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# Histopathological Alterations in the Liver and Kidney of the Fish *Chrysichthys nigrodigitatus* due to Heavy Metals in Niger River

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**Abstract**
Surveillance and monitoring of concentrations of heavy metals in water bodies may serve as an early alert system on bioaccumulation of heavy metals in aquatic food chain therein. Histopathological examination of target tissues of the fish would reveal harmful effects of chemical pollutants like heavy metals in fish. This study determined the concentrations of heavy metals in water columns and in the fish *Chrysichthys nigrodigitatus* of Niger River at Onitsha as well as pathological alterations in liver and kidney tissues of the fish. Atomic Absorption Spectrophotometer was used in determination of concentrations (mg/l) of heavy metals namely, Zinc (Zn), Iron (Fe), Copper (Cu), Lead (Pb), Mercury (Hg), Cadmium (Cd), and Arsenic (As) in water and fish samples from the Niger River during the dry season of 2011 and proceeding wet season of 2012. Annual mean concentrations of Zn, Fe, Cu, Hg, Cd, and Arsenic in water columns were significantly lower ($P<.05$) than those in the fish. Annual mean concentrations of Zn, Cu, Cd, Hg, and Arsenic in fish however complied with WHO Standards in Aquatic Foods but Fe and Pb exceeded the WHO Standards. Extensive hyperaemia, oedematous sinusoids, hepatocytes in apoptosis with pyknotic nuclei, and wide spread necrotic hepatocytes with mononuclear leucocytes infiltrations and pigment deposits in liver tissues, as well as severe hyperaemia of the interstices with degenerating and necrotic tubular epithelial cells in kidney tissues were the major characteristic histopathological findings in the fish. Bioaccumulation of heavy metals in the fish indicated that Niger River at Onitsha was experiencing impairment, and that prolonged exposure of fish to heavy metals in the river may result in increased morbidity and mortality as well as reduced productivity in fish. Potential health risks associated with human consumption of heavy metal-contaminated fish and other aquatic food chain need not be over emphasized. Similar studies should be carried out in other Nigerian water bodies in order to increase the national information bank on chemical pollution which will help in the formulation of evidence-based policy decisions on methods of achieving sustainable environment.
**Keywords:** Niger River, pollution, heavy metals, fish, bioaccumulation, liver and kidney, histopathology

## 1. Introduction

The section of the Niger near its confluence with Anambra River at Onitsha in south-eastern Nigeria is economically important for irrigation, fishing, transportation and recreation. Increasing human population, rapid industrialization and commercial activities in Onitsha Metropolis and the resultant continuous discharge of domestic and industrial effluents into the Niger are responsible for the increase in concentrations of heavy metal in the river (Nsofor & Aguiwo 2005). This is also responsible for bioaccumulation of heavy metals in the fish *Macrobrachium rosenbergi* caught in the river (Nsofor *et al.* 2014). All heavy metals found in surface waters exist in colloidal, particulate and dissolved phases; while the colloidal and particulate metals are found as hydroxides, oxides or sulfides or mixed with clay, silica or organic matter, the soluble forms are generally ions or unionized organo-metallic chelates which, according to Pandy & Corney (1997), enter the systems of aquatic organisms. Metals in the aquatic food chain eventually get into man on consumption of contaminated aquatic foods like fish. Bioaccumulation of heavy metals in fish is hazardous to both man and fish (Nsofor *et al*. 2014; Olojo *et al*. 2005) because of the histopathological changes they cause in vital organs and tissues of the body. Hence histopathological alterations in the tissues of liver, kidney and gills of fishes have been used to evaluate the health of fish exposed to heavy metals and other pollutants under laboratory (Nosakhare *et al*. 2013) and field conditions (Schwaiger *et. al.* 1997). Higher concentrations of heavy metals that occur in fish and other bioaccumulator organisms provide better representation of biologically available metals in the environment (Negin & Mehdi 2012; Fabris *et al*. 1994; Philips, 1981). In view of the ever-increasing importance of fish as a source of high quality animal protein in Nigeria (Nwuba & Ikpeze 2009; Nwuba *et al*. 2009) it is necessary to make a comprehensive assessment of the hazards posed by these metals to fish in Nigerian water bodies. There is a dearth of information on the pathologic effects of heavy metals in fish from the polluted Niger River. Hence the major aim of the study was to determine the presence of heavy metals in surface water and one of the dominant fish species *Chrysichthys nigrodigitatus* of Niger River as well as investigate pathological changes in the liver and kidney tissues of the fish. Results from this study will create public awareness on the hazards of

