EVALUATION OF ONUEBUM AXIS RIVER NUN WATER FOR HEAVY METAL-ASSOCIATED KIDNEY AND LIVER TOXICITY IN RATS

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DECEMBER, 2019

CERTIFICATION

We certify that this work was carried out by Emmanuel UCHE in the Department Of Biochemistry, University Of Benin, Benin City.

____________________ ____________________

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(Project Supervisor)

____________________ ____________________

Prof.(Mrs.) E.S.Omoregie Date
(Head of Department)

____________________ ____________________

External Examiner Date

DEDICATION

I dedicate this work to the Almighty God for his blessings, guidance and protection. For giving me a sound mind and a sound body to start and complete this work I am indeed grateful.

ACKNOWLEDGEMENTS

It is very important that I express my immense gratitude to the Almighty God and to all those who have played one role or another to bring about the successful completion of this work. I thank Prof F.O Obi, for the attention and criticisms he generously gave to me to ensure that the work was executed and the report written. I am also indebted to my senior colleague, Isabel Obadoni, in Prof F.O Obi's lab, who ensured that the instruments are used properly and data properly analyzed statistically. I show my immeasurable gratitude to my parents, Mr. and Mrs. Uche for their unwavering moral and financial support. Special thanks to my lecturers who taught me during the masters'degree coursework and were also my teachers at BSc level, for imparting me with enough knowledge to carry out such a mental task. They include; Prof M.A Adaikpoh, Prof N.E.J Orhue, Prof Uadia, Prof Nwanze, Prof Onoagbe, Prof K.E. Imafidon, Prof N.P Okolie, Prof E.S Omoregie, Prof Campbell and Dr.(Mrs) Uadia. To my other research colleagues; Moses Ayeni, Ejovwoke Emerhirhi, Mrs. Abigail Isojie, Mrs. Ruth Osemhengbe Usifo, as well as my classmates Sunday Ekperusi, Osayemen Osaigbovo, Henry Namuna, Great Ajayi and Igoudala Aigbe and to Mr Akpeh, my ever cooperative and patient lab technologist, I say thank you for contributing to the congenial atmosphere in which we had our lectures and did the research.

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ABBREVIATIONS

AAS	Atomic Absorbance Sprectrophotometry
APHA	American Public Health Association
BOD	Biochemical oxygen demand
Cd	Cadmium
CDC	Centre for Disease Control
Cr	Chromium
Cu	Copper
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organisation
Fe	Iron
Hg	Mercury
IOC	International Oil Companies
Mn	Manganese
MT	Metallothionein
Pb	Lead
RBC	Red blood cell
RNA	Ribonucleic acid
USEPA	United States Environmental Protection Agency

ABSTRACT

This study evaluated the chemistry and biological consequences of heavy metal contamination of water from River Nun at Onuebum town, Ogbia Local Government Area of Bayelsa State, Nigeria. The pH of the water, acidity, alkalinity, turbidity, sulphides, total nitrogen and total hydrocarbon content in the river water were identified. The toxicity of the water itself and that of the heavy metals in the river water were investigated using rats.

Sixty rats divided into twelve groups of five rats each were used. Group A, the control rats, received distilled water only (42.86 ml/rat/day by gavage). Group B received the boiled river water. Group C received the unboiled river water. Group D received a solution of the salts of all the metal ions identified in the river water (1.24 mg Fe, 0.018 mg Cu, 0.001 mg Hg, 0.001 mg Cd, 0.001 mg Pb, 0.001mg Mn, 0.001 mg Cr/L of distilled water). Group E received a solution containing 1.24 mg Fe/L. Group F received a solution containing 0.018 mg Cu/L. Group G received a solution containing 1.24 mg Fe/L and 0.001 mg Hg/L in distilled water. Group H received a solution containing 0.001 mg Hg/L. Group I received a solution containing 0.001 mg Cr/L. Group J received a solution containing 0.001 mg Cd/L. Group K received a solution containing 0.001 mg Mn/L. Group L received a solution containing 0.001 mg Pb/L. Each rat received 42.86 ml/kg bd wt. of the appropriate solution by gavage daily, 7 days a week for 3 months.

After an exposure duration of three months, there were significant increases ($p \leq 0.05$) in serum urea, creatinine, chloride, LDH, total and direct bilirubin levels of the groups H (mercury only), J (cadmium only) and L (lead only) rats compared to the rats in the control and the other eight groups. There were significant increases ($p \leq 0.05$) in liver GGT, LDH, total bilirubin, ALT, AST, and total protein levels of groups H (mercury only), J (cadmium only) and L (lead only) rats as compared to the control and other eight groups. There were significant increases ($p \leq 0.05$) in kidney sodium, chloride, urea and creatinine levels of groups H (mercury only), J (cadmium only) and L(lead only) rats as compared to the rats in the control and other eight groups. There was also significant ($p \leq 0.05$) increase in the group G when compared to control in terms of serum AST, ALT, Na^+ and cholesterol levels. However, these increases were not as profound as those observed for mercury when administered singly. The adverse effects, evidenced by changes in these parameters in the test rats relative to the control group, was not observed in the groups (B and C) that consumed the river water directly. The results obtained here show that heavy metals antagonize the effects of each other when administered concurrently, a pattern of behavior which would not be evident when each is examined in

isolation. The findings also show that the River Nun water, although containing several toxic elements, may be fairly safe for human consumption.

CHAPTER ONE

INTRODUCTION

Background of the Study

Water is very essential to life in all its forms and ramifications. The importance of water has been widely acknowledged in works highlighting how a deviation in water quality or quantity can translate into a drastic decline in productivity and an increase in mortality of living species (Garba *et al.,* 2008; Garba *et al.,* 2010). Also, there have been published works by the Food and Agricultural Organisation (FAO) showing that basic developmental strategies and human empowerment in African countries is significantly impeded by water related diseases (FAO, 2007). These diseases are apparently a direct consequence of pollution of these water bodies which may be as a result of human activities such as mining, farming and industrialization in general or natural processes such as weathering and acid rain. These processes release all sorts of contaminants into water bodies thereby dropping the overall potability of the waters which is evidenced by the increased turbidity, altered odour, taste, and several other estimable parameters such as its biochemical oxygen demand (BOD), phenol content, and heavy metal content. The latter (heavy metal content) is central to this study and report.

Heavy metals are essentially metallic elements with relatively high density, usually within 3.5 g/cm^3 to $7.0g/cm^3$. Heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), zinc (Zn), nickel (Ni), copper (Cu) and lead (Pb), iron (Fe), and manganese (Mn). They are non-biodegradable. This unique quality increases their toxic potential as they tend to bioaccumulate in the food chain (Ravindra *et al.,* 2015). These toxic metals are common in industrial runoffs in urban areas. Of the 50 elements categorized as heavy metals, 17 are ubiquitous and highly toxic. Among these 17 metallic elements are lead, mercury, arsenic and cadmium which are most commonly linked to human poisoning. Several factors influence the toxicity manifested by these metals such as the type of metal, their biological role, if any; the

nature or kind of organisms to which they are exposed, the duration of exposure, their chemical state (typically oxidation state) and even physical state (the shape and size). Copper, zinc, and chromium, are termed essential microelements which are actually required by the body in small amounts, but exhibit toxic effects upon larger doses (Abdollahi, 2013).

When these metals occur in water bodies such as lakes, in streams and rivers they do so concurrently even though at different individual concentrations. In this combined state, the level of toxicities they exert on the individuals drinking the water or consuming fish from such waters may be different from their individual toxicities or may be non-existent. In some cases, the resultant toxicity experienced is additive such that several organs are functionally compromised. However, in some other cases, these metals may have a somewhat antagonistic effect on each other such that the health on the individual remains largely minimized when the individual is exposed to all the metals simultaneously, unlike individual exposure to metallic entity.

Justification of the study

Worries about water pollution are a legitimate concern since its pollution affects the health and overall socioeconomic development of a people. Water samples from the River Nun at Onuebum town in Ogbia L.G.A., Bayelsa State was collected and analyzed to ascertain, among other parameters, its heavy metal content. The purpose was to find out if individuals drinking that River water or eating fish from it are unknowingly endangering their lives. However, it would be rather hasty to conclude that the water is toxic and unsafe just because of its heavy metal content and concentrations. This is so because these metals do not exist in isolation in the environment and so there is always simultaneous exposure wherein they may enhance or antagonize each other. Therefore, it is imperative to delineate the toxic potential of a heavy metal when alone, from that which it exhibits when coexisting in the environment with other heavy metals. This is the essence of this study.

Aim and Objectives

The study was aimed at evaluating the extent to which the consumption of water from Onuebum Axis of River Nun would cause adverse health effect as evidenced by liver and kidney health.

The objectives of this study include the:

1. Determination of the presence of heavy metal contaminants in the water at the sampling point
2. Determining the concentrations at which these heavy metal contaminants occur in it
3. Evaluating the effects they produce at such concentrations on liver and kidney function parameters when they occur singly
4. Determining the effects they produce at such concentrations on liver and kidney function parameters when they occur concurrently by estimation of;
    a. Serum aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase activities as well as cholesterol and bilirubin levels will be determined for the purpose of evaluating liver toxicity and,
    b. Serum chloride ion, potassium ion, sodium ion as well as urea and creatinine will be evaluated to ascertain nephrotoxicity.
5. Evaluation of the effect of the River water (boiled and unboiled) on kidney and liver functions.
6. Evaluating the histological changes associated with exposure to the metals.

CHAPTER TWO

LITERATURE REVIEW

2.1 POLLUTION OF NIGERIAN RIVERS

Water pollution is defined as an emergence of certain constituents into water sources, either directly or otherwise, which are capable of both causing deterioration in the quality of the water and adversely impacting on life forms (UN Report, 1972). Pollutants may exist either in the liquid or solid form but irrespective of their form, they adversely affect the quality of water (Owa, 2014). Niger Delta is Nigeria's oil-producing region. The region is made up of six states namely Akwa Ibom, Bayelsa Cross River, Delta, Edo and Rivers known for vast deposit of crude oil. According to Aghalino (2002), a large amount of high quality crude oil is extracted daily from the region with severe cases of oil spills. Oil exploration in the region has led to the problem of contamination of natural water sources (streams, rivers), aquatic habitat, destruction of farmland and mangroves (Olaniran, 1995; David and Omoogun 2016). Besides exploration itself, oil spillage in the region is a major problem (Oloruntegbe *et al.,* 2009). The spill affects land and water. Water contamination is the predominant environmental issue in the Niger Delta region (Raji and Abejide, 2013) and this has been attributed to oil exploration, extraction and spillage (UNEP Report, 2011). However, there are other sources of contamination. These other sources include indiscriminate disposal of refuse, leakages of sewage, channeling of industrial wastes and pesticides wash-off from farmlands. It is presently recognized that in this region, access to safe drinking water is gradually reduced due to the activities associated with oil exploration and the attendant spills (World Bank, 2008). The effect of oil spill-related water pollution is felt in the number of cases of water-borne illness reported annually (Nwilo and Badejo, 2005).

2.1.1 Correlation between water contamination and community health

The quality of life a group of people in a community enjoy, is almost directly linked to the quality of water they access (David and Omoogun, 2016). Studies have also revealed that sewage contributes to significant cases of poisoning of aquatic organisms (Alens, 2014). These findings reveal not only that water bodies are polluted but also that the fishes as well as other aquatic organisms consumed have unacceptably high burden of the pollutants. Among these pollutants are heavy metals. The allowable water limits of these metals are 0.01mg Pb/L, 0.03 mg Cd/L, 0.001mg Hg/L, 2.0 mg Cu/L, 0.05mg Cr/L, 0.01mg Fe/L, 0.1mg Mn/L and 0.07mg Ni/L (W.H.O, 1994). Most exposure to sewage-infested water in the Niger Delta occurs during swimming, bathing and use of stream water for cooking and drinking.

Contamination in water can also be due to the presence of algae and nitrates (David and Omoogun 2016). Algae and nitrates have been found to contribute to health problems ranging from skin rashes to other complex cases like respiratory, stomach or liver disorders (EPA, 2015). A phenomenon of current concern is the algal bloom being experienced by rivers in the Niger Delta which is responsible for the diminishing quality of water needed for drinking, recreation and other domestic activities (Alens, 2014). Ignorance of the effect of algal bloom and/or the lack of any other viable alternative makes the people insist on utilizing it for swimming, washing, bathing, cooking and sometimes drinking. It has been posited that consumption and dermal exposure to algal-infested water bodies have led to increased cases of hepatitis B and liver damage (Carmichael *et al.,* 2013).

2.2 RIVER NUN

River Nun marks the south-western end of Yenegoa city, 5 km downstream of a major bifurcation. It flows behind the city in a south-westerly direction after receiving polluted recharge from the Epie Creek which runs through Yenegoa town. The River Niger bifurcates into the Nun and Forcados Rivers about thirty-two kilometres downstream from Aboh, Delta

State, Nigeria (Okuyade and Abbey 2016). The Nun River is a stretch of fresh water flowing into the Gulf of Guinea, a wide inlet of the Atlantic Ocean at Akassa. It is about one hundred and sixty kilometres in length. Associated with oil exploration and exploitation is the accidental discharge of oil into the river. The serious environmental damage caused by frequent oil spills and their impacts on human and marine lives made living in the polluted area very inconvenient (Enegide, 2018). Apart from receiving polluted discharge from the Epie Creek which is a major sink for solid and liquid waste generated in the town (Fig 2.1) (Ezekwe *et al.,* 2014), markets, homes, industries and plants are all situated on the banks of the River Nun in southern Yenegoa and serve as additional pollution sources as wastes from here are directly deposited into the river. Compounding these pollution sources is the direct channeling of untreated urban storm water and sewage into the River Nun system. Essentially waters flowing from Otuedo, Otuaba and Kolo as shown in Fig 2.2. The area is populated by fishing and farming communities and settlements that depend on nature directly for their livelihoods (Okuyade and Abbey, 2016).

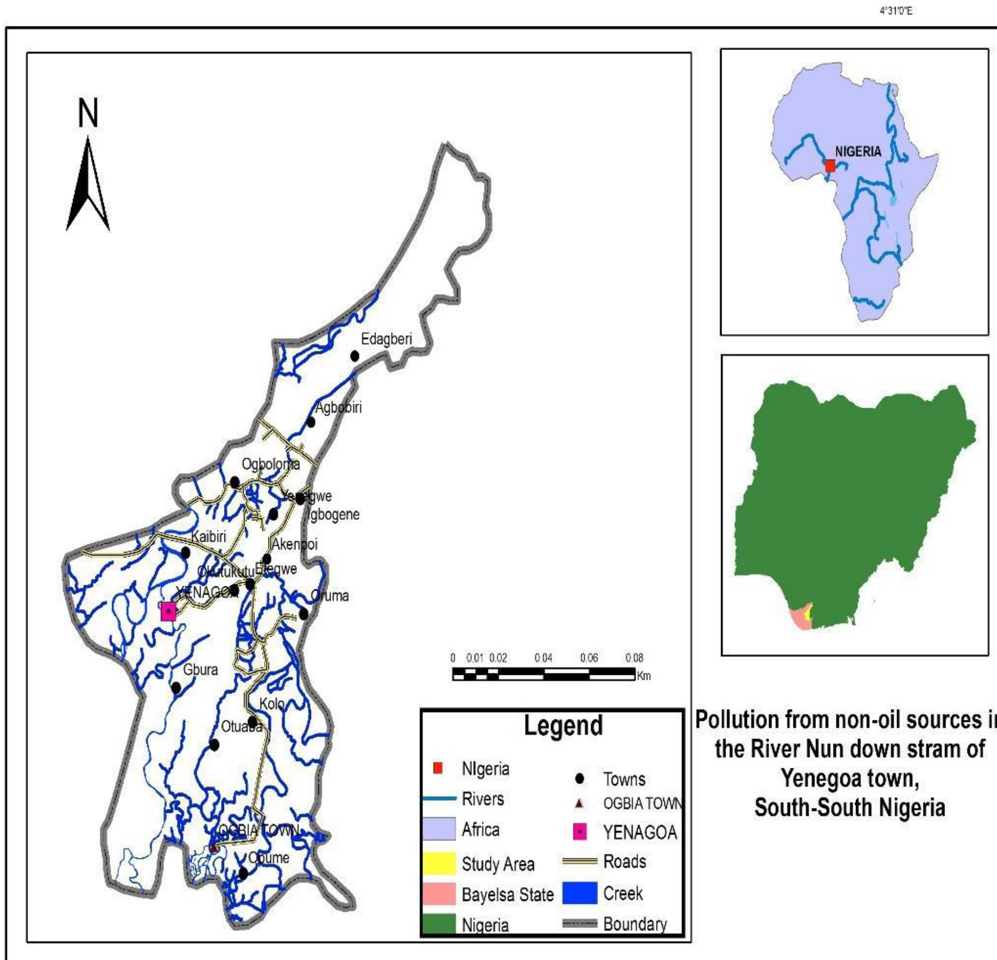


Fig 2.1 A Map Showing River Nun

Source: (Ezekwe *et al.*, 2014)

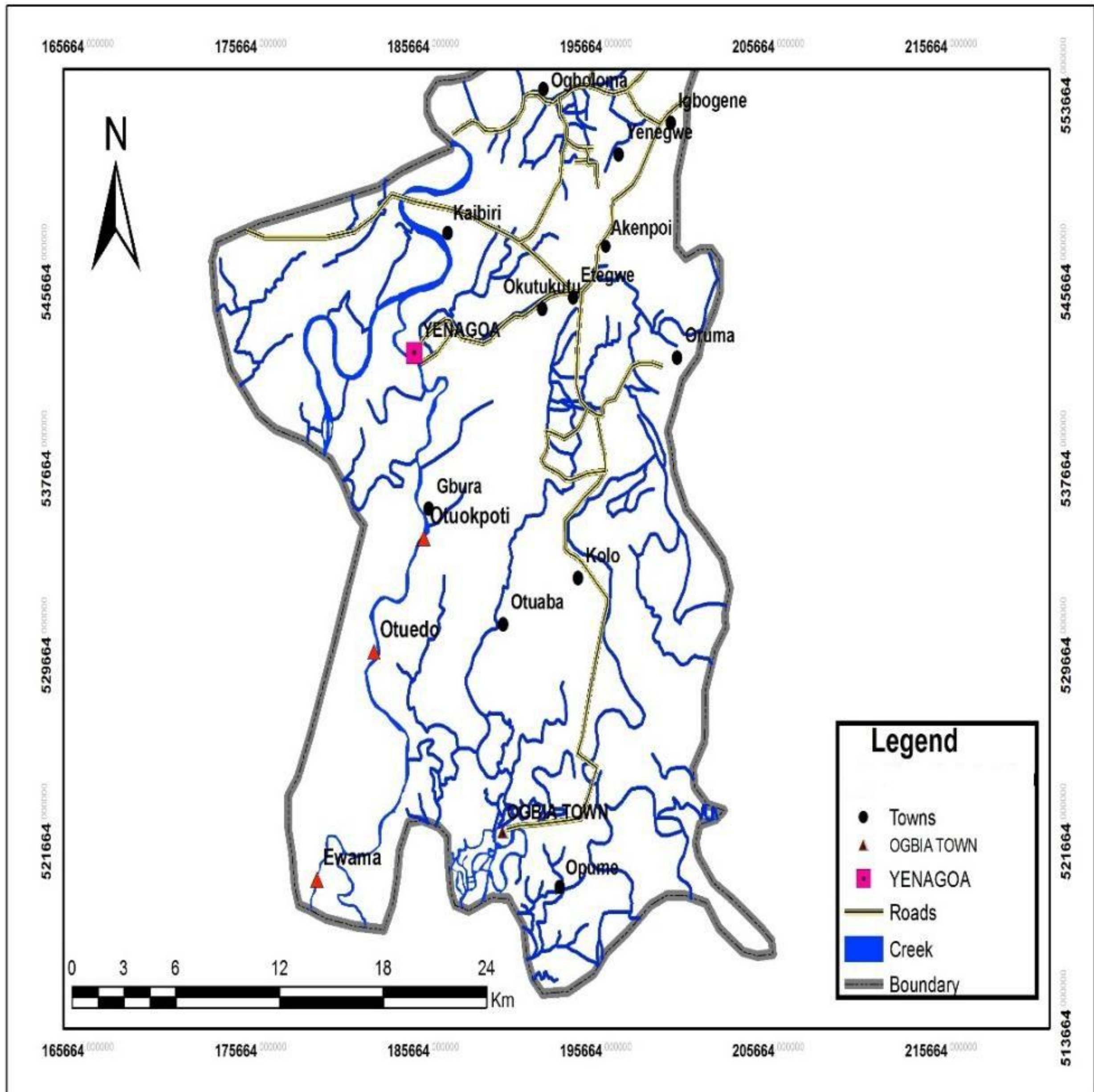


Fig 2.2 Map of the study area

Source: (Ezekwe *et al*., 2014)

2.3 DESCRIPTION OF HEAVY METAL CONTAMINANTS DISCOVERED

2.3.1 IRON

Iron is the most abundant element by mass (34.6%) that makes up the earth and is essential to most living things (Hurrel, 2010). It is a metal that is lustrous, malleable and ductile with a silver-grey appearance. It exists in one of two oxidation states, the ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions. Owing to its unique ability to serve as both an electron acceptor and donor in biological redox reactions, it can act as both an essential trace element in terms of being a cofactor, as well as producing toxicity by generating free radicals. Once induced by ions of transition metals, Fe^{2+} produces lipid peroxidation after binding of Fe^{2+} to negatively charged phospholipids. The binding results in alteration of the physical properties of the phospholipid bilayer, which is followed by the initiation and propagation of free radical chain reactions of lipid peroxidation (Gutteridge *et al* ., 1984; Tadolini and Hakim 1996; Oteiza *et al*., 2004).Iron at the intestinal surface competes with other deleterious metals for uptake. Hence its intracellular concentration and toxicity are low, when organisms are exposed to iron orally by way of potable water and/or diet.

