments. The specimens subjected to 26 mg/L, 79 mg/L and 61 mg/L had mean values that were significantly higher than other treatments in the 8th, 10th and 12th weeks of exposure, respectively. The highest ALT mean value produced in the gill was 70.43±0.24 nM/mL obtained in the highest concentration of the toxicant at the end of the 10th week of exposure. (Table 4.64).

Table 4.62: ALT Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	48.48±0.20[b]	8.73±0.15[a]	66.67±0.05	65.17±0.05[d]	48.90±0.15[b]	23.14±0.15[a]
26	56.02±0.05[d]	77.12±0.20[e]	0.00±0.00	22.63±0.15[a]	61.87±0.10[c]	48.90±0.15[d]
44	41.87±0.20[a]	23.14±0.15[c]	0.00±0.00	44.07±0.10[b]	26.36±0.15[a]	27.20±0.05[c]
61	55.51±0.05[c]	18.14±0.10[b]	0.00±0.00	74.07±0.20[e]	0.00±0.00	56.02±0.15[e]
79	48.22±0.05[b]	63.56±0.20[d]	0.00±0.00	50.34±0.10[c]	64.75±0.10[d]	24.83±0.15[b]

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.63: ALT Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	65.68±0.15[e]	21.87±0.10[c]	69.39±0.15	12.54±0.10[a]	54.32±0.05[a]	16.10±0.10[c]
26	34.75±0.20[d]	17.88±0.05[b]	0.00±0.00	13.05±0.10[b]	62.29±0.15[c]	46.70±0.05[d]
44	16.78±0.10[b]	84.75±0.10[d]	0.00±0.00	54.66±0.15[d]	0.00±0.00	10.00±0.10[b]
61	27.37±0.15[c]	0.00±0.00	0.00±0.00	23.05±0.10[c]	0.00±0.00	84.83±.0.15[e]
79	7.31±0.19[a]	16.02±0.05[a]	0.00±0.00	60.00±0.10[e]	54.41±0.10[a]	7.34±0.24[a]

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.64: ALT Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin A for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	26.61 ± 0.20^{a}	10.68 ± 0.20^{b}	7.80 ± 0.10	19.32 ± 0.10^{b}	77.37 ± 0.05^{e}	24.49 ± 0.05^{c}
26	51.44 ± 0.15^{c}	26.78 ± 0.10^{d}	0.00 ± 0.00	59.49 ± 0.10^{e}	56.19 ± 0.05^{a}	23.82 ± 0.05^{b}
44	33.56 ± 0.10^{b}	26.01 ± 0.15^{d}	0.00 ± 0.00	6.19 ± 0.15^{a}	64.15 ± 0.05^{b}	10.93 ± 0.15^{a}
61	33.31 ± 0.05^{b}	0.76 ± 0.05^{a}	0.00 ± 0.00	56.61 ± 0.10^{d}	69.92 ± 0.05^{c}	54.83 ± 0.15^{e}
79	63.05 ± 0.10^{d}	18.98 ± 0.20^{c}	0.00 ± 0.00	55.00 ± 0.15^{c}	70.43 ± 0.24^{d}	31.87 ± 0.10^{d}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

From the results of the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, and supplemented with vitamin C, the ALT production levels in the liver indicated that the highest concentration of the toxicant had mean values in both 2nd and 4th weeks of exposure, respectively that were significantly higher than other treatments. Also, the samples subjected to 61 mg/L, 26 mg/L and 44 mg/L produced mean values that were significantly higher than other treatments at the end of the 8th, 10th and 12th weeks of exposure. The highest ALT mean value produced in the liver was 86.53±0.05 nM/mL obtained in in the peak concentration of he toxicant at the end of the 4th week of exposure. (Table 4.65). On the other hand, the specimens treated with 44 mg/L had mean values in the kidneys of the fish that were significantly higher than other treatments in both 2nd and 4th week of exposure. The fishes exposed to 26 mg/L, 79 mg/L and 44 mg/L produced mean values in the 8th, 10th and 12th weeks of exposure which were significantly higher than other treatments. The highest ALT produced in the kidney was 63.48±0.15 nM/mL obtained from samples treated with 44 mg/L at the end of the 12th week of exposure. (Table 4.66). Furthermore, treatments with 26 mg/L and 44 mg/L samples' mean values produced in the gill in the 2nd and 4th weeks of exposure, respectively were significantly higher than other treatments. The samples subjected to 44 mg/L had mean values in both 8th and 12th weeks of exposure, respectively that were significantly higher than other treatments. The highest ALT mean value produced in the gill was 66.53±0.15 nM/mL obtained in treatment with 44 mg/L concentration at the end of the 12th week of exposure. (Table 4.67).

Table 4.65: ALT Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	48.48±0.20^{e}	8.73±0.15^{b}	66.70±0.05	65.17±0.05^{c}	48.90±0.15^{a}	23.14±0.15^{a}
26	8.90±0.15^{a}	0.34±0.20^{a}	0.00±0.00	21.10±0.05^{a}	66.36±0.15^{d}	42.20±0.10^{d}
44	21.19±0.10^{c}	14.32±0.05^{c}	0.00±0.00	46.27±0.20^{b}	0.00±0.00	68.39±0.15^{e}
61	12.88±0.10^{b}	23.98±0.15^{d}	0.00±0.00	65.34±0.05^{c}	54.41±0.10^{b}	33.73±0.10^{c}
79	41.87±0.10^{d}	86.53±0.05^{e}	0.00±0.00	0.00±0.00	59.66±0.10^{c}	25.93±0.10^{b}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.66: ALT Production levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	65.68±0.15^{e}	21.87±0.10^{c}	68.39±0.15	12.54±0.10^{a}	54.32±0.05^{a}	16.10±0.10^{a}
26	17.37±0.15^{c}	26.10±0.10^{d}	0.00±0.00	62.12±0.15^{d}	60.41±0.15^{b}	23.05±.0.10^{b}
44	18.39±0.15^{d}	59.58±0.15^{e}	0.00±0.00	27.37±0.15^{b}	0.00±0.00	63.48±0.15^{d}
61	5.51±0.05^{a}	16.87±0.05^{a}	0.00±0.00	48.48±0.10^{c}	62.63±0.15^{b}	41.19±0.10^{c}
79	16.61±0.20^{b}	19.41±0.15^{b}	0.00±0.00	0.00±0.00	63.05±0.10^{d}	0.00±0.00

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.67: ALT Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin C for a Period of 12 Weeks.

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	26.61 ± 0.20^{e}	10.68 ± 0.20^{a}	7.80 ± 0.10	19.32 ± 0.10^{c}	77.37 ± 0.05^{b}	24.49 ± 0.05^{a}
26	17.23 ± 0.11^{d}	10.40 ± 0.23^{a}	0.00 ± 0.00	20.96 ± 0.11^{d}	57.12 ± 0.17^{a}	39.21 ± 0.06^{c}
44	16.02 ± 0.15^{c}	42.04 ± 0.10^{d}	0.00 ± 0.00	35.59 ± 0.10^{e}	0.00 ± 0.00	66.53 ± 0.15^{e}
61	9.75 ± 0.05^{a}	26.19 ± 0.05^{b}	0.00 ± 0.00	7.12 ± 0.10^{a}	0.00 ± 0.00	49.83 ± 0.10^{d}
79	11.10 ± 0.05^{b}	32.20 ± 0.10^{c}	0.00 ± 0.00	15.76 ± 0.20^{b}	0.00 ± 0.00	36.27 ± 0.10^{b}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

From the results of the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, and supplemented with vitamin E, the ALT production levels in the liver indicated that samples subjected to 44 mg/L and 79 mg/L had mean values in both 2nd and 4th weeks of exposure, respectively that were significantly higher than other treatments. Also, the specimens exposed to 79 mg/L, 26 mg/L, 79 mg/L and 44 mg/L produced mean values in the 6th, 8th, 10th and 12th weeks of exposure, respectively which were significantly higher than other treatments. The highest ALT mean value produced in the liver was 73.82±0.15 nM/mL obtained in the maximum concentration of the toxicant at the end of the 10th week of exposure. (Table 4.68). On the other hand, the fishes exposed to 26 mg/L and 79 mg/L generated mean values in the kidneys of the fish that were significantly higher than other treatments in both 2nd and 4th week of exposure. There were gradual increases in the levels of production from the 2nd to the 6th weeks of exposure in both 44 mg/L and 61 mg/L samples. The fishes treated with 61 mg/L, 79 mg/L, 79 mg/L and 61 mg/L generated mean values at the end of the 6th, 8th, 10th and 12th weeks of exposure which were significantly higher than other treatments. The highest ALT produced in the kidney was 78.05±0.15 nM/mL obtained in 79 mg/L at the end of the 4th week of exposure. (Table 4.69). Furthermore, 44 mg/L, 26 mg/L and 44 mg/L had mean values produced in the gill in the 2nd, 4th and 6th weeks of exposure, respectively that were significantly higher than other treatments. The sample exposed to 61 mg/L produced mean values in both 10th and 12th weeks of exposure, respectively that were significantly higher than other treatments. There were gradual increases in the levels of production of ALT from the 2nd to the 6th weeks of exposure in samples exposed to the highest concentration of the toxicant. The highest ALT mean value

produced in the gill was 73.31±0.05 nM/mL obtained from samples subjected to 61 mg/L at the end of the 10th week of exposure. (Table 4.70).

Table 4.68: ALT Production Levels in the Liver of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	**4**	**6**	**8**	**10**	**12**
C	48.48±0.20^{b}	8.73±0.15^{b}	66.70±0.05^{c}	65.17±0.05^{d}	48.90±0.15^{b}	23.14±0.15^{c}
26	46.61±0.10^{a}	4.07±0.10^{a}	39.24±0.05^{a}	54.32±0.05^{c}	73.31±0.15^{d}	11.10±0.05^{a}
44	54.58±0.10^{e}	35.68±0.05^{d}	66.78±0.20^{c}	0.00±0.00	44.15±0.05^{a}	53.39±0.10^{d}
61	52.04±0.10^{c}	10.43±0.15^{c}	58.22±0.15^{b}	0.00±0.00	64.58±0.10^{c}	0.00±0.00
79	53.90±0.10^{d}	36.44±0.10^{e}	67.20±0.05^{d}	50.34±0.20^{a}	73.82±0.15^{d}	20.17±0.10^{b}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.69: ALT Production Levels in the Kidney of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	65.68±0.15^{e}	21.87±0.10^{a}	68.39±0.15^{d}	12.54±0.10^{a}	54.32±0.05^{d}	16.10±0.10^{a}
26	53.14±0.05^{d}	75.17±0.05^{d}	15.93±0.39^{a}	48.98±0.10^{b}	53.48±0.15^{c}	19.49±0.10^{b}
44	41.70±0.20^{b}	56.10±0.10^{b}	57.88±0.15^{b}	0.00±0.00	25.85±0.15^{a}	28.48±0.10^{c}
61	52.37±0.10^{c}	58.31±0.10^{c}	68.39±0.05^{d}	0.00±0.00	47.63±0.10^{b}	57.71±0.15^{e}
79	15.00±0.15^{a}	78.05±0.15^{e}	66.61±0.10^{c}	68.14±0.10^{c}	59.58±0.15^{e}	42.63±0.05^{d}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

Table 4.70: ALT Production Levels in the Gill of *C. gariepinus* Exposed to Sub-lethal Concentrations of $Pb(NO_3)_2$ and Supplemented with Vitamin E for a Period of 12 Weeks

Ts	Exposure Duration (Weeks)					
	2	4	6	8	10	12
C	26.61±0.20^{b}	10.68±0.20^{c}	7.80±0.10^{a}	19.32±0.10^{a}	77.37±0.05^{e}	24.49±0.05^{d}
26	56.10±0.10^{d}	63.82±0.05^{e}	14.07±0.20^{b}	24.66±0.15^{b}	69.32±0.10^{c}	2.37±0.10^{a}
44	56.36±0.15^{d}	8.65±0.10^{b}	61.27±0.15^{e}	0.00±0.00	32.37±0.10^{a}	13.98±0.15^{b}
61	37.37±0.05^{c}	2.46±0.15^{a}	56.78±0.10^{d}	0.00±0.00	73.31±0.05^{d}	69.49±0.10^{e}
79	12.54±0.20^{a}	18.90±0.15^{d}	31.19±0.10^{c}	0.00±0.00	46.70±0.05^{b}	16.27±0.10^{c}

Mean values and standard errors (nM/mL) with different alphabets along the column are significantly different from each other at P<0.05. Ts indicate the treatments (mg/L) and C stands for the control. (n=4).

4.1.7 Histopathological parameters of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ toxicant and the supplemented treatments with Vitamin E

In the livers of the samples of *C. gariepinus* exposed to sub-lethal concentration of Pb, the 26 mg/L concentration samples displayed aggregation and lumping together of the hepatocytes. The 26 mg/L samples of PbVE treatment group on the other hand, showed preserved hepatocytes, reduced aggregation and vacoulation of the cells. In samples treated with 44 mg/L of the Pb only group there were massive necrosis and shattering of the hepatocytes. However, in the 44 mg/L concentration samples of the PbVE group there were also preserved hepatocytes and vacoulation (but not as prominent as PbVE' 26 mg/L concentration above). There is gradual recovery of the cell nucleus and cytoplasm. The 61 mg/L concentration samples of the Pb only group indicated massive necrosis, lumping of hepatocytes as well as vacoulation. In the 61 mg/L samples of the PbVE treatment group on the other hand, there were normal tissue architecture and hepatocytes similar to control samples with hepatocytes displaying prominent nucleoli. In the 79 mg/L samples of the Pb only group there were shattering of the hepatocytes. However, in the peak concentration samples of the PbVE treatment group, there was aggregation and lumping of the hepatocytes and normal hepatocyte gradually returning. (Plate I).

In the kidneys of the samples exposed to sub-lethal concentrations of Pb, the 26 mg/L concentration displayed massive necrosis and vacoulation of the cells. The shattering occurred with loss of nucleus and cytoplasm. However, in the 26 mg/L samples of the PbVE treatment there were reduced necrosis and cells show signs of aggregating together coupled with reduced vacoulation. These cells displayed preserved cells and

cellular swelling. Likewise, in 44 mg/L samples there were massive necrosis and shattering of cells with greater severity than 26 mg/L. In the samples of 44 mg/L exposed to PbVE treatment group there were preserved cells, reduced vacoulation with slight recovery of the cells. The 61 mg/L samples exposed to Pb only group also displayed massive necrosis and severe shattering of cells. Upon supplementation with vitamin E there were cellular swelling and aggregation of cells. The viable areas with cellular swelling took in much of the stains; and cells with cytoplasm returning to normal. Furthermore, the 79 mg/L samples showed massive necrosis, tissue oedema and massive lumping of cells togetr. The 79 mg/L samples exposed to PbVE treatment on the other hand displayed massive necrosis and shattering of the cells with minimal effects of the vitamin. (Plate II).

Furthermore, gills of the samples of 26 mg/L concentration in the Pb only group there were rarefied gill filament with ruptured lamellae. Those supplemented with vitamin E displayed how the gill arch and filaments were restored to certain extent similar to the control. In 44 mg/L samples of the Pb only group, there were shattered gill arch and filaments, ruptured primary and secondary lamellae. On the other hand, in the 44 mg/L samples of the PbVE treatment group there were ruptured gill arch; and gill arch and filament slightly different from the Pb only group. In the 61 mg/L samples, the primary and secondary lamellae have been destroyed or appear shattered. However, in the PbVE group the 61 mg/L samples displayed gradual restoration of the primary and secondary lamellae. In the 79 mg/L samples of the Pb only treatment group, there were shattered filaments. On the other hand, in the 79 mg/L samples of the PbVE treatment group there were re-alignment of the gill arch and filaments. (Plate III).

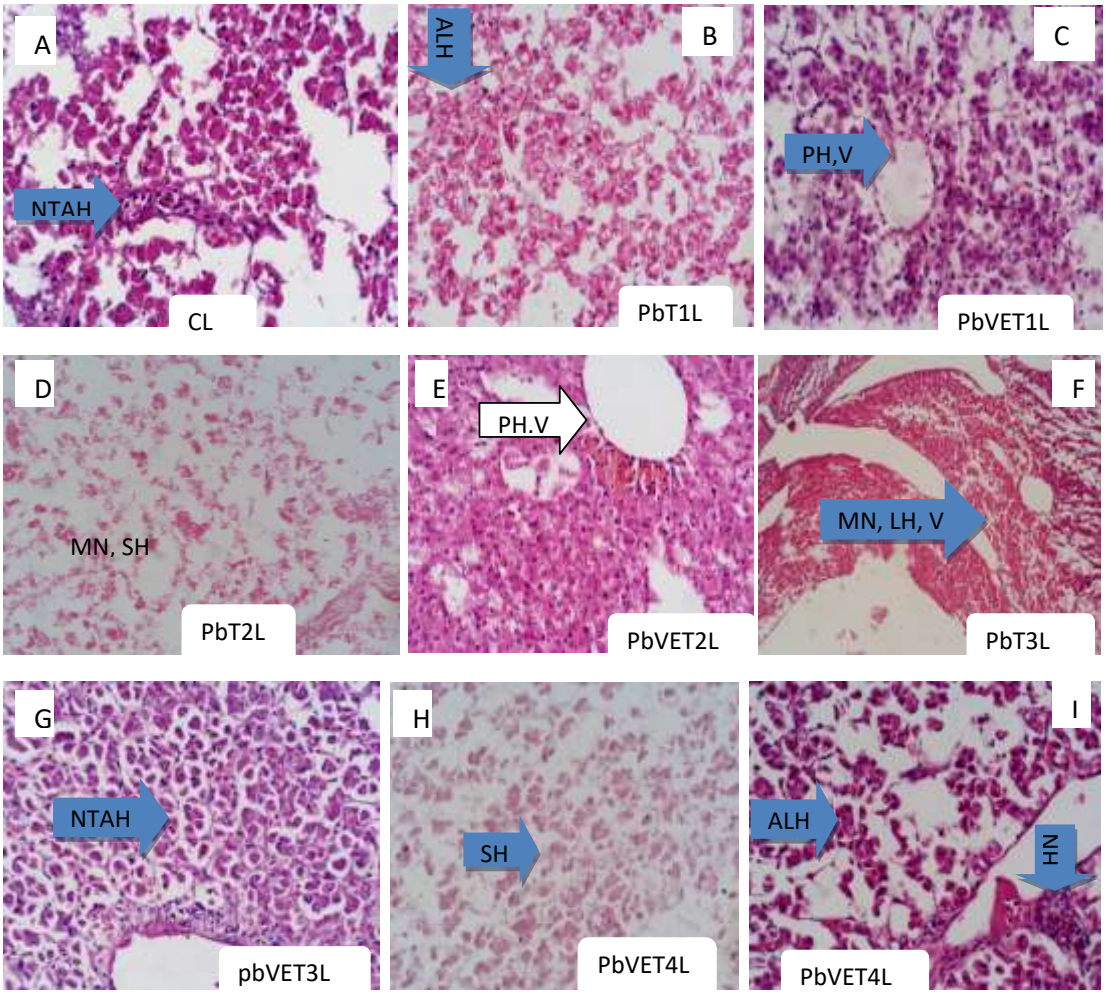

Plate I: Photomicrographs (×400) of Liver of samples of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E for a period of 12 weeks.

The phomicrographs are labeled A to I. CL represents the Control, PbT1L-PbT4L stands for treatments 1-4 (26, 44, 61 and 79 mg/L) in the liver of the samples of the Pb only group; PbVET1L-PbVET4L: stands for treatments 1-4 (26, 44, 61 and 79 mg/L) in Pb supplemented with vitamin E treatment group. NTAH stands for Normal Tissue Architecture and Hepatocytes, ALH: Aggregation and Lumping together of the Hepatocytes, MN: Massive Necrosis, SH: Splitting of Hepatocytes, V: Vacoulation, PH: Preserved Hepatocytes and NH: Normal Hepatocytes.

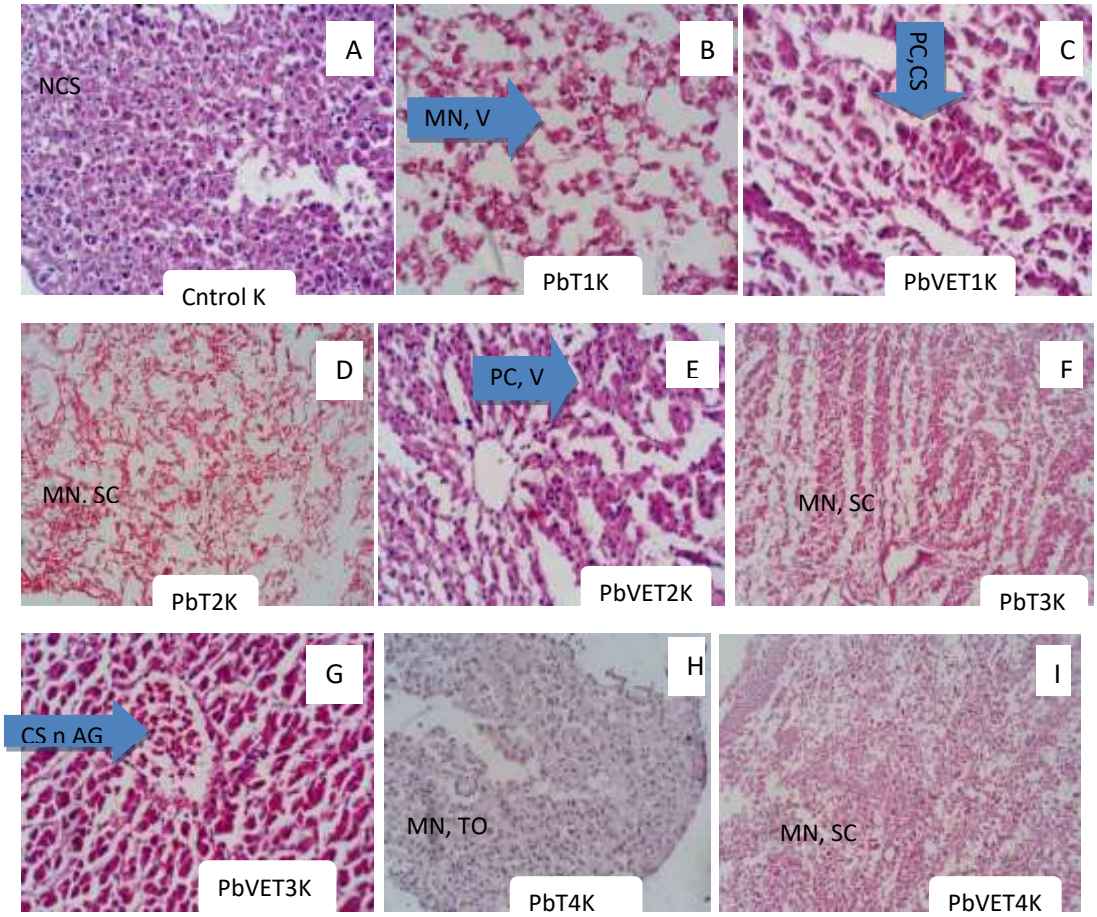

Plate II: Photomicrographs (×400) of Kidneys of samples of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E for a period of 12 weeks.