heavy metals in aquatic foods. It will also help in the formulation of evidence-based policy decisions for effective pollution management of the aquatic environment to protect humans from potential toxicity associated with heavy metals in fishes from polluted waters.

## 2. Material and methods

2.1 Area of study

The study was carried out during the dry season of 2011 and proceeding wet season of 2012 with water and fish from the Niger River near its confluence with Anambra River at Onitsha (Latitude 6º 09'N and Longitude 6º 46'E), Anambra State, south-eastern Nigeria. The river serves as a sink for industrial and domestic wastes from Onitsha and environs through surface and underground channels which discharge into them. Fish-landing sites are within the point sources of agricultural and industrial effluents and waste dumps by the river banks.

2.1.1 Collection and preservation of water and fish samples

Water samples were collected at the Fish-landing sites at 20cm below water surface in 250ml capacity plastic bottles with cork stopper. The bottles were treated with 10% nitric acid and rinsed with de-ionized water before use, to avoid metal adsorbing on the plastic bottles (Laxen and Harrison, 1981). The samplings were carried out between 8am and 12noon and taken to the Fishery Unit, Department of Zoology, Nnamdi Azikiwe University Awka where the samples were fixed with 10% nitric acid and stored in refrigerator until analysis. The 90 fishes (mean weight 200.5±10g and length 22.5±2.5cm) procured from artisanal fishers at the fish landing sites at the bank of the river were transported in ice chests to the Fishery Lab of Department of Zoology, Nnamdi Azikiwe University Awka. The fish samples were washed with clean water and the gut opened to enhance drying. The samples were dried separately to a constant weight at 80ºC in Arkson scientific oven. The dried whole fish samples were ground in a porcelain mortar and homogenized to fine powder with an electric blender. Two grams of each sample were weighed into a 50ml Kjedal Flask for digestion using a*qua regia* for one hour. *Aqua regia* is a mixture of concentrated nitric acid ($HN0_3$) and hydrochloric acid (HCl) in the ratio of 1:3. The mixture was swirled gently and allowed to digest at 80ºC under a fume hood. Digestion continued for about fifteen minutes after disappearance of the crystals leaving a clear solution. The digester was filtered through a Whatman No. 42 filter paper into a 250ml volumetric flask, and made up to 20ml with de-ionized water. The digested sample was stored in low density polythene plastic bottles prior to analysis for heavy metals.

2.1.2 Analysis of water and fish samples for heavy metals

Water and fish samples were analyzed for Zinc (Zn), Iron (Fe), Copper (Cu), Lead (Pb), Magnesium (Mg), Mercury (Hg), Cadmium (Cd) and Arsenic (As) using Atomic Absorption Spectrophotometer (AAS) Unicam 969 Model according to manufacturer's instructions. The use of AAS is a popular method of analyzing a broad range of heavy metal concentrations in the liquid state. The AAS has a hollow cathode ray discharge lamp used to produce radiation at a wavelength specific for the metal being assayed. The principle of the AAS is based on the fact that in the process of excitation and decay of atoms to ground state, energy could be absorbed or emitted. By measuring the amount of light absorbed, a quantitative determination of the concentration of analyte present in a sample can be measured. The absorption occurring is proportional to the concentration of the analyte in the sample. The calibration curve for each metal is usually obtained by plotting on a linear graph paper, the absorbance of the standard against their concentrations. The concentrations of the metal assayed are obtained from the calibration curve by interpolating the sample absorbance to the appropriate concentration (Burrell, 1975).