At higher concentrations as quite commonly present, it overcomes competition with other heavy metals for binding sites to carrier proteins and enzymes. At high concentrations of iron, the increased susceptibility of δ-aminolevulinic acid dehydratase (δ-ALAD) and ferrochelatase to lead inhibition (Ahamed *et al.,* 2007; Skoczynska, 2008) is grossly reduced.

2.3.2 LEAD

Lead occurs in a high concentration on the earth. Its ubiquity is attributed to its low melting point and high malleability which combine to make it industrially important. It is important to over 900 industries, including mining, smelting, refining, battery manufacturing, and so on (Malekirad *et al.,* 2010; Karrari *et al.,* 2012). From the foregoing, especially with regard to its abundance and indispensability in industry, lead is still regarded as an environmental and

occupational toxicant, quite particularly in urban climes (Arif *et al.,* 2015). Quite a number of heavy metals, especially those belonging to the transition series, possess some significant biological functions. However, lead has no known biological function and has a safety limit set by the WHO at 0.01mg/L (10 µg/L) (Karrari *et al.,* 2012). The most likely route of exposure to lead is through inhaling lead-containing dust particles and ingestion through contaminated food or water (Arif *et al.,* 2015). Upon exposure, absorption follows; which occurs at the intestinal interface (for exposure via food and water). Upon absorption lead is transported by the blood to be deposited in soft tissues such as the kidneys, liver, lungs and even mineralizing tissues such as bone and teeth. The kidneys may successfully excrete some of the lead while the stored remainder constitutes what is termed body burden. Lead has a half-life of about thirty days in bone although it could stay for even decades in it (Arif *et al.,* 2015). It has been shown to be a very hazardous substance with a potential for producing irreversible organ/tissue damage in exposed humans and animals. Foetuses and breastfeeding infants suffer brain damage due to exposure to lead, besides its established effects on the erythrocyte (Gulson *et al.,* 2003). These effects are classically produced via induction of oxidative stress but in the case of the erythrocytes, it interferes with heme biosynthesis (Arif *et al*., 2015). Lead-induced oxidative stress leads to the oxidative breakdown of unsaturated fatty acids in biological membranes. Damaged membranes become more permeable as its architectural integrity and function are compromised. In addition to the oxidative stress effect, it has been reported that lead can produce an increase in the level of total cholesterol, triacylglycerols, and low-density lipoproteins which could go on to render the exposed individual more susceptible to cardiovascular problems. One of such problems is arterial hypertension (Poreba *et al.,* 2011). Lead could also disrupt crucial cellular processes such as cell adhesion, intra- and inter-cellular signalling, apoptosis, ionic transportation, protein folding, enzyme regulation, and release of

neurotransmitters by simply displacing Ca^{2+} and $Mg,^{2+}$ two cations crucially important in these cellular processes (Mathew *et al.,* 2011; Flora *et al.,* 2012).

2.3.3 COPPER

Copper is a member of the third transition series of elements, with atomic number 29 and atomic weight 63.546. Other members of this transition series include chromium, iron, cobalt, manganese, nickel, and zinc. All of them possess a partially filled d orbital, except zinc (Bonnie *et al.,* 2007). Copper can exist in any one of four oxidation states; 0, +1, +2, +3. However, the cuprous ion Cu^{+} and Cupric ion Cu^{2+} are most common in biological systems. The ability of copper to cycle between these two oxidation states confers on it a special relevance in redox reactions such as in mitochondrial respiration, synthesis of melanin, and cross-linking of collagen (International Programme on Chemical Safety, 1998). Copper is also an integral part of the antioxidant enzyme, copper-zinc superoxide dismutase, and has a role in iron homeostasis as a cofactor in ceruloplasmin (Ralph and McArdle, 2001). It is important for the maturation of cytoplasmic cuproproteins and assembly of enzymes in different cell organelles (ceruloplasmin and tyrosinase in case of Golgi apparatus and cytochrome c oxidase with respect to mitochondria) (Chapman and Chan 2000; Ahmed *et al.,* 2013; Pisoschi and Pop 2015). Other roles of copper are evident in its involvement in fetal/infant development and growth, brain development and function, immune function, bone strength, cholesterol and glucose metabolism, myocardial contractility, maintenance of hair and skin, and the formation of pigments (Bonnie *et al.,* 2007). At high concentrations copper produces oxidative damage to biological systems, including peroxidation of lipids or other macromolecules (Bremner, 1998). Conversely, copper deficiency alters the role of other cellular constituents involved in antioxidant activities, such as iron, selenium, and glutathione, and thus plays an important role in diseases in which oxidant stress is elevated (Johnson *et al.,* 1992).

2.3.4 CHROMIUM

Chromium is a chemical element with the symbol Cr and atomic mass 51.99. It is a steely-grey, lustrous, hard and brittle transition metal (Brandes *et al* 1956). Cr is widely used in industry, paints manufacture and metal platings as corrosion inhibitor. Its ions enter the aquatic system through effluents discharged from tanneries, textiles, and electroplating, mining, dyeing, printing, photographic and pharmaceutical industries (Pandey and Madhuri, 2014). As a consequence of rapid industrialization and poor waste management strategies, industries pour wastes containing chromium and large amount of chromates, dichromate, and other chromium-containing compounds into waterways and bodies. Cr exists primarily in the trivalent and hexavalent forms. In natural water however, its more toxic hexavalent form is more commonly encountered than the trivalent form. Owing to its increased anthropogenic use, contamination of natural water by Cr bearing industrial wastes is quite commonly observed (Pandey and Madhuri 2014).

2.3.5 CADMIUM

Cadmium (Cd) is a naturally occurring metal that is located between the elements zinc (Zn) and mercury (Hg) in the periodic table, chemically it behaves like Zn (Bernhoft, 2013). Cadmium (Cd) is considered a nonessential heavy metal that is dangerous to humans and aquatic organisms. It can damage the kidneys and bones (Kaya, 2002; Cinar, 2003; Kaplan *et al.,* 2011). The toxicity of cadmium can be observed in both animal and human tissues (Kaya, 2002). Cadmium may enhance the production of reactive oxygen species (ROS) when its free form is taken up by cells of the kidney. Altered ROS level leads to kidney damage evidenced by glycosuria, proteinuria, and aminoaciduria (Ibrahim *et al.,* 2013). In the cell, Cd does not necessarily circulate in this free form. It may exist therein bound to carrier molecules/proteins such as albumin, metallothioneins and glutathione (Ibrahim *et al.,* 2012).

2.3.6 MANGANESE

Manganese (Mn) is a chemical element occupying position 25 on the periodic table and exists freely in nature but often found in combination with iron. Its industrial use is particularly evident in alloys, most especially stainless steel. Manganese phosphating on steel serves to prevent its rust and/or corrosion. Generally, the permanganates of alkali are powerful oxidizers. This general oxidizing ability is dependent on the oxidation state which could range from -3 to +7 in the number line fashion with +2, +4, and +7 being most prevalent (Pan *et al.,* 2018).This confers oxides of Mn with the acidic, basic or amphoteric oxide property. Manganese dioxide is used as the cathode material in zinc-carbon and alkaline batteries (Santamaria, 2008). With regard to its biological significance, Mn^{2+} ion functions as cofactor for several enzymes; from energy metabolizing enzymes like kinases, to its crucial role in free radical scavenging enzyme for the detoxification of superoxide free radicals (Roth *et al.,* 2013).

2.3.7 MERCURY

Mercury, with atomic number 80 and atomic mass 200 is considered the most toxic heavy metal as it quite easily gains entry into and is toxic to the nervous and reproductive systems. It could exist in the elemental (Hg), inorganic (Hg^{+} and Hg^{2+}) and organic (Hg-Organic) forms which could go as far-reaching as to determine the degree of toxicity it exerts. Its two oxidation states; Hg^{+} and Hg^{2+} are termed the mercurous and mercuric forms respectively. Once the elemental form (Hg) attains either of these states, its solubility in water is highly increased therefore conferring a much higher capability to produce toxicity. Exposure to this toxic substance is commonly via inhalation, ingestion or absorption through the skin. Within the body, it has a half-life of 40 days (Clarkson, *et al.,*2003). Consequent upon the severe toxicity mercury portends, the Environmental Protection Act (EPA) and World Health Organization prescribed Hg level for drinking water are 0.002 mg/L and 0.001 mg/L, respectively (Arif *et al.,* 2015). Mercury can produce very severe adverse effects with the Minamata disease being a classical example; where accidental poisoning of waterways in Japan by methylmercury produced

symptoms typical of a neurological syndrome known as `Hunter Russell syndrome'. Generally, increased exposure to mercury is known to alter physiological functions in humans, essentially leading to pulmonary toxicity (elemental form), renal dysfunction (inorganic) and severe neurologic abnormalities and other intellectual disorders (organic forms).

2.4 BIOCHEMICAL PARAMETERS INVESTIGATED

In order to capture a holistic view of the overall health effect of this river water, parameters routinely estimated for liver and kidney function as well as other indices of toxicity were estimated. Parameters such as alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase, creatinine, total and direct bilirubin, cholesterol, urea, electrolytes (sodium, potassium and chloride) and lactate dehydrogenase were investigated.

2.4.1 ALANINE AMINOTRANSFERASE

Alanine aminotransferase (ALT) is an enzyme that catalyzes the transfer of an amino group from L- alanine to α- ketoglutarate. The products of this reversible transamination reaction being pyruvate and L- glutamate, which are critical to a fundamental metabolic process termed the alanine cycle. In a bid to clinically evaluate liver health perhaps following likelihood of exposure to hepatotoxic substances, serum ALT levels, serum AST levels and their ratio to one another are routinely measured as a subset of other liver function tests and typically recorded in international units/Liter (Lala, 2018; Ghouri *et al.,* 2010)

2.4.2 LACTATE DEHYDROGENASE

The enzyme is mostly linked to cellular metabolic functions and has a major energy metabolic role. It functions as a glycolytic pathway and TCA cycle link enzyme and catalyzes the transformation of pyruvate to lactate during anaerobic period (Nelson and Cox, 2005). During stress, anaerobic oxidation is preferably utilized for supply of energy needs (Wallice, 1998). The

enzyme therefore functions as an important biomarker in evaluating toxicity of water constituents or any substance upon exposure.

2.4.2 TOTAL PROTEIN

Proteins are huge molecules essentially composed of amino acids, required by cells for proper functioning. The structure and function of an organism depends on proteins. Hence, the control of cells, tissues, and organs require proteins to take place. They are major constituents in animal metabolism, and heavy metal contaminations may interfere with the proper functioning of proteins. Hence, it is imperative to determine the variations in metabolism of proteins caused by exposing cells to heavy metals. Possible variations are the rise in the synthesis or degradation of proteins as well as the activation or inhibition of some enzyme type (Canli, 1996). Too low plasma total protein in experimental animals shows transmittable diseases, kidney damages and nutritional imbalances while too high values specify hemoconcentration and impaired H_20 balance (Gary and Williams, 1977)

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 REAGENTS AND CHEMICALS

All reagents were of analytical grade. The reagents and their sources include; Sodium hydroxide (BDH Chemicals, Poole, England), Formalin (Merck, Darmstadt, Germany), Sodium chloride (JHD, China), and randox diagnostic kits were used to carry out assays for AST,ALT, bilirubin, GGT, urea and cholesterol. For the assays for sodium, potassium, chloride and LDH, fortress diagnostic kits were used. The following are the salts of the heavy metals used:

Ferric chloride (Kermel, China)

Cadmium chloride (Kermel, China)

Copper sulphate (Kermel, China)

Lead chloride (Kermel, China)

Chromium chloride (Kermel, China)

Manganese chloride (Kermel, China)

Mercuric iodide (Kermel, China)

3.1.2 EQUIPMENT

Equipments employed were of standard quality and working condition. They include: Digestion-block/heater (Moergold, China), Spectrophotometer (Techmel & Techmel, U.S.A), Weighing balance (Mettler pgn and Mettler H80, England), pH meter (Gallenkamp, England), Centrifuge (S23a Techmel & Techmel, U.S.A), Constant temperature waterbath (Brain Scientific and Instrument Company, England) and Atomic absorbance spectrophotometer (211 Accusys Atomic Absorption Spectrophotometer, Buck Scientific, East Norwalk Connecticut, U.S.A).

3.1.3 ANIMALS

Sixty albino rats (Wistar strain) with an average weight of 100 ± 6.23g were used for the distilled and river water mediated heavy metal exposure. They were obtained from the Animal House of the Department of Biochemistry University of Benin, Benin City. Rats for both studies were maintained on growers mash (Bendel Feed and Flour Mills, Ewu) and tap water for three weeks before the study commenced. Rats were exposed to heavy metals in water orally by gavage and thereafter to the heavy metal solution and fed *ad libitum* throughout the study period.

3.1.4 WATER COLLECTION

Water was collected from the Nun River at Onuebum town in Ogbia Local Government Area of Bayelsa State, Nigeria.

3.2 METHODS

3.2.1 Water Analysis

Water from river Nun was analyzed for physicochemical properties such as pH, total suspended solids, turbidity, total alkalinity, chemical oxygen demand, biochemical oxygen demand, sulphide, nitrate-nitrogen, phenol, free oil, total hydrocarbon content and the concentration of selected heavy metals like iron, copper, mercury, chromium, cadmium, manganese and lead using the standard methods for the examination of water and wastewater (APHA, 1995)

3.2.2 Preparation of Experimental water

The effect of drinking the heavy metal contaminated River Nun water was evaluated by tainting distilled water with salts of the heavy metals observed to be present in River Nun water; iron, copper, mercury, chromium, cadmium, manganese and lead at the concentrations in which each was present in the river water, as determined using atomic absorption spectroscopy.

3.2.2.1 Iron tainted water (1.24mg/L)

The salt used was $FeCl_2 . 4H_20$ with molar mass 198.753g/mol

Exploiting the law of constant composition we say;

Since, 198.753mg of salt contains 55.847mg Iron

Therefore, 1.24mg Iron is present in $\frac{198.753}{55.847} \times 1.24$ or 4.40mg of salt

Dissolving 0.0044g of Iron in 1L of distilled water gives equivalent of 1.24mg Fe/L

3.2.2.2 Copper tainted water (0.18mg/L)

The salt used was $CuSO_4 . 5H_2O$ with molar mass 249.68 g/mol

since, 249.68 mg of salt contains 63.546 mg copper

Therefore, 0.18 mg Copper is present in $\frac{249.68}{63.546} \times 0.18$ or 0.707 mg of salt

A stock solution was made by dissolving 0.707 g of salt in 1 L of distilled water. One milliliter of this was then transferred into a 1000 ml flask and made up to the 1000 ml mark to give an equivalent of 0.18 mg Cu/L

3.2.2.3 Mercury tainted water (0.001 mg/L)

The salt used was HgI with molar mass 454.4 g/mol

Exploiting the law of constant composition it was conceived that;

since, 454.4 mg of salt contains 200.59 mg mercury,

0.001 mg Mercury will be present in $\frac{454.4}{200.59} \times 0.001$ or 0.0022 mg of salt

A stock solution was made by dissolving 0.0022 g of salt in 1 L of distilled water. One milliliter of this was then transferred into a 1000 ml flask and made up to the 1000 ml mark to give an equivalent of 0.001 mg Hg/L

3.2.2.4 Iron and mercury tainted water (1.24 mg/L + 0.001 mg/L)

One milliliter from the stock solution of iron and 1 ml of the stock solution of mercury were transferred into a 1000 ml flask and made up to the 1000 ml mark.

3.2.2.5 Chromium tainted water (0.001 mg/L)

The salt used was $CrCl_2 . 6H_2O$ with molar mass 230.994 g/mol

Exploiting the law of constant composition it was conceived that;

since, 230.994 mg of salt contains 51.99 mg chromium

0.001 mg Chromium is present in $\frac{230.994}{51.99} \times 0.001$ or 0.0044 mg of salt

A stock solution was made by dissolving 0.0044 g of salt in 1 L of distilled water. One milliliter of this was then transferred into a 1000ml flask and made up to the 1000ml mark to give an equivalent of 0.001mg Cr/L

3.2.2.6 Cadmium tainted water (0.001 mg/L)

The salt used was $CdCl_2 . 5H_2O$ with molar mass 183.31 g/mol

Exploiting the law of constant composition it was conceived;

Since, 183.31 mg of the salt contains 112.41 mg cadmium

Therefore, 0.001 mg of cadmium will be present in $\frac{193.31}{112.41} \times 0.001$ or 0.0016 mg of salt

A stock solution was made by dissolving 0.0016 g of salt in 1 L of distilled water. One milliliter of this was then put into a 1000 ml flask and made up to the 1000 ml mark to give an equivalent of 0.001 mg Cd/L.

3.2.2.7 Manganese tainted water (0.001 mg/L)

The salt used was $MnCl_2 . 4H_2O$ with molar mass 197.91 g/mol

Based on the law of constant composition, it was conceived that;

Since 197.91 mg of the salt contains 54.938 mg Manganese

0.001 mg of manganese will be present in $\frac{197.91}{54.938} \times 0.001$ or 0.0036 mg of salt

A stock solution was made by dissolving 0.0036 g of salt in 1 L of distilled water. One milliliter of this was then put into a 1000 ml flask and made up to the 1000 ml mark to give an equivalent of 0.001 mg Mn/L.

3.2.2.8 Lead tainted water (0.001 mg/L)

The salt used was $PbCl_2$ with molar mass 278.1 g/mol

Based on the law of constant composition it was conceived that;

since 278.1 mg of the salt contains 207.2 mg Lead

Therefore, 0.001 mg of Lead will be present in $\frac{278.1}{207.2} \times 0.001$ or 0.0013 mg of salt

A stock solution was made by dissolving 0.0013 g of salt in 1 L of distilled water. One milliliter was then put into a 1000 ml flask and made up to the 1000 ml mark to give an equivalent of 0.001 mg Pb/L.

3.2.2.9 All identified metals tainted water (1.24 mg Fe/L + 0.018 mg Cu/L +0.001 mg of other metals)

One milliliter from the stock solution of each of the salts of all metals (Fe, Cu, Hg, Cr, Cd, Mn and Pb) were transferred into a 1000 ml flask and made up to the 1000 ml mark. This solution mimicked the Onuebum axis of River Nun water in terms of metal composition.

3.2.3 Treatment of rats

3.2.3.1 Exposure of rats via drinking water

Sixty rats with an average weight of 100 $\pm$ 6.23g were divided randomly into twelve experimental groups of five rats per group, housed in wood frame iron mesh cages. Group A rats were maintained on feed and untainted water. Group B rats were given the boiled River Nun water. Group C rats were given unboiled River Nun water. Group D rats were maintained on a solution containing all the heavy metal ions in distilled water. Group E rats were given a

solution of iron. Group F rats were given a solution of copper. Group G rats were maintained on a solution containing ions of iron and mercury. Group H rats were given a solution of mercury. Group I rats were given a solution of chromium. Group J rats were given a solution of cadmium. Group K rats were given a solution of manganese. Group L rats were given a solution of lead. Rats in each group received the equivalent of 3 litres of the appropriate solution in water/70kg body weight/day by gavage (which was essentially 42.86 ml/kg b.wt). Following the administration, all rats were allowed free access to feed and the appropriate solution. This treatment was maintained for 90 days.

3.2.4: Animal Sacrifice and Sample Collection

Immediately after the ninety days stipulated for the study had elapsed, each rat was anaesthetized in a chloroform saturated chamber. While under anaesthesia the abdominal and thoracic regions were opened and blood was obtained by heart puncture using hypodermic syringe and needle. The blood collected from the heart was transferred to anticoagulant-free tubes. The kidneys and liver were excised and left standing on ice.

3.2.4.1: Preparation of samples

Each blood sample was centrifuged at 3000 rpm for 10 minutes and the serum separated using a Pasteur pipette and stored at -20°C until required for analysis.

3.2.4.2: Preparation of tissue homogenates

A portion of the liver of known weight and one of the kidneys after weighing were separately homogenized in ice-cold saline (1:5 w/v) to give a 20% homogenate using ice-cold mortar and pestle. Each homogenate was centrifuged at 3000 rpm for 10 minutes. The supernatant was separated with Pasteur pipette and stored frozen at -20°C until required for biochemical assays.

3.2.4.3: Histopathology samples

A portion of the liver and a kidney from each group were fixed in 10% formalin and were processed, stained and examined.

3.2.5: Biochemical Assays

3.2.5.1 Estimation of serum sodium ion concentration

Sodium concentration in serum was determined according to the method described by Maruna (1958) using Fortress diagnostic kit.