The slides are labeled from A to I, respectively. CL is the Control, PbT1K-PbT4K stands for treatments 1-4 (26, 44, 61 and 79 mg/L) in the kidney of the samples of the Pb only group; PbVET1K-PbVET4K: stands for treatments 1-4 (26, 44, 61 and 79 mg/L) in Pb supplemented with vitamin E. CS stands for Cellular Swelling, AG: Aggregation of cells, NCS: Normal Cells and Structure, MN: Massive Necrosis, V: Vacoulation of cells, PC: Preserved Cells, SC: Splitting of Cells and TO: Tissue Oedema.

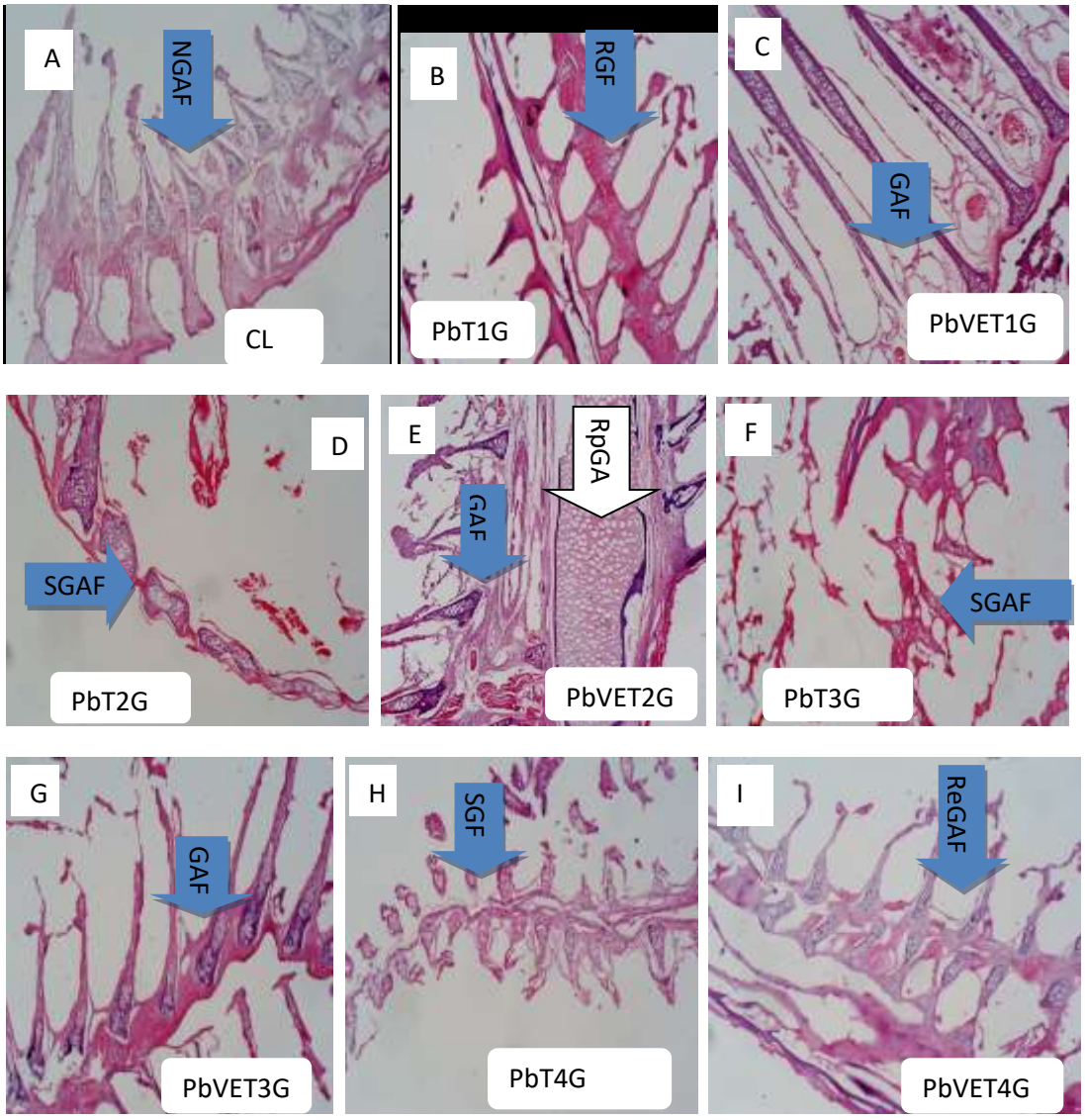

Plate III: Photomicrographs (×400) of Gills of samples of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E for a period of 12 weeks.

The photomicrographs of the gills are labeled as A to I, respectively. CL stands for the control, PbT1G-PbT4G stands for treatments 1-4 (26, 44, 61 and 79 mg/L) in the gill of the samples of the Pb only group; PbVET1G-PbVET4G: depicts treatments 1-4 (26, 44, 61 and 79 mg/L) in Pb supplemented with vitamin E. NGAF stands for Normal Gill Arch and Filament, RGF: Rarefied Gill Filament, GAF: Gill Arch and Filament, SGAF: Shattered Gill Arch and Filaments, RpGAF: Ruptured Gill Arch, SGF: Shattered Gill Filament and ReGAF: Re-alignment of the Gill Arch and Filaments.

4.2 Discussion

4.2.1 Acute toxicity and LC_{50} of *Clarias gariepinus* exposed to lethal concentrations of Pb

The ability of heavy metals to bioaccumulate, biomagnify and the difficulty in eliminating them from the body by the ordinary metabolic activities make them one of the most dangerous sources of chemical water pollution to fish, causing big losses to fish and effects on the fish consumers (Mirghaed *et al*., 2018). This is so due to the fact that, fish safety just as food safety is an important public health issue because there are numerous diseases acquired by humans on the consumption of contaminated fish (Kassa *et al*., 2010). From the results of the lethal exposure of *C. gariepinus* to Pb it was quite evident that higher concentrations of the Pb compound were required to elicit both behavioural and physical responses of one kind and the other. This is probably indicative of the fact that Pb is less toxic; that is why larger concentration of the toxicant was required to elicit cascades of physiological reactions. The presence of Pb toxicant elicited physical and behavioural responses in *C. gariepinus* fingerlings such as lassitude, emaciation, frequent gulping and gasping for air at the surfaces of the troughs, attempts to jump out of the trough; lacerations and exuding of blood from sides of the opercular. These responses were concentration and duration (time) dependent. These responses were displayed by samples in those treatments with higher concentrations and became much more prominent over time. This is probably because one of the most important factors that influence the aquatic toxicity of Pb is its free ionic concentration and availability to organisms (Baysal *et al*., 2013). These physical and behavioural responses are in conformity with the findings of Samuel *et al*. (2018) on the same species. Similar findings were also reported by Adebayo and Fapohunda

(2016) in which they indicated that, as concentration and time of exposure increased, the fish showed restlessness, rapid opercula movement, erratic swimming, discolouration and loss of reflex before death. In like manner, Aghoghovwia *et al*. (2019) reported that the fish samples exposed to paraquat exhibited change in swimming, opercular movement, body pigmentation, surfacing and air gulping; and that mortality rate increased significantly ($P<0.05$) as the concentration of the toxicant increased as well as the exposure period. Furthermore, the physical and behavioural responses observed in this research are also in agreement with the findings of Kaushal and Mishra (2011) when they observed erratic swimming and excessive mucus production on the opercular surface of the fish, *Channa punctatus*. When these fishes are consumed in one way or the other as staple food by higher vertebrates especially from toxicants occurring in these organisms over a long period of time in their natural environment there is high tendency of bioaccumulation and biomagnifications. When this happens the effects could be deleterious and multi-dimensional even in the most advanced vertebrates. For instance, recent studies indicated that 0-23 months old children in Kabwe, Zambia and three-quarter of the population are estimated to have more than 5µg of Pb per deciliter in their blood, and the children exhibit behavioural problems, learning disabilities and lower IQs (Yamada *et al*., 2020).

The LC_{50} of Pb on *C. gariepinus* in this research was 174.71 mg/L. Higher and lower values were obtained for this species as well as other fish species in previous researches. For instance, the LC_{50} of Cd and Pb were 16.3±0.5 mg/L and 80.6±0.6 mg/L, respectively on *C. gariepinus* fingerlings (Okareh and Akande, 2015). In the same vein, the 96 hrs LC_{50} of Cd and Pb against *C. gareipinus* were 8.280 mg/L and

70.183 mg/L, respectively (Ezeonyejiaku *et al*., 2019). Also, Singh and Ansari (2017) reported the 96 hr LC_{50} of lead as 21.63 mg/L and for cobalt as 69.83 mg/L, and indicated that lead was more toxic to zebrafish as compared to cobalt. The LC_{50} value at 96 hr was found to be 17.33 mg/L to *Oreochromis mossambicus* and that mortality was directly proportional to the concentration of the toxicant (Arya *et al*., 2018). In another research, Ullah *et al*. (2016) reported 96 hr LC_{50} of Lead Nitrate on the fish *Oreochromis niloticus* as 44 mg/L. In another development, the LC_{50} value at 96 hr on *Oreochromis mossambicus* was found to be 17.33 mg/L (Arya *et al*., 2018). In essence, the 96 hrs LC_{50s} are species dependent.

4.2.2 Growth parameters of *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with vitamins A, C and E

The marked improvement in standard lengths of samples exposed to PbVE treatements in comparison to other treatments with the peak at 26 mg/L (19.4 cm) probably indicated the ability of the vitamin to ameliorate the effects of the toxicant such that the fishes in this particular treatment performed beter than even the samples of the control. This finding is in line with Osfor *et al*. (2010) when they demonstrated that vitamin E could improve daily food intake, body weight gain and feed efficiency ratio. It is also known that, fish fed diet supplemented with Vitamin E exhibited protective effects by minimizing the atrazine induced toxicity on female *C. gariepinus*, through measured values more or less similar to control group fish (Kadry *et al*., 2012). Furthermore, intake of vitamin E in lead-exposed fish has been reported to prevent the accumulation of lead in tissues, and enhance the growth factor of fish (Abdalla, 2009).

Also, in the samples exposed to PbVE there was general improvement in weight gain in all treatments with marked growth in 26 mg/L- 61 mg/L with exceptional performance in in those treated with 26 mg/L. The highest weight gain of the samples was recorded in the lowest concentration of the toxicant at the 12^{th} week with 83.26g. These marked increases in weight gain, %WG (429 %) and SGR (4.59 g/day) were in sharp contrast to what were obtained in the PbVA and PbVC treatments. This probably buttresses the effectiveness of vitamine E in neutralizing or at best attenuating the effects of the toxicant, and consequently, culminating in improved growth. It is also likely that the vitamine serve as a nutrient booster for the survival of the fish as evident in 26 mg/L with the lowest concentration of the toxicant. There were poor performance in the Pb only group and not so good performances in the PbVC and PbVA treatment groups in terms of weight and its derivatives. It was evident in this research that, unlike essential elements that are required in diet for optimal growth, functioning and sustenance of the internal environment (Isibor and Imoobe, 2017) the presence of Pb in the environment of the samples was deleterious and created a lot of oxidation imbalances culminating in oxidative stress.Trace elements (especially lead) are naturally deleterious and are capable of eliciting myriads of physiological effects which are sometimes manifested physically. For instance, Ayegbusi *et al*. (2018) also reported growth performance decline and obtained SGR value of 1.95±0.015 mg/L when *C. gariepinus* samples were exposed to sub-lethal concentrations of $PbCl_2$ for a period of 21 days. In addition to this, Oluwatosin *et al*. (2018) reported that the sub-lethal exposure showed a decrease in growth with an increase in $PbCl_2$ concentration and the specific growth rate in the control was higher than other treatments with $PbCl_2$. In another development, Han *et al*. (2019) found that growth and hematological

parameters measured decreased with increasing arsenic concentration, while the concentration of plasma components measured increased. Also, fish exposed to 10, 50 and 100 µgHg/L showed a significant decrease in growth rates as from days 14 to 35 (Pratap, 2016). He attributed this to utilization of energy to overcome physiological stress induced by the toxicant, thus affecting fish growth. As the duration increases, there was lethargy and loss of apetite. This probably account in part for the lack of weight gain in the later stages of the experiment as emaciation of the samples set in after utilizing the available energy to overcome stress. In line with this, Puvaneswari and Karuppasamy (2007) posited that exposure duration evidently affected sensitivity of fish larvae and influence the weight gain in *Heteropneutes fossilis*. This could also be due to the fact that, growth reduction under metal contamination increased the energy costs due to increased metabolism (Sherwood *et al*., 2000); and it is known that growth inhibition is a prominent effect of metal accumulation following chronic exposure (Zebral *et al*., 2018).

4.2.3 Haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with Vitamins A, C and E

Haematological indices are of different sensitivity to various environment factors and chemicals (Akinrotimi *et al*., 2013). In this regard, samples of *C. gariepinus* exposed to sub-ethal concentrations of Pb environment exhibited increased WBC production in all treatments after 4 weeks of exposure. There were drastic decreases in blood platelets in all treatments which were significantly different when compared with the control. This probably buttresses the need for the body to react and elicit defense

against the xenobiotic in its environment. The same thing played out in the 8th week where there were drastic decreases in PLT except in 26 mg/L and 44 mg/L (lower concentrations), increased level of WBC, reduced RBC, Hb and PCV. This scenario also continued in the 12th week. The Hb and PCV mean values in the control were significantly higher than 26 mg/L -79 mg/L. As duration and concentration increase the need for engagement of the body's defense mechanisms may also have increased. The ability of the body's defense mechanisms to utilize platelets in combating the effects of the toxicant may have been overwhelmed especially in higher concentrations, hence, the drastic reduction. In line with this, Mohamed *et al*. (2019) reported how polluted main basin of Lake Mariout environment clearly indicate a significant decrease in RBCs, Hb, Hct, and platelet counts while a significant increase in MCH, MCHC, and WBCs in the *C. gariepinus* samples obtained from them. The findings from this research are also in conformity with Ikeogu *et al*. (2016) when they found that RBC counts, Hb, and Ht of *C. gariepinus* decreased following exposure to 20 and 35 mg/L of $Pb(NO_3)_2$; and that, haematological parameters, such as red blood cells (RBCs), haemoglobin (Hb), and hematocrit (Hct) showed significant ($P< 0.05$) concentration-dependent decreases in fish exposed to $Pb(NO_3)_2$ during both periods (Abdel-Warith *et al*., 2020). At week 8, the WBC mean values in 44 mg/L were significantly higher than 26 mg/L, 61 mg/L and 79 mg/L. Likewise, WBC mean values in 79 mg/L were significantly higher than 26 mg/L-61 mg/L and Control at week 12. The control values in RBC, Hb, PCV, MCHC and PLT were significantly higher than 26 mg/L-79 mg/L. This probably indicates the utilization of these parameters in combating the effects of the toxicant. There also seemed to be struggle for survival of the fish especially at the 12th week of exposure culminating in increased generation of

the white blood cells to counteract the effects of the toxicant. In line with the findings of this research, Abdel-Warith *et al*. (2020) also reported that RBCs, haemoglobin (Hb) and Haematocrit (Hct) showed significant ($P<0.05$) concentration dependent decreases in fish exposed to $Pb(N0_3)_2$. Likewise, Zaki *et al*. (2014) reported that long term exposure of *C. lazera* to Pb and Hg caused a gradual increase in WBCs count. Furthermore**,** Verma *et al*. (2020) reported how PCV decreased drastically relative to control after 28 days of exposure of *Heteropneustes fossilis* to the highest concentration of Pb and that, there were fluctuations in the values of MCV in all treated groups with the highest value of 195.12 μm^3 recorded at he initial stage (7 days) of exposure; and a maximum decline in MCH (7.09 %) observed in 2.65 ppm concentration after 21 and 28 days of exposure.

In samples exposed to sub-lethal concentrations of Pb supplemented with vitamin A, there were increased WBC values, increased values of PLT (which were nearly constant in the 8^{th} and 12 th weeks), reduced values of RBC, Hb and PCV after the 4^{th} and 8^{th} weeks of exposure. The blood platelets in samples exposed to 79 mg/L were also significantly higher than 26 mg/L-61 mg/L and control. The increased WBC and the significance of PLT in 79 mg/L probably points to the fact that there has to be up-regulation of the body's defense mechanisms to deal with the effects of the toxicant in the presence of the vitamin. The RBC, Hb and PCV of the control mean values were significantly higher than 26 mg/L-79 mg/L. These findings are in conformity with Olatunji *et al*. (2015) who reported an increase in blood cell count, and Platelet while there was a decrease in Haematocrit, MCV, MCH, LYMH, HMB, and RBC in higher concentration of 750 mg/L throughout the test in juvenile catfish, *Clarias gariepinus.*

Also, Verma *et al*. (2020) showed how erythrocyte sedimentation rate (ESR) increased relative to control while the PCV decreased drastically in comparison to control in the samples of *H. fossilis* exposed to varying concentrations of lead. In like manner also, Ugwuja *et al*. (2020) reported how PCV, Hbc were significantly reduced whie the total WBC, MCH, MCHC and platelets were significantly elevated in treatments with Pbc group. The presence of vitamin A in the environment of the fish perhaps, created the avenue for the utilization of the body's defense systems especially the blood platelets which increased in conjunction with the WBC in combating the effects of the toxicant unlike in the Pb only treatments where the patelets decreased.

Vitamin C is an important chain-breaking antioxidant and enzyme co-factor against heavy metals. In samples exposed to sub-lethal concentration of Pb supplemented with vitamin C, there were increased values of production of WBC and PLT, and higher values of MCV and MCH after the 4^{th} week of exposure. Also at the 8^{th} week there were increased WBC and reduced RBC. There was probably the need for the repair of the damaged tissue due to haemolysis and up-regulation of the defense system of the fish to deal with the deleterious effects of the toxicant. Abdulkareem *et al*. (2017) also attributed the reduction in the level of RBC, Hb and HCT in the fish to the destruction of RBC and haemolysis caused by the presence of dichlorvos; while increased level of WBC could be due to defence mechanism exhibited by the fish. Increased values of MCV and MCH may have been occasioned by the presence of vitamin C. The PLT values in other treatments were nearly at par with those of the control most likely because of the presence of the vitamin; since vitamin C supplementation in animals exposed to heavy metals not only affects the reduction of oxidative stress, but also

significantly reduces the levels of these metals in different tissues such as blood, liver, kidney, muscle, gills, brain (Sahiti *et al*., 2018; Donpunha *et al*., 2011 and Shahsavani *et al*., 2017). Also, Vitamin C treatment was successful in increasing the haemoglobin and haematocrit levels to normal levels (Xhyrel *et al*., 2016). Furthermore, Saraswati *et al*. (2014) reported how vitamin C ameliorated the effects of dimethoate on *C. batrachus*.

The PLT values in 26 mg/L-79 mg/L were significantly higher than the control at week 4. The production mean values of RBC in the control and 26 mg/L were also significanty higher than 44 mg/L-79 mg/L at week 8. This is probably because the effects became more over bearing in higher concentrations. Mahmoud *et al*. (2013) reported that *C. gariepinus* exposed to 7 mg/L of lead exhibited a significant decrease in their RBCs, Hb, and Hct. Also, similar findings were reported by Abdulkareem *et al*. (2017) when they showed that the values of RBC, Hb and HCT of *C. gariepinus* exposed to different concentrations of dichlorvos for 96 hrs decreased as the concentration of the toxicant increased in comparison to the control and that the, WBC and platelets increased in the fish as the concentration of the toxicant increases. Kim and Kang (2016) also reported significant decrease in the haematocrit and haemaglobin level of *Sebastes schlegelli* exposed to chromium.

Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Li *et al*., 2010). From the results of the samples exposed to sub-lethal concentrations of Pb and supplemented with vitamin E, there were increased WBC and PLT values (which are significantly lower in 26 mg/L-79 mg/L than in the control), reduced RBC, Hb and PCV after the 4^{th} week of

exposure. The same scenario played out in the parameters indicated except PLT in the 8 and 12th week of exposure. The body defence system probably fought with these parameters to combat the deleterious effects of the toxicant. The RBC, MCH, MCHC, Hb and PCV mean values in 26 mg/L-79 mg/L were significantly lower than the mean values of the control. This is in conformity with the findings of Adeyemo (2007) when he reported that, the packed cell volume (PCV) and RBC of the treatments decreased significantly in comparison to that of the control, while their platelet counts increased compared with the control; but the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) increased considerably in all treatments exposed to Pb compared to the control. In like manner, Satish *et al*. (2018) reported significant decrease in the RBC counts, WBC counts, Hb and PCV levels after exposure of the fish, *Channa punctata* to acephate and the MCV, MCH and MCHC levels of the blood on the other hand, were significantly increased. Also, Gaafar *et al*. (2010) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be due to pathological condition in fish exposed to toxicant. The WBC mean values in 79 mg/L were significantly higher than 26 mg/L-61 mg/L and the control at the end of the 12th week of exposure probably because the need for up-regulation of body's defense system to ensure survival as the concentration and duration increased has become more important than ever. WBC, MCV and MCHC showed increasing trend at sub-lethal exposure to cypermethrin (Neelima *et al*., 2015). The MCHC mean values in 26 mg/L were significantly higher than 44 mg/L-79 mg/L and the control. This is probably due to the presence of vitamin E in the environment of the fish samples. As earlier stated, the physical gain in weight and lengths in this

treatment may also have manifested physiologically. Given that, this is the lowest concentration of the exposure the effects of the toxicant may have been diminished to the point of out-performing the control. Vitamins E and C have been shown to have chelating abilities, affecting the reduction in the amount of accumulated metals in different tissues to various organisms, including fish (Donpunha *et al*., 2011). Likewise, the total antioxidant capacity in erythrocytes also returned to values between 58.9 % and 67.7 % in workers exposed to Pb after treatment with vitamins E and C supplements for a year, a level similar to those in exposed non-Pb workers (Rendón-Ramírez *et al*., 2014). Also, Suleiman *et al*. (2010) reported that fish pretreated with vitamins C and E significantly suppressed the adverse haematological effects of the toxicant. In the same vein, Ebuehi *et al*. (2012) indicate that oral administration of vitamins C and E significantly reduced the blood lead concentration, ameliorates the hepatic damage and significantly reduced the oxidative stress in the brain of rats.

