

HEPATOTOXICITY OF LEAD ACETATE AND MERCURY CHLORIDE ON THE LIVER OF AFRICAN CATFISH

* ¹AYANDA, O.I., ¹OLASEHINDE, G.I., ¹AJAYI, A.A., ¹OLUKANMI, I.B. & ¹FATIREGUN, A.F.

¹Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria.

ABSTRACT

This study assayed the potential hepatotoxicity of lead and mercury, administered as lead acetate and mercury chloride respectively in *Clarias gariepinus*. One hundred and twenty juveniles of the catfish per toxicant were used for the experiment. The test media which was prepared using tap water as solvent was renewed once every 24 hours at the same concentration in a static renewal bioassay. Three concentrations 24 mg/l, 12 mg/l, 6 mg/l of lead acetate and 2.5 mg/l, 1.25 mg/l, 0.625 mg/l mercury chloride, and a control, 0 mg/l were used for the definitive test. Ten fishes were distributed in each fish tank, for each test concentration (including the control) in replicates. The subacute test was conducted for fifteen days. Six fishes were sacrificed from each treatment group and the control every fifth day. The liver of fishes were removed and prepared for histopathological observation. The results showed several histopathological damages ranging from vacuolar degeneration of the hepatocytes, to variably-sized cytoplasmic vacuoles of the hepatocytes. This suggests that lead and mercury are toxic to *Clarias gariepinus*.

Keywords: Hepatotoxicity, lead acetate, HgCl, African catfish

INTRODUCTION

Pollution of the aquatic environment via sources such as chemical spills, discharge from industries and from agricultural lands can result in an increase in the concentration of heavy metals while also affecting the health of fish (Misha *et al.*, 2007; Satheeshkumar and Kumar, 2011). Serious concerns about their hazards on humans, animals and plants have been raised due to the degree of this widespread contamination (Adedeji and Okocha, 2011).

According to Lenntech (2004), a metallic chemical element, poisonous or toxic at low concentrations and with a relatively high density is a heavy metal. The potential for heavy metals to bioaccumulate and biomagnify has been reported as one major problem associated with its persistence (Zhuang *et al.*, 2009). The presence of heavy metals in some organisms is greater than the quantity present in the environment alone naturally. This is because of the tendency of heavy metals bioaccumulate, hence, heavy metals are very dangerous. The accumulation of compounds in living organisms whenever they are taken up and stored is faster than the time it takes for it to break down (metabolized) and/or excreted (Glover-Kerkvliet, 1995). Aquatic organisms such as coastal fishes and seabirds can be monitored for the presence of these contaminants (Akenova, 2000; Beljaeva, 2000).

Heavy metals are natural, essential components of our environment. Generally, they are present in trace quantities in natural water bodies. In addition to the natural sources, many other human-related sources contributes to concentrations of metals in the environment (Aderinola *et al.*, 2009). Recently, activities of industries have increased the natural concentrations of these metals leading to serious problems in the environment. The organisms inhabiting such polluted sites are usually at the

mercy of very high concentrations of these pollutants (Woo *et al.*, 1993). The bioaccumulation and bio magnification of metals in living systems explains the pathways and processes of these pollutants from one stage of the food chain to another, showing the greater ability for bioaccumulation in the concerned organisms. The retention time of toxic substances is higher, as a result of increasing concentrations through trophic levels when compared with other food components. Hence, different species of fish are used often to indicate heavy metal contamination (Svobodova *et al.*, 2004).

The major reason for heavy metal toxicity both in the aquatic environment and also to organisms who rely on aquatic food products is that heavy metals cannot be destroyed through biodegradation. Heavy metal bioaccumulation in the tissues of aquatic animals make the tissue concentrations of heavy metals an issue of public health concern to humans and even animals (kalay *et al.*, 1999; Ashraf 2005).

Mercury is one persistent pollutant in the environment, with the ability to bioaccumulate in fish and other organisms including humans, is mercury (Chang *et al.*, 2009). Mercury salts and Organomercury compounds and salts of mercury rank amongst highly poisonous substances in our environment. Mercury and its compounds are toxic even in little quantities, which are hazardous to the health of man. Poisoning from mercury manifests in the form of renal and neurological disruptions, easily by-passing the barrier between the blood and brain thereby impacting on the brain (Resae *et al.*, 2005).

Another heavy metal that is common in the environment is Lead. It can be derived from industrial discharges, urban waste waters, and agricultural runoff. Contamination of the

environment with Lead is a problem of global importance. It is included in gasoline as an anti-knock which contributes to its presence in the air, and then further transported to the rivers and streams via runoffs where it is taken up and incorporated into the bodies of fish and other aquatic organisms (Olojo *et al.*, 2005).