Principle

In the assay, sodium ion is precipitated as the triple salt, sodium magnesium uranyl acetate, and the excess uranium then, allowed to react with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium ion in the test specimen.

Procedure

Filtrate Preparation: Test tubes were labeled namely, blank, standard, and samples (serum) after which 1.0 mL of filtrate reagent (uranyl acetate 2.1 mM + magnesium acetate 20 mM in ethyl alcohol) was added to all tubes. This was followed by the addition of 50 µl of each sample to appropriate sample tube, 50 µl of standard (sodium chloride solution) were put in the test tube labeled standard and 50 µl of distilled water to the blank. All the tubes were shaken vigorously and continuously for 3 minutes. After which they were centrifuged at high speed (3500 rpm) for 10 minutes.

Color development: Into a set of new, labeled test tubes corresponding to the above filtrate tubes, 1.0 mL of acid reagent (dilute acetic acid) was added followed by the addition of 50 µL of supernatant to respective tubes. Color Reagent (potassium ferrocyanide + non-reactive stabilizers + fillers) 50 µL was then added to the mixtures, shaken for even mixing and absorbance read with a spectrophotometer at 550 nm using distilled water as blank.

Calculation

Sodium concentration= $\frac{change\ in\ Asample}{change\ in\ Astandard}$ x Standard concentration

3.2.5.2 Estimation of serum potassium ion concentration

Estimation of serum potassium concentration was based on the method described by Tietz (1995) using Fortress diagnostic kits.

Principle

The amount of potassium ion was determined by using the sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of the colloid is usually proportional to the potassium concentration.

Procedure

Test tubes were labeled, blank, standard, and sample after which 1.0 mL of potassium reagent was pipetted into all tubes. Aliquots (10 µL) of standard, distilled water and samples were added to the various tubes after which the contents of each test-tube were mixed and left to stand for 3 minutes at room temperature. The absorbance was read at 500 nm read against reagent blank.

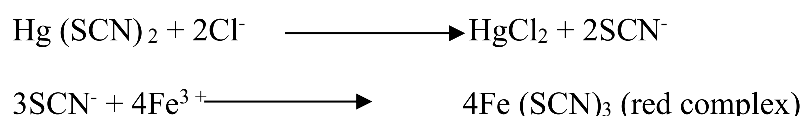
Calculation

Potassium concentration= $\frac{Asample}{Astandard}$ x Standard concentration

3.2.5.3 Estimation of serum chloride ion concentration

Estimation of serum chloride ion concentration was based on the method described by Tietz (1995) using Fortress diagnostic kits.

Principle



$Hg(SCN)_2 + 2Cl^- \longrightarrow HgCl_2 + 2SCN^-$

$3SCN^- + 4Fe^{3+} \longrightarrow 4Fe(SCN)_3$ (red complex)

Chloride ions form a soluble, non-ionized compound with mercuric ions and displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react

with ferric ions to form a red colored complex that absorbs light at 456 nm. The intensity of the color produced is directly proportional to the chloride ion concentration.

Procedure

Test tubes were labelled blank, calibrator, and sample after which 1 mL of chloride reagent was pipetted into all tubes. Aliquots (10 µL) of standard, distilled water and samples were added to the various tubes appropriately after which the contents of each test-tube were mixed and left to stand for 5 minutes at room temperature. The absorbance of each sample was thereafter, read against reagent blank at 456 nm.

Calculation

Chloride concentration= $\frac{Abs\ sample}{Abs\ standard}$ x Standard concentration

3.2.5.4 Estimation of serum creatinine concentration

Estimation of creatinine concentration was carried out according to the method described by Bartels and Bohmer (1972) using Randox diagnostic kits.

Principle

Creatinine in alkaline solution reacts with picric acid to form a deep yellow complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Procedure

Aliquots (1.0 mL) of the working reagent (a mixture of 35mmol/L picric acid and 0.32 mol/L of sodium hydroxide) were added to 0.1 mL of each sample. Similarly 1.0 mL of the standard solution was added to 1.0 mL of the sample in a different test tube. The mixture was equilibrated and absorbance A_1 of the standard and sample were read after 30 seconds at 492 nm. Exactly two minutes later, absorbance A_2, of the standard and sample were taken.

Calculation

$A_2 - A_1 = \Delta Asample$ or $\Delta Astandard$

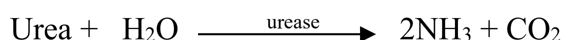
$$\text{Creatinine concentration} = \frac{Asample}{Astandard} \times \text{Standard concentration}$$

3.2.5.5 Estimation of serum urea concentration

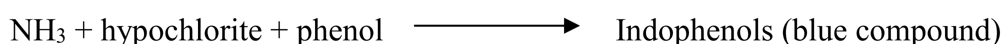
Estimation of urea concentration was carried out according to the method described by Fawcet and Scott (1960) using Randox diagnostic kits.

Principle

Urea in serum is hydrolysed to ammonia by urease. The ammonia formed is then measured photometrically by Berthelot's reaction.



$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$



$$NH_3 + \text{hypochlorite} + \text{phenol} \longrightarrow \text{Indophenols (blue compound)}$$

Procedure

Three sets of test tubes labelled blank, standard and sample were used. Into appropriate test tubes, 10 µl each of distilled water, standard solution and the serum samples respectively, were added. Then, 0.1 ml of Reagent 1 (sodium nitroprusside and urease) was added to each test tube. The contents of each tube were mixed and incubated at 37°C for 10 minutes. Then, to the test tubes 2.50 ml each of Reagent 2 (phenol solution) and Reagent 3 (sodium hypochlorite solution) were added, mixed and incubated at 37°C for another 15 minutes. The absorbance of each sample was measured at 546 nm against the blank.

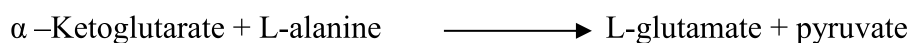
Manual Urea calculation

$$\text{Urea concentration} = \frac{Asample}{Astandard} \times \text{Standard concentration}$$

3.2.5.6: Assay for Alanine Aminotransferase Activity (ALT)

The activity of this enzyme in the samples was evaluated by the method described by Reitman and Frankel (1957).

Principle


α –Ketoglutarate + L-alanine → L-glutamate + pyruvate

Pyruvate + 2,4 dinitrophenylhydrazine → Pyruvate hydrazone

ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed, when pyruvate reacts with 2,4 dinitrophenylhydrazine.

Procedure

Test tubes were labelled blank and sample after which 0.1 ml of distilled water and sample were added to appropriate tubes. To each test tube, 0.5 ml of working reagent 1 (phosphate buffer + L-alanine + α-oxoglutarate) was added after which the contents of each test-tube were mixed and incubated at 37°C for 30 minutes. This was followed by the addition of 0.5 ml of reagent 2 (2,4-dinitrophenylhydrazine) to the test tubes and incubation at room temperature for 20 minutes. Thereafter, 5.0 ml of NaOH was added, mixed properly and left for 5 minutes. Absorbance of sample against reagent blank was taken at 546 nm.

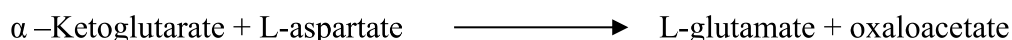
Calculation

The Randox kit provided values corresponding to standard concentrations with which to plot a standard curve from which concentrations obtained from the assay were extrapolated.

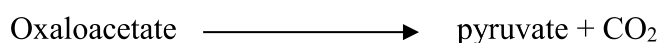
3.2.5.7: Assay for Aspartate Aminotransferase Activity (AST)

The activities of AST were assayed according to the procedure of Reitman and Frankel (1957) using Randox kit.

Principle


α –Ketoglutarate + L-aspartate → L-glutamate + oxaloacetate

The unstable oxaloacetate formed is then spontaneously decarboxylated to form pyruvate as the equation below shows.


Oxaloacetate → pyruvate + CO_2

AST activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4 dinitrophenylhydrazine.

Procedure

Test tubes were labelled blank and sample after which 0.1 mL of distilled water and sample were added to appropriate tubes. To each test tube, 0.5 mL of reagent 1(phosphate buffer + L-aspartate + α-oxoglutatate) was added after which the contents of each tube were mixed and incubated at 37°C for 30 minutes. This was followed by the addition of 0.5 mL of reagent 2 (2,4-dinitrophenylhydrazine) to the test tubes and left at room temperature for 20 minutes. Thereafter, 5.0 mL of NaOH was added, mixed properly and incubated for 5 minutes. Absorbance of each sample was read against reagent blank at 546 nm.

Calculation

The Randox kit provided values corresponding to standard concentrations with which to plot a standard curve from which concentrations obtained from the assay were extrapolated.

3.2.5.8: Estimation of total and direct bilirubin

Estimation of total and direct bilirubin was carried out according to the method reported by Sherlock (1951), using Randox kit.

Principle

Direct (conjugated) bilirubin reacts with diazotised sulphanilic acid in alkaline medium to form a blue colored complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, allowing the released bilirubin to react with diazotised sulphanilic acid.

Procedure for total bilirubin assay

Aliquots (200 µL) of sulphanilic acid, 1000 µL of caffeine and 200 µL of sample were measured into test tubes labelled sample and sample blank respectively. Nitrate solution (50 µL) was added to sample tubes alone and the tubes were mixed properly and left at room temperature for 10 minutes. Tartrate reagent (1000 µL) was then added into both test tubes, mixed thoroughly

and left at room temperature for 30 minutes. Thereafter, absorbance of each sample was read at 578 nm against the sample blank.

Calculation

Total Bilirubin= 10.8 x Asample

Procedure for direct bilirubin assay

Aliquots (200 µL) of sulphanilic acid, 2000 µL of 0.9% NaCl and 200 µL of sample were measured into test tubes labelled sample and sample blank respectively. Nitrate solution (50 µL) was added to the sample tubes alone and all the tubes were mixed properly and left for 10 minutes at room temperature. Absorbance of each sample was read at 546 nm against the sample blank.

Calculation

Direct Bilirubin= 14.4 x Asample

3.2.5.9 Estimation of lactate dehydrogenase Activity (LDH)

The lactate dehydrogenase (LDH) activity was assayed based on UV ray absorption method. This is an optimized standard method according to the recommendations of Deutsche Gesellschaft fur Klinische Chemie (1993).

Principle

LDH catalyses the interconversion of pyruvate to L-lactate using NADH as the reducing equivalent (i.e NADH is oxidized to NAD^+ and vice-versa). The rate of decrease in NADH is directly proportional to the LDH activity and is determined by measurement of the rate of absorbance change at 340nm due to the following reaction:

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-lactate} + NAD^+$$

Procedure

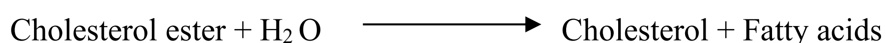
The assay for lactate dehydrogenase was done using LDH test kit which contained reagent R1a (buffer/substrate) and reagents R1b (NADH). The vials of freeze dried NADH (R1b) were each reconstituted with 3 mL of R1a (buffer/substrate) and mixed gently. The sample (0.1 mL) and 3 mL of the rehydrated NADH solution were pipetted into each test tube. The contents of the tube mixed gently, transferred into a quartz cuvette and the initial absorbance was read at 340 nm. The clock was started instantaneously and the absorbance was taken again after exactly 1, 2 and 3minutes.

3.2.5.11: Estimation of cholesterol concentration

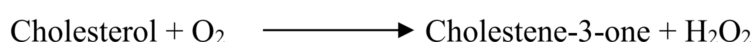
Estimation of cholesterol concentration was carried out according to the method described by Fawcet and Scott (1960) using Randox diagnostic kits.

Principle

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase



Cholesterol ester + $H_2O \longrightarrow$ Cholesterol + Fatty acids



Cholesterol + $O_2 \longrightarrow$ Cholestene-3-one + H_2O_2

$2H_2O_2$ +phenol + 4-aminoantipyrine $\longrightarrow$ quinoneimine + $4H_2O$

Procedure

Three sets of test tubes labelled blank, standard and sample were used. Into appropriate test tubes, 10 µL each of distilled water, standard solution and the serum samples were separately added. Then, 0.1 mL of the cholesterol Reagent (pipes buffer, 4-aminoantipyrine, phenol, peroxidase and cholesterol esterase) was added to each test tube. The contents of each tube were mixed and incubated at 25°C for 10 minutes. The absorbance of each sample was measured at 500 nm against the blank.

3.2.5.12: Estimation of gamma glutamyl transferase Activity (GGT)

Estimation of gamma glutamyl transferase activity was carried out according to the colorimetric method described by Tietz (1986) using Randox diagnostic kits.

Principle

The substrate L- Ɣ -glutamyl-3-carboxy-4-nitroanilide in the presence of glycylglycine is converted by Ɣ - GT in the sample to 5- amino-2-nitrobenzoate which can be measured at 405nm.

L- Ɣ - glutamyl-3- carboxy-4-nitroanilide + glycylglycine

L- Ɣ - glutamylglycylglycine + 5-amino-2-nitrobenzoate

Procedure

The assay for gamma glutamyl transferase was done using GGT test kit which contained reagent R1a (buffer/glycylglycine) and reagents R1b (NADH). The vials of freeze dried NADH (R1b) were each reconstituted with 3 mL of R1a (buffer/substrate) and mixed gently. The sample (0.1 mL) and 1 mL of the rehydrated NADH solution were pipetted into each test tube. The mixture was mixed gently, transferred into a cuvette and the initial absorbance was read at 405 nm. The clock was started simultaneously and the absorbance was taken again after exactly 1, 2 and 3 minutes.

Calculation

Determination of GGT concentration

$$\text{GGT concentration} = 1158 \times \textit{change in A}$$

3.3: Histopathological Evaluation

Histopathological evaluation was carried out according to the method described by Kiernan (2008).

Procedure

Tissues (kidney and liver) were excised from animals and preserved using 10% neutral buffered formalin placed in pre-labelled universal containers and sent to the histopathology laboratory. Using standard operating procedures, the tissues were sliced and placed in labelled tissue cassettes. The thickness of tissues did not exceed 3-5 mm. Tissues were subjected to automatic tissue processing using the Leica TP2010 automatic tissue processor for 18 hours passing them through the four stages of tissue processing namely: fixation (using 10% neutral buffered formalin) dehydration (using ascending grades of isopropyl alcohol), clearing or dealcoholisation (using xylene) and finally impregnation or infiltration (using molten paraffin wax). The tissues were then embedded in paraffin wax using the Leica automated tissue embedder and sectioned to get ultra-thin sections at five (5) microns; using the thermoscientific semi-automated rotary microtome. Tissues were floated out from the thermoscientific digital floating bath on frosted end pre-labelled slides and dried on the thermoscientific digital slimline hot plate. Tissues were further dried in the hot air oven overnight and subjected to haematoxylin and eosin staining to demonstrate the general tissue structure. Stained slides were mounted in DPX and allowed to dry before viewing under the microscope using x10 and x40 magnification.

3.4: Statistical analysis

The results were presented as mean ± standard error of mean. In order to establish whether the mean values are statistically different from each other, analysis of variance (ANOVA) was done using SPSS (Statistical Package for Social Sciences) software version 21. In order to know which means have differences that are significant, LSD multiple range test was done and values were considered significantly different at $p \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1 Nephrotoxic Effects

4.1.1: Effects of mercury, cadmium and lead on serum urea levels

The effects of mercury, cadmium, and lead on rat serum urea levels after exposure are presented in Table 4.1. Following a three-month oral exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in serum urea levels when compared to the control and the other eight group's serum urea values.

4.1.2: Effects of mercury, cadmium and lead on serum creatinine levels

The effects of mercury, cadmium, and lead on rat serum creatinine levels after 3 months exposure are presented in Table 4.1. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in serum creatinine level when compared to the control and other eight groups. However, the mercury treated group (group H) did not differ significantly from group I, the chromium treated group.

4.1.3: Effects of a solution containing all metal ions identified in river water on serum urea levels

The effects of a solution containing all identified heavy metals (Fe, Cu, Hg, Cd, Cr, Mn and Pb) ions on rat serum urea levels after exposure, are presented in Table 4.1 Following a three-month exposure by gavage, the composite solution of all identified metal ions in distilled water caused a significant ($p \leq 0.05$) increase in serum urea level when compared to the control and group B, the boiled river water group.

4.1.4: Effects of a solution of the binary of iron and mercury on serum urea levels

The effects of a solution of the binary of iron and mercury on serum urea levels after exposure, is presented in Table 4.1. Following a three-month exposure by gavage, the solution containing

all metal ions identified in river water caused a significant ($p \leq 0.05$) decrease in serum urea level as compared to group E, the Fe only group.

4.1.5: Effects of mercury, cadmium and lead on kidney urea levels

The effects of mercury, cadmium, and lead on rat kidney urea levels after exposure, are presented in Table 4.2. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in kidney urea levels as compared to the control and the other eight groups

4.1.6: Effects of mercury, cadmium and lead on kidney creatinine levels in rats

The effects of mercury, cadmium, and lead on rat kidney creatinine levels after exposure, are presented in Table 4.2. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in kidney creatinine level compared to the control and the other eight groups.

4.1.7: Effects of unboiled river water on kidney urea levels

The effects of unboiled river water on rat kidney urea levels after exposure are presented in Table 4.2. Following a three-month exposure by gavage, unboiled river water caused a significant ($p \leq 0.05$) increase in kidney urea level compared to the control.

4.1.8: Effects of a solution containing all metal ions identified in river water on kidney urea levels

The effects of a solution containing all metal ions identified in river water on rat kidney urea levels after exposure are presented in Table 4.2. Following a three-month exposure by gavage, the solution containing all metal ions identified in river water caused a significant ($p \leq 0.05$) increase in kidney urea level compared to the control and group B, the boiled river water group.

4.1.9: Effects of iron tainted water on kidney urea levels in rats

The effects of iron tainted water on rat kidney urea levels after exposure are presented in Table 4.2. Following a three-month exposure by gavage, iron tainted water caused a significant ($p \leq 0.05$) increase in kidney urea level compared to the control.

4.1.10: Effects of a solution of the binary of iron and mercury on kidney urea levels

The effects of solution of iron and mercury binary on kidney urea levels after exposure are presented in Table 4.2. Following a three-month exposure by gavage, the solution of the binary of iron and mercury caused a significant ($p \leq 0.05$) decrease in kidney urea level as compared to the control and group D, the all metals group.

4.1.11: Effects of chromium tainted water on kidney urea levels

The effects of chromium tainted water on rat kidney urea levels after exposure are presented in Table 4.2. Following a three-month exposure by gavage, chromium tainted water caused a significant ($p \leq 0.05$) increase in kidney urea level as compared to the control.

Table 4.1: Changes in serum urea and creatinine status

Group	Treatment	Parameters	
		Urea(mg/dl)	Creatinine(mg/dl)
A	Control	42.21 ± 3.87	4.02 ± 0.28
B	Boiled river water	42.66 ± 2.68	5.06 ± 0.92
C	Unboiled river water	45.32 ± 2.52	6.11 ± 1.23
D	All metals	51.66 ± 2.58^{a,b}	6.33 ± 0.79
E	Iron(1.24mg/L)	44.14 ± 1.72	5.39 ± 0.99
F	Copper(0.18mg/L)	45.36 ± 3.66	5.93 ± 1.23
G	Iron+ Mercury	42.05 ± 2.41^{d}	4.85 ± 1.13
H	Mercury(0.001mg/L)	63.21 ± 2.81^{a,b,c,d,e,f,g,i,k}	10.03 ± 0.91 ^{a,b,c,d,e,f,g,k}
I	Chromium(0.001mg/L)	44.57 ± 2.27	7.24 ± 0.70
J	Cadmium(0.001mg/L)	63.10 ± 3.71 ^{a,b,c,d,e,f,g,i,k}	11.79 ± 1.42 ^{a,b,c,d,e,f,g,i,k}
K	Manganese(0.001mg/L)	42.74 ± 2.92	5.31 ± 0.99
L	Lead(0.001mg/L)	62.73 ± 2.21 ^{a,b,c,d,e,f,g,i,k}	11.59 ± 2.79 ^{a,b,c,d,e,f,g,i,k}

Values are presented as means ± SEM; n= 3
*Values with superscripts a,b,c,d,e,f,g,h,i,j,k or l are significantly different from the value of the group with the corresponding uppercase letter A,B,C, D,E,F,G,H,I,J,K or L ($p \leq 0.05$).