4.2.4 Physico-chemical parameters of the test media of *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with vitamins A, C and E

The physico-chemical parameters of the test media tested for were Dissolved Oxygen (DO), pH, Electrical Conductivity (EC), Total Dissolved Solids and the Temperature. From the results there were slight variations from one treatment group to the other which were all within the acceptable limit by relevant authorities for rearing and culturing in aquaculture. For instance, the optimal dissolved oxygen concentration for growth of eggs and juveniles of African catfish (*C. gariepinus*) is 9 mg/L, while adults would survive in water of at least 3 mg/L of dissolved oxygen (FAO, 2013). This may

have direct bearing on the fact that changes and effects witnessed in the whole research may not have arisen from the slight variations in the physico-chemical parameters of the test media but from the toxicant and, or supplements. Also, the ranges of values obtained are within the range for optimal fish production (Boyd and Lickotoper, 2014).

4.2.5 Antioxidants levels of *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with Vitamins A, C and E

4.2.5.1 GSH production levels in C. gariepinus exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with Vitamins A, C and E

Antioxidants serve as superior therapy compared to conventionally-used chelating agents which may have attendant side effects in treatment of lead toxicants. Antioxidants possess both chelating and ROS scavenging capacity enabling elimination of lead from intracellular sites and blood stream which are effective while the subject is still exposed (Lamidi and Akefe, 2017). Also, GSH is the most important non-protein thiol in all living cells and has vital role in protection of intracellular body against toxins such as Cu and Zn through the action of GR, GST and GPx (Saddick *et al*., 2017). From the analysis of the results of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$, the mean values of the glutathione (GSH) produced in the liver of the control samples were significantly higher than other treatments in the first two weeks of exposure to the toxicant. However, 79 mg/L samples' mean values in the 4^{th} week of exposure were significantly higher than the other treatments including the control. This is most likely because at the initial stages of the exposure the samples were adapting to the changing environmental condition

but at the 4th week of exposure there was an increase in the level of production in 79 mg/L probably to counteract the deleterious effects posed by the presence of the toxicant. As the duration of the exposure increased, the effects were probably being felt such that 44 mg/L and 26 mg/L mean values became significantly higher in the 6th and 8th week of exposure, respectively than other treatments. This is also probably the reason why at the end of the 12th week, 26 mg/L samples' mean values were significantly higher than other treatments; and also had the highest mean value of 82.04±0.13 µg/ml in the liver of the fish. Ibrahim (2019) reported that liver enzymes (such as AST and ALT) showed a significant increase in malathion exposed groups; and the activity of SOD, CAT, GSH and GST showed a significant increase ($P<0.05$) when compared to the control groups of *Oreochromis niloticus* exposed to malathion. Also, pendimethalin has been reported to induce leukocytosis, hyperglobulinemia, hyperglycemia and increased lipid peroxidation and decreased levels of glutathione, SOD, catalase, glutathione reductase in the liver tissues (Zaahkook *et al*., 2016). At lower concentrations of the toxicant, the effects are probably minimal. In line with this, Jhamtani *et al*. (2017) reported that hepatic GSH levels in Zebra fish were not altered when exposed to 2 ppm of $Pb(N0_3)_2$. Also in line with the assertions above, increase in reduced glutathione (GSH) level in fish tissues was attributed to presence of defense system to protect the fish from the oxidative stress or could appear as an antioxidant adaptation to metal exposure (Marzouk *et al*., 2017). In like manner, Hermenean *et al*. (2015) observed that the liver has a higher capacity and adaptability to counteract ROS compared to kidney.

In the kidneys of the samples reactions of the fish to the presence of the toxicant indicated that 79 mg/L and 26 mg/L samples' mean values were significantly higher

than other treatments including the control in the first 2 weeks of exposure. This is probably an initial surge in the production level of the antioxidant to curtail the effects of the toxicant. The 61 mg/L samples' mean values (of 30.84±0.10 µg/ml) in the kidney of the fish exposed to the sub-lethal concentration of the toxicant was the highest at the end of the 8th week most likely because the body' defense mechanisms have to be up-regulated to deal with the prevailing conditions. This also probably became evident when the GSH mean values in the 10th week of exposure in the treatments were significantly lower than the control when the production capacity of the body must have dwindled. In line with this, Samuel *et al*. (2017b) reported that GSH production levels were significantly lower in the kidneys of *C. gariepinus* exposed to sub-lethal concentrations of lead nitrate at 28 mg/L and 43 mg/L, respectively. Also, Saliu and Bawa-Allah (2012) reported reduced values of GST, GSH, SOD, CAT and MDA in comparison with the control when *C. gariepinus* was exposed to sub-lethal concentration of lead nitrate for 28 days.

Furthermore, in the gills of the samples exposed to sub-lethal concentration of the toxicant, there were general low production levels at the end of the 4th week of exposure. This is probably because the principal organs of detoxification such as liver and kidney were engaged much more than the gills that served as entrance to the toxicant. The highest GSH production level in the gill of the fish was 31.30±0.10 µg/ml in 44 mg/L samples at the 6th week of exposure. This is probably because at this period of the exposure there is the need to up-regulate the body's defense mechanisms to counteract the effects of the influx of the toxicant. In line with this, Pb has been reported to have significantly increased G-6-PDH activity and decreased GSH level in the gill, both Pb and Cd significantly increased MDA levels in liver and kidneys while

Pb increased its level in gills of the fish and that the combination of Pb and Cd increased MDA level in livers and decreased GSH level in gills (Elarabany and Bahnasawy, 2019).

Vitamin A is a fat-soluble vitamin that plays an important role in vision, bone growth, reproduction, cell division, cell differentiation, growth and general maintenance in animals. From the analysis of the results of the fish samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin A, 44 mg/L samples' mean values were significantly higher than other treatment in the 8^{th} week of exposure. The 26 mg/L samples' mean values in the 10^{th} and 12^{th} weeks were significantly higher than other treatments. In the lower concentrations (26 mg/L and 44 mg/L) the GSH production levels were high probably due to the interventions of the presence of vitamin A coupled with the lower concentrations. The highest mean value of 23.57±0.10 µg/ml in the Liver of the fish also obtained in 26 mg/L at the 10^{th} week of exposure probably because as the duration of exposure increases there is constant need for the up-regulation of the body's defense mechanisms even at lower concentrations to ensure better physiology and survival. Udo (2017) demonstrated how dietary supplementation with vitamin A led to improved growth rate in *C. gariepinus* and recommended that 833-1666 IU/Kg units of vitamin A should be included in the diet for optimum growth and efficient feed utilization. Also, feed consumption and conversion efficiency, protein efficiency ratio, growth, percentage growth, relative growth rate, assimilation and metabolism were greatly improved in feeds supplemented with 400 mg/Kg units of vitamin A (Jeyaraj *et al*., 2012).

Similar scenario played out in the kidneys of the fish such that T1 mean values were significantly higher than other treatments at the 2nd week of exposure. Likewise, 44 mg/L and 26 mg/L samples' mean values were significantly higher than other treatments in the 4th and 8th week of exposure, respectively. However, 79 mg/L samples' mean values in the 10th and 12th weeks of exposure were significantly higher than other treatments. Also, the highest mean value of 58.74±0.07 µg/ml in the kidney was obtained in 79 mg/L samples at the 12th week of exposure. This is probably because as the duration and concentration increased the initial succor provided by the vitamin may have been overwhelmed; hence, the need for sustained increase in the production of the antioxidant to contain the deleterious effects to manageable level. In addition to the foregoing, the gills of the samples of the fish indicated that there were general low GSH production levels in the gills of the fish at the 4th week of exposure. The 44 mg/L and 26 mg/L samples' mean values were significantly higher than other treatments at the 8th and 10th weeks of exposure, respectively. As the duration of exposure increases, the part of the body in constant touches and has larger surface area with the immediate environment, probably up-regulated its defense mechanism to deal with the effects at that point. This is also probably why 26 mg/L samples' mean values were significantly higher than other treatments at the 12th week of exposure and produced the highest mean value of 52.72±0.07 µg/ml.

In the samples of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C, 26 mg/L samples' mean values in the liver of the fish were significantly higher than other treatments at the end of the second week of exposure. The 44 mg/L and 26 mg/L samples' mean values in the 4th and 8th weeks of

exposure, respectively were significantly higher than other treatments. This is probably because the rate of production of the antioxidant elicited by the presence of the toxicant out-weighs the effects at these stages in the treatments with the lower concentrations. However, as the duration of exposure increases there were probably constant needs for the up-regulation of the defense system as evident when the 44 mg/L and 79 mg/L samples' mean values in the 10^{th} and 12^{th} weeks of exposure, respectively were significantly higher than other treatments; and thus the highest mean value of 25.79±0.07 µg/ml in the liver of the fish in this case, was obtained in 79 mg/L at the 12^{th} week of exposure. This is also probably because, fish accumulate pollutants preferentially in their fatty tissues like liver and the effects become apparent when concentrations in such tissues attain a threshold level (Omar *et al*., 2014). In addition to this, Vitamins C and E, or in combination (as antioxidants) has been reported to have ameliorated the hepato-renal and testicular toxicity of abamectin, but were not completely protective, especially in liver tissue (Magdy *et al*., 2016).

On the other hand, 44 mg/L samples' mean values at the 8^{th} week of exposure were significantly higher than other treatments. Also, the 79 mg/L and 26 mg/L samples' mean values were significantly higher than other treatments in the 10^{th} and 12^{th} weeks of exposure. The GSH production level triggered in 44 mg/L probably continued through-out the experiment. This up-regulation of the defense system is probably why the highest mean value of 28.40±0.13 µg/ml was obtained in 44 mg/L at the 12^{th} week of exposure. In line with this, Shaymaa *et al*. (2019) reported that supplementation of group III with vitamin C ameliorated the toxic effect induced by engine oil through improvement of kidney function parameters, elevation of the antioxidant status of tissue and improvement of the histological images of renal and muscle tissues.

Furthermore, the same scenario played out in the gills as the 79 mg/L samples' mean values were significantly higher than other treatments in the 2nd, 4th, 8th and 10th weeks of exposure. The elicited GSH production levels were sustained till the end of the research probably in order to combat the effects of the toxicant and ensure survival. This is also probably why the highest mean value of 37.55±0.03 µg/ml in 79 mg/L samples was obtained in the gills of the fish in this case at the 8th week of exposure for susteined up-regulation of the defense system of the fish at the highest level of concentration and duration; and that the presence of the vitamin may have reduced the burden in lower concentrations. In line with this, Sahiti *et al*. (2020) reported how supplementation of vitamins C and E either alone or jointly had significantly decreased ($P<0.01$; $P<0.05$) levels of accumulated heavy metals in investigated tissues compared to the control and exposed groups. However, GOT and GTP were altered, GSH was significantly reduced and haemoglobin and haematocrit were substantially decreased in juvenile olive flounders (*Pratichthys olivaceus*) exposed to zinc for a period of 2 weeks (Kim and Kang, 2019).

From the results of the samples of fish exposed to sub-lethal concentrations of Pb(NO3)2 and supplemented with vitamin E, the 26 mg/L and 44 mg/L samples' mean values were significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. There may be probably low utilization of this antioxidant in the lower concentrations than in higher concentrations. The 44 mg/L and 61mg/L samples' mean values in the 10th and 12th weeks, respectively were significantly higher than other treatments. The highest mean value of 57.21±0.03 µg/ml in the liver of the exposed samples of the fish was obtained in 61 mg/L at the 12th week of

exposure probably because the initial utilization may have been overtaken by constant susteined production of the antioxidant. In line with this, Kadry *et al*. (2012) posited that oxidative damage in the liver tissues was evident in the increased levels of lipid peroxidation (LPO) and reduced glutathione content (GSH) in the samples of *C. gariepinus* exposed to chronic toxicity of Atrazine. The 26 mg/L and 61 mg/L samples' mean values in the kidneys of the samples were significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. Likewise, the 44 mg/L and 79 mg/L samples' mean values in the 6th and 8th weeks of exposure, respectively were significantly higher than other treatments. This is probably because the level of utilization varies with the concentration and duration of exposure to the toxicant.

Also, the 26 mg/L and 61 mg/L samples' mean values in the 10th and 12th weeks of exposure, respectively were significantly higher than other treatments. The highest mean value of 83.51±0.07 µg/ml in the kidneys of the sample was obtained in 26 mg/L at the 10th week of exposure. This could also be as a result of low utilization of the available antioxidant as well as the constant need for the up-regulation of the defense system to deal with the challenges posed by the toxicant. Furthermore, 79 mg/L and 61 mg/L samples' mean values in the gills of the fish were also significantly higher than other treatments in the 6th and 10th weeks of exposure, respectively. The 61 mg/L mean value of 63.29±0.07 µg/ml obtained at the 12th week was the highest and was significantly higher than other treatments. Also, the need for constant up-regulation of the defense system may have come to play. In line with this, Azeez and Braimah (2020a) reported how the depleted endogenous antioxidants such as GPx, GST and GSH were restored in fish fed vitamin E-supplemented feeds when *C. gariepinus* were

exposed to potassium dichromate. In like manner, Azeez and Braimah (2020b) reported how the depleted endogenous antioxidants such as GPx, GST and GSH were restored in fish fed vitamin E- supplemented feeds when *C. gariepinus* were exposed to copper sulphate.

4.2.5.2 MDA production levels in C. gariepinus exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with Vitamins A, C and E

In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, the MDA liver mean values in 44 mg/L and 61 mg/L in the 2nd and 4th weeks of exposure, respectively, were significantly higher than other treatments including the control. The 44 mg/L samples' mean values in both 6th and 8th weeks of exposure were significantly higher than other treatments including the control. The need to increase the production of MDA as a defense mechanism was probably elicited in 44 mg/L and as the duration of the exposure increases the necessity becomes more pertinent in dealing with the deleterious effects of the toxicant. The same also probably goes for 61 mg/L and 79 mg/L samples' mean values in the 10th and 12th weeks of exposure as they were significantly higher than other treatment including the control to curtail the effects. This is also probably why the highest MDA production level (56.65±0.06 nM/mg) in the liver of the samples was obtained in 61 mg/L at the 10th week of exposure. In line with this, the exposure of rainbow trout to pollutants led to an increase in MDA (Calapoglu *et al*., 2017). This is also, in conformity with the finding of Markiewicz-Górka *et al*. (2015) when they reported that malondialdehyde (MDA) significantly increased in both heart and liver of the animals after combined exposure to metals (Cd,

Pb and Mn); given the fact that liver is the primary organ for detoxification of xenobiotics (Maurya and Malik, 2016).

In another development, the MDA produced in the kidneys of the fish indicated that 79 mg/L samples' mean values were significantly higher than other treatments including the control in the 2nd week of exposure. The mean values of the 4^{th,} 8th and 10th weeks of exposure in the treatments were significantly lower than the control. The effects of the toxicant at the initial stage probably led to the surge in MDA production in the highest concentration. However, this surge may not have been sustained subsequently as the duration of exposure increased. In like manner, Neeratanaphan *et al*. (2020) reported that the levels of plasma MDA in the catfish from the reservoir near the Khon Kaen municipal landfill (polluted) revealed a decrease in plasma MDA and no significant difference as compared with the reference fish. The 79 mg/L samples' mean values in both 6th and 12th weeks of exposure were however, significantly higher than other treatments. When the fish have probably adapted to the prevailing environmental conditions and when the body's defense system must have overcome the initial effects of the toxicant the production level increased; and perhaps, the susteinance of this high level of production of the antioxidant was necessary to ensure survival as the fishes were becoming increasingly emaciated as the duration of the exposure increased. This is also probably why the highest MDA production level (40.33±0.06 nM/mg) in this case was obtained in 79 mg/L at the end of the 10th week of exposure but not higher than the control at this stage of exposure. In line with this, Adeogun *et al*. (2012) also observed significant increase in MDA activity in the kidney and attributed it to high antioxidant (CAT, SOD and GSH) activities recorded in the study.

In the gills of the sample, the control mean values were significantly higher than other treatments in the 2nd week of exposure. The 44 mg/L samples' mean values in both 4th and 6th weeks were significantly higher than other treatments including the control. This is probably because the need for up-regulation of the body's defense system was elicited in 44 mg/L as the duration of exposure increased. This same reason probably goes for 61 mg/L, 44 mg/L and 79 mg/L samples' mean values in the 8th, 10th and 12th weeks of exposure, respectively which were significantly higher than other treatments including the control. The highest MDA mean value of 80.28±0.06 nM/mg in the gills of the samples was obtained in 44 mg/L in the 10th week of exposure probably indicated sustained production of MDA to counteract the effects of the toxicant in this treatment. In line with this, Elarabany and Bahnasawy (2019) reported that both Pb and Cd significantly increased MDA levels in liver and kidneys while Pb increased its level in gills of the fish.

In another development, the samples of fish exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin A, the MDA production in the liver indicated close ranges of values in 26 mg/L-61 mg/L with 44 mg/L samples significantly higher than other treatments in the 2nd week of exposure. The initial spike in the production levels of MDA in the $Pb(NO_3)_2$ only treatments was not witnessed here instead a close range. This is probably arising from the presence of vitamin A in the water matrix of the fish. At the 4th and 8th weeks of exposure, the 44 mg/L samples' mean values were also significantly higher than other treatments. This is probably because the succor provided in this treatment cannot be sustained at higher concentrations of the toxicant especially as the duration of the exposure increased.

Hence, 79 mg/L samples' mean values in the 10th and 12th weeks of exposure were significantly higher than other treatments; and also, the highest mean value of 40.10±0.03 nM/mg at the 12th week was obtained in 79 mg/L specimens. This is probably because the need for up-regulation at the highest concentration must be resuscitated and susteined for the survival of the fish. This is also probably partly due to the fact that the concentration of the vitamin in the water matrix may not have been sufficient to overcome the effects of the concentration of the toxicant present in the water. On the other hand, the MDA produced in the kidneys indicated that 79 mg/L samples' mean values in the 2nd, 10th and 12th weeks of exposure were significantly higher than other treatments. Similar reasons adduced for the responses of liver to the toxicant and the presence of the supplement would probably be plausible for the responses of the kidney perhaps, with greater sensitivity. This is because the highest MDA production in this case was 58.41±0.02 nM/mg obtained in 79 mg/L samples at the 10th week. Furthermore, in the gills of the fish samples, the 79 mg/L samples' mean values in both 2nd and 4th weeks of exposure were significantly higher than other treatments. The gill, portal of entrance to the toxicant probably had to respond first to the onslaught of the toxicant and up-regulate the body's defense in order to counteract the deleterious effects of the toxicant. Subsequently, the lower concentrations responded accordingly as the duration of exposure increases. This is probably why the 26 mg/L samples' mean values in both 10th and 12th weeks of exposure were significantly higher than other treatments, and the highest MDA production in the gill in this case was 80.30±0.05 nM/mg; which was obtained in 26 mg/L samples at the 10th week of exposure. Literatures depicting the ameliorative roles of vitamin A in catfishes; and attenuating the effects of toxicants is rare at the moment. However, Ali

et al. (2019) reported how chitosan, calcium and vitamins A and E combinations attenuated the adverse effects caused by HFD (High Fat-Diets) intake in rats.

From the results of the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C, the 61 mg/L and 79 mg/L samples' MDA mean values in the liver of the samples in the 2nd and 4th weeks of exposure, respectively were significantly higher than other treatments. The higher concentrations probably elicited increased level of production of MDA in order to counteract the effects of the toxicant. This is also probably why the highest MDA production in the liver of the fish was 46.60±0.06 nM/mg, which was obtained in 61 mg/L samples after the 2nd week of exposure. At the initial periods of exposure the effects of the vitamin C supplement were probably felt in the lower concentrations, hence, low level of MDA production. In the long-run however, 44 mg/L samples' mean values in both 8th and 10th weeks of exposure were significantly higher than other treatments probably when the initial succor could no longer be sustained. Vitamin C has been reckoned to have ameliorative or mitigative effects. For instance, administration of 500 mg/day of vitamin C for one month in battery-manufacturing workers has been shown to reduce MDA concentration and nitrite levels improving antioxidant status including erythrocyte osmotic fragility and activities of endogenous antioxidant enzymes by scavenging the ROS generated due to high blood lead levels; vitamin C restores and sustains bones, teeth and cartilage acting as a major antioxidant (Ganesh *et al*., 2016).

In another development, the MDA mean values in the kidneys of the samples indicated that 61 mg/L samples' mean values in the 2nd and 12th week of exposure

were significantly higher than other treatments. The highest mean value in this regard was 40.21±0.02 nM/mg obtained in 61 mg/L samples at the end of 8th week of exposure. In this treatment for optimal physiological balance that would ensure survival of the fish there must probably be up-regulation of the defense system. Furthermore, the 61 mg/L samples' mean values in both 4th and 10th weeks of exposure were significantly higher than other treatments. The effects of this toxicant were probably more deleterious in this treatment much more than in other treatments. Hence, high production rate at one stage of the exposure or the other. This is likely why the highest mean value of 45.07±0.05 nM/mg was obtained from 61 mg/L samples in the 4th week of exposure. Also, the 26 mg/L samples' mean values in the 12th week of exposure were significantly higher than other treatments probably because the extended duration of exposure may have overwhelmed the mitigative effects of the vitamin at earlier stages. In line with this, Sahiti *et al*. (2018) reported how the exposure of *Cyprinus carpio* to mixture of Pb, Cd and Cr induced significant increase in MDA content in their gills; and that, upon administration of vitamin C there were significant decrease in MDA level in the gills of fish that received mixture of the heavy metals.