Physiological changes in fish can serve as indicators of pollution in the environment, as such fishes are widely used to evaluate the health status of aquatic ecosystems (Kock *et al.*, 1996).

The fish *Clarias gariepinus* is an economically important hardy freshwater fish; it has the ability to withstand both poor and oxygen-rich waters and is highly valued in Nigeria. Though the adverse effects of toxicity arising from lead has been well elucidated in man (Klaassen, 1992), the rate and prevalence of lead poisoning in Nigeria are currently on the increase (CDC, 2011), necessitating review and study of its effects on fish. Therefore this study attempts to determine the effects of lead, administered as lead acetate; and mercury, in the form of mercury chloride in *Clarias gariepinus*, by evaluating their histopathological response to the chemical.

METHODOLOGY

The fish samples used for this study were purchased from a fish farm in Ota, Ogun State. The increased sensitivity in juveniles when compared to adults also informed their use in this study (Odiete, 1999; Solbe, 1995). Two hundred and forty samples, one hundred and twenty for each toxicant, of *C. gariepinus* with mean weight 13.4 ± 1.2 g were used for the experiment. They were transported to the laboratory in oxygenated polyvinyl chloride (PVC) materials. In the laboratory, the fishes were held in 30-litre capacity plastic tanks containing water, during which they were allowed to acclimatize for about two weeks and fed on commercial floating pellet feed at 5% of their body weight.

Chemicals

Technical grade lead acetate $Pb(C_2H_3O_2)_2$ and mercury chloride $HgCl_2$ were purchased for this study. Proper care was taken to ensure the chemicals remained within the prescribed storage conditions prior to and during use.

Toxicity Tests

Prior to the main experiment, a range finding toxicity test was conducted to determine the concentrations suitable for the study. Three concentrations were chosen for each toxicant. A static renewal bioassay was adopted in which the test media was renewed once every 24 hours, and at the

same concentration to remove unconsumed feed. After the acclimatization period, the juveniles were divided randomly into four groups (three treatments and a control) of thirty fish and each group subdivided into a set of three subgroups consisting of ten fish each. The subgroups were kept in twenty-four different tanks in 25 liters of water. This was done for each toxicant.

A stock solution was prepared for each of the toxicant and from the stock solution, appropriate volumes that represents each of the concentrations for both chemical was drawn out and added to tap water in the tanks and the volume of water was made up to 25 liters. The different groups were respectively exposed to 0.625 mg/l, 1.25 mg/l, 2.5 mg/l, 0 mg/l of mercury chloride ($HgCl_2$), and 6 mg/l, 12 mg/l, 24 mg/l, 0 mg/l of lead acetate representing groups A to D. The fishes were exposed to 12:12 period of light and darkness respectively. Eight fishes were removed (two each from each subgroup) and sacrificed every five days. The experiment was run for fifteen days.

Histopathology

The liver of sacrificed fishes was excised.

They were fixed in physiological saline for 24hr, washed with 70% ethanol and dehydrated through a graded series of ethanol (Kelly, 1979; Schalm *et al.*, 1975). They processed tissues were examined under the microscope at low and high magnification and photomicrographed (Keneko, 1989).

RESULTS

The result of the present study revealed that mercury and lead are toxic to fish. The liver of fishes exposed to the different concentrations of both chemicals showed different degrees of pathological changes; however, the major cyto-architectural alteration observed is vacuolation of the hepatocytes. Fish liver exposed to different concentrations of lead acetate showed liver damages such as vacuolar degeneration of the hepatocytes, multiple foci of hepatocytes and aggregates of heterophils in hepatic parenchyma containing several variably-sized and large-sized cytoplasmic vacuoles in the hepatocytes, dissociated hepatic cords and perivascular accumulation of lymphocytes were observed in liver cells. Liver of fishes exposed to 0.625 mg/l of mercury chloride was observed to show damage as diffuse severe vacuolar degeneration of hepatocytes with variably-sized vacuoles after five days, which continued until the tenth day and by the fifteenth day, the vacuolation had been more pronounced (Fig 1-3).