Table 4.2: Changes in kidney urea and creatinine status

Group	Treatment	Parameters	
		Urea(mg/dl)	Creatinine(mg/dl)
A	Control	45.61 ± 1.23	5.68 ± 0.16
B	Boiled river water	53.57 ± 3.06	7.00 ± 1.10
C	Unboiled river water	52.53 ± 2.71[a]	7.81 ± 0.82
D	All metals	63.08 ± 2.36[a,b]	7.52 ± 0.64
E	Iron(1.24mg/L)	56.57 ± 1.23[a]	6.77 ± 0.85
F	Copper(0.18mg/L)	57.31 ± 4.26	7.86 ± 1.33
G	Iron+ Mercury	53.49 ± 3.12[d]	6.46 ± 0.79
H	Mercury(0.001mg/L)	73.43 ± 3.57[a,b,c,d,e,f,g,i,k]	12.95 ± 0.98 [a,b,c,d,e,f,g,i,k]
I	Chromium(0.001mg/L)	56.03 ± 1.71[a]	8.34 ± 0.81
J	Cadmium(0.001mg/L)	76.83 ± 6.39[a,b,c,d,e,f,g,i,k]	13.88 ± 1.45 [a,b,c,d,e,f,g,i,k]
K	Manganese(0.001mg/L)	54.41 ± 3.13	6.65 ± 0.41
L	Lead(0.001mg/L)	74.24 ± 2.17[a,b,c,d,e,f,g,i,k]	14.06 ± 1.68 [a,b,c,d,e,f,g,i,k]

Values are presented as means ± SEM; n= 3
*Values with superscripts a,b,c,d,e,f,g,h,I,j,k or l are significantly different from the value of the group with the corresponding uppercase letter A,B,C, D,E,F,G,H,I,J,K or L ($p \leq 0.05$).

4.1.12: Effects of mercury, cadmium and lead on serum sodium ion levels in rats following daily oral exposure

The effects of mercury, cadmium, and lead on rat serum sodium ion levels after exposure, are presented in Table 4.3. Following a three-month exposure by gavage, mercury, cadmium, and lead caused a significant ($p \leq 0.05$) increase in serum sodium ion level as compared to the control and other eight groups. This trend was however not particularly so, when compared to group G. Group G, rats exposed to the binary of iron and mercury, caused a significant ($p \leq 0.05$) increase in sodium level compared to the control.

4.1.13: Effects of mercury, cadmium and lead on serum potassium levels

The effects of mercury, cadmium, and lead on serum potassium levels after exposure, are presented in Table 4.3. Following a three-month exposure by gavage, mercury, cadmium, and lead caused a significant ($p \leq 0.05$) increase in serum potassium level compared to the control and other eight groups. However, the mercury treated group (group H) potassium level did not differ significantly from group I, the chromium treated group.

4.1.14: Effects of mercury, cadmium and lead on serum chloride levels

The effects of mercury, cadmium, and lead on rat serum chloride levels after exposure, are presented in Table 4.3. Following a three-month exposure by gavage, mercury, cadmium, and lead caused a significant ($p \leq 0.05$) increase in serum chloride level when compared to the control and other eight groups.

4.1.15: Effects of a solution of iron and mercury binary on serum sodium levels

The effect of solution of the binary of iron and mercury on rat serum sodium levels after exposure, are presented in Table 4.3. Following a three-month exposure by gavage, the solution of the binary of iron and mercury caused a significant ($p \leq 0.05$) increase in serum sodium level compared to the control.

4.1.16: Effects of a solution containing all metal ions identified in river water on serum chloride levels

The effects of a solution containing all metal ions identified in river water on serum chloride levels after exposure, are presented in Table 4.3. Following a three-month exposure by gavage, the solution containing all metal ions identified in river water caused a significant ($p \leq 0.05$) increase in serum chloride level when compared to the control

4.1.17: Effects of the solution of the binary of iron and mercury on serum chloride levels

The effect of the solution of the binary of iron and mercury on serum chloride levels after exposure, are presented in Table 4.3. Following a three-month exposure by gavage, the solution of the binary of iron and mercury a significant ($p \leq 0.05$) decrease in serum chloride levels when compared to the control.

4.1.18: Effects of mercury, cadmium and lead on kidney sodium levels

The effects of mercury, cadmium, and lead on rat kidney sodium levels after exposure, are presented in Table 4.4. Following a three-month exposure by gavage, mercury, cadmium, and lead caused a significant ($p \leq 0.05$) increase in kidney sodium level compared to the control and other eight groups.

4.1.19: Effects of manganese on kidney potassium levels in rats

The effect of manganese on rat kidney potassium levels after exposure, are presented in Table 4.4. Following a three-month exposure by gavage, Group K, the manganese treated group, caused a significant ($p \leq 0.05$) increase in kidney potassium level compared to the control and other ten groups. Although manganese was significantly disruptive, mercury, cadmium and leadeven had a more pronounced effect.

4.1.20: Effects of mercury, cadmium and lead on kidney chloride levels

The effects of mercury, cadmium, and lead on kidney chloride levels after exposure, are presented in Table 4.4. Following a three-month exposure by gavage, mercury, cadmium, and

lead caused a significant ($p \leq 0.05$) increase in kidney chloride level compared to the control and other eight groups.

4.1.21: Effect of copper tainted water kidney sodium levels

The effect of copper tainted water on rat kidney sodium levels after exposure, are presented in Table 4.4.

Following a three-month exposure following gavage, copper tainted water caused a significant ($p \leq 0.05$) increase in kidney sodium level as compared to the control.

4.1.22: Effect of manganese tainted water on kidney sodium levels

The effect of manganese tainted water on rat kidney sodium levels after exposure, are presented in Table 4.4. Following a three-month exposure by gavage, manganese tainted water caused a significant ($p \leq 0.05$) increase in kidney sodium level compared to the control and group G, the binary of Fe and Hg treated group.

4.1.23: Effects of unboiled river water on kidney chloride levels

The effect of unboiled river water on rat kidney chloride level after exposure, are presented in Table 4.4. Following a three-month exposure by gavage, unboiled river water caused a significant ($p \leq 0.05$) increase in kidney chloride level as compared to the control.

4.1.24: Effects of a solution containing all metal ions identified in river water on kidney chloride levels

The effect of a solution containing all metal ions identified in river water on rat kidney chloride levels after exposure, are presented in Table 4.4. Following a three-month exposure by gavage, the solution containing all metal ions identified in river water caused a significant ($p \leq 0.05$) increase in kidney chloride level as compared to the control

4.1.25: Effects of iron tainted water on kidney chloride levels

The effect of iron tainted water on rat kidney chloride level after exposure, are presented in Table 4.4. Following a three-month exposure by gavage, iron tainted water caused a significant ($p \leq 0.05$) increase in kidney chloride level as compared to the control.

4.1.26: Effects of copper tainted water on kidney chloride levels

The effect of copper tainted water on kidney chloride levels after exposure, are presented in Table 4.4. Following a three-month exposure by gavage, copper tainted water caused a significant ($p \leq 0.05$) increase in kidney chloride level when compared to the control.

4.1.27: Effects of chromium tainted water on kidney chloride levels

The effect of chromium tainted water on rat kidney chloride levels after exposure, are presented in Table 4.4. Following a three-month exposure by gavage, chromium tainted water caused a significant ($p \leq 0.05$) increase in kidney chloride level compared to the control but a decrease compared to groups H, J and L the Cd, Hg and Pb treated groups respectively.

Table 4.3: Changes in serum sodium, potassium and chloride status

Group	Treatment	Parameters		
		Sodium(mEq/L)	Potassium(mEq/L)	Chloride(mEq/L)
A	Control	131.65 ± 2.01	8.87 ± 0.39	72.15 ± 2.96
B	Boiled river water	136.53 ± 2.14	10.07 ± 0.77	69.39 ± 5.12
C	Unboiled river water	133.55 ± 4.40	10.97 ± 1.43	75.24 ± 2.87
D	All metals	134.67 ± 3.91	11.50 ± 0.90	81.39 ± 3.00[b]
E	Iron (1.24mg/L)	135.63 ± 0.29	10.38 ± 0.94	74.70 ± 2.10
F	Copper(0.18mg/L)	134.86 ± 4.48	11.13 ± 1.19	75.33 ± 3.70
G	Iron+ mercury	155.76 ± 21.81[a]	9.58 ± 0.61	70.49 ± 3.40[d]
H	Mercury(0.001mg/L)	172.08 ± 9.86[a,b,c,d,e,f,i,k]	15.03 ± 0.76[a,b,c,d,e,f,g,k]	93.00 ± 2.89[a,b,c,d,e,f,g,i,k]
I	Chromium(0.001mg/L)	142.17 ± 2.34	12.25 ± 0.68[a]	74.48 ± 2.27
J	Cadmium(0.001mg/L)	175.09 ± 5.69[a,b,c,d,e,f,i,k,]	16.83 ± 1.54[a,b,c,d,e,f,g,i,k]	93.09 ± 3.12[a,b,c,d,e,f,g,i,k]
K	Manganese(0.001mg/L)	148.33 ± 4.75	10.68 ± 0.77	73.03 ± 2.81
L	Lead(0.001mg/L)	173.31 ± 6.24 [a,b,c,d,e,f,i,k,]	16.54 ± 1.89 [a,b,c,d,e,f,g,i,k]	94.49 ± 2.96 [a,b,c,d,e,f,g,i,k]

Values are presented as means ± SEM; n= 3

*Values with superscripts a,b,c,d,e,f,g,h,i,j,k or l are significantly different from the value of the group with the corresponding uppercase letter A,B,C,D,E,F,G,H,I,J,K or L ($p \leq 0.05$).

Table 4.4: Changes in kidney sodium, potassium and chloride status

Group designation	Treatment	Parameters		
		Sodium(mEq/L)	Potassium(mEq/L)	Chloride(mEq/L)
A	Control	141.55 ± 3.68	10.37 ± 0.17	75.70 ± 1.55
B	Boiled river water	156.53 ± 8.47	12.09 ± 1.16	84.21 ± 2.93
C	Unboiled river water	155.43 ± 5.95	12.65 ± 1.14	87.37 ± 2.64[a]
D	All metals	152.19 ± 5.09	12.66 ± 0.60	93.20 ± 2.34[a]
E	Iron (1.24mg/L)	153.47 ± 2.46	11.86 ± 0.82	86.30 ± 1.43[a]
F	Copper(0.18mg/L)	159.66 ± 2.93[a]	12.68 ± 1.28	87.15 ± 4.16[a]
G	Iron+ mercury	143.67 ± 1.83	11.34 ± 0.49	83.91 ± 2.75
H	Mercury(0.001mg/L)	192.88 ± 7.18[a,b,c,d,e,f,g,i,k]	16.85 ± 1.06	103.06 ± 3.84[a,b,c,d,e,f,g,i,k]
I	Chromium(0.001mg/L)	155.66 ± 5.58	13.49 ± 0.86	86.38 ± 1.49[a,j]
J	Cadmium(0.001mg/L)	194.47 ± 5.48[a,b,c,d,e,f,g,i,k]	18.18 ± 1.21	105.44 ± 7.58[a,b,c,d,e,f,g,i,k]
K	Manganese(0.001mg/L)	161.43 ± 9.24[a,g]	45.20 ± 33.18[a,b,c,d,e,f,g,h,i,j]	85.67 ± 2.47[a]
L	Lead(0.001mg/L)	192.08 ± 6.38[a,b,c,d,e,f,g,i,k]	18.45 ± 0.97	110.13 ± 1.93[a,b,c,d,e,f,g,i,k]

Values are presented as means ± SEM; n= 3
*Values with superscripts a,b,c,d,e,f,g,h,i,j,k or l are significantly different from the value of the group with the corresponding uppercase letter A,B,C, D,E,F,G,H,I,J,K or L ($p \leq 0.05$).

4.2 Hepatotoxic Effects

4.2.1: Effects of mercury, cadmium and lead on serum ALT activity

The effects of mercury, cadmium, and lead on rat serum ALT activity after exposure, are presented in Table 4.5. Following a three-month exposure by gavage, mercury, cadmium, and lead caused a significant ($p \leq 0.05$) increase in serum ALT activity compared to the control and the other groups except group G, the iron and mercury combined group, in which a significant ($p \leq 0.05$) increase in serum ALT activity was also observed compared to the control.

4.2.2: Effects of mercury, cadmium and lead on serum AST activity

The effects of mercury, cadmium, and lead on serum AST activity after exposure, are presented in Table 4.5. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in serum AST activity compared to the control and the other groups except group G, the iron and mercury combined group, in which a significant ($p \leq 0.05$) increase in serum AST activity was observed as well, relative to the control.

4.2.3: Effects of mercury, cadmium and lead on liver ALT activity

The effects of mercury, cadmium, and lead on liver ALT activity after exposure, are presented in Table 4.6. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in liver ALT activity compared to the control and the other eight groups.

4.2.4: Effect of copper on liver ALT activity

The effect of copper on liver ALT activity after exposure is presented in Table 4.6.

Following a three-month exposure by gavage, copper caused a significant ($p \leq 0.05$) increase in liver ALT activity compared to the control.

4.2.5: Effect of manganese on liver ALT activity

The effect of manganese on liver ALT activity after exposure is presented in Table 4.6.

Following a three-month exposure by gavage, manganese caused a significant ($p \leq 0.05$) increase in liver ALT activity when compared to the control and group G, the binary of Fe and Hg treated group.

4.2.6: Effects of mercury, cadmium and lead on liver AST activity

The effects of mercury, cadmium, and lead on liver AST activity after exposure, are presented in Table 4.6. Following a three-month exposure by gavage, mercury, cadmium, and lead caused a significant ($p \leq 0.05$) increase in liver AST activity compared to the control and the other eight groups.

4.2.7: Effect of copper on liver AST activity

The effect of copper on liver AST activity after exposure is presented in Table 4.6.

Following a three-month exposure by gavage, copper caused a significant ($p \leq 0.05$) increase in liver AST activity compared to the control.

4.2.8: Effect of manganese on liver AST activity in rats following daily oral exposure

The effect of manganese on liver AST activity after exposure is presented in Table 4.6.

Following a three-month exposure by gavage, manganese caused a significant ($p \leq 0.05$) increase in liver AST activity compared to the control and group G, the binary of Fe and Hg treated group.

Table 4.5: Changes in serum ALT and AST activities

Group	Treatment	Parameters	
		ALT(U/L)	AST(U/L)
A	Control	141.52 ± 2.08	159.65 ± 2.01
B	Boiled river water	146.37 ± 2.10	164.53 ± 2.14
C	Unboiled river water	143.78 ± 4.41	161.55 ± 4.40
D	All metals	145.26 ± 4.47	162.67 ± 3.91
E	Iron(1.24mg/L)	145.79 ± 0.23	163.63 ± 0.29
F	Copper(0.18mg/L)	144.94 ± 4.74	162.86 ± 4.48
G	Iron+ Mercury	165.66 ± 21.86[a]	183.76 ± 21.80[a]
H	Mercury(0.001mg/L)	182.30 ± 9.92[a,b,c,d,e,f,i,j,k,l]	200.08 ± 9.86[a,b,c,d,e,f,i,k]
I	Chromium(0.001mg/L)	152.14 ± 2.31	170.17 ± 2.34
J	Cadmium(0.001mg/L)	186.97 ± 6.54 [a,b,c,d,e,f,i,k]	203.69 ± 5.69 [a,b,c,d,e,f,i,k]
K	Manganese(0.001mg/L)	158.43 ± 4.95	176.33 ± 4.75
L	Lead(0.001mg/L)	183.01 ± 6.15 [a,b,c,d,e,f,i,k]	201.31 ± 6.24 [a,b,c,d,e,f,i,k]

Values are presented as means ± SEM; n= 3

*Values with superscripts a,b,c,d,e,f,g,h,I,j,k or l are significantly different from the value of the group with the corresponding uppercase letter A,B,C, D,E,F,G,H,I,J,K or L ($p \leq 0.05$).

Table 4.6: Changes in liver ALT and AST activities

Group	Treatment	Parameters	
		ALT(U/L)	AST(U/L)
A	Control	151.42 ± 3.70	173.55 ± 3.68
B	Boiled river water	166.62 ± 8.58	188.53 ± 8.47
C	Unboiled river water	165.27 ± 5.82	187.43 ± 5.95
D	All metals	162.91 ± 5.43	184.19 ± 5.09
E	Iron(1.24mg/L)	163.18 ± 2.39	185.47± 2.46
F	Copper(0.18mg/L)	169.66 ± 2.93[a]	191.66 ± 2.93[a]
G	Iron+ Mercury	153.67 ± 1.83	175.67 ± 1.83
H	Mercury(0.001mg/L)	202.88 ± 7.18[a,b,c,d,e,f,g,i,k]	224.88 ± 7.18[a,b,c,d,e,f,g,i,k]
I	Chromium(0.001mg/L)	165.66 ± 5.58	187.66 ± 5.58
J	Cadmium(0.001mg/L)	204.47 ± 5.48 [a,b,c,d,e,f,g,i,k]	226.47 ± 5.48 [a,b,c,d,e,f,g,i,k]
K	Manganese(0.001mg/L)	171.43 ± 9.24[a,g]	193.43 ± 9.24[a,g]
L	Lead(0.001mg/L)	202.08 ± 6.36 [a,b,c,d,e,f,g,i,k]	224.08 ± 6.36 [a,b,c,d,e,f,g,i,k]

Values are presented as means ± SEM; n= 3
*Values with superscripts a,b,c,d,e,f,g,h,I,j,k or l are significantly different from the value of the group with the corresponding uppercase letter A,B,C, D,E,F,G,H,I,J,K or L ($p \leq 0.05$).

4.2.9: Effects of mercury, cadmium and lead on serum direct bilirubin levels

The effects of mercury, cadmium, and lead on rat serum direct bilirubin levels after exposure, are presented in Table 4.7. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in serum direct bilirubin levels compared to the control and the other eight groups. However, the mercury treated group (group H) did not differ significantly ($p \geq 0.05$) from group I, the chromium treated group.

4.2.10: Effects of mercury, cadmium and lead on serum total bilirubin levels

The effects of mercury, cadmium, and lead on serum total bilirubin levels after exposure, are presented in Table 4.7. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in serum total bilirubin levels compared to the control and the other eight groups.

4.2.11: Effects of mercury, cadmium and lead on liver direct bilirubin levels

The effects of mercury, cadmium, and lead on liver direct bilirubin levels after exposure, are presented in Table 4.7. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in liver direct bilirubin levels compared to the control and other eight groups. However, the mercury treated group (group H) value did not differ significantly ($p \leq 0.05$) from group I (the chromium treated group) value.

4.2.12: Effects of mercury, cadmium and lead on liver total bilirubin levels in rats following daily oral exposure

The effects of mercury, cadmium, and lead on liver total bilirubin after exposure, are presented in Table 4.7. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in liver total bilirubin compared to the control and the other eight groups.

4.2.13: Effects of mercury, cadmium and lead on serum GGT activity.

The effects of mercury, cadmium, and lead on rat serum GGT activity after exposure, are presented in Table 4.8. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in serum GGT activity compared to the control and the other groups except group G, the iron and mercury combined group, in which a significant ($p \leq 0.05$) increase in serum GGT activity was also observed compared to the control.

4.2.14: Effects of mercury, cadmium and lead on serum LDH activity.

The effects of mercury, cadmium, and lead on serum LDH activity after exposure, are presented in Table 4.8. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in serum LDH activity compared to the control and the other eight groups.

4.2.15: Effects of mercury, cadmium and lead on liver GGT activity.

The effects of mercury, cadmium, and lead on liver GGT activity after exposure, are presented in Table 4.8. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in liver GGT activity compared to the control and the other groups except group F, the copper only group, in which a significant ($p \leq 0.05$) increase in liver GGT activity was also observed compared to the control.

4.2.16: Effects of mercury, cadmium and lead on liver LDH activity

The effects of mercury, cadmium, and lead on liver LDH activity after exposure, are presented in Table 4.8. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in liver LDH activity compared to the control and the other eight groups.

4.2.17: Effects of mercury, cadmium and lead on serum cholesterol levels.

The effects of mercury, cadmium, and lead on rat serum cholesterol levels after exposure, are presented in Table 4.9. Following a three-month exposure by gavage, mercury, cadmium, and

lead caused significant ($p \leq 0.05$) increases in serum cholesterol level as compared to the control and the other eight groups except group G, the binary of iron and mercury group, in which a significant ($p \leq 0.05$) increase in serum cholesterol level was demonstrated also, when compared to the control.

4.2.18: Effects of mercury, cadmium and lead on liver cholesterol levels.

The effects of mercury, cadmium, and lead on liver cholesterol levels after exposure, are presented in Table 4.9. Following a three-month exposure by gavage, mercury, cadmium, and lead caused significant ($p \leq 0.05$) increases in liver cholesterol level compared to the control and the other eight groups except group G the iron and mercury combined group which caused a significant ($p \leq 0.05$) increase in liver cholesterol level compared to the control, also.

Table 4.7: Changes in serum and liver total and direct bilirubin levels.