In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E, the MDA production levels in the liver of the fish indicated that 79 mg/L samples' mean values in the 2nd week of exposure were significantly higher than other treatments. This is probably because at the early stage of the exposure the need for high levels of production of MDA was only necessary at the highest concentration. Subsequently, as the duration of exposure continued other lower concentrations began to feel the impact of the toxicant which may have died down in higher concentrations

due to ability to adapt to the situation. This is probably why the 44 mg/L samples' mean values in both 4th and 6th weeks of exposure were significantly higher than other treatments; as well as 26 mg/L samples' mean values in the 8th and 10th weeks of exposure were significantly higher than other treatments. Also, the highest mean value of MDA in the liver was 40.19±0.03 nM/mg obtained in 26 mg/L samples' at the 10th week of exposure. On the other hand, the 26 mg/L and 61 mg/L samples' mean values in the kidney of the fish were significantly higher than other treatments in the 2nd and 6th weeks of exposure, respectively. The 26 mg/L samples' mean values were significantly higher than other treatments only at 12th week of exposure. The highest mean value in this regard was 40.17±0.47 nM/mg obtained in 26 mg/L samples at the 12th week of exposure. The response of the kidney to the toxicant took a different turn with the lowest concentration out-performing the higher concentrations in terms of high production of MDA. This is probably because the fishes have become bigger far more than other fishes in other treatments. Perhaps, this shows how sensitive the kidney is in responding to the presence of the toxicant and the vitamin in its environment. Furthermore, in the gills of the sample, the 61 mg/L samples' mean values in both 2nd and 4th weeks of exposure were significantly higher than other treatments. The 79 mg/L and 26 mg/L samples' mean values in the 6th and the 12th weeks of exposure were significantly higher than other treatments. The initial increases recorded in 61 mg/L and 79 mg/L samples may have arisen from the need to counter the effects of the toxicant where the concentration of the vitamin may not have been sufficient to do same. The highest MDA mean value produced in the gill in this case was 77.04±0.06 nM/mg obtained in 26 mg/L samples at the end of the 12th week of exposure. This is probably because the fishes have grown bigger at this stage and

particularly in this treatment. Humayun *et al*. (2015) demonstrated how supplementation of intoxicated bird with vitamin E displayed an amelioration and improvement in the tissue integrity of the samples treated with Pb. Likewise, the serum MDA level and CAT activity were positively correlated with blood levels of Pb and Cd treated samples; and that MDA levels were significantly higher in glazers exposed to Pb and Cd treatments than that of the control (Hormozi *et al*., 2018). Furthermore, the administration of vitamin E resulted in significantly decreased MDA and nitrogen oxide concentrations in the cerebral cortex of rats; significantly increase the brain GSH levels and activities of CAT and SOD when compared to lead only groups indicative of the capability of vitamins to reduce or mitigate ROS-induced negative effects elicited by the presence of Pb (Ebuehi *et al*., 2012).

4.2.5.3 AST production levels in C. gariepinus exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with Vitamins A, C and E

In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, the AST production levels in the liver of the fish indicated that the control mean values in the 2nd and 4th weeks of exposure were significantly higher than other treatments. The 26 mg/L samples' mean values in the 6th and 8th weeks of exposure were significantly higher than other treatments including the control. The production rate of AST is high in lower concentrations than in higher concentration in the liver of the samples probably because at lower concentrations lesser concentration of AST is needed in combating the effects of the toxicant; hence greater availability in the already elicited production. This is also probably why the 26 mg/L and 44 mg/L samples' mean values in the 10th week of exposure were significantly higher than other treatments including the control,

as well as the production of the highest mean value of AST (124.68±0.19 nM/mL) in the liver which was obtained from 26 mg/L samples at the 6th week of exposure. In line with this, Adeyemi *et al*. (2014) reported that the liver aspartae aminotransferase activity (AST) showed a significant reduction after exposure to either Pb or cypermethrin alone. However, the mean values of 61 mg/L samples in the 12th week of exposure were significantly higher than other treatments including the control; probably because, at this stage of the exposure there was the need for the up-regulation of the body's defense system to counter the effects of the toxicant. Similar report was given by Abdel-Warith *et al*. (2020) when they reported that hepatic enzyme activities of AST and ALT displayed a significant increase with increasing concentrations and exposure time. Also, sub-lethal concentrations of lead acetate (28.2 and 14.1 ppm) caused an increase of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities in fish *Cirrihinus mrigala* which indicate liver damage (Chavan and Muley, 2014).

On the other hand, the 61 mg/L, 79 mg/L and 26 mg/L samples' mean values in the kidney of the fish were significantly higher than other treatments including the control in the 2nd, 4th and 6th weeks of exposure, respectively. However, the mean values of 79 mg/L samples were significantly higher than other treatments including the control in the 8th week of exposure. There is probably constant build up to upstage the deleterious effects of the toxicants. The highest mean value in this regard was 141.40±0.10 nM/mL obtained in 26 mg/L at the 12th week of exposure. This value is probably indicative of the fact that there was less utilization of AST in the lowest concentration especially towards the end of the research in the kidney of the fish. In line with this, Michael *et al*. (2018) reported that AST, ALT and LDH in fish exposed to atrazine were

significantly increased with increasing atrazine across the treatments relative to control. Furthermore, in the gills of the sample, the 26 mg/L samples' mean values in the 2nd week of exposure were significantly higher than other treatments including the control. This is probably due to differences in the concentration produced and the concentration utilized in combating the effects of the toxicant after initial elicitation. However, the control mean values in the 4th, 6th and 10th weeks of exposure were significantly higher than other treatments. This may be due to the utilization of the AST produced at these stages of the exposure such that the concentrations in the un-exposed samples were higher. The highest AST mean value produced in the gill in this case was 124.21±0.28 nM/mL obtained in 79 mg/L samples at the end of the 12th week of exposure. At this stage of exposure, there is probably the need for sustained production of AST to counteract the effects of the toxicant. Markiewicz-Górka *et al*. (2015) reported that aspartate aminotransferase (AST) activity and bilirubin concentration also increased significantly in the animal group exposed to all three metals and correlated positively with blood Cd, Pb, and Mn. Also, GOT and GPT levels increased significantly in starry flounder, *Platichthys stellatus* exposed to hexavalent chromium (Ko *et al*., 2019).

In another development, the samples of fish exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin A, the AST production in the liver indicated that T1 mean values were significantly higher than other treatments in the 2nd week of exposure. This is probably because at lower concentration in the presence of the vitamin and at early stage of the exposure the utilization is minimal after being triggered by the presence of the toxicant. At the 4th and 8th weeks of exposure,

respectively the 79 mg/L and 26 mg/L samples' mean values were significantly higher than other treatments. At higher concentrations and at later stages of the exposure, 61 mg/L and 79 mg/L samples' mean values in the 10th and 12th weeks of exposure were significantly higher than other treatments probably depicting the constant need for improvement in the immune system of the body to counter the effects of the toxicant. This is also probably why the highest mean value of 113.75±0.19 nM/mL at the 10th week was obtained from 61 mg/L samples. On the other hand, the AST mean values produced in the kidneys indicated that 26 mg/L and 44 mg/L samples' mean values in the 2nd and 4th weeks of exposure were significantly higher than other treatments. This is probably because the rate of utilization in higher concentration is higher than in lower concentration. Also, the 44 mg/L samples' mean values in both 8th and 12th week of exposure were significantly higher than other treatments and the highest AST production value in this case (139.86±0.19 nM/mL) was also obtained in 44 mg/L samples at the 12th week. This concentration (44 mg/L) probably proved to be the threshold of the elicitation of the effects of the toxicant in the kidney of the fish. Furthermore, in the gills of the fish samples, the 79 mg/L samples' mean values in the 2nd week of exposure were significantly higher than other treatments. This probably suggests early up-regulation of the body's immune system especially at the portal of entry to the toxicant. At later stages of the exposure the need for sustenance of the defense system was probably why the 26 mg/L, 61 mg/L and 79 mg/L samples' mean values in the 8th, 10th and 12th weeks of exposure, respectively were significantly higher than other treatments; and the highest AST production value in the gill in this case (115.78±0.10 nM/mL) was also obtained in 79 mg/L samples at the 12th week of exposure. The presence of vitamin A probably must have mitigated the effects of the

toxicant in lower concentrations in the gills of the fish. Shokrzadeh *et al*. (2012) reported that, the rats exposed to diazinon in combination with vitamin A, E and C separate groups displayed significant reduction in ALT and AST activities compared to diazinon group. Also, toxic effects of As exposure on *P. stellatus* indicate how GOT and GPT values increased with increasing arsenic concentration as well as the duration increases (Han *et al*., 2019)**.**

From the results of the analysis of the samples of fish exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin C, the 61 mg/L and 26 mg/L samples' mean values in the 4^{th} and 8^{th} weeks of exposure, respectively were significantly higher than other treatments. At the early stage of exposure there was probably the need for up-regulation of the defense system in the higher concentrations. The presence of the vitamin most likely ensured less utilization of AST produced due to the onslaught of the toxicant. This is probably why they are produced in greater concentrations at later stages of the exposure. Similarly, the same scenario played out in 26 mg/L and 44 mg/L samples' mean values in the 10^{th} and 12^{th} week of exposure which were significantly higher than other treatments and the highest mean value (129.06±0.37 nM/mL) was also obtained in 26 mg/L samples at the 10^{th} week of exposure. In line with this, Mirona *et al*. (2013) showed how glucose, hepatic alanine transaminase (ALT) and aspartate transaminase (AST) levels along with erythrone profile are more convenient biomarkers of water pollution and can be used for early detection for pollution effects on fishes. At these stages of the exposure the fish samples have also grown bigger in lower concentrations than in higher ones. In another development, the AST mean values produced in the kidneys indicated that

the 61 mg/L samples' mean values in both 2nd and 4th weeks of exposure, respectively were significantly higher than in other treatments. The mean values increased in 61 mg/L samples at early stages of the exposure probably because the immune system of the body needs to be increased at this concentration in order to deal with the effects of the toxicant. In addition to this, at lower concentrations and increased duration of the exposure the other treatments come to play perhaps, when the effects have overwhelmed the initial succor provided by the vitamin. This is probably why the 44 mg/L samples' mean values in the 8th week were significantly higher than in other treatment. Also, the mean values of 26 mg/L and 61 mg/L samples in the 10th and 12th weeks of exposure were significantly higher than other treatments. The highest AST mean value in the kidneys (112.81±0.19 nM/mL) of the fish was also obtained in 26 mg/L samples at the 10th week of exposure. Furthermore, the gills' AST production levels indicated that 44 mg/L samples' mean values in the 2nd and 4th weeks of exposure, respectively were significantly higher than other treatments. The need for up-regulation of the immune system in the gill was probably elicited at these stages of exposure; the 44 mg/L samples' mean values in the 12th week of exposure were also significantly higher than other treatments when the effects of the toxicant had probably overwhelmed the effects of the vitamin. Likewise, 79 mg/L samples' mean values in the 8th and 10th weeks of exposure, respectively were significantly higher than other treatments, and the highest AST mean value in the gill was 125.78±0.28 nM/mL obtained in 79 mg/L samples at the 8th week of exposure. At these later stages when the duration and concentration increased the need for sustained production of AST probably became more pertinent. In conformity with this, Abdel-Warith *et al*. (2020) reported that Hepatic enzyme activities of aspartate amino transferase (AST)

and alanine aminotransferase (ALT) displayed a significant increase with increasing concentrations and exposure time.

In the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$ and supplemented with vitamin E, the AST production in the liver of the fish indicated that, 44 mg/L and 26 mg/L in the 2nd and 4th weeks of exposure were significant and the highest mean value (135.78±0.28 nM/mL) was also obtained in 26 mg/L samples at the 4th week. The AST production levels were probably not utilized once triggered or elicited but retained in the lower concentrations till the end of the research. The 79 mg/L, 79 mg/L, 61 mg/L samples' mean values in the 6th, 8th and 10th weeks of exposure were significantly higher than other treatments in their respective treatments probably due to the need to up-regulate the defense system to counter the effects of the toxicant. Arenas *et al*. (2017) posited that AST and ALT are highly conservative indicators in liver, and are commonly located in hepatic cytoplasm and would release into the circulation when hepatocytes necrotize. In addition to the forgoing, the 26 mg/L samples mean values in the kidneys of the samples in 2nd week of exposure are significantly higher than other treatments. The 44 mg/L samples mean values in both 4th and 6th weeks of exposure were significantly higher than other treatments. At the early stages of the exposure the production levels of AST were high. The initial surge and elicitation of AST were not as high as in the higher concentration treatments probably due to the presence of the vitamin in the water matrix. This is probably why 61 mg/L and 79 mg/L samples' mean values in the 10th and 12th week of exposure were significantly higher than other treatments and the highest mean value of AST produced in the kidney (123.90±0.10 nM/mL) was also obtained in 79 mg/L at the end

of the 12th week of exposure. In line with these observations, Mohamed *et al*. (2019) reported a marked significant decrease in AST, ALT, urea, creatinine ($P \leq 0.05$) in DMSA groups and also how administration of DMSA improved the histopathological alterations in fish liver and kidney. On the other hand, the 26 mg/L, 44 mg/L and 26 mg/L samples' mean values of AST produced in the gill at the end of the 2nd, 4th and 6th weeks of exposure, respectively were significantly higher than other treatments and the highest mean value (153.12±0.19 nM/mL) in this case was also obtained in 44 mg/L at the end of the 4th week. This is probably because at these lower concentrations the AST produced were not so much engaged in dealing with the deleterious effects of the toxicant unlike in the higher concentrations; hence its availability and significance. The presence of the vitamin especially in the lower concentrations was probably also a contributing factor in the ability of the fish to deal with the effects of the toxicant.

4.2.5.4 ALT production levels in C. gariepinus exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with Vitamins A, C and E

From the results of the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, the ALT production levels indicated that 26 mg/L and 79 mg/L samples' mean values in the 2nd and 4th weeks of exposure, respectively were significantly higher than other treatments including the control. The need for up-regulation of the defense system was probably elicited from the beginning of the exposure especially in the lowest concentration; given that, AST and ALT are highly conservative indicators in liver, and are commonly located in hepatic cytoplasm and would release into the circulation when hepatocytes necrotize (Arenas *et al*., 2017). Subsequently, the higher concentrations witnessed increased production of ALT. This is probably why the 79

mg/L samples' mean values of both 6th and 8th weeks of exposure were significantly higher than other treatments including the control and the highest mean value of ALT produced in the liver of the samples(87.20±0.15 nM/mL) was also obtained in 79 mg/L samples at the 4th week of exposure. In order to counter the effects of the toxicant, the immune system probably had to be improved upon. This up-regulation was also probably necessary in 61 mg/L samples' mean values in the 10th and 12th weeks of exposure, respectively which were significantly higher than other treatments including the control. This could also be due to the fact that, ALT is a cytoplasmic enzyme found in very high concentration in the liver (Arbonnier, 2004); and that AST is less specific than ALT as marker of liver damage, but elevation in the serum levels of the two enzymes is an indicator of tissue damage and altered membrane ability (Satpal and Punnia, 2010). Also, reduction in plasma protein levels may be due to impaired protein synthesis or metabolism (Ramesh *et al*., 2014). On the other hand, the 26 mg/L samples' mean values in the kidneys of the fish were significantly higher than other treatments including the control in both 2nd and 4th week of exposure. This is probably because the production of the enzyme was triggered in this treatment by the toxicant but minimally utilized due to the low concentration of the toxicant. The sensitivity of the kidney in producing ALT in response to the effects of the toxicant is also probably minimal. This is probably why the control mean values in the 6th, 8th and 10th weeks of exposure were significantly higher than other treatments. The 79 mg/L samples' mean values in the 12th week of exposure were significantly higher than other treatments. This 79 mg/L samples' mean value (65.76±0.20 nM/mL) in the 12th week of exposure was also the highest ALT produced in the kidney. At higher concentration however, there must probably be an up-regulation of the defense system

to deal with the effects of the toxicant. In line with this, Al-Balawi *et al*. (2011) reported how exposure of *C. gariepinus* to lead acetate at all concentrations caused reduced growth rate, had significant effects on erythrocyte count, haemoglobin concentration and haematocrit values, increased plasma GOT and GPT; and sperm motility was also hampered after 4 weeks. Furthermore, 26 mg/L, 61 mg/L and 79 mg/L samples' mean values produced in the gill in the 2^{nd}, 4^{th} and 6^{th} weeks of exposure, respectively were significantly higher than other treatments including the control. In the gill, the first point of call, there were elevation of ALT production level especially in lower concentration and subsequently in 61 mg/L and 79 mg/L. This is again probably because of the need to regulate the body's defense mechanisms. The 26 mg/L and 61 mg/L samples' mean values in the 8^{th} and 12^{th} weeks of exposure, respectively were significantly higher than other treatments including the control. The highest ALT mean value produced in the gill was 69.92±0.05 nM/mL obtained in 61 mg/L at the end of the 12^{th} week of exposure probably due to the same reason given above. Similarly, Kim and Kang (2015) reported a significant increase in the GOT and GPT of Korean rockfish, *Sebastes schegelli* exposed to dietary lead. Likewise, Muralisankar *et al*. (2014) reported that dietary zinc exposure increases the GOT and GPT in the fresh water prawn, *Macrobranchium rosenbergii*.

From the results of the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, and supplemented with vitamin A, the ALT production levels in the liver indicated that 26 mg/L mean values in both 2^{nd} and 4^{th} weeks of exposure, respectively were significantly higher than other treatments. This is probably because there is less utilization of the enzyme unlike in other higher concentrations. This is also probably

why the highest mean value of ALT produced in the liver of the samples (77.12±0.20 nM/mL) was also obtained in 26 mg/L samples at the 4th week of exposure. However, as the duration of the exposure and the concentrations of the treatments increased there were probably the needs for the up-regulation of the body's immune system to counteract the deleterious effects of the toxicant. In line with this, the 61 mg/L, 79 mg/L and 61 mg/L samples mean values of 8th, 10th and 12th weeks of exposure are significantly higher than other treatments. Okonkwo and Ejike (2012) and Olojo *et al*. (2012) similarly reported that elevations in ALT and AST concentrations in serum of the catfish may be attributed to disruption of hepatic cells as a result of necrosis or altered membrane permeability after exposure to lead. On the other hand, the 26 mg/L samples' mean values in the kidneys of the fish were significantly higher than other treatments in 2nd week of exposure. The 44 mg/L and 61 mg/L samples' mean values in the 4th and 8th weeks of exposure were significantly higher than other treatments. This is probably because as the concentration and duration of exposure increased there was constant need for the up-regulation of the body's defense system as in other cases. The 26 mg/L and 61 mg/L samples' mean values in the 10th and 12th weeks of exposure were significantly higher than other treatments. The highest mean value of ALT produced in the kidney was 84.75±0.10 nM/mL in 61 mg/L samples at the 12th week of exposure probably due to the same reason stated above. Furthermore, 79 mg/L and 26 mg/L samples' mean values produced in the gill in the 2nd and 4th weeks of exposure, respectively were significantly higher than other treatments. At the portal of entry, the highest concentration elicited the significant production of the enzyme at early stage of the exposure probably to ensure survival and avoid being overwhelmed by the effects of the toxicant. As the duration of the exposure increased other

concentrations followed suit. This is probably why the 26 mg/L, 79 mg/L and 61 mg/L samples' mean values in the 8th, 10th and 12th weeks of exposure, respectively were significantly higher than other treatments. The highest ALT mean value produced in the gill was 70.43±0.24 nM/mL obtained in 79 mg/L samples at the end of the 10th week of exposure. At this stage and concentration the deleterious effects of the toxicant may have elicited the up-regulation of the body's immune system to put it in check.

From the results of the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, and supplemented with vitamin C, the ALT production levels in the liver indicated that 79 mg/L samples' mean values in both 2nd and 4th weeks of exposure, respectively were significantly higher than other treatments. In the highest treatment the effects of the vitamin may not have been able to thoroughly deal with the effects of the toxicant especially at the early stages of the exposure. This is probably why the effects were elicited and sustained in 79 mg/L samples which also produced the highest ALT mean value (86.53±0.05 nM/mL) in the liver at the end of the 4th week of exposure. The production levels of the enzyme in lower concentrations were probably not much at early stage due to the presence of the vitamin. However, at later stages of the exposure there were probably the needs for up-regulation of the immune system especially in the treatments with lower concentrations. This is probably why the 61 mg/L, 26 mg/L and 44 mg/L samples' mean values of 8th, 10th and 12th weeks of exposure were significantly higher than other treatments. Similar findings by Ellakany and Gaafar (2002) indicated that in *Oreochromis niloticus*, the ALT activities in liver and muscle were found to increase during the time course of endogenous cortisol elevation induced by ochratoxin intoxication and the results also indicated that the tissue injury

in toxicated fish recovered when they were fed dietary ascorbic acid because the AST and ALT activities in fish exposed to the lower or higher dose of ochratoxin + vitamin C became similar to those of control fish. Also, vitamin E and C can reduce Pb and Cu levels in serum and tissues of liver and kidney as well as reduce Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), urea and creatinine levels in Pb and Cu intoxicated male rats (Osfor *et al*., 2010). On the other hand, the 44 mg/L samples' mean values in the kidneys of the fish were significantly higher than other treatments in both 2nd and 4th week of exposure. Perhaps, the ALT elicited at these stages may not have been put to much utilization and hence, its availability and significance coupled with the presence of the vitamin. This is also probably why the highest ALT produced in the kidney (63.48±0.15 nM/mL) was also obtained in 44 mg/L samples at the end of the 12th week of exposure which was also significant at this stage. Similar finding by Ikeogu *et al*. (2020) indicated significant increase in ALT and urea when *C. gariepinus* was exposed to sub-lethal concentrations of glyphosphate but there were decreases in AST, ALT and urea in the treatments supplemented with vitamin C. Furthermore, 26 mg/L and 44 mg/L samples' mean values produced in the gill in the 2nd and 4th weeks of exposure, respectively were significantly higher than other treatments. In the lower concentrations and in the presence of vitamin C there are usually high production levels of the enzyme or antioxidant in question. This is probably because the presence of the vitamin normally mitigates the effects of the toxicant; and as such, leads to under utilization of the already elicited production of the enzyme and or, the antioxidant. This is also probably why the 44 mg/L samples' mean values in both 8th and 12th weeks of exposure, respectively were significantly higher than other treatments and the highest ALT mean value produced in the gill (66.53±0.15

nM/mL) was also obtained in 44 mg/L samples at the end of the 12th week of exposure. This is probably because Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular damage or disruption (Yolanda and Maria, 2012).