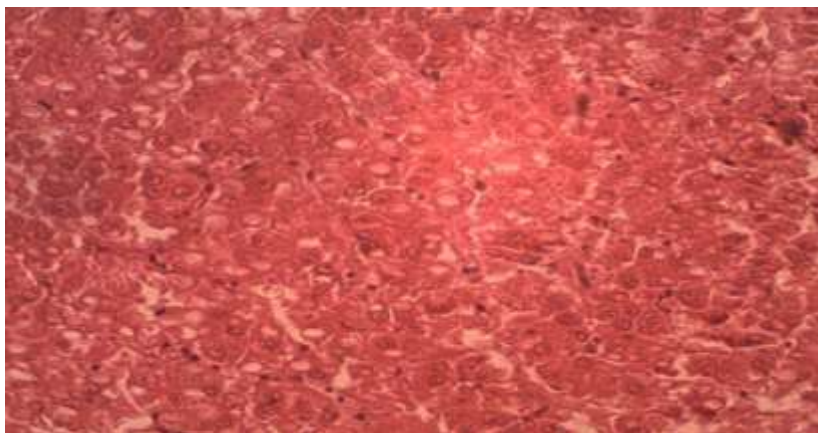


Fig 1 Liver of fish exposed to 0.625 mg/l of mercury chloride after five days shows moderate vacuolar degeneration of the hepatocytes

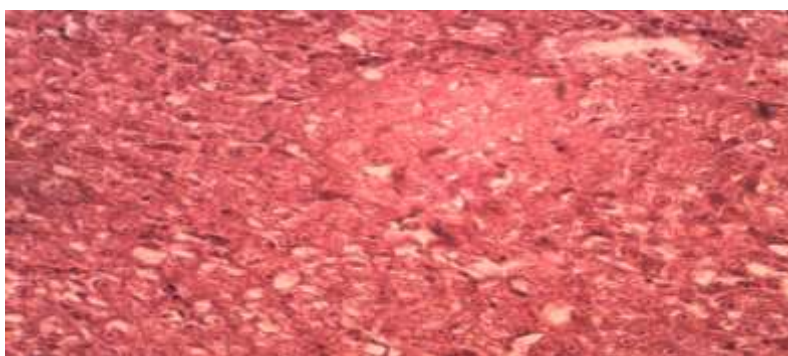


Fig 2 Liver of fish exposed to 0.625 mg/l of mercury chloride after ten days shows random vacuolar degeneration of the hepatocytes; as they contain variably-sized vacuoles; there is moderate dissociation of hepatic cords.

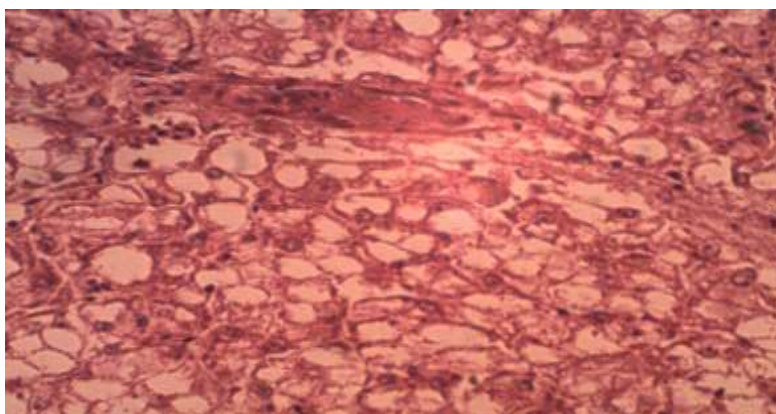


Fig 3 Liver of fish exposed to 0.625 mg/l of mercury chloride fifteen days shows multiple foci of hepatocytes containing variably-sized cytoplasmic vacuoles; there are also several foci of aggregates heterophils in the hepatic parenchyma lymphocytes in the hepatic

The hepatic tissue of the fishes exposed to 1.25 mg/l of mercury chloride (Fig 4-5) showed diffuse vacuolar degeneration of hepatocytes, perivascular accumulation of lymphocytes and variably-sized

cytoplasmic vacuoles in the hepatocytes giving rise to moderate, random vacuolar cytoplasmic vacuoles in the degeneration of the hepatocytes.

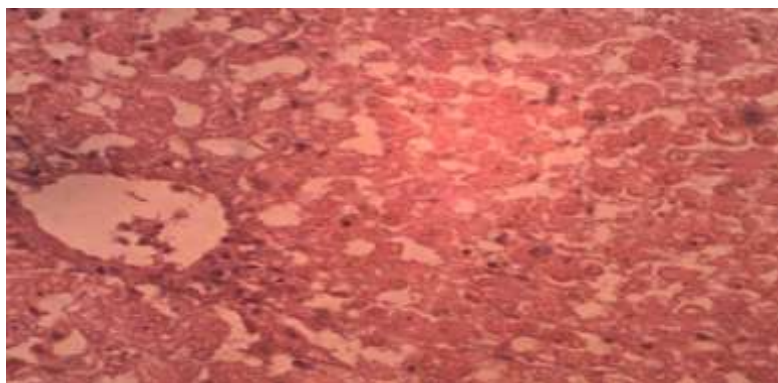


Fig 4 Liver of fish exposed to 1.25 mg/l of mercury chloride fishes after ten days shows diffuse vacuolar degeneration of hepatocytes. The vacuoles are variably-sized.