Group	Treatment	Serum		Liver	
		Direct bilirubin (mg/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total bilirubin (mg/dl)
A	Control	0.40 ± 0.02	0.56 ± 0.02	0.50 ± 0.03	0.71 ± 0.02
B	Boiled river water	0.50 ± 0.09	0.70 ± 0.11	0.63 ± 0.11	0.87 ± 0.13
C	Unboiled river water	0.61 ± 0.12	0.78 ± 0.08	0.76 ± 0.15	0.97 ± 0.10
D	All metals	0.63 ± 0.07	0.75 ± 0.06	0.79 ± 0.09	0.94 ± 0.08
E	Iron(1.4mg/L)	0.53 ± 0.09	0.67 ± 0.08	0.67 ± 0.12	0.84 ± 0.10
F	Copper(0.18mg/L)	0.59 ± 0.12	0.78 ± 0.13	0.74 ± 0.15	0.98 ± 0.16
G	Iron + Mercury	0.48 ± 0.11	0.64 ± 0.07	0.68 ± 0.14	0.80 ± 0.09
H	Mercury(0.001mg/L)	1.00 ± 0.09[a,b,c,d,e,f,g,k]	1.29± 0.09[a,b,c,d,e,f,g,i,k]	1.25 ± 0.11[a,b,c,d,e,f,g,k]	1.61 ± 0.12[a,b,c,d,e,f,g,i,k]
I	Chromium(0.001mg/L)	0.72 ± 0.07	0.83 ± 0.08	0.90 ± 0.08	1.04 ± 0.10
J	Cadmium(0.001mg/L)	1.17 ± 0.14[a,b,c,d,e,f,g,i,k]	1.38± 0.14[a,b,c,d,e,f,g,i,k]	1.47 ± 0.17[a,b,c,d,e,f,g,i,k]	1.73 ± 0.18[a,b,c,d,e,f,g,i,k]
K	Manganese(0.001mg/L)	0.53 ± 0.09	0.66 ± 0.04	0.66 ± 0.12	0.83 ± 0.05
L	Lead(0.001mg/L)	1.15 ± 0.27[a,b,c,d,e,f,g,i,k]	1.41± 0.16[a,b,c,d,e,f,g,i,k]	1.44 ± 0.34[a,b,c,d,e,f,g,i,k]	1.75 ± 0.21[a,b,c,d,e,f,g,i,k]

Values are presented as means ± SEM; n= 3
*Values with superscripts a,b,c,d,e,f,g,h,i,j,k or l are significantly different from the value of the group with the corresponding uppercase letter A,B,C, D,E,F,G,H,I,J,K or L ($p \leq 0.05$).

Table 4.8: Changes in serum and liver GGT and LDH activities

Group	Treatment	Serum		Liver	
		GGT (U/L)	LDH (U/L)	GGT (U/L)	LDH (U/L)
A	Control	161.65 ± 2.01	321.86 ± 22.93	171.55 ± 3.68	397.83 ± 11.72
B	Boiled river water	166.53 ± 2.14	404.53 ± 73.96	186.53 ± 8.47	490.23 ± 37.14
C	Unboiled river water	163.55 ± 4.40	489.06 ± 98.76	185.43 ± 5.95	546.93 ± 57.52
D	All metals	164.67 ± 3.91	506.66 ± 63.18	182.19 ± 5.09	526.86 ± 45.30
E	Iron(1.24mg/L)	165.63 ± 0.29	431.73 ± 49.90	183.47 ± 2.46	474.13 ± 59.85
F	Copper(0.18mg/L)	164.86 ± 4.48	474.66 ± 98.42	189.66 ± 2.93^{a}	550.43 ± 93.79
G	Iron + Mercury	185.76 ± 21.80^{a}	388.26 ± 90.72	173.67 ± 1.83	452.20 ± 55.84
H	Mercury(0.001mg/L)	202.08 ± 9.86^{a,b,c,d,e,f,i,k}	802.40 ± 73.33^{a,b,c,d,e,f,g,k}	222.88 ± 7.18^{a,b,c,d,e,f,g,i,k}	906.50 ± 68.89 ^{a,b,c,d,e,f,g,k}
I	Chromium(0.001mg/L)	172.17 ± 2.34	579.73 ± 56.28	185.66 ± 5.58	583.30 ± 56.91
J	Cadmium(0.001mg/L)	205.69 ± 5.69^{a,b,c,d,e,f,i,k}	943.73 ± 114.14^{a,b,c,d,e,f,g,i,k}	224.47 ± 5.48^{a,b,c,d,e,f,g,i,k}	971.60 ± 102.13^{a,b,c,d,e,f,g,i,k}
K	Manganese(0.001mg/L)	178.3 ± 4.75	425.33 ± 79.76	191.43 ± 9.24^{a,g}	465.50 ± 29.12
L	Lead(0.001mg/L)	203.31 ± 6.24^{a,b,c,d,e,f,i,k}	926.93 ± 222.51^{a,b,c,d,e,f,g,i,k}	222.08 ± 6.36^{a,b,c,d,e,f,g,i,k}	984.20 ± 117.90^{a,b,c,d,e,f,g,i,k}

Values are presented as means ± SEM; n= 3
*Values with superscripts a,b,c,d,e,f,g,h,i,j,k or l are significantly different from the value of the group with the corresponding uppercase letter A,B,C,D,E,F,G,H,I,J,KorL($p \leq 0.05$).

Table 4.9: Changes in serum and liver cholesterol levels

Group	Treatment	Serum cholesterol (mg/dl)	Liver cholesterol (mg/dl)
A	Control	153.65 ± 2.00	165.65 ± 2.00
B	Boiled river water	158.53 ± 2.14	170.53 ± 2.14
C	Unboiled river water	155.55 ± 4.40	167.55 ± 4.40
D	All metals	156.67 ± 3.91	168.67 ± 3.91
E	Iron(1.24mg/L)	157.63 ± 0.29	169.63 ± 0.29
F	Copper(0.18mg/L)	156.86 ± 4.48	168.86 ± 4.48
G	Iron+ Mercury	177.76 ± 21.80[a]	189.76 ± 21.80[a]
H	Mercury(0.001mg/L)	194.08 ± 9.86[a,b,c,d,e,f,i,k]	206.08 ± 9.86[a,b,c,d,e,f,i,k]
I	Chromium(0.001mg/L)	164.17 ± 2.34	176.17± 2.34
J	Cadmium(0.001mg/L)	197.69 ± 5.69[a,b,c,d,e,f,i,k]	209.69 ± 5.69 [a,b,c,d,e,f,i,k]
K	Manganese(0.001mg/L)	170.33± 4.75	182.33 ± 4.75
L	Lead(0.001mg/L)	195.31 ± 6.24[a,b,c,d,e,f,i,k]	207.31 ± 6.24[a,b,c,d,e,f,i,k]

Values are presented as means ± SEM; n= 3
*Values with superscripts a,b,c,d,e,f,g,h,I,j,k or l are significantly different from the value of the group with the corresponding uppercase letter A,B,C, D,E,F,G,H,I,J,K or L ($p \leq 0.05$).

HISTOPATHOLOGY

4.3.1 Effects of cadmium, mercury and lead on rat kidney ultrastructure

The photomicrographs of the control and the Cd only, the Hg only and Pb only exposed rat kidney sections after a three-month oral exposure are presented in Plates 4.1, 4.16, 4.20 and 4.24 respectively.

The kidney section from rat in the control group showed normal histological features. The cortical parenchyma (B) and the renal corpuscles, which appeared as dense rounded structures (A) are evident (Plate 4.1).

Sections of kidney from a rat in the Cd only group revealed visible atrophied renal corpuscle (A) with the tubules appearing not so distinct (B) (Plate 4.16). Sections of the kidney from a rat in the Hg only group revealed round renal corpuscle (A) with the tubules (B) (Plate 4.20). Sections of kidney from the Pb only group revealed enlarged renal corpuscle (A) with the tubules appearing distorted (B) (Plate 4.24).

4.3.2 Effects of cadmium, mercury and lead on rat liver ultrastructure

The photomicrographs of the control, the Cd only, the Hg only and Pb only exposed rat liver sections after a three-month oral exposure are presented in Plates 4.2, 4.15, 4.19 and 4.23 respectively.

The control rat liver section showed normal liver histology with centrioles (A), hepatocytes and nucleus (B), and sinusoidal space (C) (Plate 4.2)

Sections of liver from a rat in the Cd only group revealed thickened centriole wall (A) with mild focal inflammation. The hepatocytes revealed nucleus with mild steatosis (Plate 4.15). Sections of liver from a rat in the Hg only group revealed visible centriole (A). The hepatocytes revealed nucleus that appears slightly pyknotic (B)(Plate 4.15). Sections of liver from a rat in the Pb only group revealed portal triad (A) with congested sinusoids, however, the hepatocytes revealed nucleus that appears pyknotic (B)(Plate 4.23).

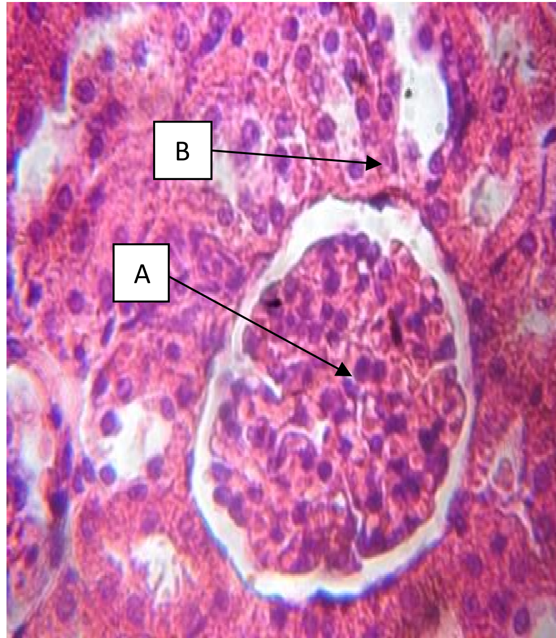


Plate 4.1: Photomicrograph of control rat kidney at the end of three-month exposure. (H and E staining x400).

*The cortical parenchyma (B) and the renal corpuscles, which appeared as dense rounded structures (A) are evident

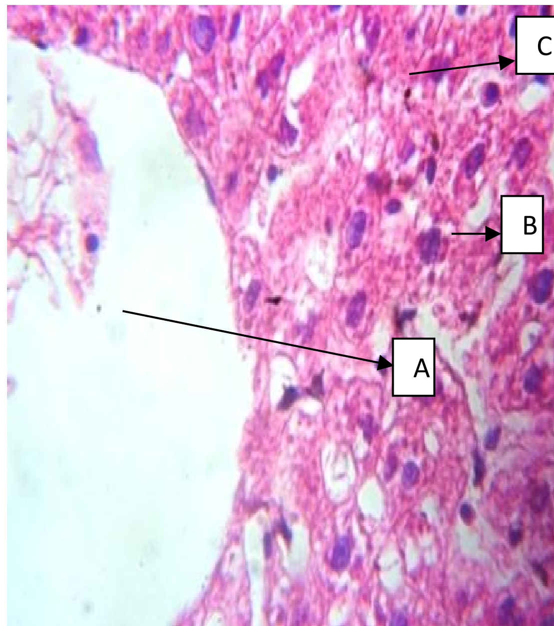


Plate 4.2: Photomicrograph of control rat liver at the end of three-month exposure. (H and E staining x400).

* The plate reveals normal liver histology with centrioles (A), hepatocytes and nucleus (B), and sinusoidal space (C).

4.3.3 Effect of boiled heavy metal contaminated water from river Nun on rat liver ultrastructure

The photomicrograph of rat liver section after a three-month oral exposure to boiled heavy metal contaminated water from river Nun is presented in in Plate 4.3. The plate reveals centriole (A) with noticeable mild mononuclear infiltrates in the sinusoids (B). The hepatocytes bear evidence of pyknotic nucleus.

4.3.4 Effect of boiled heavy metal contaminated water from river Nun on rat kidney ultrastructure

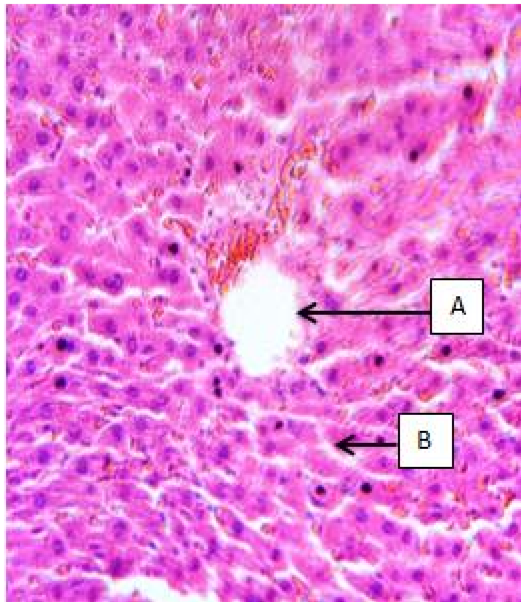
The photomicrograph of rat kidney section after a three-month oral exposure to boiled heavy metal contaminated water from river Nun is presented in Plate 4.4. Photomicrograph reveals visible renal corpuscle (A) with diffused inflammatory cells in the interstitial parenchyma (B). The tubules appear not so distinct.

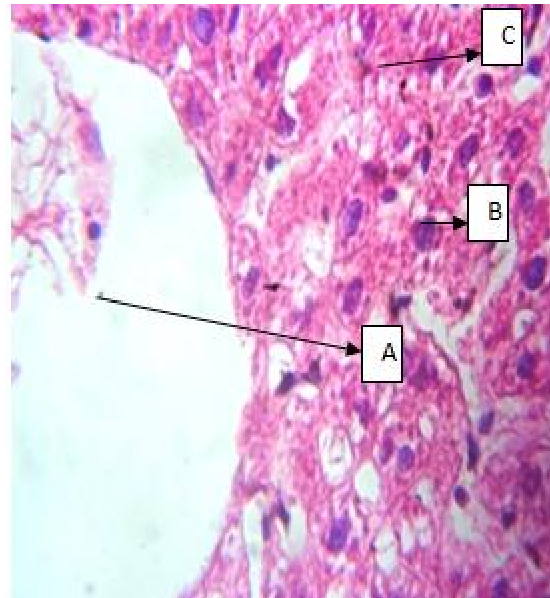
4.3.5 Effect of heavy metal contaminated unboiled water from river Nun on rat liver ultrastructure

The photomicrograph of rat liver section after a three-month oral exposure to the unboiled river water is presented in Plate 4.5. The plate reveals thickened centriole wall (A) with mild focal inflammatory cells (B). The hepatocytes reveal nucleus that appears slightly distinct.

4.3.6 Effect of heavy metal contaminated unboiled water from river Nun on rat kidney ultrastructure

The photomicrograph of rat kidney section after a three-month oral exposure to unboiled river water is presented in plate 4.6. Photomicrograph reveals visible renal corpuscle (A) with the tubules appearing not so distinct (B) (Plate 4.6).

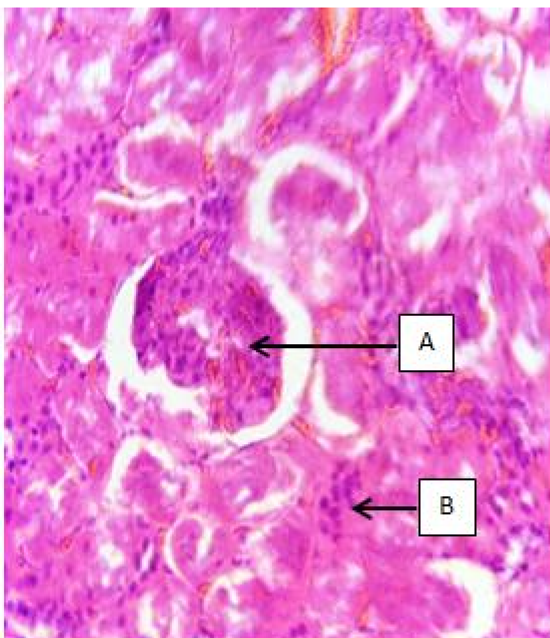


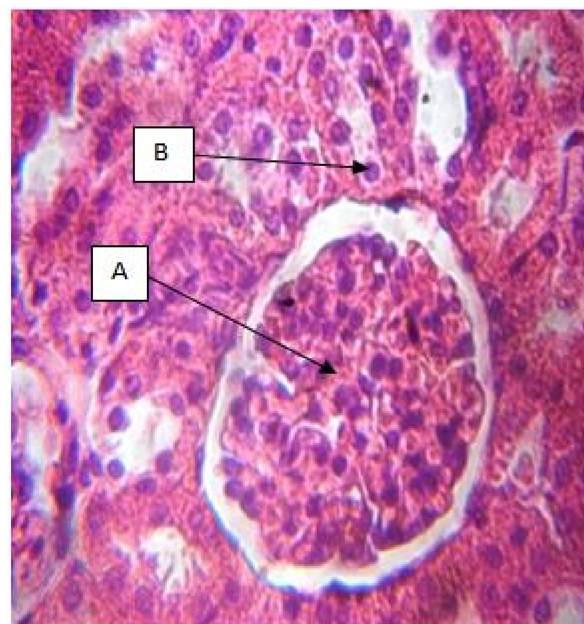


(a) **(b)**

Plate 4.3: Photomicrograph of the liver of rat treated with boiled river water at the end of three-month exposure (a) and that of the control (b). (H and E staining x400).

*The plate (a) reveals centriole (A) with noticeable mild mononuclear infiltrates in the sinusoids (B) while (b) shows normal features

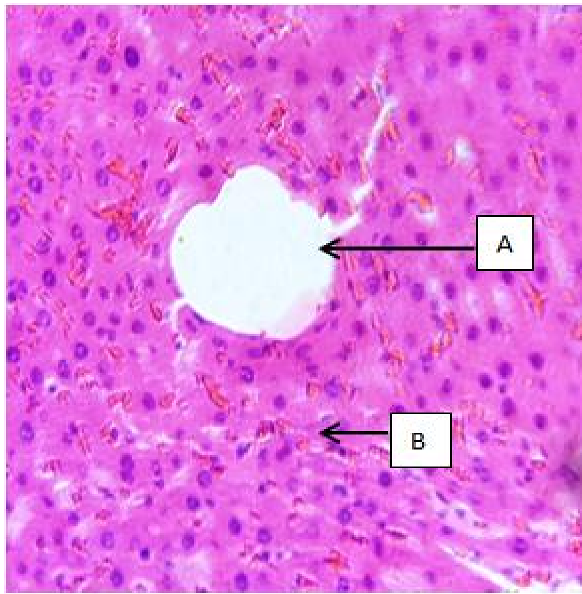


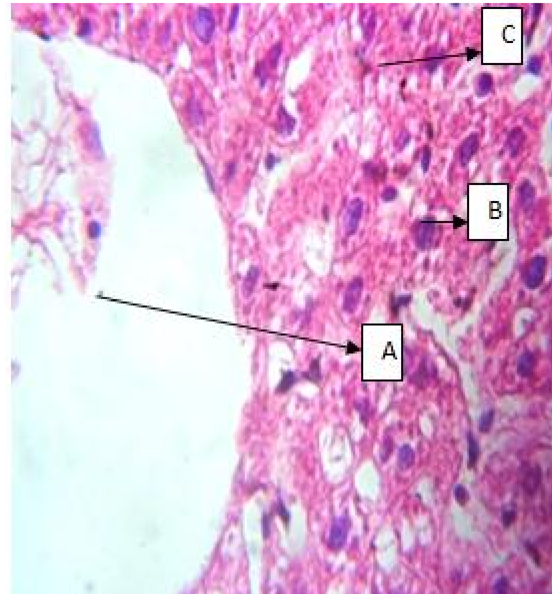


(a) **(b)**

Plate 4.4: Photomicrograph of the kidney of rat treated with boiled river water at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals visible renal corpuscle (A) with diffused inflammatory cells in the interstitial parenchyma (B) while the control (b) shows normal features

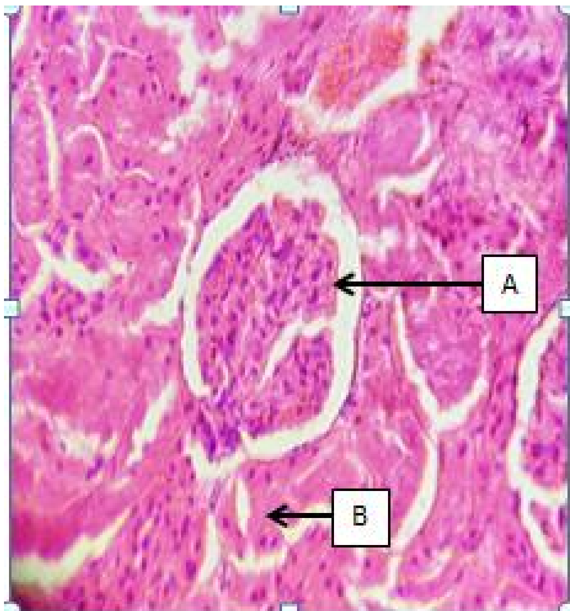


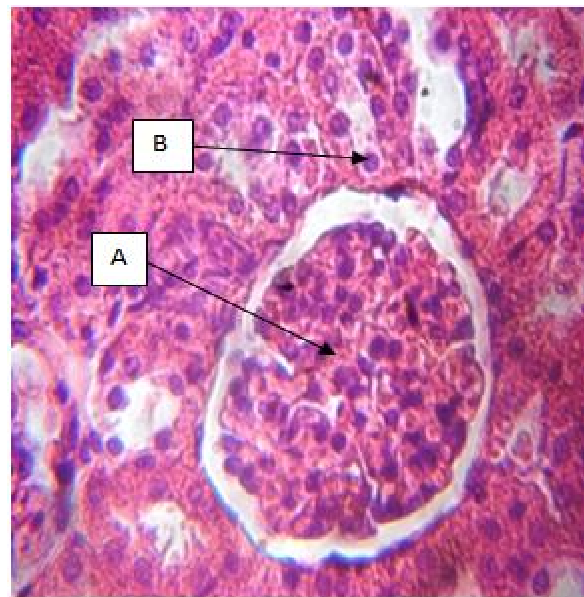


(a) **(b)**

Plate 4.5: Photomicrograph of the liver of rat treated with unboiled river water at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals thickened centriole wall (A) with mild focal inflammatory cells (B) while (b) shows normal features.