From the results of the samples exposed to sub-lethal concentrations of $Pb(NO_3)_2$, and supplemented with vitamin E, the ALT production levels in the liver indicated that 44 mg/L and 79 mg/L samples' mean values in both 2nd and 4th weeks of exposure, respectively were significantly higher than other treatments. This is probably because there were responses to overcome first, the elicitation threshold that was reached in 44 mg/L and at early stage of the exposure; and second, the urgent need to up-regulate the production of the enzyme to counteract the onslaught of the toxicant in the highest concentration in the 4th week. Also, this is probably why the 79mg/L samples' mean values in both 6th and 10th weeks of exposure, respectively were significantly higher than other treatments and the highest ALT mean value produced in the liver (73.82±0.15 nM/mL) was also obtained in 79 mg/L at the end of the 10th week of exposure. Apart from the elicitation of the enzyme production in 44 mg/L in the 2nd week of exposure the effects of the toxicant only became evident in lower concentrations at later stages of the exposure as 26 mg/L and 44 mg/L samples' mean values of 8th and 12th weeks of exposure, respectively were significantly higher than other treatments probably courtesy of the presence of the vitamin. In line with this, Mahmoud *et al*. (2012) reported that supplementation of selenium and vitamin E decreases the toxic effects of mercury, significantly increased the mean values of Na+, urea, creatinine, AST, ALT and ALP in comparison to control values; since, vitamins C and E are natural non-enzymatic antioxidants that are able to scavenge free radicals

and decrease lipid peroxidation (Zhai *et al*., 2015). On the other hand, the T1 and T4 mean values in the kidneys of the fish were significantly higher than other treatments in both 2nd and 4th week of exposure. The same reason and explanation given above may also be tenable here. There were gradual increases in the levels of production from the 2nd to the 6th weeks of exposure in both 44 mg/L and 61 mg/L samples. This trend may have been occasioned by the increasing need for the up-regulation of the body's defense systems to counter the effects of the toxicant as the duration of exposure increased. The 61 mg/L, 79 mg/L, 79 mg/L and 61 mg/L samples' mean values in the 6th, 8th, 10th and 12th weeks of exposure were significantly higher than other treatments; and highest ALT produced in the kidney (78.05±0.15 nM/mL) was also obtained in 79 mg/L samples at the end of the 4th week of exposure probably buttressing the fact that the responses are concentration and duration dependent banking on the sensitivity of the kidney in detecting the effects. Similar report was given by Azeez and Braimah (2020a) when they showed how plasma ALT and AST and ALP activities were increased when *C. gariepinus* was exposed to varying concentrations of copper sulphate. Also, TL, AST and ALT in *Channa punctata* exposed to lead acephate were significantly increased (Satish *et al*., 2018). Furthermore, 44 mg/L, 26 mg/L and 44 mg/L samples' mean values produced in the gill in the 2nd, 4th and 6th weeks of exposure, respectively were significantly higher than other treatments. In this scenario, the ALT production levels in the gills indicate the elicitation and sustenance in lower concentration at the early stages of the exposure. At later stages however, the 61 mg/L samples' mean values in both 10th and 12th weeks of exposure, respectively were significantly higher than other treatments; and the gradual increases in the levels of production of ALT from the 2nd to the 6th

weeks of exposure in 79 mg/L samples probably express the need for constant up-regulation of the immune system to deal with the changing environment. This is also probably why the highest ALT mean value produced in the gill (73.31±0.05 nM/mL) was also obtained in 61 mg/L samples at the end of the 10^{th} week of exposure. Similarly, Mahmoud *et al*. (2013) found that *C. gariepinus* exposed to Pb exhibited increased AST and ALT levels; which is also in line with Olojo *et al*. (2012) who stated that there was an increase in AST and ALT values for *C. gariepinus* after exposure to lead.

4.2.6 Histopathological parameters of *C. gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ toxicant and the supplemented treatments with Vitamin E

In the livers of the samples of *C. gariepinus* exposed to sub-lethal concentration of Pb, the 26 mg/L samples displayed aggregation and lumping together of the hepatocytes. This is probably because the presence of the toxicant elicited some physiological changes that culminated in tissue distortions; but upon administration of vitamin E with the same concentration of the toxicant such physiological perturbations were probably alleviated. Hence, there were preserved hepatocytes, reduced aggregation and vacoulation of the cells. Ahmad *et al*. (2011) reported that the most common changes in liver of fishes exposed to cadmium chloride were loosening of hepatic tissue, vacuolated cell cytoplasm, enucleation and eccentric nuclei. Abdulkareem *et al*. (2017) reported reduction in the pathological damages in the liver of the fishes in the group fed on 5 % *Moringa oleifera* leaves which was an indication that 5 % *M. oleifera* leaves in fish diet could minimize liver damage. Also in line with the ameliorative

capacity of vitamin E it has been shown that lead acetate combined with vitamin C plus vitamin E supplemented rats showed mild congestion of the interstitial blood vessels and the seminiferous tubules with its components appeared normal compared to DMSA (dimercaptosuccinic acid) treated rats; and that treatment with DMSA combined with vitamin C plus vitamin E showed more or less normal histological appearance of the testes in lead acetate induced histopathological changes in the affected organ (El-Sayed *et al*., 2015). The massive necrosis and distorsion of the hepatocytes in 44 mg/L samples of the Pb only group were probably due to the increased concentration of the toxicant. These effects were however, probably ameliorated to certain extent in the 44 mg/L samples of the PbVE group which displayed preserved hepatocytes and vacoulation but not as prominent as the amelioration witnessed in PbVE, 26 mg/L samples. Similarly, Deore and Wagh (2012) reported vacoulation in cytoplasm, degeneration of nuclei, vacoulation in stroma, cloudy swellings, pycnotic nuclei, necrosis, rupture of blood sinusoids and disarray of hepatic cods and loss of shape of hepatocytes in the liver when *C. gachua* was exposed to Cu. Also, Maurya *et al*. (2019) reported that histopathological changes in liver, intestine, gill, muscle and heart showed increasing degrees of damages in the tissues in correlation with accumulation pattern of pesticides while normal architecture of these organs were observed in the control.

Similarly, the 61 mg/L samples of the Pb only group indicated massive necrosis, lumping of hepatocytes as well as vacoulation which were probably attenuated in the 61 mg/L samples of the PbVE treatment group that displayed normal tissue architecture and hepatocytes similar to control samples with hepatocytes displaying prominent nucleoli. The sucouring effects of the vitamin may have been brought to

bear in restoring the normal tissue architecture. Similar results were reported by Kadry *et al*. (2012) on how histopathological examination of the liver of exposed fish showed dilatation and congestion of blood vessels, fatty degeneration, necrosis and pyknotic nuclei of hepatocytes; and that fish fed diet supplemented with vitamin E exhibited protective effect by minimizing the atrazine-induced toxicity, through measured values more or less similar to the control group fish. Also, Mahmoud *et al*. (2018) demonstrated the protective effects of propolis and vitamin C against histolopathological changes in the liver of *C. gariepinus* treated with cypermethrin. Co-administration of vitamin C with Pb acetate has been shown to diminish the severity of pathological changes and reduced the number of affected organs compared to intoxicated rats (El-Neweshy and El-Sayed, 2011). Furthermore, in the 79 mg/L samples of the Pb only group there were shattering of the hepatocytes. The increased concentration of the toxicant may have accounted for this. At this concentration the amelioration of the vitamin was minimal as there was aggregation and lumping of the hepatocytes. Similar results were reported by Osisiogu and Aladesanmi (2019) on how cadmium caused degeneration of the liver hepatocytes, congestion of central vein, area of necrosis, cytoplasmic vacuolation, vascular dilation and dilation of sinusoids in the hepatic cells in exposed fish (*C. gariepinus*) as compared to that of the control fish. In addition to this, Chavan and Muley (2014) reported that there was loss of cellular architecture in hepatocytes, along with haemolysis due to destruction of erythrocytes and prominent focal necrosis in *Cirrhinus mrigala* was due to the presence of heavy metals**.**

There have been various reports on the adverse effects of heavy metal contamination on fish health, which include histopathological alterations in their internal organs (Javed and Usmani, 2019). In the kidneys of the samples exposed to sub-lethal concentrations of Pb, the 26 mg/L samples displayed massive necrosis and vacoulation of the cells. The shattering occurred with loss of nucleus and cytoplasm.These alterations may have arisen from the effects elicited by the toxicant altering the physiological status of the fish, which now manifested in the the distortion of the tissue architecture. These alterations were probably ameliorated in the presence of vitamin E since the T1 samples of the PbVE treatment group showed reduced necrosis and cells show signs of aggregating together coupled with reduced vacoulation. In line with this, Abd-Elghaffar *et al*. (2015) reported that the kidneys treated with lead acetate showed severe tubular necrosis, periglomerular lymphoid cell reaction, dilatation of renal tubule, hyaline tubular cast associated with hemorrhage. Similarly, in 44 mg/L samples there were massive necrosis and shattering of cells with greater severity than 26 mg/L samples probably due to increased concentration of the toxicant. In the same vein, these effects were probably alleviated by the presence of the vitamin because the samples of 44 mg/L sample exposed to PbVE treatment group displayed preserved cells, reduced vacoulation with slight recovery of the cells. Similar report by Ahmad *et al*. (2011) also indicated how the kidney of *C. batrachus* exposed to cadmium chloride were characterized by loosening of haemopoietic tissue, uriniferous tubules have lost their original appearance, vacuolated cytoplasm, degeneration in the epithelial cells of renal tubule, narrowing of the tubular lumen and damaged glomeruli.

The 61 mg/L samples exposed to Pb only group also displayed massive necrosis and severe shattering of cells probably due to increasing concentration of the toxicant**.** In

line with this, Odo and Ododeyi (2017) reported that exposure of juveniles of *C. gariepinus* to selenium toxicity led to hyperplasia and haemorrhage of the gill lamellar, vacoulation of the kidney and mucosal eruption of the skin which increased with increase in the selenium concentration. Upon supplementation with vitamin E there were cellular swelling and aggregation of cells. The viable areas with cellular swelling took in much of the stains; and cells with cytoplasm returning to normal. The vitamin probably ensured quick recovery and improvement in the tissue architecture. Meanwhile, the 79 mg/L samples showed massive necrosis, tissue oedema and massive lumping of cells together. This is probably due to the high concentration of the toxicant which may have overwhelmed the immune status of the fish; culminating in physiological imbalances that manifested in the destruction of the tissue architecture. Vitamin administration at this high concentration of the toxicant may have had little or no effects on the kidney of the fish since the 79 mg/L samples exposed to PbVE treatment group displayed massive necrosis and shattering of the cells. As the concentration of the toxicant increased there were probably corresponding increase in the the deleterious effects experienced by the fish. Likewise, Odo *et al*. (2016) indicated that the toxic effect of Cyperdicot is clear on the behavioural and histopathological aspects of the fish gills, liver, and kidney tissues and that vitamin E had no amelioration effects on them. Similar histopathological changes were also reported by Nsofor *et al*. (2014) on how heavy metals like Zn, Fe, Cu, Hg, Cd, Pb and Arsenic detected in River Niger around Onitsha elicited extensive hyperaemia, oedematous sinusoids, hepatocytes in apoptosis with pyknotic nuclei, and wide spread necrotic hepatocytes with mononuclear leucocytes infilterations and pigment deposits

in liver tissues, as well as severe hyperaemia of the interstices with degenerating and necrotic tubular epithelial cells in kidney tissues of *Chrysichthys nigrodigitatus*.

Histology provides a rapid method to detect the effects of irritants in various tissues at different level, and so, the harmful effect is indicated among histopathological change in fish organs (Olojo *et al*., 2005). In the gills of the samples of 26 mg/L samples in the Pb only group there were rarefied gill filaments with ruptured lamellae. Those supplemented with vitamin E displayed how the gill arch and filaments were restored to certain extent similar to the control. These alterations in the gill architecture were probably due to the intake of the toxicant which was apparently remedied in the treatments with vitamin supplements probably due to the low concentration of the toxicant since the main location for ion transfer, gas exchange and acid base control is the gill. This is in conformity with the findings of Olojo *et al*. (2005) who reported that after 9 days of treatment with 0.006 mg/L the gills showed a gradual process of cytoarchitectural distortion of the lamellae with primary and secondary lamellae overlapping, as there was a decrease in the size of gill because of shrinkage in cartilaginous supporting mass in *C. gariepinus* exposed to lead. Also, the major histological effects reported by Adebayo and Fapohunda (2016) were hypertrophy, necrosis of hepatocyte and secondary lamella of the liver, gills and kidney when *C. gariepinus* was exposed to premium motor spirit. In this research, the primary lamellae are intact in 80 % o the photomicrographs. However, there is obvious degradation of respiratory epithelia cells leading to obvious osmoregulatory and ionregulatory dysfunctions. The 44 mg/L samples of the Pb only group displayed shattered gill arch and filaments, ruptured primary and secondary lamellae which were probably

alleviated to certain extent in the 44 mg/L samples of the PbVE treatment group which showed slight differences in ruptured gill arch and filament. This may be due to the increased concentration of the toxicant. This reason may also be plausible in 61 mg/L samples with higher concentration since the samples displayed shattered primary and secondary lamellae of the gill. These were probably improved upon in the PbVE group where the samples displayed gradual restoration of the primary and secondary lamellae. Similar report was given by Omirinde *et al*. (2017) when they showed how grades of chlorpyrifos induced several gill histo-architectural damages such as: moderate to severe gill epithelia sloughing, primary and secondary lamellar hyperplasia and central veinous congestion in the parenchyma with pronounced severity in fish exposed to higher concentrations; and the gill morphometrics (secondary lamellar length, width, interlamellar distance and surface area) were markedly altered by the graded concentrations of chlorpyrifos. Likewise, Osisiogu and Aladesanmi (2019) reported that histopathological changes in the muscle was dependent on the concentration of the toxicant and increased with increase in concentration. Furthermore, Kumar *et al*. (2015) reported severe histological alterations in the gills of *C. batrachus* which include mucus cells hyperplasia, bulging of the taste buds, and formation of interlamellar and sub-epithelial spaces in the primary and secondary gill lamellae.

Subsequently, in the 79 mg/L samples of the Pb only treatment group, there were shattered filaments which were probably ameliorated in the PbVE treatment group in which there were re-alignment of the gill arch and filaments. In line with this, Chavan and Muley (2014) reported that the gills of *Cirrhinus mrigala* exposed to heavy metals showed lamellar degeneration, epithelial lifting, dilation with congestion in blood

vessels of primary filaments and necrosis of lamellar epithelial cells. Also, Mshelbwala *et al*. (2017) reported that the pathologic changes observed in mussel were represented by branchial and intestinal epithelial cells vacuolization, intestinal lipofuscinosis, lamellar necrosis, and mononuclear cell infiltration.

CHAPTER FIVE

CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION OF RESEARCH TO KNOWLEDGE

5.1 Conclusion

Samples of *C. gariepinus* exposed to the varying concentrations of Pb toxicant displayed varying physical and behavioural responses in proportion to their concentrations in each case. The observed responses include vigorous and frequent gasping for air and attempts to jump out of the trough. Subsequenty, there were lethargic swimming, lassitude, emaciation and oozing out of blood at the opercular ends especially in higher concentrations, and eventual death.The 96 hours LC_{50} was 174.71 mg/L.

The growth parameters of *C. gariepinus* in terms of total and standard lengths as well as the weight of the samples displayed differing levels of responses and improvements in the various treatment groups. In the supplemented treatments, the improvements were majorly evident in lower concentrations than in higher ones. There were marked increases in the standard length throughout the Pb+Vitamin E treatment group. The significant improvements were recorded in 26 mg/L, the lowest concentration of the toxicant with 19.40 cm as the highest. The total lengths of Pb and their supplemented treatments also recorded improvements. The highest total length was recorded in the lowest concentration at the 12^{th} week of exposure with 22.30 cm. The weight parameters also towed the same lines. In the samples exposed to PbVE there was general improvement in weight values in all treatments with marked growth in 26 mg/L-61 mg/L concentration of toxicant with exceptional performance in the lowest

concentration. The highest weight gain of the samples was recorded in 26 mg/L samples at the 12th week with the value of 83.26 g. This % WG was 429 %.There were also increased % WG (Weight gain) and SGR (Specific Growth Rate) in 26 mg/L and 61 mg/L, respectively. The relationships between length and weight of *C. gariepinus* were linear throughout the period of exposure. The 44 mg/L samples had the highest R^2 values (0.6796) depicting how length and weight are interdependent.

The results from the samples exposed to Pb toxicant and then supplemented subsequently with vitamins A, C and E displayed different levels of ameliorations and improvements in mitigating the effects of the toxicant on the blood parameters. There were increased White Blood Cells count (WBC) and drastic reduction in blood platelets in the Pb only group at the end of the 12th week especially in higher concentrations of the toxicant. In PbVA treatment group after the 12th week, the PLT, RBC, Hb and PCV mean values in the highest concentration (79 mg/L) were significantly higher than other treatments. In the higher concentrations the blood parameter were mostly elevated where there were needs to up-regulate the defence systems to counter the effects of the toxicant. In sample subjected to PbVC, at the end of the 12th week of exposure, only the 44 mg/L samples' mean values in all the parameters and treatments were significantly lower than other treatments. In this treatment group the buffering capacity of the vitamins was evident in lower concentrations. Samples exposed to PbVE treatments displayed higher values of WBC, MCV, MCH, MCHC, PLT at various stages of the sampling and concentrations of the toxicant throughout the period of the exposure ensuring adaptation and survival of the fish to the onslaught of the xenobiotic manifesting the ameliorative roles of the vitamins mostly at lower concentrations of the toxicant.

The responses of the organs of *C. gariepinus* to $Pb(NO_3)_2$ in terms of GSH, MDA, AST and ALT were organ, concentration and duration dependent through-out the periods of exposure. The ameliorave roles of vitamins A, C and E were manifested mostly in the lower concentrations in terms of production levels of the antioxidant parameters of interest. The liver of the samples exposed to Pb only group displayed higher level of response to the toxicant with the highest GSH produced in the lowest concentration in comparison to other fish organs. The GSH highest mean value produced in the liver was 82.04±0.13 µg/ml. In the PbVA group the response was more in the kidney in the highest concentration with 58.74±0.07 µg/ml as the maximum. There were general low levels of production in all organs of the fish in the PbVC group. The highest GSH value was produced in the gill with 37.55±0.03 µg/ml.The kidneys of the PbVE group exhibited the highest level of GSH production in comparison to other organs.The highest GSH mean value in the kidney was 83.51±0.07 µg/ml.The MDA production levels displayed varying values with the highest obtained in the gills of the samples of the Pb only, PbVA and PbVE treatment groups while the PbVC group recorded its highest in the liver of the samples. The highest mean values of MDA were 80.28±0.06 nM/mg (Pb only group), 80.30±0.05 nM/mg (PbVA), 77.04±0.06 nM/mg (PbVE). On the other hand, the PbVC highest mean values was 46.60±0.06 nM/mg obtained in the liver.The AST production levels in the kidneys of the samples of the Pb only and PbVA groups recorded the highest. The liver and the gills in the PbVC and PbVE groups, respectively produced the highest values of AST. The highest mean values of AST were 141.40±0.10 nM/mg (Pb only), 139.86±0.19 nM/mg (PbVA), 129.06±0.37 nM/mg (PbVC) and 153.12±0.19 nM/mg (PbVE). Similarly, the samples of the fish exposed to sub-lethal concentrations

of Pb toxicant displayed varying levels of production of ALT with higher production levels mostly at higher concentrations of the toxicant. In the Pb only and PbVC groups the liver of the samples produced the highest ALT while the kidneys did same in the PbVA and PbVE groups. The high levels of production of the enzyme especially in higher concentrations suggest physiological imbalances due to the presence of the toxicant. The highest ALT mean values produced in each treatment groups were: Pb only (87.20±0.15 nM/mg), PbVA (84.75±0.10 nM/mg), PbVC (86.53±0.05 nM/mg) and PbVE (78.05±0.15 nM/mg). The outcome of this research buttresses the relevance of liver and kidneys in the detoxification of xenobiotics in the environment of living organisms.

The livers of the samples of *C. gariepinus* exposed to sub-lethal concentrations of Pb displayed varying alterations of the tissue architecture which include aggregation and lumping together of the hepatocytes, massive necrosis and shattering of the hepatocytes as well as vacoulation. These were ameliorated at varying degrees to near normal tissue architecture in the PbVE treatment group samples especially in samples exposed to 61 mg/L where there were preserved hepatocytes, reduced aggregation and vacoulation of the cells similar to control samples. Similar trends were established in the kidneys of the samples exposed to sub-lethal concentrations of Pb which displayed massive necrosis and vacoulation of the cells with loss of nucleus and cytoplasm; and tissue oedema and massive lumping of cells together in the highest concentration. There were also varying levels of ameliorations in the tissues of the samples of the treatments supplemented with vitamin E.The various histopathological alterations in the gills of the samples of the Pb only group were rarefied gill filament with ruptured lamellae, shattered gill arch and filaments, ruptured primary and secondary lamellae.

These were remedied for in the samples supplemented with vitamin E in which the gill arch and filaments were restored and re-aligned to certain extent similar to the control.

5.2 Recommendations

From the results obtained in this research, the following are recommended:

i. There should be routine check on this important staple food (*C. gariepinus*) especially those obtained from the wild to ensure that the level of contamination is within permissive limit by relevant authority considering how deleterious Pb toxicant is.

ii. For full effects of the ameliorative roles of the vitamins, the concentrations or the amount administered should be increased substantially in relation to the concentration of the toxicant in each treatment.

iii. Further research should also take into cognizance, the inclusion of the vitamins in separate control treatments without the toxicant to give a clearer picture of how the vitamins work on the immunity and the nutritive value of the fish.

iv. The out-come of the research established the impacts of the vitamins in ameliorating the effects of the toxicant in the immediate environment of the fish. Therefore, the administration of the vitamins should be considered as a remedy in dealing with heavy metals intoxication given the fact that the responses are concentration and duration dependent both in terms of the toxicant and vitamins.

v. The elicitation and utilization of the antioxidants at one point or the other were adopted by the fish in dealing with the effects of the toxicant. Higher concentrations of the vitamins as well could facilitate the understanding of

the effects of the vitamins in mitigating the effects of the toxicant especially in terms of the production levels of the antioxidants.

vi. The high levels of production of the antioxidant suggest that AST and ALT are good biomarkers of the oxidative stress elicited by the presence of the toxicant. AST and ALT can therefore, be adopted as quick check of the oxidative stress elicited by the presence of the toxicant.

vii. The kidneys and liver of *C. gariepinus* in this research were fully engaged in mitigating the effects of the toxicant in the presence of the vitamins with varying production levels of the antioxidants at one point or the other. Hence, these organs should be adopted in quick biomarking of oxidative stress elicited by the presence of xenobiotics.

viii. The out-come of the histopathological analyses of the tissues indicated how deleterious Pb toxicant is and how they can be ameliorated to certain extent by administering certain concentration of vitamin E; therefore, can serve as baseline information in exploring other vitamins especially at concentrations proportional to the concentrations of the toxicant of interest.

ix. The vitamins supplemented treatments displayed varying levels of ameliorations far better than the Pb only group. Amongst these, the PbVE fared better than others including the control. Vitamin E therefore, can be adopted in boosting the immune systems; rapid growth and nutritional improvement of *C. gariepinus* in fish farming.

5.3 Contributions to Knowledge

The exposure of *Clarias gariepinus* to lead (Pb) led to the following observed responses: vigorous and frequent gasping for air and attempts to jump out of the trough, lethargic swimming, lassitude, emaciation, oozing out of blood at the opercular ends especially in higher concentrations and eventual death. This showed how deleterious the toxicant was to *C. gariepinus*. The application of vitamins A, C and E led to improved growth performance in the fish especially in samples supplemented with vitamin E which was concentration and duration dependent. The highest weigth value of 83.26 g was obtained in the lowest dose of the toxicant with vitamin E.