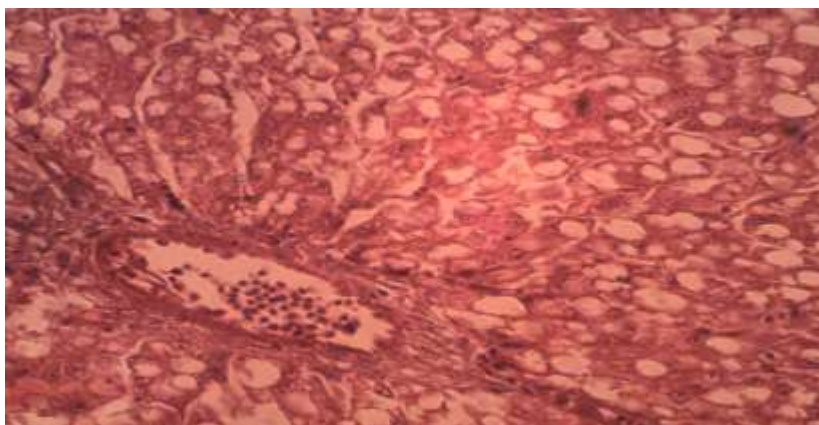


Fig 5 Liver of fish exposed to 1.25 mg/l of mercury chloride after fifteen days shows perivascular accumulation of lymphocytes and variably-sized cytoplasmic vacuoles in the hepatocytes giving rise to moderate, random vacuolar cytoplasmic vacuoles in the degeneration of the hepatocytes.

The hepatic tissues of fishes exposed to the highest concentration of mercury chloride, i.e. 2.5 mg/l (Fig 6) shows dissociated hepatic cords.

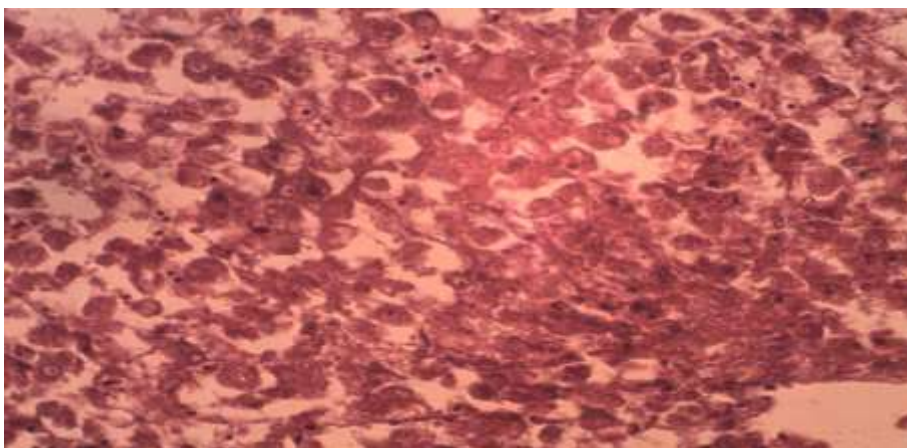


Fig 6 Liver of fish exposed to 2.5 mg/l of mercury chloride after fifteen days shows hepatic cords are dissociated

Liver tissues of fish exposed to lead acetate showed similar forms of alterations to those observed in the liver of mercury-exposed fishes. These ranges from vacuolar degeneration in the hepatocytes,

aggregations of inflammatory, dissociated hepatic cords, black necrotic spots and perivascular accumulation of lymphocytes were observed in liver cells. Liver of fishes in exposed to 6mg/l of

lead acetate showed damage in the form of diffuse vacuolar degeneration of hepatocytes with

variably-sized vacuoles and moderate dissociation of hepatic cords (Fig 7-8).