(a) **(b)**

Plate 4.6: Photomicrograph of the kidney of rat treated with unboiled river water at the end of three-month exposure (a) with control (b). (H and E staining x400).

*The plate (a) reveals visible renal corpuscle (A) with the tubules appearing not so distinct (B) while the control (b) shows normal features

4.3.7 Effect of a solution containing all metal ions identified in the river Nun water on rat liver ultrastructure

The photomicrograph of rat exposed to a solution containing all metal ions identified in River Nun water after three-month oral consumption is presented in Plate 4.7. The plate reveals liver centriole (A) with mild focal inflammatory cells. The hepatocytes reveal nucleus that appears slightly distinct.

4.3.8 Effect of a solution containing all metal ions identified in the river Nun water on rat kidney ultrastructure

The photomicrograph of rat kidney section of rat exposed to a solution containing all metal ions identified in River Nun water after three-month oral consumption is presented in Plate 4.8.

The ultrastructure reveals visible renal corpuscle (A) with tubules and interstitial flooded with diffuse mononuclear infiltrates (B).

4.3.9 Effect of Iron tainted water on rat liver ultrastructure

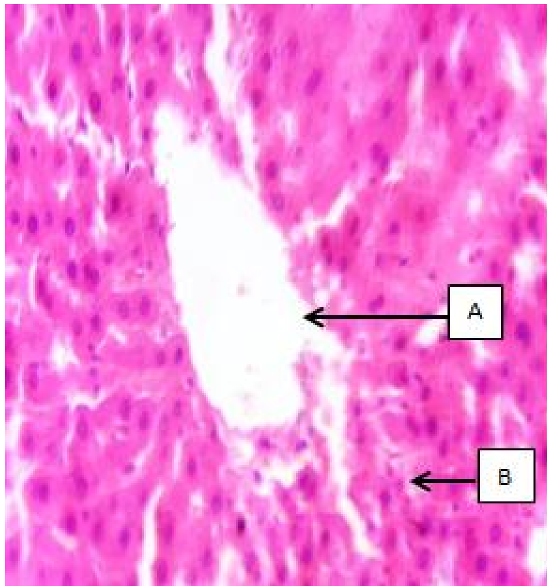
Photomicrograph of rat liver section after three months oral exposure to iron-tainted water is presented in Plate 4.9

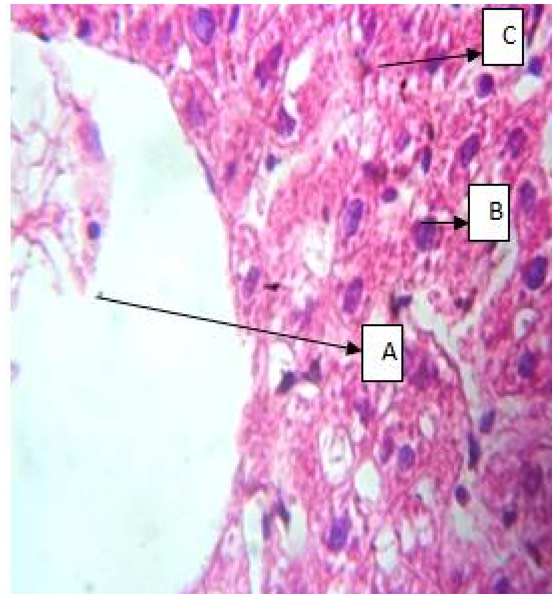
Photomicrograph reveals visible liver centriole (A) with noticeable hepatocytes showing vacuolated nucleus (B) and well fenestrated sinusoids.

4.3.10 Effect of Iron tainted water on rat kidney ultrastructure

Photomicrograph of rat kidney section after three months oral exposure to iron-tainted water is presented in Plate 4.10

Photomicrograph reveals visible renal corpuscle (A) and interstitial space (B) and tubules

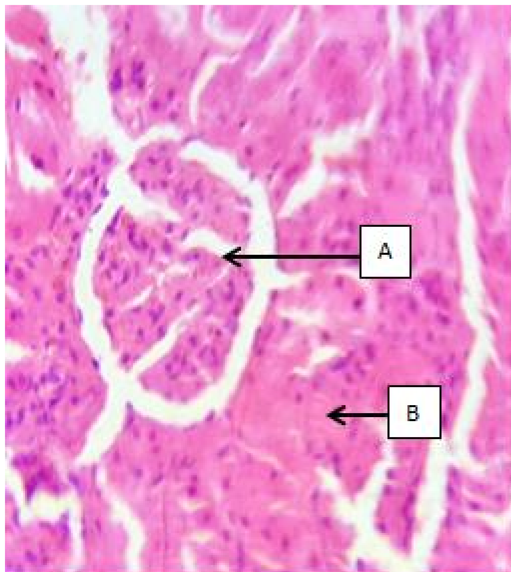


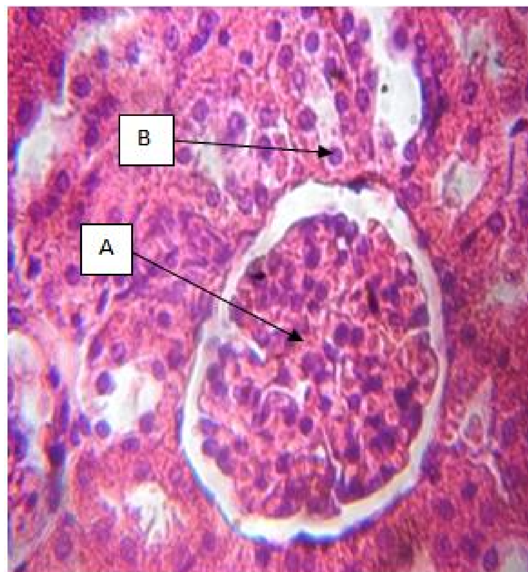


(a) **(b)**

Plate 4.7: Photomicrograph of the liver of rat treated with mixture of all metals at the end of three-month exposure (a) with the control (b). (H and E staining x400).

* The plate (a) reveals liver centriole (A) with mild focal inflammatory cells while the control (b) shows normal features.

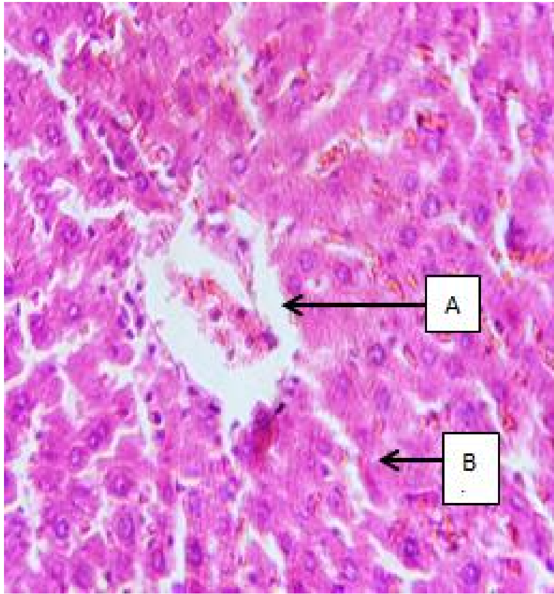


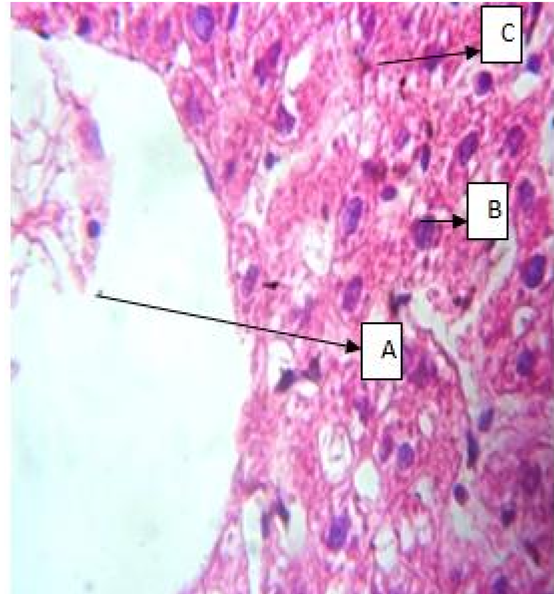


(a) **(b)**

Plate 4.8: Photomicrograph of the kidney of rat treated with mixture of all metals at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals visible renal corpuscle (A) with tubules and interstitial flooded with diffuse mononuclear infiltrates (B) while the control shows normal features.

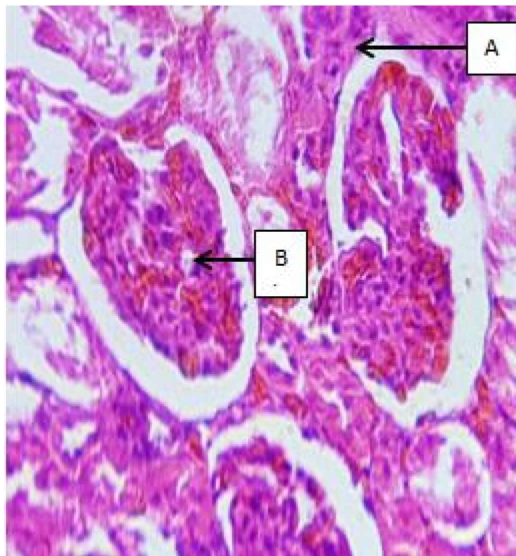


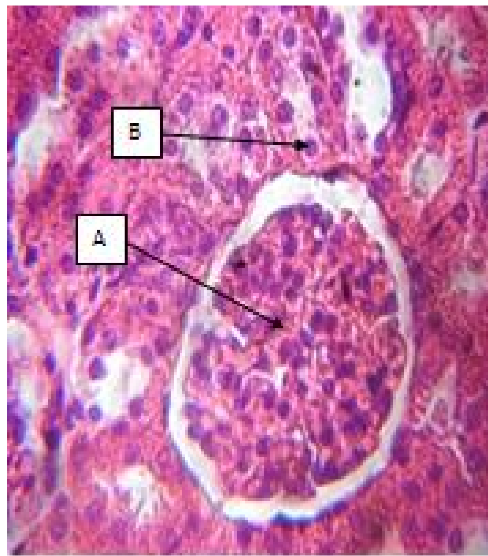


(a) **(b)**

Plate 4.9: Photomicrograph of the liver of rat treated with iron at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals visible liver centriole (A) with noticeable hepatocytes showing vacuolated nucleus (B) and well fenestrated sinusoids while the control (b) shows normal features





(a) **(b)**

Plate 4.10: Photomicrograph of the kidney of rat treated with iron at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals visible renal corpuscle (A) and interstitial space (B) and tubules while the control (b) shows normal features

4.3.11 Effect of copper tainted water on rat liver ultrastructure

Photomicrograph of rat liver section after three months oral exposure to copper-tainted water is presented in Plate 4.11. Photomicrograph reveals liver centriole (A) the hepatocytes and sinusoids reveal mild congestion (B).

4.3.12 Effect of copper tainted water on rat kidney ultrastructure

Photomicrograph of rat kidney section after three months oral exposure to copper-tainted water is presented in Plate 4.12. Photomicrograph reveals slightly atrophied renal corpuscle (A) the tubules which appear not so distinct (B).

4.3.13 Effect of Iron and mercury binary treatment on rat liver ultrastructure

The photomicrograph of rat liver section after three months exposure to the combination of iron and mercury tainted water is presented in Plate 4.13. The photomicrograph reveals liver portal triad (A) with congested sinusoids however the hepatocytes reveal nucleus that appears pyknotic (B).

4.3.14 Effect of the binary of Iron and mercury treatment on rat kidney ultrastructure

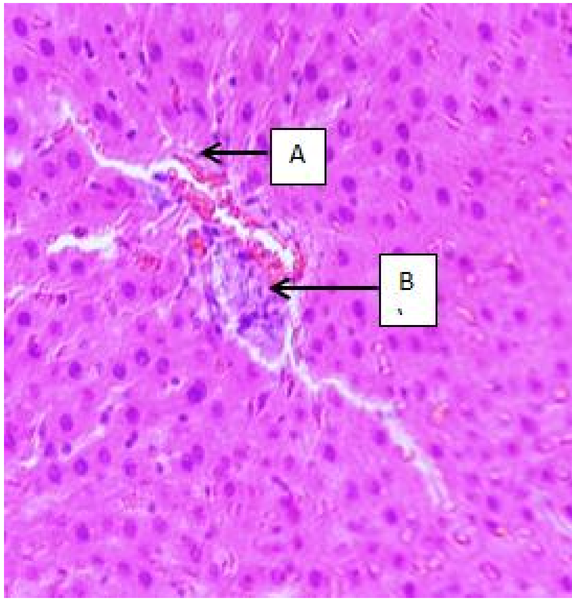
The photomicrograph of rat kidney section after three months exposure to the binary of iron and mercury tainted water is presented in Plate 4.14. Photomicrograph reveals enlarged renal corpuscle (A) with the tubules appearing distorted (B).

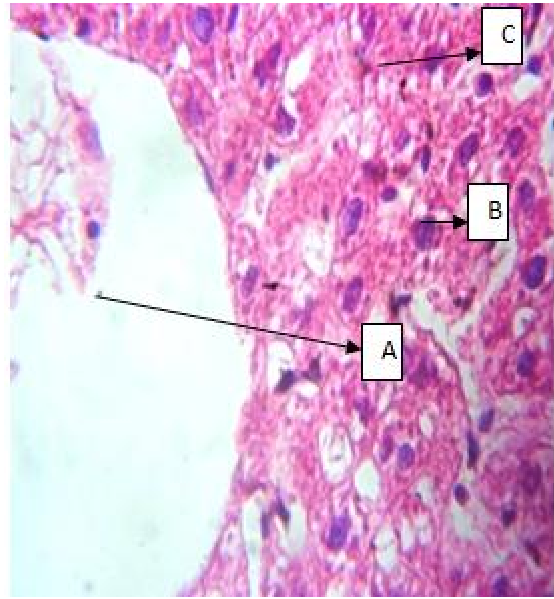
4.3.15 Effect of mercury tainted water on rat liver ultrastructure

Photomicrograph of rat liver section after three months oral exposure to mercury-tainted water is presented in Plate 4.19. Photomicrograph of the liver reveals visible centriole (A). The hepatocytes reveal nucleus that appears slightly pyknotic (B).

4.3.16 Effect of mercury tainted water on rat kidney ultrastructure

The photomicrograph of rat kidney section after three months oral exposure to mercury-tainted water is presented in Plate 4.20. Photomicrograph reveals round renal corpuscle (A) with the tubules (B).

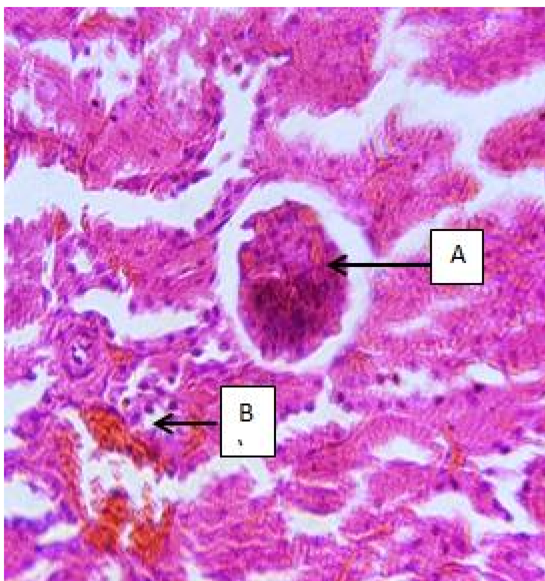


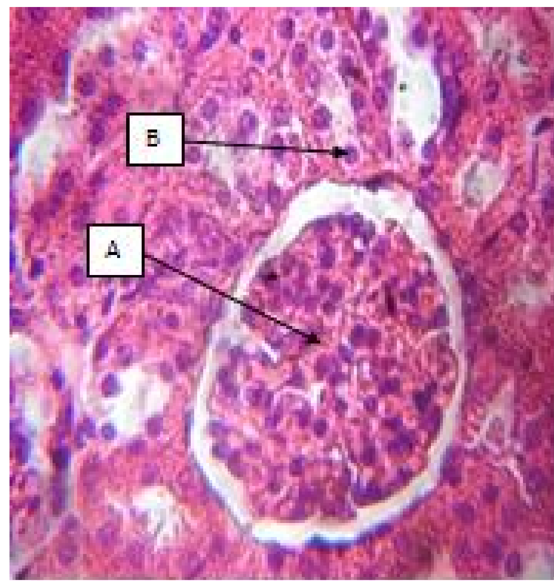


(a) **(b)**

Plate 4.11: Photomicrograph of the liver of rat treated with copper at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals liver centriole (A) the hepatocytes and sinusoids reveal mild congestion (B) while the control (b) shows normal features

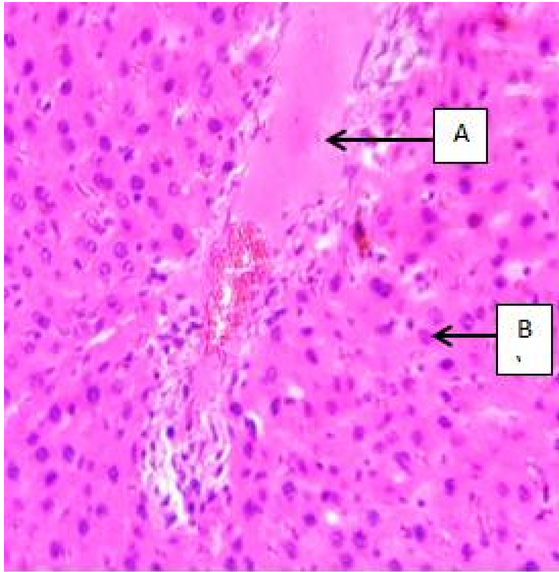


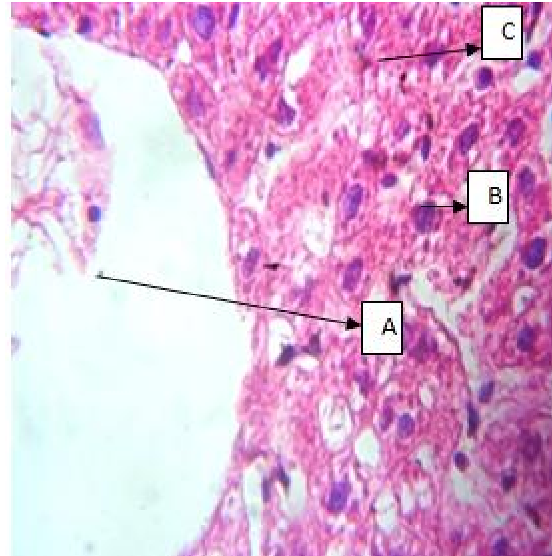


(a) **(b)**

Plate 4.12: Photomicrograph of the kidney of rat treated with copper at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals slightly atrophied renal corpuscle (A) the tubules which appear not so distinct (B) while the control (b) shows normal features

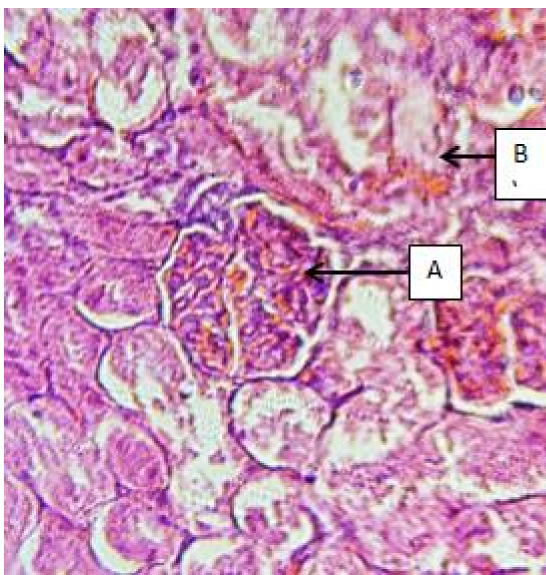


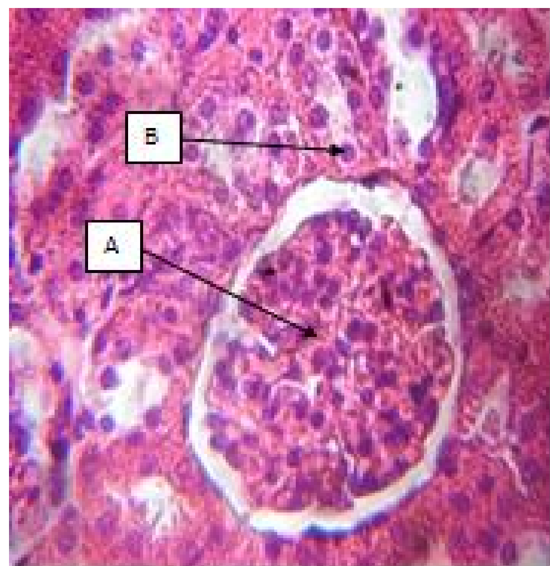


(a) **(b)**

Plate 4.13: Photomicrograph of the liver of rat treated with iron and mercury binary at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals liver portal triad (A) with congested sinusoids however the hepatocytes reveal nucleus that appears pyknotic (B) while the control (b) shows normal features