There were varying responses of the organs of the fish (gill, kidney and liver) in the production levels of the antioxidants of interest from the various treatment groups. The liver and gills of the samples exposed to Pb only (82.04±0.13 µg/ml) and PbVC (37.55±0.03 µg/ml) groups respectively; kidneys of the samples of PbVA (58.74±0.07 µg/ml) and PbVE (83.51±0.07 µg/ml) groups had higher levels of production of reduced glutathione (GSH), respectively. The MDA production levels displayed varying values with the highest obtained in the gills of the samples of the Pb only (80.28±0.06 nM/mg), PbVA (80.30±0.05 nM/mg) and PbVE (77.04±0.06 nM/mg); while the PbVC group recorded its highest in the liver of the samples with 46.60±0.06 nM/mg. The kidneys of the samples of the Pb only (141.40±0.10 nM/mg) and PbVA groups (139.86±0.19 nM/mg) recorded the highest AST; whereas the maximum values of the antioxidant were obtained in the liver and gills of the PbVC (129.06±0.37 nM/mg) and PbVE (153.12±0.19 nM/mg). In the Pb only (87.20±0.15 nM/mg) and PbVC groups (86.53±0.05 nM/mg) the liver of the samples produced the peak ALT while the kidneys did same in the PbVA (84.75±0.10 nM/mg) and PbVE groups PbVE

(78.05±0.15 nM/mg). The responses of the organs of *C. gariepinus* to $Pb(NO_3)_2$ in terms of GSH, MDA, AST and ALT were organ, concentration and duration dependent through-out the periods of exposure.

A method of collection of blood adopted involved the insertion of the syringe in between the opercula end and the pectoral fin on the ventral surface of the fish. The syringe was held perpendicularly and blood drawn out with suction pressure. Larger quantity of blood was drawn with this method in comparison to the little quantity available from the caudal end of the fish.

There were likewise, varying responses in the blood parameters analysed at various stages of the research; and the effects of the toxicant were ameliorated to certain degrees especially in the PbVE treatment groups in which the immune systems of the fish were boosted to the levels equivalent to the control. PbVE treatments displayed higher values of WBC, MCV, MCH, MCHC, PLT with the values of 16.50±0.29 (10^9cells/L) in the highest concentration of the toxicant, 95.00±2.89 (Fl), 32.50±0.87 (Pg) and 32.00±1.16 (g/dl) in the lowest dose; and 231.00±5.78 (Cmm), in the sequence listed.

Various architectural alterations of the organs of fish exposed to Pb toxicant such as aggregation and lumping together of the hepatocytes, tissue oedema, gill arch and filaments degenerations, massive necrosis and shattering of the hepatocytes as well as vacoulation were observed; and these were remedied for in the samples supplemented with vitamin E in which the liver cells, gill arch and filaments were restored and re-aligned to certain extent similar to the control.

REFERENCES

Abdalla, A. E. (2009). The role of antioxidant (vit E) in the control of lead (Pb) pollution and enhancement of growth within Nile Tilapia (*Oreochromis niloticus*). *International Journal of Applied Research in Veterinary Medicine*, 7, 97.

Abd-Elghaffar, S. K. H., El-Sayed, M. F., Adly, M. A., & Abdel-Samei, W. M. (2015). The protective effects of DMSA and some vitamins against toxicity induced by lead in male Albino rats. *Journal of Pharmaceutical and Applied Chemistry*, 1(1), 1-8.

Abdel-Tawwab, M., Shalaby, A. M. E., Ahmed, M. H, & Khattab, Y. A. (2001). Effect of supplement dietary L- ascorbic acid (vitamin C) on mercury intoxication and growth performance of Nile tilapia (*Orechromis niloticus* L.). *Annals of Agricultural Science Moshtoher*, 39 (2), 961- 973.

Abdel-Tawwab, M., Mousaad, N. M. M., Sharafeldin, K. M. (2013). Changes in growth and biochemical status of common carp, *Cyprinus carpio* L. exposed to water-borne zinc toxicity for different periods. *International Journal of Aquatic Research*, 5(1), 11.

Abdel-Warith, A. A., Younis, E. M., Al-Asgah, N. A., & Wahbi, O. M. (2011). Effect of zinc toxicity on liver histology of Nile tilapia, *Oreochromis niloticus*. *Scientific Research Essential*, 6(17), 3760-3769.

Abdel-Warith, A. A., Younis, E. M. I., Al-Asgah, N. A., Rady, A. M., & Allam, H. Y. (2020). Bioaccumulation of lead nitrate in tissues and its effects on hematological and biochemical parameters of *Clarias gariepinus. Saudi Journal of Biological Science*, 27(3), 840–845. doi: 10.1016/j.sjbs.2020.01.015.

Abdulkareem, S. I., Oyediran, O. A., Shaibu, R. D., & Raji, M. (2017). Toxicological effects of Dichlorvos on haematology and histological changes in *Clarias gariepinus* juveniles and ameliorative potentials of *Moringa oleifera* leaves. *Journal of Aquatic Sciences*, 32(2), 303-315. Doi: http://dx.doi.org/10.4314.jas.v32i2.9.

Adebayo, I. A., & Fapohunda, O. O. (2016). Haematological and Histological Responses of *Clarias gariepinus* Fingerlings Exposed to Premium Motor Spirit (PMS). *Nigerian Journal of Fisheries and Aquaculture*, 4(2), 1 – 7.

Adeogun, A. O., Babatunde, T. A., & Chukwuka, A.V. (2012). Spatial and temporal variations in water and sediment quality of One river, Ibadan, Southwest Nigeria. *European Journal of Scientific Resources*, 74, 186-204.

Adewolu, M. A., Adeniji, C. A., & Adejobi, A. B. (2008). Feed utilization, growth and survival of *Clarias gariepinus* (Burchell, 1822) fingerlings cultured under different photoperiods. *Aquaculture*, 283, 64-67.

Adeyemi, J. A., Adewale, O. O., & Oguma, A. Y. (2014). Mortality, oxidative stress and hepatotoxicity in Juvenile African catfish, *Clarias gareipinus* (Burchell, 1822) exposed to Lead and cypermethrin. *Bulletin of Environmental Contamination and Toxicology*, 92, 529-533.

Adeyemo, O. K. (2007). Haematological Profile of *Clarias gariepinus* (Burchell, 1822) Exposed to Lead. *Turkish Journal of Fisheries and Aquatic Sciences*, 7, 163-169.

Agarwal, R., Goel, S. K., Chandra, R., & Behari, J. R. (2010). Role of vitamin E in preventing acute mercury toxicity in rat. *Environmental Toxicology and Pharmacology*, 29, 70-78.

Aghoghovwia, O. A., Morgan, P. I., & Izah, S. C. (2019). Behavioral Response and Acute Toxicity of Fingerlings of African Cat Fish, *Clarias gariepinus* Exposed to Paraquat Dichloride. *Journal of Plant and Animal Ecology*, 1(3), 13-20. Doi:10.14302/issn.2637-6075.jpae-19-2779.

Ahamefula, N. J., Doherty, F. J., Renner, K. O., Igbo, J. K., & Ajani, G. E. (2014). Spatiotemporal variations in hepatosomatic index and antioxidant enzyme activity in Brackish Water Fish species in the Lagos Coastal Zone. *Journal of environment and human*, 1, 1-5.

Ahmad, I., Maria, V. L., Oliveira, M., Serafim, A. Bebianno, M. J., Pacheco, M., & Santos, M. A. (2008). DNA damage and lipid peroxidation vs. protection responses in the gill of *Dicentrarchus labrax* L. from a contaminated coastal lagoon (Ria de Aveiro, Portugal). *Science of the Total Environment*, 406, 298-307.

Ahmad, B., Qureshi,T. A., Manohar, S., Kaur, P., & Khaliq, R. (2011). Effect of Cadmium Chloride on the Histoarchitecture of Liver and Kidney of a freshwater Catfish, *Clarias batrachus*. *International Journal of Environmental Sciences*, 2 (2), 543-548.

Ahmad, Z. (2012). Toxicity bioassay and effects of sub-lethal exposure of malathion on biochemical composition and haematological parameters of *Clarias gariepinus*. *African Journal of Biotechnology*, 11(34), 8578 -8585.

Ahmed, N. F., Sadek, K. M., Soliman, M. K., Khalil, R. H., Khafaga, A. F., Ajarem, J. S., Maodaa, S. N., & Allam, A. A. (2020). *Moringa Oleifera* Leaf Extract Repairs the Oxidative Misbalance following Sub-Chronic Exposure to Sodium Fluoride in Nile Tilapia *Oreochromis niloticus*. *Animals*, 10, 626.

Akani, N. P., & Gabriel, U. U. (2016). Enzymatic responses of *Clarias gariepinus* (Burchell, 1822) exposed to sub-lethal concentrations of an oilfield wastewater. *Journal of Applied Biology and Biotechnology*, 4 (05), 026-032. DOI: 10.7324/JABB.2016.40504.

Akinrotimi, O. A., Orlu, E. E., & Gabriel, U. U. (2013). Haematological Responses of *Tilapia guineensis* treated with industrial effluents. *Applied Ecological and Environmental Sciences*, 1, 10-13.

Al-Attar, A. M. (2011). Vitamin E attenuates liver injury induced by exposure to lead, mercury, cadmium and copper in albino mice. *Saudi Journal of Biological Sciences*, 18, 395–401.

Al-Balawi, H. F. A., Ahmad, Z., Al-Akel, A. S., & Al-Misned, F. (2011). Toxicity bioassay of lead acetate and effects of its sub-lethal exposure on growth, haematological parameters and reproduction in *Clarias gariepinus*. *African Journal of Biotechnology*, 10(53), 11039-11047.

Al-Balawi, H. F. A., Al-Akel, A. S., & Al-Misned, F. (2013). Effects of sub-lethal exposure of lead acetate on histopathology of gills, liver, kidney and muscle and its accumulation in these organs of *Clarias gariepinus*. *Brazilian Archives of Biology and Technology*, 56(2), 45–56.

Al-Busaidi, M., Yesudhanon, P., Al-Mughairi, S., Al-Rahbi, W.A.K., Al-harthy, K.S., Al-Mazrooei, N. A., & Al-Habsi, S.H. (2011). Toxic metals in commercial marine fish in Oman with reference of national and international standards. *Chemosphere*, 85, 67–73.

Ali, S., Hussain, S., & Ulhaq, M. (2019). Renal toxicity of heavy metals (Cadmium and Mercury) and their amelioration with ascorbic acid in rabbits. *Environmental Science and Pollution Research*, 26, 3909-3920.

Alkallak, S. N. H. (2013). A Comparative Study of Accumulation of Cadmium and Lead in the Tapeworm, *Postgangesia inarmata* (De Chambrier; Alkallak and Mariaux, 2003) and some Tissues of the Catfish *Silurus glanis* in the Governorate of Nineveh, North Iraq. *University Journal for Basic Sciences*, 29, 1-5.

Almedia, J. A., Barreto, R. E. Novelli, L. B., Castro, F. J., & Moron, S. E. (2009). Oxidative stress biomarkers and aggressive behavior in fish exposed to aquatic cadmium contamination. *Neotropical Ichthyol*ogy, 7(1), 103-108.

Almroth, B. C., Sturve J., Berglund, A., & Forlin, L. (2005). Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. *Aquatic Toxicology*, 73, 171-180.

Annabi, A., Said, K., & Messaoudi, I. (2013). Cadmium: Bioaccumulation, Histopathology and Detoxifying Mechanism in Fish. *American Journal of Research Communication*, 1 (4), 2-6.

Arbonnier, M. (2004). *Trees, shrubs and lianas of West African dry zones*. CIRAD, Coted'Ivorie: Margra Publishers, GMBH MNHN. 194p.

Arenas, J. J., Villafranca, M., Nieto-Guindo, E., ´Alvaro S. M., Moreno, S. M., Garrido, S., & Abil´es, J. (2017). Effects of cyclic parenteral nutrition on parenteral associated liver dysfunction parameters. *Nutrition Journal*, 16, 1-8.

Arya, P. D., Joseph, M. L., & Mathew, A. (2018). Determination of LC_{50} of lead acetate for a fish, *Oreochromis mossambicus* (Peters). *International Journal of Fisheries and Aquatic Studies*, 6(6), 180-182.

Atamanalp, M., Keles, M. S., Halilorglu, H. I., & Aras, M. S. (2002). The effects of cypermethrin (A synthetic pyrethroid) on some biochemical parameters of (Ca, P, Na, and TP) of Rainbow trout (*Oncorhynchus mykiss*). *Turkish Journal of Biology*, 26, 1157 - 1160.

Awodele, O., Olayemi, S. O., Alimba, C. G., Egbejogu, C., & Akintonwa, A. (2010). Protective Effect of Vitamin C and or Vitamin E on Micronuclei Induction by Rifampicin in Mice. *Tanzania Journal of Health and Research*, 12 (2), 2-10.

Ayegbusi, E. O., Aladesanmi, T. O., Kosemani, E. O., & Adewusi, A. O. (2018). Effects of lead chloride on growth performance of *Clarias gariepinus* (Burchell, 1822). *International Journal of Bioassays*, 7(5), 5638-5644.

Ayoola, S. O., Idowu, A. A., Babalola, O. E., & Ademoye, M. K. (2014). Heavy metals accumulation and Enzymatic Biomarker in Commercially Important Fish Species (*Hemichromis fasciatus* and *Chrysichthys nigrodigitatus*) at the Landing Site, Bariga, Lagos Lagoon. *World Applied Sciences Journal*, 29 (2), 298-306. DOI: 10.5829/idosi.wasj.2014.29.02.1628.

Ayyat, M. S., Ayyat, A. M., Naiel, M. A., & Al-Sagheer, A. A. (2020). Reversal effects of some safe dietary supplements on lead contaminated diet induced impaired growth and associated parameters in Nile tilapia. *Aquaculture*, 515, 734580.

Azeez, O. I., & Braimah, S. F. (2020a). Mitigating effect of vitamin-E on copper sulphate-induced toxicity in African catfish (*Clarias gariepinus*). *European Journal of Medical and Health Sciences*, 2(4). https:doi.org/10.24018/ejmed.2020.2.4.411.

Azeez, O. I., & Braimah, S. F. (2020b). Protective effects of vitamin E on potassium dichromate-induced haemotoxicity and oxidative stress in African catfish (*Clarias gariepinus*). *Asian Journal of Environment & Ecology*, 13(2), 18-31. http;//doi.org/10.9734/ajee/2020/v13i230177.

Bakare, A. A., Patel, S., Pandey, A.K., Bajpayee, M., & Dhawan, A. (2012). DNA and oxidative damage induced in somatic organs and tissues of mouse by municipal sludge leachate. *Toxicology and Industrial Health*, 28, 614–623.

Balint, T., Ferenczy, J., Katai, F., Kiss, I., Kroaz, L., Lang, G., Polyhos, C., Szabo, I., & Nemesok, O. (1997). Similarities and differences between the massive eel

(*Amguilla anguilla*) devastation that occurs in the lake Ablation in 1991 and 1995. *Ecotoxicology and Environmental Safety*, 37, 17 -23.

Banaee, M. (2013). Physiological dysfunction in fish after insecticides exposure. In S. Trdan (Ed), *Insecticides Development of Safer and More Effective Technologies.* London, UK: IntechOpen. Doi:10.5772/54742.

Baysal, A., Ozbek, N., & Akman, S. (2013). Determination of trace metals in waste water and their removal processes. In Einschlag, F. S. G., & Carlos, L. (Eds)., *Waste Water-Treatment Technologies and Recent Analytical Developments*. INTECH Europe. Doi:10.5772/52025.

Bharrhan, S., Chopra, K., & PraveenRishi, P. (2010). Vitamin E supplementation modulates endotoxin-induced liver damage in a rat model. *American Journal of Biomedical Sciences*, 2, 51–62.

Bijoy, N. S., & Nimila, P. T. (2011). Lindane toxicity: histopathological, behavioural and biochemical changes in *Etroplus maculatus*. *Marine Environmental Research*, 30, 1-8.

Bolognesi, C., & Cirillo, S. (2014). Genotoxicity biomarkers in aquatic bioindicators. *Current Zoology*, 60 (2), 273–284.

Borane, V. R. (2010). Protective role of ascorbic acid on the cadmium induced changes in hematology of the freshwater fish, *Channa orientalis* (Schneider). *Journal of Chemo and Biosphere*, 29-32.

Borane, V. R. (2013). Protective role of ascorbic acid on the cadmium induced changes in hematology of the freshwater fish, *Channa orientalis* (Schneider). *Advances in Applied Science Research*, 4(2), 305-308.

Boyd, C. E., & Lickotoper, F. I. (2014). *Water quality in pond for aquaculture.* Alabama Agriculture Experimenta station, Auburn University, Alabama, pp 30.

Calapoglu, M., Sevinc, Z., Togany, V. A., & Kalyoncu, H. (2017). Evaluation of oxidative stress and genotoxicity for environmental monitoring using farmed rainbow trout. *Fresenius Environmental Bulletin*, 26, 7105–7113.

Chavan, V. R., & Muley, D. V. (2014). Effects of heavy metals on liver and gill of fish *Cirrhus mrigala*. *International Journal of Current Microbiology and Applied Science*, 3, 277-287.

Costa, P. M., Caeiro, S., & Costa, M. H. (2013). Multi-organ histological observations on juvenile Senegalese soles exposed to low concentrations of water borne cadmium. *Fish Physiology and Biochemistry*, 39, 143–158.

Costantini, D., Marasco,V., & Moller, A. P. (2011). A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *Journal of Comparative Physiology*, B 181, 447–456.

Dahunsi, S. O., Oranusi, S. U., & Ishola, R. O. (2012). Bioaccumulation Pattern of Cadmium and Lead in the Head Capsule and Body Muscle of *Clarias gariepinus* (Burchell, 1822) Exposed to Paint Emulsion Effluent. *Research Journal of Environmental and Earth Sciences*, 4(2), 166-170.

Dalton, T. P., Shertzer, H. G., & Puga, A. (1999). Regulation of gene expression by reactive oxygen. *Annual Review of Pharmacology and Toxicology*, 39, 67-101.

Das, P. C., Ayyappan, S., Das, B. K., & Jena, J. K. (2004). Nitrite toxicity in Indian major carps: sublethal effect on selected enzymes in fingerlings of *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*. *Comparative Biochemistry and Physiology*, 138, 3 – 10.

Deore, S., & Wagh, S. (2012). Heavy metal induced histopathological alterations in liver of *Channa gachua* (Ham.). *Journal of Experimental Sciences*, 25, 481-489.

De Silva, P. M. C. S., Kirby, J., & Samoyowarhhen, L. A. (2002). Low concentrates of lorsban in water result in far reaching behavioural and histological effects in early life stages in guppy. *Ecotoxicology and Environmental Safety*, 53, 248 - 254.

DelRio, D., Pellegrini, N., Colombi, B., Bianchi, M., Serafini, M., & Torta, F. (2003). Rapid fluorimetric method to detect total plasma malondialdehyde with mild derivatization conditions. *Clinical Chemistry*, 49, 690–692.

Di Giulio, R. T., Benson, W. H., Sanders, B. M., & Van Veld, P. A. (1995). Biochemical mechanisms: Metabolism, adaptation and toxicity. In: G. M. Rand (Eds.), *Fundamentals of aquatic toxicology: Effects, environmental fate and risk assessment* (pp. 523-561). 2nd ed. Washington, D.C: Taylor & Francis.

Doherty, V. F., Ogunkuade, O. O., & Kanife, U. C. (2010). Biomarkers of oxidative stress as indicators of Environmental pollution in some selected fishes in Lagos, Nigeria. *American-Eurasian Journal of Agriculture and Environmental Science*, 7(3), 359-365.

Donpunha, W., Kukongviriyapan, U., Sompamit, K., Pakdeechote, P., Kukongviriyapan, V., & Pannangpetch, P. (2011). Protective effect of ascorbic acid on cadmium-induced hypertension and vascular dysfunction in mice. *Bio Metals*, 24, 105.

Dotan, Y., Lichtenberg, D., & Pinchuk, I. (2004). Lipid peroxidation cannot be used as a universal criterion of oxidative stress. *Progress in Lipid Research*, 43, 200–227.

Ebuehi, O. A., Ogedegbe, R. A., & Ebuehi, O. M. (2012). Oral administration of vitamin C and E ameliorates Lead-induced hepatotoxicity and oxidative stress in the rat brain. *Nigerian Quarterly Journal of Hospital Medicine*, 22, 85-90.

Eissa, I. A. M., Derwa, H. I., Mona, I., Ramadan, R. A., Mona, Z., & Nashwa, M. (2014). Use of enzyme activities as biomarkers for oxidative stress induced by metacercarial affections in some cultured tilapia species. *Life Science Journal*, 11(3), 8-15.

Elarabany, N., & Bahnasawy, M. (2019). Comparative and interactive biochemical effects of sub-lethal concentrations of cadmium and lead on some tissues of the African catfish (*Clarias gariepinus*). *Toxicological Research*, 35(3), 249-255. Doi:10.5487/tr.2019.35.3.249.

El-Hack, M. E. A., Abdelnour, S. A., Arif, M., Khafaga, A., Shaheen, H., Samak, D., & Swelum, A. A. (2019). Putative impacts of phytogenic additives to ameliorate lead toxicity in animal feed. *Environmental Science and Pollution Research*, 26, 23209–23218.

Ellakany, H., & Gaafar, H. (2002). Effects of combined aflatoxicosis and ochratoxicosis on immunological, biochemical and histopathological measurements in broilers. 6th Vet. Zag. Conference (7-9 Sept. 2002), Hurghada.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archive Biochemistry and Biophysics*, 82,70–77.

El-Neweshy, M., & El-Sayed, Y. (2011). Influence of VitC supplementation on lead-induced histopathoogical alterations in male rats. *Experimental and toxicologic pathology*, 63(3), 221-227. Doi: 10.1016/j.etp.2009.12.003.

El-Sayed, M. F., Abdel-Ghafar, S. K., Adly, M. A., Salim, A. A., & Abdel-Samei, W. A. (2015). The ameliorative effects of DMSA and some vitamins against toxicity induced by lead in the testes of albino rats. *The Journal of Basic and Applied Zoology*, 71, 60–65.

El-Shehawi, A. M., Fagr, K. A., & Mohamed A. S. (2013). Estimation of water pollution by genetic biomarkers in tilapia and catfish species shows species-site interaction. *African Journal of Agriculture*, 1 (1), 001-007.