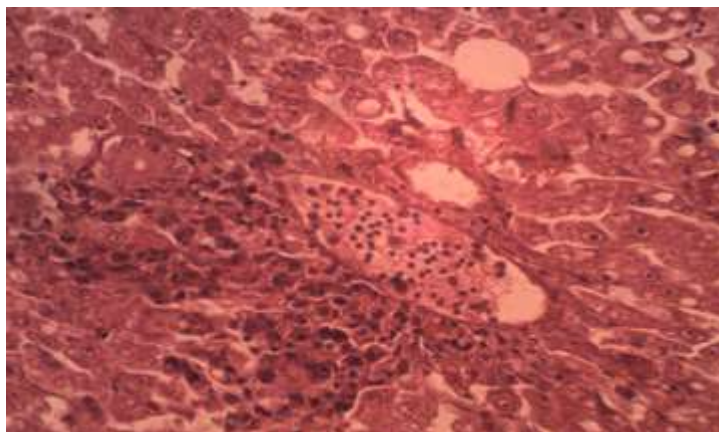


Fig 7 Liver of fish exposed to 6 mg/l of lead acetate after five days shows diffuse vacuolar degeneration of hepatocytes.

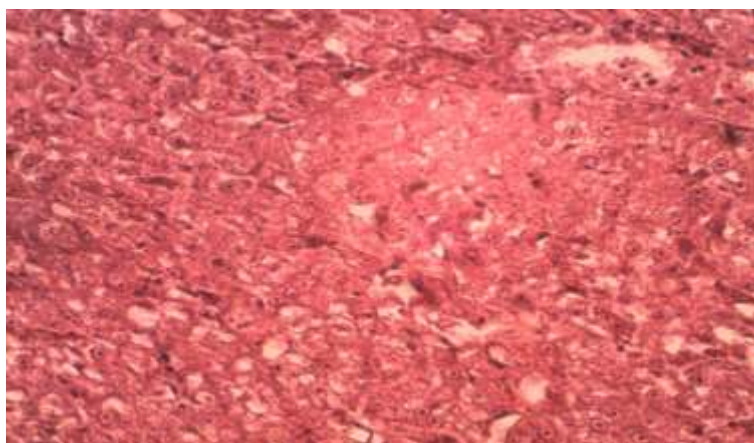


Fig 8 Liver of fish exposed to 6 mg/l of lead acetate after fifteen days shows random vacuolar degeneration of the hepatocytes; as they contain variably-sized vacuoles; there is moderate dissociation of hepatic cords

The hepatic tissue of the fishes treated with 12 mg/l lead acetate (Fig 9) revealed extensive vacuolar degeneration of hepatocytes while liver of fishes

receiving the highest concentration of the chemical showed variably sized to large-sized vacuoles in the hepatocytes (Fig 10-11).

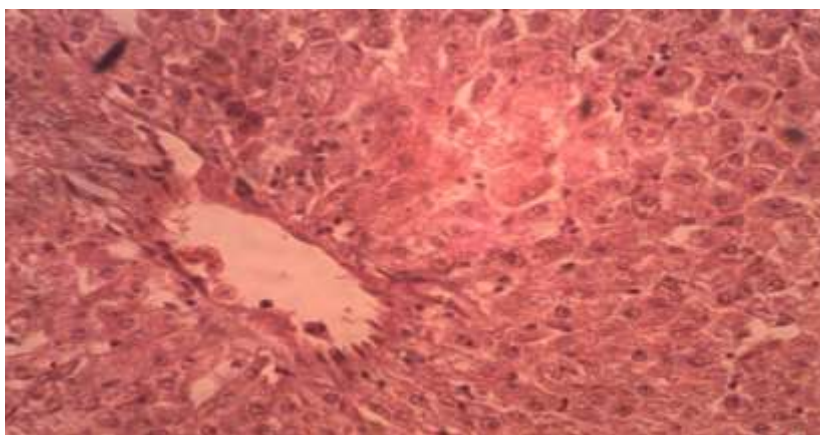


Fig 9 Liver of fishes exposed to 12mg/l of lead acetate after ten days shows locally extensive vacuolar degeneration of hepatocytes

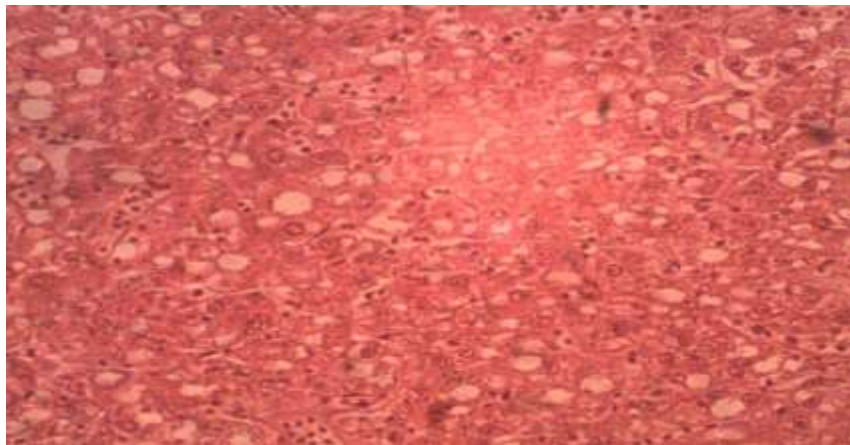


Fig 10 Liver of fishes exposed to 24 mg/l of lead acetate after ten days shows variably-sized cytoplasmic vacuoles in the hepatocytes.