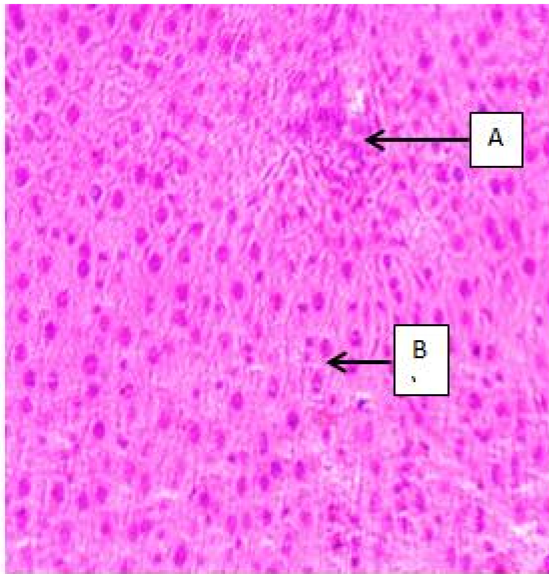


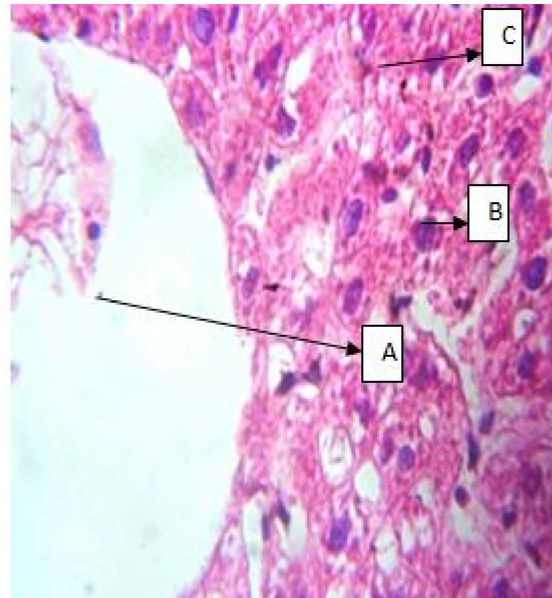


(a) **(b)**

Plate 4.14: Photomicrograph of the kidney of rat treated with iron and mercury binary at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals enlarged renal corpuscle (A) with the tubules appearing distorted (B) while the control (b) shows normal features

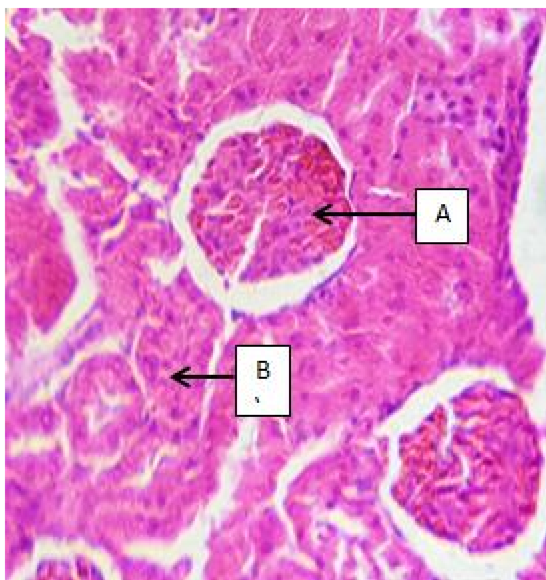


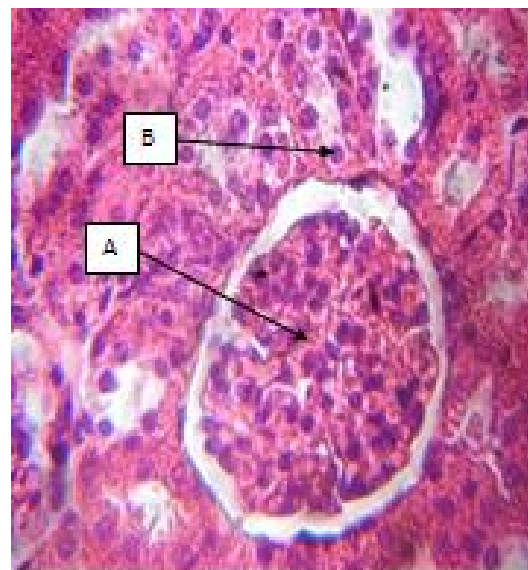


(a) **(b)**

Plate 4.19: Photomicrograph of the liver of rat treated with mercury at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals visible centriole (A). The hepatocytes reveal nucleus that appears slightly pyknotic (B) while the control (b) shows normal features





(a) **(b)**

Plate 4.20: Photomicrograph of the kidney of rat treated with mercury at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals round renal corpuscle (A) with the tubules (B) while the control (b) shows normal features

4.3.17 Effect of chromium tainted water on rat liver ultrastructure

The photomicrograph of rat liver section after three months oral exposure to chromium-tainted water is presented in Plate 4.17. Photomicrograph reveals liver centriole (A) surrounded by infiltrates. The sinusoids, however, appear congested. The hepatocytes reveal nuclei that appear pyknotic (B).

4.3.18 Effect of chromium tainted water on rat kidney ultrastructure

Photomicrograph of rat kidney section after three months oral exposure to chromium-tainted water is presented in Plate 4.18. Photomicrograph reveals enlarged renal corpuscle with atrophied glomerulus (A) with the tubules appearing distorted (B).

4.3.19 Effect of cadmium tainted water on rat liver ultrastructure

Photomicrograph of rat liver section after three months oral exposure to cadmium-tainted water is presented in Plate 4.15. Photomicrograph reveals thickened centriole wall (A) with mild focal inflammatory cells. The hepatocytes reveal distinct nuclei as well as mild steatosis (B)

4.3.20 Effect of cadmium tainted water on rat kidney ultrastructure

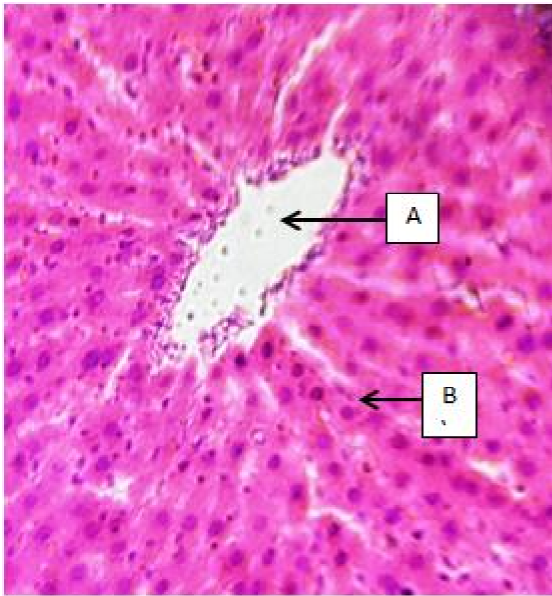
Photomicrograph of rat kidney section after three months oral exposure to cadmium-tainted water is presented in Plate 4.16. Photomicrograph reveals visible atrophied renal corpuscle (A) with the tubules appearing not so distinct (B).

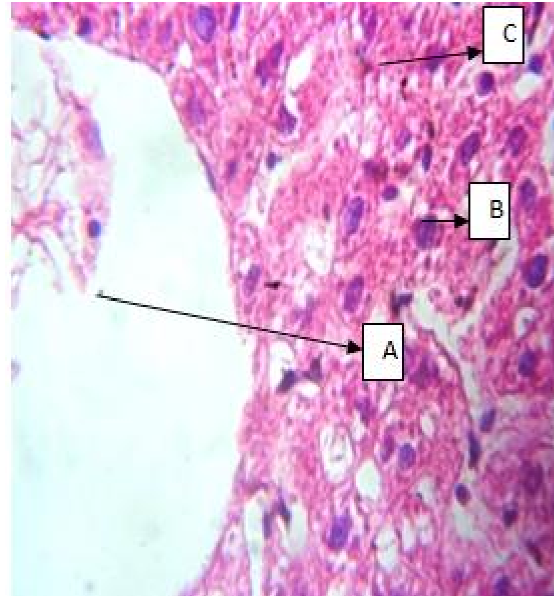
4.3.21 Effect of manganese tainted water on rat liver ultrastructure

Photomicrograph of rat liver section after three months oral exposure to manganese-tainted water is presented in Plate 4.21. Photomicrograph reveals liver portal triad (A) with noticeable mild inflammatory cells surrounding it. The hepatocytes shows vacuolated nuclei (B).

4.3.22 Effect of manganese tainted water on rat kidney ultrastructure

Photomicrograph of rat kidney section after three months oral exposure to manganese-tainted water is presented in Plate 4.22. Photomicrograph reveals visible renal corpuscle (A) with diffused inflammatory cells in the interstitial parenchyma (B). The tubules appear not so distinct.

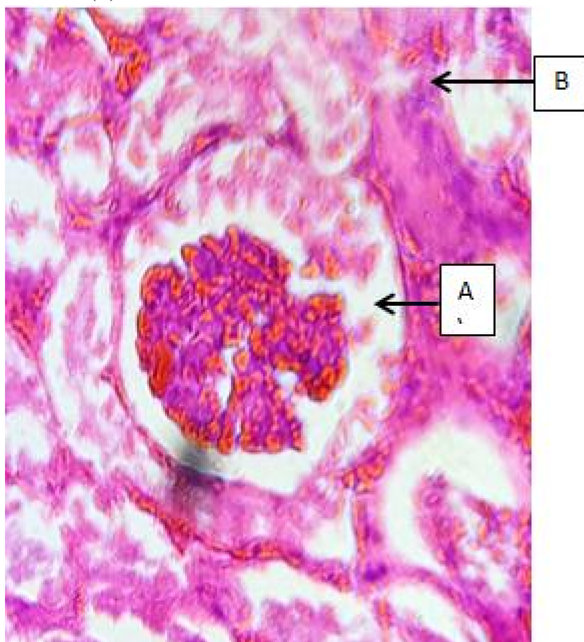


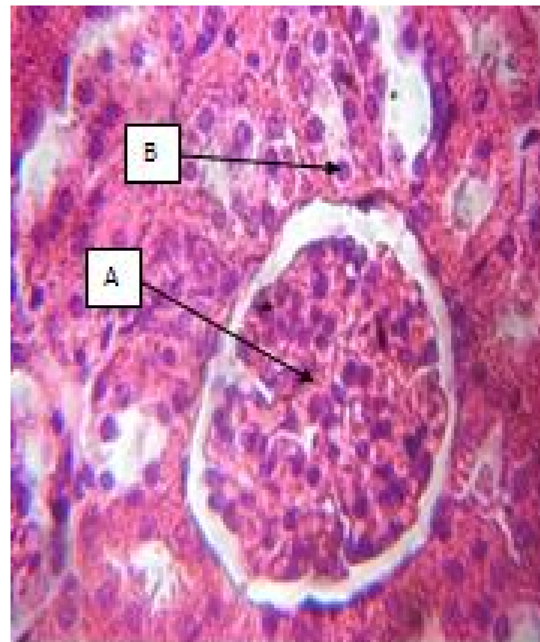


(a) **(b)**

Plate 4.17: Photomicrograph of the liver of rat treated with chromium at the end of three-month exposure (a) with the control (B). (H and E staining x400).

*The plate (a) reveals liver centriole (A) surrounded by infiltrates with nuclei that appear pyknotic (B) while the control (b) shows normal features

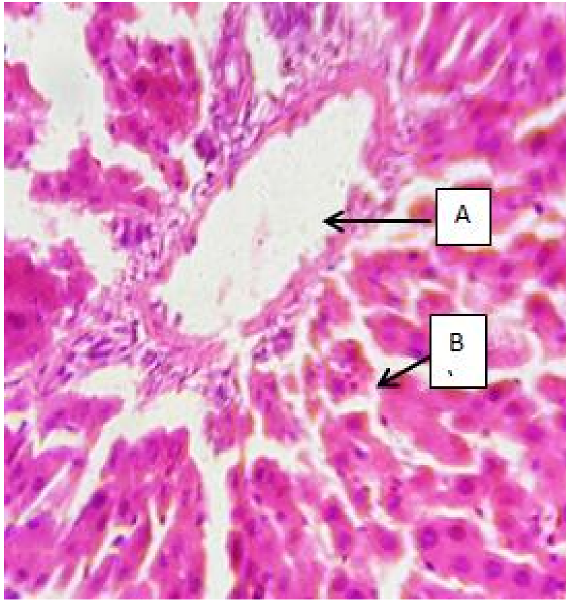


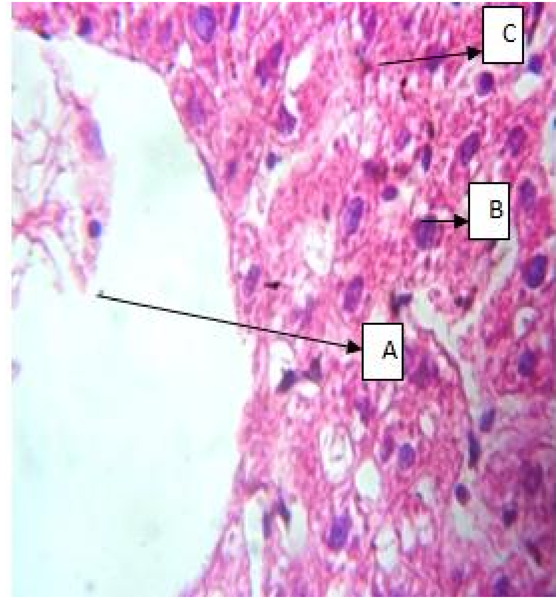


(a) **(b)**

Plate 4.18: Photomicrograph of the kidney of rat treated with chromium at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals enlarged renal corpuscle with atrophied glomerulus (A) with the tubules appearing distorted (B) while (b) shows normal features.

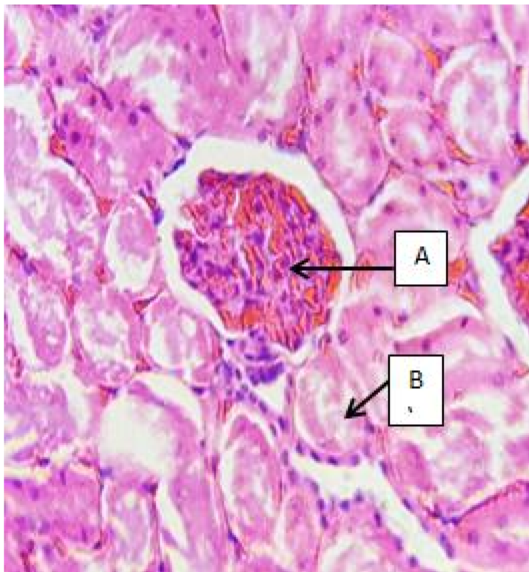


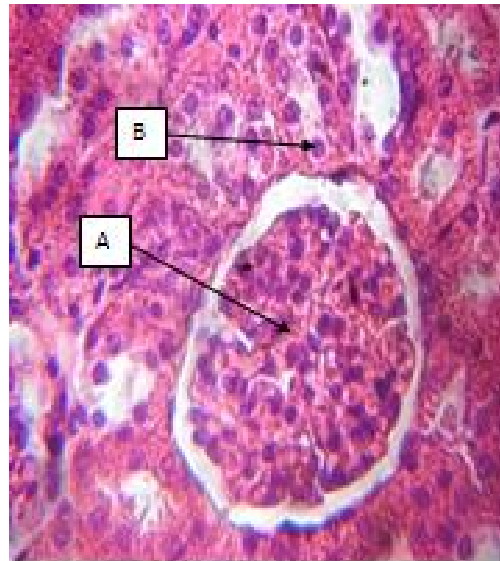


(a) **(b)**

Plate 4.15: Photomicrograph of the liver of rat treated with cadmium at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals thickened centriole wall (A) with mild focal inflammatory cells. The hepatocytes reveal distinct nuclei as well as mild steatosis (B) while the control (b) shows normal features.

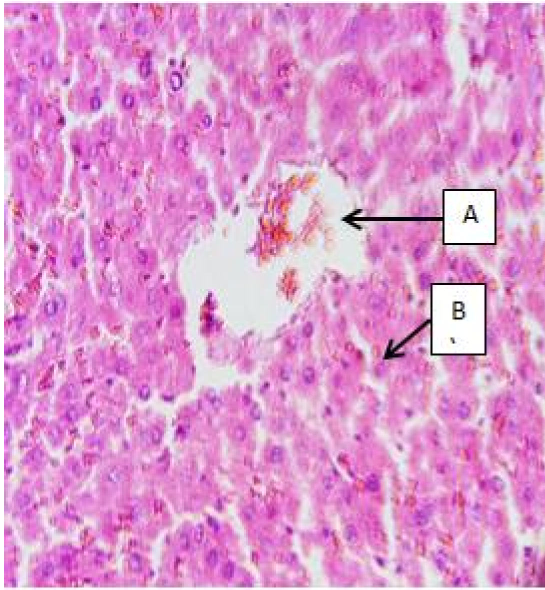


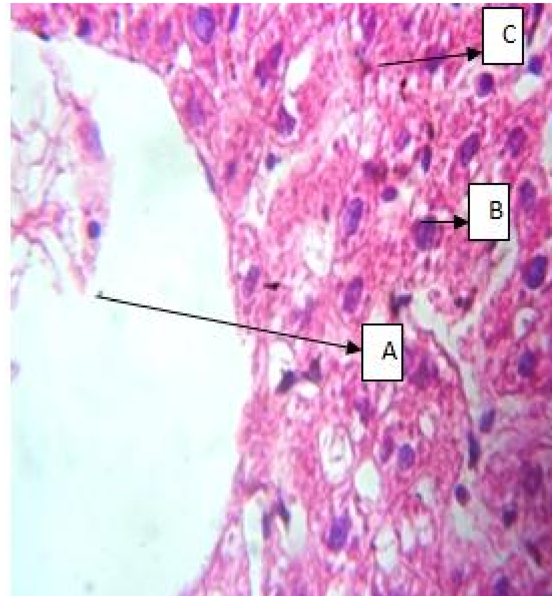


(a) **(b)**

Plate 4.16: Photomicrograph of the kidney of rat treated with cadmium at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals visible atrophied renal corpuscle (A) with the tubules appearing not so distinct (B) while the control (b) shows normal features

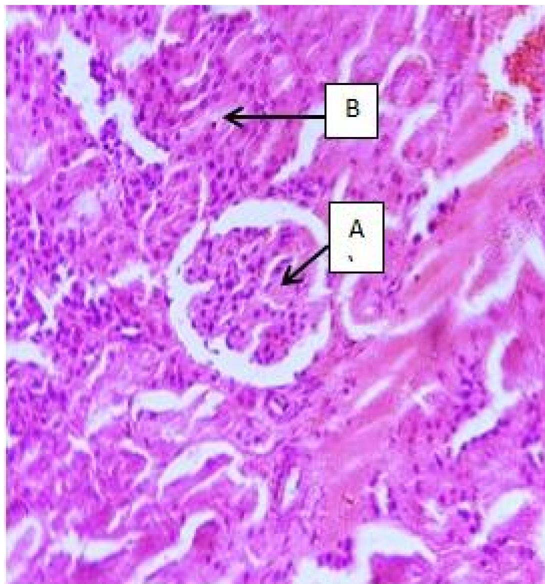


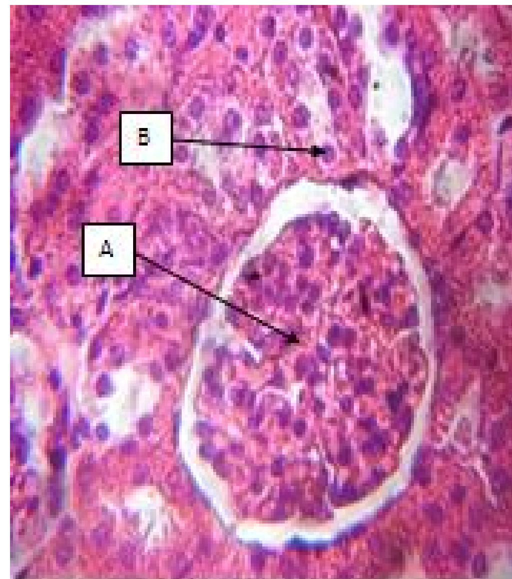


(a) **(b)**

Plate 4.21: Photomicrograph of the liver of rat treated with manganese at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals liver portal triad (A) with noticeable mild inflammatory cells surrounding it. The hepatocytes shows vacuolated nuclei (B) while the control (b) shows normal features





(a) **(b)**

Plate 4.22: Photomicrograph of the kidney of rat treated with manganese at the end of three-month exposure (a) with the control (b). (H and E staining x400).