El-Shenawy, N. S., & Al-Ghamdi, O. A. (2014). Phenthoate induced oxidative stress in fresh isolated mice hepatocytes: Alleviation by ascorbic acid. *Toxicology, Environment and Health Science*, 6 (2), 67–80.

Ercal, N., Gurer-Orhan, H., & Aykin-Burns, N. (2001). Toxic metals and oxidative stress Part I: mechanisms involved in metal induced oxidative damage. *Current Topics in Medical Chemistry*, 1, 529–539.

Ezeonyejiaku, C. D., Okoye, C. O., & Ezenwelu, C. O. (2019). Toxicity and Bioaccumulation Studies of Heavy Metals on a Freshwater Fish. *International Journal of Oceanography & Aquaculture*, 3(3), 000169. DOI: 10.23880/ijoac-16000169.

FAO (2003). Food Security: concepts and measurement. Rome: Food and Agriculture Organization of the United Nations. In FAO (Ed.), *Trade Reforms and Food Security*. pp. 25-34.

FAO (2010). Food and Agricultural Organization of the United Nations: World Review of Fisheries and Aquaculture, http: // www. fao. org / docrep / 013 /i1820e / i1820e01.pdf. Accessed 22/04/2019.

FAO (2012). The state of the world fisheries and aquaculture. Fisheries and Aquaculture Department, Rome, 2012.

FAO. (2013). Improving pond water quality. Training Series for Aquaculture; Available:ftp://ftp.fao.org/fi/cdrom/fao_training/FAO_Training/General/x6709e/x6709e02.htm.

FAO Fish Stat (2019). Cultured Aquatic Species Information Programme, *Clarias gariepinus* (Burchell, 1822). Accessed 8/29/2019.

Feng, Y., Huang, X., Duan, Y., Fan, W., Duan, J., Wang, K., Chen, D., & Yang, S. (2018). The Effects of Vitamin E and Metallothionein on the Antioxidant Capacities of Cadmium-Damaged Liver in Grass Carp, *Ctenopharyngodon idellus*. *BioMed Research International*,1-8. https://doi.org/10.1155/2018/7935396.

Finney, D. J. (1971). *Probit Analysis*. (3rd ed.). UK: Cambridge University Press. Pp.333.

Friedman, L. S., Martin, P., & Munoz, S. J. (1996). Liver function tests and the objective evaluation of the patient with liver disease. In D. Zakin, T. D. Boyer (Eds), *Hepatology: A Textbook of Liver Disease*, (3rd ed.). (pp. 791–833). Philadelphia: WB Saunders.

Gaafar, A. Y., El-Manakhly, E. M., Soliman, M. K., Soufy, H., Mona, S. Z., Mohamed, S. G., & Hassan, S. M. (2010). Some pathological, biochemical and haematological investigations on Nile Tilapia (*Oreochromis niloticus*) following chronic exposure to edifenphos pesticide. *Journal of American Science*, 6(10), 542-551.

Gabriel, U. U., Akinrotimi, O. A., & Ariweriokuma, V. S. (2012). Changes in metabolic enzymes activities in selected organs and tissues of *Clarias gariepinus* exposed to cypermethrim. *Journal of Chemical Engineering*, 1(1), 25 - 30.

Gago-Dominguez, M., & Castelao, J. E. (2006). Lipid peroxidation and renal cell carcinoma: further supportive evidence and new mechanistic insights. *Free Radical Biology and Medicine*, 40,721–733.

Ganesh, H. G., Arun, J. P., Jyotsna, A. P., Mandakini, S. K., & Ajit, S. (2016). Biochemical effects of Pb exposure on oxidative stress and antioxidant status of

battery manufacturing workers of Western Maharashtra, India. *Journal of Basic Chemical Physiology and Pharmacology*, 27, 141-146.

Guedenon, P., Edorh, A. P., Hounkpatin, A. S. Y., Alimba, C. G., Ogunkanmi, A., Nwokejiegbe, E. G., & Boko, M. (2012). Acute Toxicity of Mercury ($HgCl_2$) to African Catfish, *Clarias gariepinus. Research Journal of Chemical Sciences*, 2(3), 41-45.

Hamed, H. S. (2015). Vitamin E attenuated the oxidative stress and biochemical changes induced by endosulfan in female Catfish (*Clarias gariepinus*). *World Journal of Fish and Marine Sciences*, 7, 313-324.

Han, J., Park, H., Kim, J., Jeong, D., & Kang, J. (2019). Toxic Effects of arsenic on growth, hematological parameters, and plasma components of starry flounder, *Platichthys stellatus*, At two water temperature conditions. *Fisheries and Aquatic Sciences*, 22, 3. https://doi.org/10.1186/s41240-019-0116-5.

Hermenean, A., Damache, G., Albu, P, Ardelean, A., Ardelean, G. Ardelean, D. P….., & Dinischiotu, A. (2015). Histopatological alterations and oxidative stress in liver and kidney of *Leuciscus cephalus* following exposure to heavy metals in the Tur River, North Western Romania. *Ecotoxicology and Environmental Safety*, 119, 198–205.

Hormozi, M., Mirzaei, R., Izadi, S., Dehghan Haghighi, J., & Nakhaee, A. (2018). The biochemical effects of occupational exposure to lead and cadmium on markers of oxidative stress and antioxidant enzymes activity in the blood of glazers in tile industry. *Toxicology and Industrial Health*, 34(7), 459-467. https://doi.org/10.1177/0748233/718769526.

Hounkpatin, A. S. Y., Johnson, R. C., Guédénon, P., Domingo, E., Alimba, C. G., Boko, M., & Edorh, P. A. (2012). Protective effects of vitamin C on haematological parameters in intoxicated Wistar Rats with cadmium, mercury and combined cadmium and mercury. *International Research Journal of Biological Sciences,* 1(8), 76-81.

Humayun, Y., Aslam, A., Azeem, T., Abid, S. A., Shah, M. A. A., Munir, M. T., & Umar, S. (2015). Toxic effects of lead on biochemical and histological features and their amelioration by Vitamin E in Japanese quails (*Coturnix coturnix japonica*). *Journal of Avian Research*, 1(1), 4-9.

Ibrahim, A. T. (2019). Biochemical and histopathological response of *Oreochromis niloticus* to malathion hepatotoxicity. *Journal of Royal Science*, 1(1), 10-15.

Ikeogu, C. F., Nsofor, C. I., Igwilo, I. O., & Ngene, A. A. (2016). Haematological and serological responses of *Clarias gariepinus* to sublethal concentrations of lead nitrate. *Journal of Pharmaceutical Science and Bioscientific Research*, 6, 442–446.

Ikeogu, C. F., Ikpeze, O. O., Omobowale,T. O., & Oluwafemi, B. E. (2020). Ascorbic acid effects on glyphosphate-induced haematological and serological pathology in juveniles of the cat fish *Clarias gariepinus* (Pisces:Clariidae) Burchell, 1822. *Asian Journal of Research in Animal and Veterinary Sciences*, 6(4), 18-32.

Isibor, P. O., & Imoobe, T. O. T. (2017). Trace Metals in Water, Bottom Sediment, Shrimp and Dependent Human Blood in Ukwuani Local Government Area of Delta State, Nigeria. *Asian Journal of Environment and Ecology*, 2(3), 1–16.

Javed, M., & Usmani, N. (2019). An Overview of the adverse effects of heavy metal contamination on fish health. The National Academy of Sciences, India, 89, 389–403. doi: 10.1007/s40011-017-0875-7.

Jeyaraj, N., Rajan, M. R., & Santhanam, P. (2012). Dietary requirement of vitamin A and biochemical composition of common carp, *Cyprinus carpio* var communis. *Journal of Fisheries and Aquatic Science*, 7, 65-71.

Jhamtani, R. C., Shukla, S., Dahiya, M. S., & Agarwal, R. (2017). Evaluation of acute effects of lead at sub-lethal concentrations in zebra fish. *Research Journal of Environmental Toxicology*, 11, 97-103. Doi:10.3923/rjet.2017.97.103.

Kadry, S., Marzouk, M. S., Amer, A. M., & Hana, M. I. (2012). Vitamin E as an antioxidant in female African catfish (*Clarias gariepinus*) exposed to chronic toxicity of Atrazine. *Egyptian Journal of Aquatic Biology and Fisheries*, 16(2) (1110-6131), 83-98. Doi: 10.21608/ejabf.2012.2127.

Kaplan, M. M. (1993). Laboratory tests. In L. Schiff, E. R. Schiff (Eds.), *Diseases of the Liver*, (7th ed.). (pp. 108–144.). Philadephia, PA, USA : JB Lippinocott.

Karadag, H., Fırat, O., & Fırat, O. (2014). Use of Oxidative Stress Biomarkers in *Cyprinus carpio* L. for the Evaluation of Water Pollution in Ataturk Dam Lake (Adiyaman, Turkey). *Bulletin of Environmental Contaminant and Toxicology*, 92(3), 289–293. doi: 10.1007/s00128-013-1187-0.

Karami, A., Christianus, A., Ishak, Z., Courtenay, S. C., Sayed, M. A., Noor, A. M., & Noor, S. H. (2010). Effect of triploidization on juvenile African catfish (*Clarias gariepinus*). *Aquaculture International*, 18, 851-858.

Kassa, H., Silverman, S. G., & Baroudi, K. (2010). Effect of a manager training and certification program on food safety and hygiene in food service operations. *Environmental Health Insights*, 4, 13–20.

Kaushal, B. T., & Mishra, A. (2011). A comparative toxicity analysis of cadmium compounds on morphological and behavioral aspects in air breathing freshwater fish, *Channa punctatus*. *International Journal of Science and Nature*, 2, 266-269.

Kawabata, T. T., Chapman, M. Y., Kim, D. H., Stevens, W. D., & Holsapple, M. P. (1990). Mechanisms of In Vitro Immunosuppression by Hepatocyte-generated

Cyclophosphamide Metabolites and 4 Hydroxycyclophosphamide. *Biochemistry and Pharmacology*, 40 (5), 927.

Kim, H. S., Kwack, S. J., & Lee, B. M. (2000). Lipid peroxidation, antioxidant enzymes, and benzo[a]pyrene- quinones in the blood of rats treated with benzo[a]pyrene. *Chemistry and Biology Interaction*, 127, 139–150.

Kim, J. H., & Kang, J. C. (2015). The lead accumulation and haematological findings in juvenile rockfish, Sebastes schlegelli exposed to the dietary lead (II) concentrations. *Ecotoxicology and Environmenta Safety*, 115, 33-39.

Kim, J. H., & Kang, J. C. (2016). The chromium accumulation and its physiological effects in juvenile rockfish, *Sebastes schlegelli*, exposed to different levels of dietary chromium (Cr^{6+}) concentrations. *Environmental Toxicology and Pharmacology*, 41, 152-158.

Kim, J., & Kang, J. (2019). Toxic effects on hematological parameters and oxidative stress in juvenile olive flounder, *Platichthys olivaceus* exposed to water borne zinc. *Aquaculture Reports*, 15, 100225. https;//doi.org/10.1016/j.aqrep.2019.100225.

Ko, H., Park, H., & Kang, J. (2019). Change of growth performance, haematological parameters and plasma component by hexavalent chromium exposure in starry flounder, *Platichthys stellatus*. *Fisheries and Aquatic Sciences*, 22, 9.

Kumar, B. G., Nandan, S. B., Kumari, T. A., & Sajeevan, K. (2014). Curative effect of Vitamin C on the Variation in Biochemical and Histopathological Parameters Induced by Copper Exposure in the Teleost Fish, *Anabas testudineus,* (Bloch, 1792). *IOSR Journal of Environmental Science, Toxicology and Food Technology* (IOSR-JESTFT), 8 (8) Ver. II, 28-35.

Kumar, M., Kumar, P., & Devi, S. (2015). To study the histopathological changes in the gills of *Clarias batrachus*, an air-breathing teleost after short term exposure of copper sulphate. *Journal of Aquaculture Research and Development*, 6,1.

Kumaraguruparan, R., Kabalimoorthy, J., & Nagini, S. (2005). Correlation of tissue lipid peroxidation and antioxidants with clinical stage and menopausal status in patients with adenocarcinoma of the breast. *Clinical Biochemistry*, 38,154–158.

Lamidi, I. Y., & Akefe, I. O. (2017). Mitigative effects of antioxidants in lead toxicity. *Clinical Pharmacology and Toxicology*, 1(1), 3.

Layachi, N., & Kechrid, Z. (2012). Combined protective effect of vitamins C and E on cadmium induced oxidative liver injury in rats. *African Journal of Biotechnology*, 11(93), 16013-16020. DOI: 10.5897/AJB12.2665.

Li, Z. H., Velisek, J., Zlabek, V., Grabic, R., Machova, J., Kolarova, J., & Randak, T. (2010). Hepatic antioxidant status and haematological parameters in rainbow trout, *Onchorynchus mykiss*, after chronic exposure to carbamazepine. *Chemical and Biological Interactions*, 183, 98-104.

Lopez-Torres, M., Perez-Campo, R., Cadenas, S., Rojas, C., & Barja, G. (1993). A comparative research of free radicals invertebrates - II. Non-enzymatic antioxidants and oxidative stress. *Comparative Biochemistry and Physiology*, 105, 757-763.

Luschak, V. I. (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology*, 1, 13–30.

Magdy, B. W., Mohamed, F. E., A. Seleem, A. A., & Sarhan-Rana, S. (2016). Ameliorative effects of antioxidants (vitamins C and E) against abamectin toxicity in liver, kidney and testis of male albino rats. *The Journal of Basic and Applied Zoology*, 77, 69–82.

Mahboob, S., Al-Balawi, H. F. A., Al-Misned, F., Al-Quraishy, S., & Ahmad, Z. (2014). Tissue metal distribution and risk assessment for important fish species from Saudi Arabia. *Bulletin of Environmental Contaminants and Toxicology*, 92, 61–66.

Mahmoud, U. M., Mekkawy, I. A., & Ibrahim, A. A. (2012). Biochemical response of the African catfish, *Clarias gariepinus* (Burchell, 1822) to sub-lethal concentrations of mercury chloride with supplementation of selenium and Vitamin E. *Toxicology and Environmental Health Sciences*, 4, 218-234.

Mahmoud, U. M., Abdel-Basset, M., Ebied, S., & Mohamed, M. (2013). Effect of lead on some haematological and biochemical characteristics of *Clarias gariepinus* dietary supplemented with lycopene and vitamin E. *Egyptian Academic Journal of Biological Science*, 5, 67–89.

Mahmoud, U. M., Ebied, A. M., & Mohamed, S. M. (2018). A Protective Effect of Propolis and Vitamin C against Cypermethrin Toxicity on Liver of *Clarias gariepinus*. *Journal of Pharmacy and Biological Sciences* (IOSR-JPBS), 13(3), 61-68. DOI: 10.9790/3008-130306168.

Makinde, G. E. O., Olaifa, F. E., & Banjo, O. T. (2015). Acute Toxicity and Histopathological Changes in Gill and Liver of catfish (*Clarias gariepinus*) Juvenile exposed to 2, 4-D Amine (Herbex D SI). *Journal of Biology, Agriculture and Healthcare*, 5(4), 145-150.

Markiewicz-Górka, I., Januszewska, L., Michalak, A., Prokopowicz, A., Januszewska, E., Pawlas, N., & Pawlas, K. (2015). Effects of chronic exposure to lead, cadmium, and manganese mixtures on oxidative stress in rat liver and heart. *Arh Hig Rada Toksikol*, 66, 51-62. DOI: 10.1515/aiht-2015-66-2515.

Marzouk, M., Abdelkader, E. M., El-zayat, M., & Aboushady, A. (2017). Assessing environmental impact indicators in road construction projects in developing countries. *Sustainability*, 9(5), 843. Doi:10.3390/su9050843.

Maurya, P. K., & Malik, D. S. (2016). Bioaccumulation of xenobiotics compound of pesticides in riverine system and its control technique: a critical review. *Journal of Industrial Pollution and Control*, 32 (2), 580-594.

Maurya, P. K., Malik, D. S., Yadav, K. K., Gupta, N., & Kumar, S. (2019). Haematological and histological changes in fish, Heteropneustes fossilis exposed to pesticides from industrial waste water. *Human and Ecological Risk Assessment: An International Journal*, 1-29. Doi:10.1080/10807039.2018.1482736.

McGregor, G. P., & Biesalski, H. K. (2006). Rationale and impact of Vitamin C in clinical nutrition. *Current Clinical Nutrition and Metabolic Care*, 9, 697-703.

Mekkawy, I. A., Mahmoud, U. M., Wassif, E. T., & Naguib, M. (2011). Effects of cadmium on some haematological and biochemical characteristics of *Oreochromis niloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and vitamin E. *Fisheries, Physiology and Biochemistry*, 37(1),71-84. DOI10.1007/s10695-010-9418-3.

Mekkawy, I. A. A., Mahmoud, U. M., Wassif, E. T., & Naguib, M. (2012). Protective roles of tomato paste and vitamin e on cadmium-induced histological and histochemical changes of liver of *Oreochromis niloticus* (Linnaeus, 1758). *Journal of Fisheries and Aquatic Science*, 7, 240-265.

Merciai, R., Guasch, H., Kumar, A., & Sabater, S. (2014). Trace metal concentration and fishsize: variation among fish species in a Mediterranean river. *Ecotoxicology and Environmental Safety*, 107, 154–163.

Michael, P. O., Ogunrotimi, B. V., & Eitokpah, P. (2018). Response of *C. gariepinus* juvenile exposed to sub-ethal concentration of Atrazine. *Aquaculture Studies*, 18(1), 19-26.

Mirghaed, A. T., Hoseini, S. M., & Ghelichpour, M. (2018). Effects of dietary 1, 8-cineole supplementation on physiological, immunological and antioxidant responses to crowding stress in rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology*, 81, 182–188.

Mirhashemi, S. M., Moshtaghie, A. A., Ani, M., & Aarabi, M. H. (2010). Lead toxicity on kinetic behaviors of high and low molecular weight alkaline phosphatase isoenzymes of rat, *in vivo* and *in vitro* studies. *Journal of Biological Sciences*, 10, 341-347.

Mohamed, N. A., Ali, A. M., Bakhoum, S. A., Abdel-Kader, H. H., & Ahmed, M. A. (2019). Monitoring of Oxidative Stress Biomarkers and Toxicity of Lead and Mercury in Catfish of Lake Mariout, Egypt: The Role of Meso -2, 3-Dimercaptosuccinic Acid (DMSA). *Egyptian Journal of Aquatic Biology & Fisheries*, 23(2), 165 – 182.

Monteiro, D. A., Rantin, F. T., & Kalinin, A. L. (2010). Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the

tropical freshwater fish matrinxă, *Brycon amazonicus* (Spix and Agassiz, 1829). *Ecotoxicology*, 19, 105-123.

Morina, V., Aliko, V., Sula, E., Gavazaj, F., Cakaj, F., & Ferizi, R. (2013). Physiological response of fish to water pollution in Sitnica River (Kosovo). *Indian Streams Research Journal*, 3(1), 1-5.

Mshelbwala, F. M., Ibrahim, N. D. G., Saidu, S. N., Azeez, A. A., Akinduti, P. A., Kwanashie, C. N., Kadiri, A. K. F., Muhammed, M., Fagbamila, I. O., & Luka, P. D. (2017). Motile Salmonella serotypes causing high mortality in poultry farms in three South-Western States of Nigeria. *Veterinary Records*, 4, e000247.

Muralisankar, T., Bhavan, P. S., Radhakrishnam, S., Seenivasan, C., Manickam, N., & Srinivasan, V. (2014). Dietary supplementation of zinc nano particles and its influence on biology, physiology and immune responses of the freshwater prawn, *Macrobrachium rosenbergii*. *Biology of Trace Elements Research*, 160, 56-66.

Murtala, B. A., Abdul, W. O., & Akinyemi, A. A. (2012). Bioaccumulation Of heavy metals in fish (*Hydrocynus forskahlii*, *Hyperopisus bebe occidentalis* and *Clarias gariepinus*) Organs in downstream Ogun Coastal water, Nigeria. *Transnational Journal of Science and Technology*, 5, 2-10.

Neelima, P., Sunitha, K., GopalaRao, N., & Chandra SekharaRao, J. (2015). Haematological Alterations in *Cyprinus carpio* as biomarkers of cypermethrin toxicity. *International Journal of Current Research*, 7(8), 18864-18870.

Neeratanaphan, L., Kamollerd, C., Suwannathada, P., Suwannathada, P., & Tengjaroenkul, B. (2020). Genotoxicity and Oxidative Stress in Experimental Hybrid Catfish Exposed to Heavy Metals in a Municipal Landfill Reservoir. *International Journal of Environmental Research and Public Health*, 17 (1980), 1-14. doi:10.3390/ijerph17061980.

Nishida, Y. (2011). The chemical process of oxidative stress by copper (II) and iron (III) ions in several Neuro-degenerative disorders. *Monatshefte fur Chemie*, 142, 375–384.

Nsofor, C., Ikpeze, O. O., ngenegbo, U. C., Ikeogu, C. F., & Okonkwo, J. C. (2014). Histopathological alterations in the liver and kidney of the fish, *Chrysichthys nigrodigitatus* due to heavy metals in Niger River. *Journal of Natural Sciences Research*, 4(12), 11-18.

Odo, K. E., & Ododeyi, D. O. (2017). Responses and histopathological studies of *Clarias gariepinus* exposed to selenium toxicity. *International Journal of Marine Science*, 7(29), 284-291. Doi: 10.5376/ijms.2017.07.0029.

Odo, G. E., Agwu, J. E., Madu, J., Ezea, C. O., Nnamonu, E. I., & Eneje, V. O. (2016). Histopathological effects of Cyperdicot and vitamin E supplementation on selected organs of *Clarias gariepinus* (Burchell, 1822) reared in a tropical fish

farm in Nigeria. *African Journal of Biotechnology*, 15(9), 303-314. DOI: 10.5897/AJB2015.14801.

Ognjanović, B. I., Pavlović, S .Z., Maletić, S. D., Zikić, R.V., Stajn, A. S., Radojicić, R. M., Saicic, Z. S., & Petrović, V. M. (2003). Protective Influence of Vitamin E on Antioxidant Defense System in the Blood of Rats Treated with Cadmium. *Physiological Research*, 52 (5), 563-570.

Okareh, O. T., & Akande, F. (2015). Lead and Cadmium Levels of African Catfish (*Clarias gariepinus*) and the Effect of Cooking Methods on their Concentrations. *British Journal of Applied Science & Technology*, 11(2), 1-12. DOI: 10.9734/BJAST/2015/18781.

Okonkwo, F. O., & Ejike, C. E. (2012). Simulation of heavy metal contamination of fresh water bodies: toxic effects in thecatfish and its amelioration with cocontamination with glyphosate. *Journal of Applied Sciences and Environmental Management*, 15(2), 341 –345.