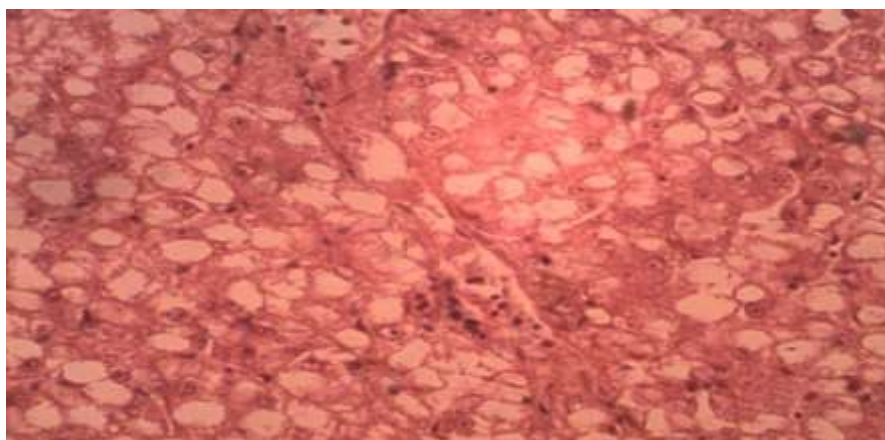


Fig 11 Liver of fishes exposed to 24 mg/l of lead acetate after fifteen days shows large-sized cytoplasmic vacuoles in the hepatocytes, it also shows perivascular accumulation of lymphocytes.

The liver architecture of fishes in the control group, (Fig 12) were intact, showing no visible lesions, no vacuolation and no necrosis indicating that the

alterations observed in the liver of the chemical-exposed fishes are as a result of the toxic effects of the chemicals.



Fig 12 Liver of fish in the control group shows no visible lesion, uniformly fine cytoplasmic vacuoles giving the hepatocytes a foamy appearance

DISCUSSIONS

Anthropogenic pollutants exert effects on organisms, fish inclusive, and the general health status a whole population can be determined from histopathological alterations of body organs (Mohamad, 2009). Histopathological indices are synonymous to other stress biomarkers as many other pollutants will still undergo metabolic activation so as to bring about cyto-alteration in the organism affected (Braunbeck et al., 2005). The liver of fish in this study showed varying degree of changes ranging from vacuolar degeneration in the hepatocytes, multiple foci of hepatocytes with variably-sized cytoplasmic vacuoles, several foci of aggregates of heterophils in the hepatic parenchyma, variably-sized cytoplasmic vacuoles in the hepatocytes giving rise to a moderate random vacuolar degeneration of hepatocytes, dissociated hepatic cords, perivascular accumulation of lymphocytes and large-sized cytoplasmic vacuoles in the hepatocytes. It is possible that the changes noticed in the liver cells are a direct effect of the toxicity of the pollutants on the hepatocytes, since the liver detoxifies chemicals and toxins of all types (Soufy, et al., 2007). Similar observations were reported by Olojo et al. (2005) in the liver of *Clarias gariepinus* exposed to Lead.

Liver lesions in aquatic organism are often as a result of aquatic pollution (Ali, et al., 2008). Hence, the pathological changes in fish tissues observed in this study are likely to be as a direct result of the heavy metals. Mohamed (2009) opined that exposing fish to pollutants of industrial and agricultural origins caused several pathological changes in different fish tissues. Abbas and Ali (2007) exposed *Oreochromis spp.* to hexavalent chromium and reported similar histopathological alterations. The way several xenobiotics work can trigger off the making of a specific enzyme that causes metabolic changes, which can further lead to cellular intoxication and probably death at the level of the cell whereas this may be seen as necrosis at the level of the tissue (Velkova-Jordanoska and Kostoski, 2005).