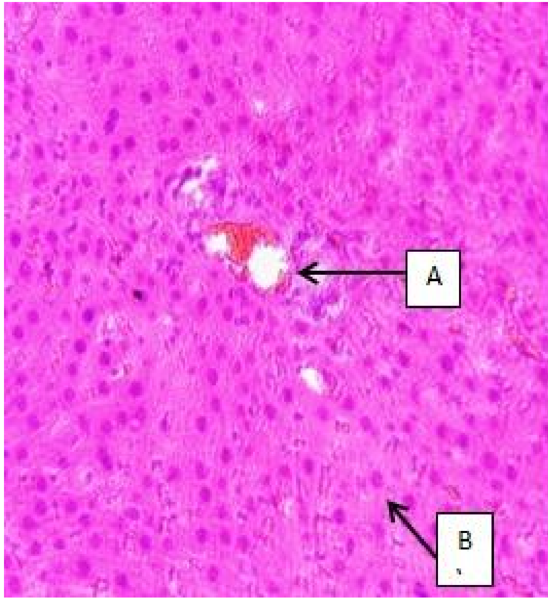
*The plate (a) reveals visible renal corpuscle (A) with diffused inflammatory cells in the interstitial parenchyma (B) while the control (b) shows normal features

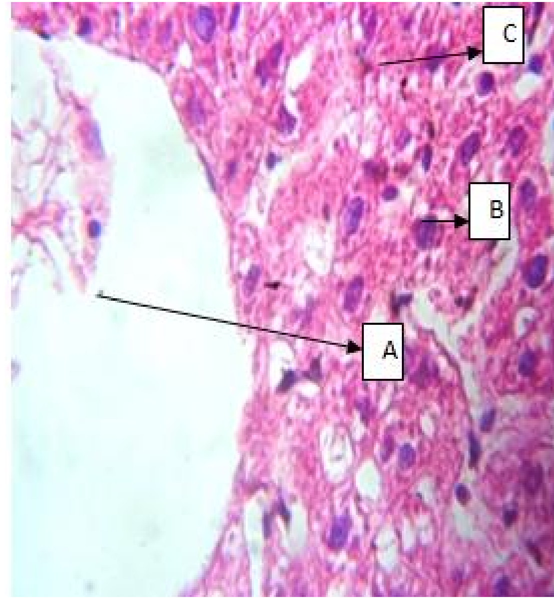
4.3.23 Effect of lead tainted water on rat liver ultrastructure

Photomicrograph of rat liver section after three months oral exposure to lead-tainted water is presented in Plate 4.23. Photomicrograph reveals liver portal triad (A) with congested sinusoids. However, the hepatocytes reveal nucleus that appears pyknotic (B).

4.3.24 Effect of lead tainted water on rat kidney ultrastructure

The photomicrograph of the lead-tainted water treated rat kidney section after a three-month oral exposure is presented in Plate 4.24. Photomicrograph reveals enlarged renal corpuscle (A) with the tubules appearing distorted (B).

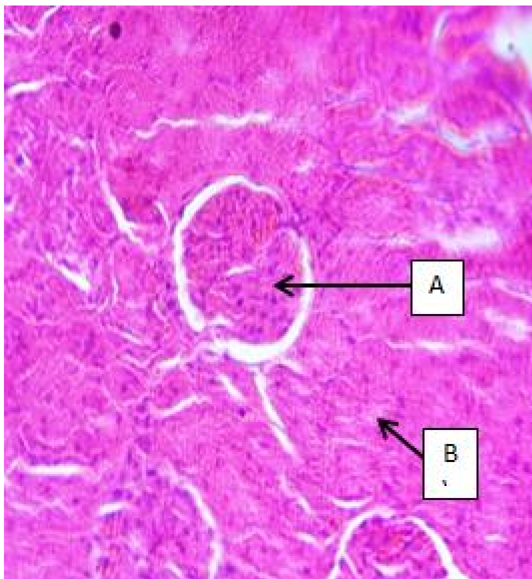


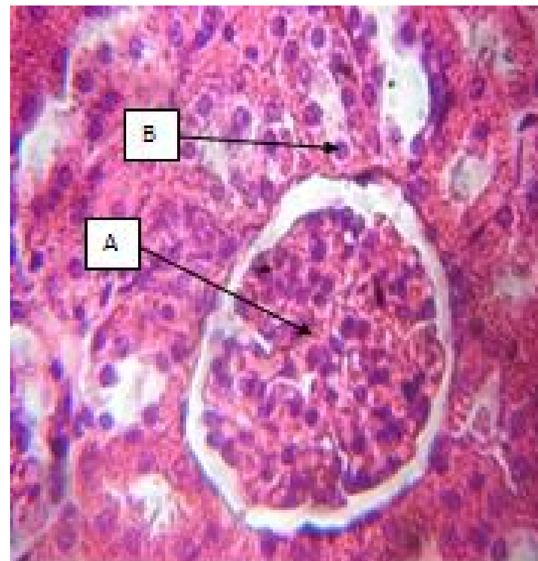


(a) **(b)**

Plate 4.23: Photomicrograph of the liver of rat treated with lead at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals liver portal triad (A) with congested sinusoids and the hepatocytes reveal nucleus that appears pyknotic (B) while the control (b) shows normal features





(a) **(b)**

Plate 4.24: Photomicrograph of the kidney of rat treated with lead at the end of three-month exposure (a) with the control (b). (H and E staining x400).

*The plate (a) reveals enlarged renal corpuscle (A) with the tubules appearing distorted (B) while the control (b) shows normal features

CHAPTER FIVE

5.1 DISCUSSION

Heavy metals are ubiquitous in the environment and are notorious for their potential to cause physical, biochemical and histological changes. Exposure to these may result from consumption of contaminated food or water, inhaling contaminated air or direct physical or dermal contact with a sufficient dose of the substance. There are numerous studies that have successfully examined the dose, duration and route of exposure as well as other physiological factors that influence the toxicity of heavy metals (Arif *et al.,* 2015; Roy, 2009).

5.1.1 Nephrotoxic Effects

5.1.1.1 Urea status

Urea is synthesized in the liver using ammonia produced during the catabolism of amino acids. The set of reactions from the incorporation of ammonia to form a carbamoyl phosphate molecule to the formation of urea is termed the urea cycle. The urea so formed is then transported to the kidneys by the blood for excretion (Rodwell, 2004). Generally, urea is considered a very relevant biochemical marker when estimating kidney dysfunction and failure (Gnanamani *et al.,* 2008).Results obtained from this study showed that mercury, cadmium and lead, when administered singly, remarkably increased the serum and kidney urea concentrations. This is in agreement with the findings of Missoun *et al.* (2010) who reported significant increases in urea concentrations in experimental animals intoxicated with lead. Also, Saxena *et al.* (2009) reported remarkable increases in urea levels upon intoxication with arsenic trioxide (ArO_3). The results also show that serum and kidney urea levels of the unboiled and boiled river water groups as well as that of the group treated with solution of all metals, were not significantly different from the control group. This is in agreement with the work of Hanafy and Soltan (2004) who concluded that combined exposure to heavy metals can diminish levels of urea and creatinine in the blood.

5.1.1.2 Creatinine status

Creatinine is the catabolic product of creatine phosphate, a high-energy compound used as a transient storage form of energy in the skeletal muscle. Creatinine level is fairly stable as it is easily excreted by the kidneys, thus, elevated levels invariably imply diminished renal function, (Hanafy and Soltan, 2004). Results obtained from this study indicate that exposure to mercury, cadmium and lead, when administered singly, significantly increased the serum and kidney creatinine concentrations. This is in agreement with the findings of Ibrahim (2013) who reported significant increases in the serum concentrations of creatinine in animals intoxicated with cadmium. A very similar result was also obtained by Preet and Dua (2011) who even showed this for a few other metals. Aisha and Elham (2000) explained such increase as likely owing to the toxic effects of these metals on the renal tubules and glomeruli leading to glomerular and renal tubular dysfunction. The present study also showed that the creatinine levels of unboiled and boiled river water treated groups as well as that of the group treated with solution of all metals were not significantly different from the control group. Evidently, these metals occurring concurrently had a way of impairing individual toxicities. Singling out Fe as an entity, this can be understood on the basis of the findings of Obiorah *et al.* (2009) which showed that iron reduces radical-induced oxidative toxicity. Generally, it has been shown using a mixture of zinc and other heavy metals, that exposure to heavy metals coexisting in a solution diminishes rather than increases urea and creatinine levels (Hanafy and Soltan, 2004).

5.1.1.3 Sodium ions

Sodium ion (Na^+) together with potassium ion (K^+), are important for establishing an electrochemical gradient in virtually all types of cell in the body. Uncontrolled elevation in plasma Na^+ induces hypernatremia. Kang *et al.* (2002) reported that hypernatremia although rare, occurs during loss of body fluids containing less sodium than plasma particularly when there is limited water intake. This invariably leads to a perturbation in the osmotic pressure of body

fluids. The results from this study show that significant increases in the level of serum and kidney Na^+ results, when mercury, cadmium and lead, are administered singly. This finding is in agreement with that of Osuala *et al.* (2013) who reported that the level of serum sodium ion in mice exposed to Cd, Mn, Pb and Zn singly for 28 days exhibited similar elevations. Sheikh *et al.* (2011) also showed that mercury could produce a significant increase in serum sodium and chloride ion concentration. Ogunka-Nnoka and Uwakwe, (2012) reported that hypernatremia is mostly a consequence of severe water loss. A conclusion shared by Abdulrahman *et al.* (2007) who reported hypernatremia as an indication of dehydration. However, owing to the consistent liberal supply of water to rats in this study, the increase in sodium ion observed here is largely due to inability of the kidney to excrete adequate sodium ion via the tubular fluid, which is tantamount to kidney damage. This study also showed that the unboiled and boiled river water groups as well as the solution of all metals were not able to cause significant increases in serum Na^+ relative to the control group. Evidently, when these metals coexist in the environment, they have a way of antagonizing individual toxicities.

5.1.1.4 Potassium ions

Excess potassium in the blood is termed hyperkalemia. It occurs in cases of renal failure because the kidneys lose their ability to excrete the mineral. Severe dehydration could also result in hyperkalemia. The consequences of this condition are muscle weakness and cardiac arrhythmias that lead to heart failure (Abdul, 2011). Results obtained from this study when rats were exposed to mercury, cadmium and lead orally, showed that the treatment caused increased level of potassium ion in all cases. This shows that oral consumption of cadmium, mercury and lead may have hyperkalemic effect. Mehmet *et al.* (2011) reported that increase in plasma K^+ level was observed in rats exposed to cadmium and zinc and attributed it to erythrocyte destruction induced by Cd and Zn. Osuala *et al.* (2013) also observed this effect at very low doses of manganese and attributed it to the free inorganic Mn^{2+} which although abundant in manganese

solutions, cannot traverse the hydrophobic milieu of membrane bilayer thus inhibiting its transport.

5.1.1.5 Chloride ions

Hypochloremic and hyperchloremic alkalosis, although rare, is a life-threatening condition that results from chlorine deficiency and elevation respectively (Rose and Post, 2001). Results from the cadmium, mercury and lead exposed groups show a significant increase in serum chloride concentrations compared to control. This increase is likely due to the improper functioning of the kidneys. This view is based on the report that elevation in chloride ion is associated with a dysfunction or hyperactivity of the parathyroid glands (Sheikh *et al.,* 2011). This result is also in agreement with the work of Osuala *et al.* (2013) who reported that the level of serum chloride ion increased upon exposure to the heavy metals Cd, Fe, Mn, Pb and Zn.

5.1.2 Hepatotoxic Effects

The liver, which is primarily responsible for the metabolism of xenobiotics, is usually the first organ to interact with heavy metals or even drugs and nutrients following ingestion as they enter via the hepatic portal vein from the digestive system. This makes the liver the primary target of exposure to heavy metals via ingestion and consequently, the organ to be studied when evaluating the toxic effects of substances. Such study usually involves evaluating the blood levels of the activities of enzymes like ALP, GGT, ALT and AST which are increased in the blood following liver damage (Osama and Mohamed 2014; Osuala *et al.,* 2013).

5.1.2.1 Direct Bilirubin

Serum bilirubin is considered one of the true tests of liver function since it is a clear reflection of the liver's ability to take up and process bilirubin into bile pigment. Elevated bilirubin levels may indicate impaired liver function. Results obtained from this study when rats were exposed to mercury, cadmium and lead via gavage, showed that the treatment caused increased level of

bilirubin in all cases. This is in agreement with the findings of (Alhazza, 2008) who reported similar increase in direct bilirubin levels upon heavy metal intoxication. Ballatori (1987) showed that biliary excretion is the mechanism used for effectively excreting or eliminating about 95% of Mn from the body, hence, its elevation is largely caused by impaired liver function. It is very likely that the observed increase in serum bilirubin levels after treatment with lead tainted water is consequent upon a mechanism involving the induction of a key enzyme in the heme catabolic pathway, heme oxygenase, responsible for converting heme to bilirubin (Seddik *et al.,* 2010; Murrey *et al.,* 2006).

5.1.2.2 Total Blirubin

Bilirubin accumulates from the breakdown of hemoglobin present in red blood cells. During normal function, the liver removes bilirubin from the blood and excretes it through bile as bile pigment. High levels of total bilirubin in groups treated with cadmium, mercury and lead only are obviously due to the hepatotoxicity of these metals. This observed increase is due to a compromise in the liver's ability to excrete bilirubin or an obstruction in the bile duct (Veena *et al.,* 2012). Another less likely possibility is the inhibition of one or more enzymes involved in bilirubin conjugation.

5.1.2.3 Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) Activities

The serum ALT and AST levels are reliable specific biochemical markers for hepatocellular necrosis (McLin and Yazigi 2011). Their levels are rapidly increased during liver damage arising from hepatitis, cirrhosis or virtually any cause. Results obtained from this study show that in the cadmium, mercury and lead groups there was increased ALT and AST activity after 3 months oral exposure via gavage when compared to the control group. This result is in agreement with the results of Al-Attar (2011) who reported that a combination of heavy metals (Pb, Hg, Cd, and Cu) induced significant elevations in plasma ALT, AST, ALP, and GGT and concluded that the elevation is clear evidence of liver damage. Likewise, Kim *et al.* (2008) in his

work regarding heavy metal toxicity, considered the heavy metals Pb, Fe, Cu and Al as main reasons for high elevations in ALT and AST. Makhlouf and Makhlouf (2012) reported significant elevation in the serum activities of ALT and AST following exposure to heavy metal. Similar observations have been reported by many other investigators who exposed animals to Pb (Liu *et al.,* 2010), and Hg (Agarwal *et al*., 2010; Bashandy *et al.,* 2011), Cd (Renugadevi and Prabu, 2009; Kumar *et al.,* 2010; Bashandy *et al.,* 2011) and Cu (Li *et al.,* 2008). Results obtained in this study did not reveal any increase in serum ALT and AST activities when the unboiled and boiled river water as well as the group treated with solution of all metals were compared to the control. This finding is similar to the work of El-Yamany *et al.* (2015) who reported reduced AST and ALT levels when Cd and Se, were administered to experimental animals simultaneously.

5.1.2.4 gamma-glutamyl transferase (GGT) and lactate dehydrogenase (LDH) activities

GGT and LDH are routinely assayed for during liver function tests. However, taken singly, none is specific for any disease. LDH is not exactly tissue specific unless in regard to its isoenzymic forms and as such could be found even in blood cells and heart muscle. Generally LDH could be elevated remarkably in cancer and several non-cancerous conditions including liver disease (Holmes and Goldberg, 2009). Breakdown of tissues release LDH thus making its estimation useful in evaluating any toxicity with attendant loss of architectural integrity. The mercury, cadmium and lead groups in this study showed a significant increase in the activities of both enzymes. This finding appears to be in harmony with that of (Seddik *et al* (2010) and Ibrahim *et al*., 2012) who treated experimental rats

LDH activities in the blood of Pb-treated mice. Other researchers have also found remarkable increases in LDH and GGT levels upon treatment of experimental animals with Pb (Bersényi *et al.,* 2003; Shalan *et al.,* 2005; Liu *et al.,* 2010), Hg (Bersényi *et al.,* 2003; Agarwal *et al.,* 2010; Bashandy *et al.,* 2011), Cd (Fouad *et al.,* 2009; Renugadevi and Prabu, 2009; Swapna and

Reddy, 2011) which they posited as likely due to the induction of free radical, and lipid peroxidation with attendant damage to biomembrane which makes it possible for enzymes to leak into the blood. The GGT increase is also in agreement with the work of Alwaleedi (2015) who showed that upon treating experimental mice with a heavy metal such as lead, a corresponding dose-dependent increase results. An increase which is likely due to hepatotoxicity and oxidative damage (Tatjana *et al.,* 2003).

5.1.2.5 Cholesterol Levels

Altered cholesterol level in the blood indicates a disorder of lipid metabolism and especially liver disease (Bernard and Grzyna, 1999). Cholesterol level was observed to increase significantly upon exposure to the metals Pb, Hg and Cd. This concurs with the findings of Osuala *et al.* (2013) where increase in cholesterol levels was observed after the 28th day of administering Pb orally. A phenomenon which Haque *et al.* (2006) attributed to the rapid absorption property of lead. This phenomenon was shown to be consequent upon the presence of Pb-binding protein in liver, blood, brain and kidney which increases its binding affinity to intracellular compartments thereby increasing its retention in the body (Han *et al* 1996; Haque *et al.,* 2006). This mechanism is true of mercury and cadmium as well. The result is in agreement with the work of Yang and Chen (2003) who reported a marked increase in cholesterol level upon exposure of experimental animals to heavy metal. Although few others have explained this increase by attributing it to necrosis of liver tissue and subsequent release of cholesterol into the blood, Hadi *et al.* (2009) proposed an alternate mechanism involving increased cholesterol stores as a defense against the stress induced by heavy metals. Kori-Siakpere (2011) in his work on *Clarias gariepinus*, opined that reduced lipoprotein lipase could be responsible for increased cholesterol levels.

5.1.2.6 Histopathological findings on rat liver and kidney exposed to heavy metals via tainted water

Histopathological results revealed relatively more altered histologic features in the groups treated with mercury, cadmium and lead when compared to the control group and the other groups. Control rat kidney revealed normal histological features with detailed cortical parenchyma and renal corpuscles appearing as dense rounded structures. Sections of liver from rats exposed to iron were quite normal but for the vacuolated nucleus, a finding previously observed in the work of Leelavinothan and Ayyasamy (2015), who reported among other ultra-structural changes microvesicular steatosis, pyknotic cell and vacuolation on exposure of rats to iron for 10 days. Rats exposed to only cadmium presented with visible atrophied renal corpuscle with loss of architecture in the renal tubules. The lead treated group also followed suit, revealing enlarged renal corpuscle with distorted tubules. However, the mercury treated group showed apparently normal features for this. The control rat liver section showed normal liver histology with well-appearing centrioles, hepatocytes, nucleus and sinusoidal space. Sections of liver from a rat in the Cd only group revealed thickened centriole wall with mild focal inflammation and hepatocytes with obvious steatosis. A phenomenon which was also found in the work of (Renugadevi and Prabu, 2009) who discovered that the liver of rat treated with cadmium produced inflammatory cell infiltration and fatty degeneration which he explained as being likely due to a cadmium-induced formation of highly reactive radicals and subsequent lipid peroxidation; An explanation which is especially plausible, given the result from the biochemical assays in this study. Kidney sections revealed atrophied renal corpuscle and degeneration of or a not so distinct tubule which was also found to be so in the work of Dardouri *et al.,* 2016. This tubular degeneration and inflammation has also been reported by Ahmed and Basma (2016) where they treated mice with cadmium. The mercury treated group as well as the lead-treated group revealed hepatocytes bearing pyknotic nuclei. This is in agreement with the

work of Amal and Fawzy (2013) who found that rats treated with cadmium, among other effects, presented with pyknotic nuclei. The photomicrographs from the boiled river water group revealed pyknotic nucleus, albeit a milder form. The kidney tubules appeared not so distinct and the renal corpuscle was very distinct albeit with diffuse inflammatory cells within the interstitial parenchyma. The unboiled river water photomicrographs revealed a very similar finding to that of the boiled river water with slight discrepancies where the mild alterations occurred. Both groups showed milder forms of metal-induced toxicity compared to situations in which they acted singly. The effect produced by the solution of all metals was more similar to the control group than any other group, with distinct nucleus, centriole and renal corpuscles. From the foregoing, it is evident that there is higher antagonizing effect as more metals are combined and the likelihood of additional substances, present in the river water, contributing to toxicity. One may allude that the supposed additional substances may act by facilitating absorption of the metals or inhibiting excretion.

5.2 Conclusion

In conclusion, heavy metals exhibit toxic effects as evidenced by the changes in electrolyte concentration, changes in hepatic and renal enzymes, and abnormalities in organ histology. However, when administered concurrently there is a remarkable decrease in manifestations of these toxic effects as the renal and hepatic enzyme activities and histopathological changes revealed.

5.3 Contribution to knowledge

The study has contributed to knowledge in the following ways:

1. The level of individual heavy metals in River Nun at Onuebum may be toxic when examined alone. However, when examined in combination with their co-pollutants as they occur in their natural environment, their toxic capacity is reduced.
2. Iron antagonizes the toxic effect of mercury when co-administered in rats.

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