Olatunji, A. E., Bekeh, A. F., Otelahu, O. L., & Cornelius, I. (2015). Haematological and Histopathological Responses of Catfish, *Clarias gariepinus* Juvenile to Coconut Water, *Cocos nucifera*. *International Journal of Fisheries and Aquatic Studies*, 3(1), 97-110.

Olojo, E. A. A., Olurin, K. B., Mbaka, G., & Oluwemimo, A. D. (2005). Histopathology of the gill and liver tissues of the African catfish, *Clarias gariepinus* exposed to lead. *African Journal of Biotechnology*, 4(1), 117-22.

Olojo, E. A. A., Abass, A. A., Olurin, K. B., & Mbaka, G. (2012). The potential use of certain protein metabolism parameters as biomarkers of heavy metal (lead) stress in the African cat fish (*Clarias gariepinus*). *Agric Journal*, 7(5), 316-322.

Oluah, N. S. (1999). Plasma aspartate aminotransferase activity in the catfish *Clarias albopunctatus* exposed to sublethal zinc and mercury. *Bulletin of Environmental Contaminants and Toxicology*, 63(3), 343–349. DOI: 10.1007/s001289900986.

Oluwatosin, A., Aladesanmi, T. O., Ayegbusi, O., & Kosemani, O. (2018). Effects of lead chloride on growth performance of *Clarias gariepinus* (Burchell, 1822). *International Journal of Bioassays*, 7(5), 5638-5644.

Omar, W. A., Saleh, Y. S., & Marie, M. A. S. (2014). Integrating multiple fish biomarkers and risk assessment as indicators of metal pollution along the Red Sea coast of Hodeida, Yemen Republic. *Ecotoxicology and Environmental Safety*, 110, 221-231.

Omirinde, J. O., Audu, B. S., Mohammed, O. M., & Gosomji, I. J. (2017). Histomorphometric changes in the gill of *Clarias gariepinus* exposed to acute concentrations of chlorpyrifos. *Journal of Morphology Science*, 34 (3), 197-202. http://dx.doi.org/10.4322/jms.105516.

Organization for Economic Cooperation and Development (OECD). (2007). *Maximum Acceptable Contaminants: guidance safety level.* Fresh water fish (RPWS 1991). Pp. 12-28.

Osfor, M. M. H., Ibrahim, H. S., Mohamed, Y. A., Ahmed, A. M., Abd El Azeem, A. S., & Hegazy, A. M. (2010). Effect of alpha lipoic acid and vitamin E on heavy metals intoxication in male albino rats. *Journal of Animal Science*, 6, 56–63.

Osioma, E., Akanji, M. A., & Arise, R. O. (2013). Hepatic enzyme markers and proteins in serum and some selected tissues in *Clarias gariepinus* from swamp around Kokori-Erhoike oil field, Nigeria. *Journal of Research in Biology*, 3(4), 984-992.

Osisiogu, C. P., & Aladesanmi, O. T. (2019). Cadmium-induced toxicity and antioxidant enzyme responses in tissues and organs of African catfish (*Clarias gariepinus*). *African Journal of Biochemistry Research,* 13(5), 63-72. DOI: 10.5897/AJBR2018.0996.

Otitoloju, A. A., & Don-Pedro, K. N. (2001). Influence of joint application of heavy metals on level of each metal accumulated in periwinkle, *Tympanotonus fuscatus* (Gastropoda Potmididae). *Revolutionary Biology and Tropical Biology*, 54(3), 803-814.

Ozden, B., & Mustafa, K. (2010). The effects of pollution on the vitamins A, E, C, b-carotene contents and oxidative stress of the fresh water crayfish, *Astacus leptodactylus*. *Ecotoxicology and Environmental Safety*, 73, 138–142.

Palace, V. P., Doebel, C., Baron, C. L., Evans, R. E., Wautier, K. G., Klaverkamp, J. F., Werner, J., & Kollar, S. (2005). Caging small-bodied fish as an alternative method for environmental effects monitoring (EEM). *Water Quality Resource Journal of Canada*, 40(3), 328–333. http://doi.org/10.2166/wqrj.2005.037.

Palaniappan, P. L. R. M., Krishnakumar, N., & Vadivelu, M., (2009). Bioaccumulation of lead and the influence of chelating agents in *Catla catla* fingerlings. *Environmental Chemistry Letters*, 7, 51-54.

Paul, N., & Sengupta, M. (2013). Lead induced overactivation of phagocytes and variation in enzymatic and non-enzymatic antioxidant defenses in intestinal macrophages of *Channa punctatus*. *Modern Research in Inflammation*, 2, 28-35. Doi: 10.4236/mri.2013.22004.

Peixoto, F. P., Carrola, J., Coimbra, A. M., Fernandes, C., Teixeira, P., Coelho, L., Conceicao, I., Olivera, M. M., & Fontainhas-Fernandes, A. (2013). Oxidative stress responses and histological hepatic alterations in barbel, *Barbus bocagei*, from Vizela River, Portugal. *Revista Internacional de Contaminacion Ambiental*, 29 (1), 29-38.

Pratap, H. B. (2016). Haematological Responses and Growth of African fresh water cichlids, *Oreochromis niloticus* exposed to ambient inorganic mercury. *International Journal of Zoological Investigations*, 2(1), 9-16.

Pratt, T. C., Cullen, F. T., Sellers, C. S., Thomas, W. L., Madensen, T. D., Daigle, L. E., Fearn, N. E. & Gau, J. (2010). The empirical status of social learning theory: a meta-analysis. *Justice Quarterly*, 27:6, 765-802. Doi: 10.1080/07418820903379610.

Priyadarshani, S., Wickramasinghe, D. D., & Udagama, P. V. (2011a). Phagocytosis as a biomarker to detect environmental pollution by heavy metals using an amphibian model *Euphlyctis hexadactylus* (Ranidae): A pilot study. *Proceedings of the 6th Biennial Scientific Sessions of Allergy & Immunology Society of Sri Lanka*, p. 40.

Priyadarshani, S., Wickramasinghe, D. D., de Silva, D. N., & Udagama-Randeniya, P. V. (2011b). Phagocytosis as a biomarker of immunotoxicity in response to selected heavy metals in *Euphlyctis hexadactylus* (Ranidae): a pilot study. *Proceedings of the 31st Annual Sessions of the Institute of Biology*, p 34.

Puvaneswari, S., & Karuppasamy, R. (2007). Accumulation of cadmium and its effects on the survival and growth of larvae of *Heteropneustes fossilis* (Bolch, 1974). *Journal of Fish and Aquatic Sciences*, 2(1), 27-37.

Radic, S., Gregorovic, G., Stipanicev, D., Cvjetko, P., Srut, M., Vujcic, V., Orescanin, V., & Klobucar, G. I. V. (2013). Assessment of Surface water in the Vicinity of Fertilizer Factory using Fish and Plants. *Ecotoxicology and Environmental Safety*, 96, 32-40. http://doi.org/10.1016/j.ecoevn.2013.06.023.

Rahman, Z., Ahmad, I., & Rashid, I. (2018). Effects of Cadmium Exposure on Growth and Survival and Accumulation in Various Organs of Nile Tilapia (*Oreochromis niloticus*, Linnaeus). *Journal of Agriculture and Aquaculture*, 1(1).

Rajagopalan, R., Kode, A., Penumatha, S. V., Kallikat, N. R., & Venugopal, P. M. (2004). Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. *Journal of Pharmacy and Pharmaceutical Science*, 83, 2747-2752.

Rajagopalan, R., Periyasamy, L., & Baskaran, M. (2010). Effects of *Phyllanthus niruri* on Alcohol and Polyunsaturated Fatty Acid Induced Oxidative Stress in Liver. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2 (4).

Ramesh, M., Sankaran, M., Veera-Gowtham, V., & Poopal, R. K. (2014). Haematological, biochemical and enzymological responses in an Indian major carp, *Labeo rohita* induced by sub-lethal concentration of water borne selenite exposure. *Chemical and Biological Interraction*, 207, 67-73.

Reitman, S., & Frankel, S. (1957). Glutamic-Pyruvate transaminase assay by colorimetric method. *American Journal of Clinical Pathology*, 28- 56.

Rendón-Ramírez, A. L., Maldonado-Vega, M., Quintanar-Escorza, M. A., Hernández, G., Arévalo-Rivas, B. I., Zentella-Dehesa, A., & Calderón-Salinas, J. V. (2014). Effect of vitamin E and C supplementation on oxidative damage and total antioxidant capacity in lead-exposed workers. *Environmental Toxicology and Pharmacology*, 37, 45.

Saddick, S., Afifi, M., & Zinada, A. O. (2017). Effects of zinc nano-particles on oxidative stress-related genes and antioxidant enzymes activity in the brain of *Oreochromis niloticus* and *Tilapia zilli*. *Saudi Journal of Biological Sciences*, 24, 1672-1678.

Saglam, D., Atli, G., Dogan, Z., Baysoy, E., Gurler, C., Eroglu, A., & Canli, M. (2014). Response of the antioxidant system of freshwater fish (*Oreochromis niloticus*) exposed to metals (Cd, Cu) in different hardness. *Turkish Journal of Aquatic Science*, 14, 43–52.

Sahiti, H., Bislimi, K., Bajgora, A., Rexhepi, A., & Dalo, E. (2108). Protective Effect of Vitamin C against Oxidative Stress in Common Carp (*Cyprinus carpio*) Induced by Heavy Metals. *International Journal of Agriculture and Biosciences*, 7, 71.

Sahiti, H., Bislimi, K., Rexhepi, A., & Dalo, E. (2020). Metal Accumulation and Effect of Vitamin C and E in Accumulated Heavy Metals in Different Tissues in Common Carp (*Cyprinus carpio*) Treated with Heavy Metals. *Polish Journal of Environmental Studies*, 29 (1), 799-805. DOI: 10.15244/pjoes/103354.

Sajitha, G. R., Regi, J., Andrews, A., Ajantha, K. G., Augustine, P., & Augusti, K. T. (2010). Garlic oil and vitamin E prevent the adverse effects of lead acetate and ethanol separately as well as in combination in the drinking water of rats. *Indian Journal of Clinical Biochemistry*, 25, 280–288.

Saliu, J. K., & Bawa-Allah, K. A. (2012). Toxicological effects of lead and zinc on the antioxidant enzyme activities of post juvenile *Clarias gariepinus*. *Resources and Environment*, 2(1), 21-26. DOI:10.5923/j.re.20120201.03.

Samuel, P. O., Adakole, J. A., Suleiman, B., & Yaro, J. D. (2017a). Histopathological alterations in kidney and liver of *Clarias gariepinus* (Burchell, 1822) studied in River Galma, Nigeria. *Applied Scientific Reports*, 1-8. doi: 10.7243/2054-9903-2-1.

Samuel, P. O., Abiola, O. P., Arimoro, F. O., Ayanwale, A. V., Mohammed, A. Z., & Auta, Y. I. (2017b). Effects of lead nitrate on glutathione levels in *Clarias gariepinus* (Burchell, 1822) post juvenile. *Journal of Aquatic Sciences*, 32(2), 223-229. Doi:https:dx.doi.org/10.4314.jas.v32i2.1.

Samuel, P. O., Okoye, L. C., Abiola, O. P., Arimoro, F. O., & Auta, Y. I. (2018). Acute toxicity assessment of lead nitrate on *Clarias gariepinus* (Burchell, 1822). *International Journal of Fisheries and Aquatic Studies*, 6(2), 459-462.

Saraswati, D., Renu, S., Rajendra, C., Arun, R., & Vinoy, K. S. (2014). Ameliorative role of vitamin C against diomethoate toxicity in air breathing fish, *Clarias batrachus*. *Global Journal of Bio-Science and Biotechnology*, 3(1), 46-50.

Satish, P. V. V., Sravani, G., Ajaybabu, B., & Sunita, K. (2018). Haematological alterations after exposure periods of Acephate in fresh water snake headed fish, *Channa punctata*. *International Journal of Zoology and Applied Biosciences*, 3(4), 302-311. https://doi.org/10.5281/zenodo.1322891.

Satpal, S. K. J., & Punnia, J. S. (2010). Studies on biochemical changes in sub-acute thiodicarb toxicity in rats. *Toxicology International*, 17, 30-32.

Sfakianakis, D. G., Renieri, E., Kentouri, M., & Tsatsakis, A. M. (2015). Effect of heavy metals on fish larvae deformities: A review. *Environmental Research*, 137, 246-255.

Shahsavani, D., Baghshani, H., & Nourian, K. (2017). Effect of thiamine and vitamin C on tissue lead accumulation following experimental lead poisoning in *Cyprinus carpio*. *Iranian Journal of Veterinary Science and Technology*, 9, 39.

Sharma, D. K., & Ansari, B. A. (2013). Effects of deltamethrin on CAT, LPO and GSH in tissues of zebra fish, *Danio rerio*. *Research Journal of Environmental Toxicology*, 7(1), 38–46.

Shaymaa, H. M., Ebtihal, M. M. E., Rehab, A. G., & Mona, K. G. (2019). Molecular, biochemical and histopathological studies on the ameliorative effects of vitamin C on the renal and muscle tissues of Nile tilapia fish (*Oreochromis niloticus*) affected by the usage of engine oil. *Aquaculture Research*, 50(11), 3357-3368. https://doi.org/10.1111/are.14294.

Sherwood, G. D., Rasmussen, J. B., Rowan, D. J., Brodeur, J., & Hontela, A. (2000). Bioenergetic cost of heavy metal exposure in yellow perch (*Perca flavescens*): insitu estimates with a radiotracer (137Cs) technique. *Canadian Journal of Fisheries and Aquatic Sciences*, 57(2), 441-450.

Shilpi, G., Shilpi, S., & Sunita, S. (2015). Tolerance Against Heavy Metal Toxicity In Cyanobacteria: Role Of Antioxidant Defense System. *International Journal of Pharmacy and Pharmaceutical Sciences*, (7) (2), 0975-1491.

Shokrzadeh, M., Shobi, S., Attar, H., Shayegan, S., Payam, S. S. H., & Ghorbani, F. (2012). Effect of vitamins A, E and C on liver enzyme activity in rats exposed to organophosphate pesticide Diazinon. *Pakistan Journal of Biological Sciences*, 15(19), 936-941. Doi: 10.3923/pjbs.2012.936941.

Siess, M. H., Le Bon, A. M., Canivenc-Lavier, M. C., & Susch, M. (2000). Mechanisms Involved in the Chemoprevention of Flavonoids. *Biofactor*, 12 (4), 193-199.

Singh, C. B., & Ansari, B. A. (2017). Toxicity of two heavy metals Lead and Cobalt on Zebrafish, *Danio rerio*. *Scholars Academic Journal of Biosciences,* 5(9), 682-687.

Singh, Z., Kaur, T., & Kaur, M. (2017). Toxicological Assessment of Barium Carbonate on Behavioral and Hematological Parameters in *Channa punctatus*. *International Journal of Medical Research and Health Sciences,* 6(5), 88-95.

Sissein, E. A., Diepreye, E. Tonkiri, A., & Asara, A. A. (2014). Nonenzymatic antioxidant levels as pollution biomarkers in liver of *Clarias gariepinus* harvested from Gbarantoru Swamp: a polluted site in Bayelsa State. *Journal of Chemical, Biological and Physical Sciences,* 4(3), 2325-2332.

Stegeman, J. J., Brouwer, M., Richard, T. D. G., Fo¨rlin, L., Fowler, B. A., Sanders, B. M., & Van Veld, P. A. (1992). Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effect. In R. J. Huggett, R. A. Kimerly, P. M. Mehrle, H. L. Bergman (Eds.). *Biomarkers: Biochemical, Physiological and Histological markers of Anthropogenic Stress*. (pp. 235/335). Chelsea, MI, USA : Lewis Publishers.

Suleiman, F. A., Dayo, O. A., Muftau, S., AbdulGaniyu, G., Olushola, O. O., & Joseph, O. A. (2010). Chlorpyrifos-induced clinical, haematological and biochemical responses in Swiss Albino mice-mitigating effect by co-administration of vitamins C and E. *Life Science Journal*, 7(3), 37-44.

Suruchi, K., & Khanna P. (2011). Assessment of heavy metal contamination in different vegetables grown in and around urban areas. *Research Journal of Environmental Toxicology,* 5, 162–179.

Thakur, V., & Kanshere, R. R. (2014). Comparative Study on the Protective Role of Vitamin C and Vitamin E on Mercury Induced Toxicity in *Heteropneusts fossilis*. *International Research Journal of Science & Engineering,* 2 (2), 37-43.

Tingman, W., Jian, Z., & Xiaoshuan, Z. (2010). Fish product quality evaluation based on temperature monitoring in cold chain. *African Journal of Biotechnology*, 9, 6146-6151.

Uchendu, C., Amtali, S. F., & Ayo, J. O. (2012). The organophosphate, chlorpyrifos, oxidative stress and the role of some antioxidant: a review. *African Journal of Agricultural Research,* 7 (18), 2720–2728.

Udo, I. U. (2017). Effects of dietary vitamin A level on growth, feed utilization and survival of juvenile North African catfish (*Clarias gariepinus*). *Livestock Research for Rural Development*, 29, (22). Retrieved October 27, 2023, from http://www.lrrd.org/lrrd29/2/udo29022.htm.

Ugwuja, E. I., Nweze, V., Ikaraoha, C. I., & Ohayi, S. R. (2020). Zinc ameliorates lead toxicity by reducing body Pb-burden and restoring Pb-induced haematological

and biochemical derangements. *Toxicological Research and Application*: https://doi.org/10.1177/2397847320956562.

Ullah, A., Rehman, H., Khan, A. Z., Rehman, Z., Ahmad, S., Rehman, M. U., Bakht, A., Abdin, Z. U., Amjad, Z., Qureshi, M. F., & Khan, Z. (2016). Determination of 96-h LC_{50} of Lead Nitrate for a Fish, *Oreochromis niloticus*. *Journal of Entomology and Zoological Studies*, 4(4), 1216-1218.

Usman, M., Umaru, J. A., & Kibgu, A. A. (2019). Acute toxicity of *Jatropha curcas* (Barbados nut) latex extracts to *Oreochromis niloticus* juveniles. *Proceedings of the 2nd Freshwater Biological Association of Nigeria*, Pg 1-9.

Van Der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology*, 13, 57-149.

Verma, V. K., Gupta, N., & Gupta, D. K. (2020). Haemotoxicity of Pb on the blood parameters of fresh water teleost, *Heteropneustes fossilis* (Bloch). *International Journal of Pharmaceutical Sciences and Research*, 11(3), 1444-1451. Doi:10.13040/IJPSR.0975-8232.11(3).1444-51.

Vinodhini, R., & Narayanan, M. (2009). The impact of toxic heavy metals on the hematological parameters in common carp (*Cyprinus carpio* L.). *Iranian Journal of Environmental Health Science and Engineering*, 6(1), 23-28.

Waheed, R., El Asely, A. M., Bakery, H., El-Shawarby, R., Abuo-Salem, M., Abdel-Aleem, N., Malhat, F., Khafaga, A., & Abdeen, A. (2020). Thermal stress accelerates mercury chloride toxicity in *Oreochromis niloticus* via up-regulation of mercury bioaccumulation and HSP70 mRNA expression. *Science of the Total Environment*, 718, 137326.

Witeska, M., Sarnowski, P., Ługowska, K., & Kowal, E. (2014). The effects of cadmium and copper on embryonic and larval development of ide, (*Leuciscus idus* L). *Fish Physiology and Biochemistry*, 40, 151-163.

World Health Organization (1995). *Health criteria & other supporting information*. In: Guidelines for drinking water quality, Geneva, 2nd edn. 2, 31–388.

Xhyrel, J. J., Nikko, L. G., & Marlon, C. P. (2016). Relative antioxidant efficacy of α-tocopherol and ascorbic acid on blood lead, haemoglobin and haematocrit level of lead-exposed *Rattus norvegicus* (albino rat). *Der Pharmacia Lettre*, 8, 169-179.

Yamada, D., Hiwatari, M., Hangoma, P.,Narita, D., Mphuka, C., Chita, B., Yabe, Y., Nakayama, S. M. M., Choongo, K., & Ishizuka, M. (2020). Assessing the population-wide exposure to lead pollution in Kabwe, Zambi: an econometric estmation based on survey data. *Scientific Reports*, 10(1), 15092. Doi:10.1038/s41598-020-71998-5.

Yancheva, V., Stoyanova, S., Velcheva, I., Petrova, S., & Georgieva, E. (2014). Metal bioaccumulation in common carp and rudd from the Topolnitsa reservoir, Bulgaria. *Archives of industrial hygiene and toxicology*, 65(1), 1-10. DOI: 10.2478/10004-1254-65-2014-2451.

Yildirin, N. C., Benzer, F., & Danabas, D. (2011). Evaluation of environmental pollution at Munzur River of tunceli applying oxidative stress biomarkers in *capoeta trutta* (Heckel, 1843). *Journal of Animal and Plant Sciences*, 21(1), 66-71.

Yolanda, M., & Maria, L. I. (2012). Use of antioxidants for the treatment of cognitive and behavioural disorders in individuals with fragile X syndrome. *WO2012080554*.

Zaahkook, S. A. M., Abdel-Raheid, H. G., Ghanem, M. H., & Al-Sharkawy, S. M. (2016). Physiological and oxidative stress biomarkers in the freshwater catfish (*Clarias gariepinus*) exposed to pendimethalin-based herbicide and recovery with EDTA. *International Journal of Advanced Research*, 4(10), 243-264. Doi:10.21474/IJAR01/1784.

Zaki, M. S., Attia, A., Zaid, A., Mostafa,F., Abdelzaher, M. F., & Shalaby, S. I. (2014). Clinicopathological changes in fish exposed to pollutants. *Life Science Journal*, 11(3).

Zebral, Y. D., Anni, I. S. A., Afonso, S. B., Abril, S. I. M., Klein, R. D., & Bianchini, A. (2018). Effects of life-time exposure to waterborne copper on the somatotropic axis of the viviparous fish, *Poecilia vivipara*. *Chemosphere*, 203, 410-417.

Zhai, Q., Narbad, A., & Chen, W. (2015). Dietary strategies for treatment of cadmium and Pb toxicity. *Nutrients*, 7(1), 552-571. Doi: 10.3390/nu.701.0552.

Zikic, R. V., Stajn, S., Pavlovic, Z., Ognjanovic, B. I., & Saicic, Z. S. (2001). Activities of superoxide dismutase and catalase in erythrocyte and plasma transaminases of goldfish (*Carassius auratus gibelio* Bloch.) exposed to cadmium. *Physiological Research*, 50, 105–111.