The liver is the organ mostly associated with the process of detoxification and biotransformation. Furthermore, it is one organ most affected by contaminants in the water by virtue of its position, function and supply of blood (Camargo and Martinez, 2007). Vacuoles in the hepatocytes might be indicative of an imbalance in the rate by which substances are synthesized in the parenchymal cells and the rate at which they are released into the circulation system (Gingerich, 1982). Increasing levels of hepatocyte vacuolation has been described as a signal to the process of degeneration, suggestive of metabolic damage which may be related to exposure to contaminated water (Pacheco and Santo 2003). Consequently, a plausible explanation for the histological changes noticed in the liver of *C.*

gariepinus in this study might be to say that they are responses to stress, as well as to direct and additive effects of the contaminants.

Liver alterations may be used as biomarkers, indicating prior exposure to environmental contaminants. The vacuolated cells in the liver of fish is evident of fatty degeneration. Necrotic portions observed in the liver tissue can be as a result of the too much work needed by the fish to eliminate the toxicant from its body in the detoxification process. Results in this are similar to the observations of Rahman et al. (2002), Fanta et al. (2003).

Some of the effects of exposure to chemicals observed in this study may be respiratory and osmoregulatory disorders. As opposed to having an irreversible toxic effect, these changes may also be interpreted to be the defensive action of the organ against contamination. However, modifications of this nature may be detrimental to the health of fish, by increasing chances of susceptibility to secondary infections and probably death (Hawkins et al., 2008).

CONCLUSION

This study has shown that exposure of *C. gariepinus* juveniles to low concentrations of mercuric chloride and lead acetate can induce various pathological damage in the form of histological degradation. Hence, pathology of these organs could serve as an important biomarker of heavy metal toxicity. Besides, changes in the main organs involved in xenobiotic metabolism could have serious consequences on the overall physiology of the exposed fish.

REFERENCES

- Abbas H.H and Ali F.K. (2007). Study the effect of hexavalent chromium on some biochemical, cytotoxicological and histopathological aspects of the *Oreochromis spp.* *Pakistan Journal of Biological Science*, 10: 3973-3982.
- Adedeji O.B, and Okocha R.C. (2011). Assessment Level of Heavy Metal in Prawns (*Macrobrachium Macrobrachion*) and Water from Epe Lagoon. *Advances in Environmental Biology*, 5(6): 1342-1345
- Aderinola O.J, Clarke E.O, Olarinmoye O.M, Kusemiju V and Anatekhai M.A. (2009). Heavy Metals in Surface Water, Sediments, Fish and Periwinkles of Lagos Lagoon. *American-Eurasian J. Agric. & Environ. Sci.*, 5(5): 609-617
- Aksenova M. (2000). Tjazelnye metaly - mehanizmy nefrotoksichnosti. *Nefrologija i dializ.* p. 56 - 58.
- Ali F.K, El-Shafai S, Samhan F and Khalil W.K.(2008). Effect of water pollution on

- expression of immune response genes of *Solea aegyptiaca* in Lake Qarun. *African Journal of Biotechnology*, 7: 1418-1425.
- Ashraf W. (2005). Accumulation of heavy metals in kidney and heart tissues of *Epinephelus microdon* fish from the Arabian Gulf. *Environmental Monitoring and Assessment*, 101:311.
- Beljaeva N. (2000). Značimost' morfoložičeskikh pokazatelej v gigeničeskikh issledovanijach. In *Gigiena a sanitarija* .p. 56-58.
- Braunbeck T, Boettcher M, Hollert H, Kosmehl T, Lammer E, Leist E, Rudolf M and Seitz N. (2005). Towards an alternative for the acute fish LC (50) test in chemical assessment: the fish embryo toxicity test goes multi-species – an update. *ALTEX* 22: 87-102
- Camargo M.M and Martinez C.B. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotropical Ichthyology*, 5: 327-336
- Centre for Disease Control (2011). Health Disparities and Inequalities Report - United States: helping to understand Lead poisoning outbreak in Nigeria. 60:1-116
- Chang T.C, You S.J, Yu B.S, Chen C.M and Chiu Y.C. (2009). "Treating high-mercury-containing lamps using full-scale thermal desorption technology," *Journal of Hazardous Materials*, 162(2-3): 967-972.
- Fanta E, Rios F, Romao S, Vianna A and Freiburger S.(2003). Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. *Ecotoxicology and Environmental Safety*, 54(2):119-130.
- Gingerich W.H. (1982). Hepatic Toxicology of Fishes. In: *Aquatic Toxicology*, Weber, L.J. (Ed.). New York: Raven Press, pp: 55-105.
- Glover-Kerkvliet J. (1995). Environmental Assault on Immunity. *Environmental Health Perspectives*, 103(3): 236-237.
- Hawkins W.E, Overstreet R.M and Provancha M.J.(2008). Effects of space shuttle exhaust plumes on gills of some estuarine fishes. *Ecology of Fish*, 57:27-39.
- Kalay M, Ay P and Canil M. (1999). Heavy metal concentration in fish tissues from the northeast Mediterranean sea. *Bulletin of Environmental Contamination and Toxicology*, 6:673-671.
- Klaassen, C.D. (1992). Heavy metals and heavy metal antagonists. In: *Pharmacological Basis of Therapeutics*, Goodman L, Gilman A, (eds) McGraw Hill Medical Publishing Division: New York, 1825-1872.
- Kelly, W.R. (1979). *Veterinary Clinical Diagnosis* (2nd ed). Balliere Tindall, London pp. 266-279.
- Keneko, J.J. (1989). *Clinical Biochemistry of domestic animals* (4th ed). Academic press: California, pp. 132-133.
- Kock G, Triendi M and Hofer R. (1996). Seasonal pattern of metal accumulation in Arctic char (*Salvelinus alpinus*) from an oligotrophic Alpine lake related to temperature. *Canadian Journal of fisheries and Aquatic Sciences*, 53:780-786.
- Lenntech Water Treatment and Air Purification (2004). *Water Treatment*, Published by Lenntech, Rotterdamseweg, Netherlands (www.excelwater.com/thp/filters/Water-Purification.htm).
- Mishra S, Bhalke S, Saradhi IV, Suseela B, Tripathi IM, Pandit C.G., Puranik V.D. (2007). Trace metals and organometals in selected marine species and preliminary risk assessment to human beings in Thane Creek area, Mumbai. *Chemosphere*, 69: 972-978.
- Mohamed F.A.S. (2009) Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. *World Journal of Fish Marine Sciences*, 1: 29-39.
- Odiere W.O. (1999). Impacts associated with water pollution, In: *Environmental physiology of Animal and pollution*. 1st Ed. Diversified Resources Ltd., Lagos, Nigeria. pp 360
- Olojo E.A, Olurin K.B, Mbaka G and Oluwemimo A. (2005). Histopathology of the gill and liver tissues of the African catfish *Clarias gariepinus* exposed to lead. *African Journal of Biotechnology*, 4: 117-122.
- Pacheco M and Santos M.A. (2002). Biotransformation, genotoxic and histopathological effect of environmental contaminants in European eel (*Anguilla anguilla*). *Ecotoxicology and Environmental Safety*, 53: 331-347
- Rahman Z, Hossain M.F, Mollah B and Ahmed G.U. (2002). Effect of Diazinon on Fish. *The ICLARM Quarterly*, 42:29-34.
- Resae A, Derayat J, Mortazavi S.B, Yamini Y and Jafarzadeh M.T. (2005). Removal of Mercury from chlor-alkali industry wastewater using *Acetobacter xylinum* cellulose. *American Journal of Environmental Sciences*, 1(2): 102-105.
- Satheeshkumar P, Senthilkumar D. (2011). Identification of heavy metals contamination by multivariate statistical analysis methods in Pondicherry mangroves, India. *Journal of Environmental Earth Science*, 1(1): 30-48
- Schalm O.W, Jain N.C and Carrol E.J. (1975). *Veterinary haematology*, (3rd Ed). Philadelphia, LeaardFebiger, pp. 15-81.

- Solbe J.F. (1995). Fresh water. In: *Handbook of Ecotoxicology* Collins P, (Ed). Black Well Science Ltd, Osneymeed, pp683.
- Soufy H, Soliman M, El-Manakhly E and Gaafa A. (2007). Some biochemical and pathological investigations on monosex Tilapia following chronic exposure to carbofuran pesticides. *Global Veterinaria*, 1: 45-52.
- Svobodova Z, Celechovska O, Kolara J, Randak T and Zlabek V. (2004). Assessment of metal contamination in the upper reaches of the Ticha Orlice River. *Czech. Journal of Animal Science*, 49:458- 64
- Velkova-Jordanoska L and Kostoski G. (2005). Histopathological analysis of liver in fresh (*Barbus meridionalis petenyi* Hekel) in reservoir Trebenista. *Natura Croatica*, 14: 147-153.
- Woo, P.T.K, Sin Y.M and Wong M.K. (1993). Effect of short-term acute cadmium exposure on blue tilapia *Oreochromis aureus*. *Environmental Biology of Fishes*, 37: 67-74.
- Zhuang P, Zou H and Shu W. (2009). Biotransfer of heavy metals along a soil-plant-insect-chicken food chain: Field study. *International Journal of Environmental Science*, 21(6): 849–853.