Standard metal solutions used for metal assay were prepared under the supervision of Professor Augustine Eboatu, a Chartered Chemist in the Department of Industrial Chemistry, Nnamdi Azikiwe University Awka. A gram of zinc wire was dissolved in 10ml of HCl and diluted to 1000ml with de-ionized water. A gram of copper chloride was dissolved in 10ml of $HNO_3$ and diluted to 1000ml with de-ionized water. A gram of iron wire was dissolved in 50ml of $HNO_3$ and diluted to 1000ml with de-ionized water; and 1.83g of lead nitrate was dissolved in 100ml de-ionized water and 10ml of concentrated nitric acid ($HNO_3$) added. Also, 4.95g of magnesium sulfate was dissolved in 200ml of de-ionized water and 1.5ml concentrated nitric acid was added and the solution made up to 1000ml with de-ionized water. Again, 0.14g of mercuric chloride was dissolved in 100mls of de-ionized water in a conical flask and 0.8ml of hydrochloric acid added. The mixture was made up to 1000ml with de-ionized water. Finally, 1.32g of arsenous oxide was dissolved in 25ml of NaOH solution. The solution was further diluted to 1000ml with de-ionized water (Reish & Oshida, 1986). The mixture was made up to 1000ml with de-ionized water. Four concentrations of each stock standard metal solution were prepared by diluting aliquots of the stock solutions to 1000ml. Each standard was aspirated into the flame of cathode ray and the absorbance recorded.

2.1.3 Preparation and examination of photomicrographs of liver and kidney sections of *C. nigrodigitatus*

Twenty live *C. nigrodigitatus* were euthanized, dissected and the livers and kidneys eviscerated for histological examination. The organs were labeled and fixed separately in small plastic containers in 10% Formal-Salin solution at room temperature for 24hrs to prevent autolysis and putrefaction. 1000mls of Formal-Salin solution

was formulated with 100ml of concentrated formaldehyde, 900ml of tap water, and 8.5g of sodium chloride crystals. The preserved tissues were left in running water for thirty minutes to remove all traces of fixative before being sectioned into pieces of 2mm diameter and then dehydrated for 30 minutes in ascending series70, 90, and 100% concentration of ethanol respectively. The tissues were cleared of ethanol overnight in chloroform and then immersed in molten paraffin wax for infiltration at 60ºC in an electric oven for one hour. The tissues were then embedded in fresh molten paraffin wax at room temperature. The embedded tissues were sectioned, each 5µm in thickness, with a microtome knife with a manual rotary microtome (American optical comp. No. 800). The serial sections were mounted on clean microscope glass slides previously subdued with egg albumen to enhance proper adhesion of the tissue on the slides. The slides were properly labeled, cleared of paraffin in two changes of Xylene for thirty minutes; and hydrated in descending grades 90, 70, and 50% concentration of ethanol for ten minutes respectively. Staining was done using aqueous solution of Harris H&E stain in glass staining trough. The stained sections were dehydrated in ascending grades 50, 70 and 90% concentration of ethanol, cleared in chloroform solution, and covered with cover slides using Canada balsam for proper adhesion. The permanent slides produced were examined and interpreted by Dr. Soyinka S.V.O.S, a Veterinary Pathologist in University of Nigeria Nsukka, who also took photomicrographs of the sections with photomicrograph microscope Model-Gallen III No. Bm 600 at varying magnifications (Drury & Wallington 1976).

## 3. Results and Discussions

Mean concentrations of heavy metals analyzed in water columns and the fish *C. nigrodigitatus* of the Niger River, compared with the internationally recommended standard guidelines for aquatic foods are shown in Table 1; and clearly illustrated in Figure 1.

Table 1. Some heavy metals in water columns and the fish *Chrysichthys nigrodigitatus* of Niger River

| | Niger River | | Aquatic foods | | |
|---|---|---|---|---|---|
| | Water column | *C. nigrodigitatus* | WHO (1984) | UNEP (1996) | FEPA (1991) |
| Zn | $0.321^{a} \pm 0.09$ | $4.35^{b} \pm 1.20$ | 5 | 5 | 5-10 |
| Fe | $1.52^{a} \pm 1.07$ | $9.73^{b} \pm 1.30$ | 1-3 | 0.3 | 0.1-1 |
| Cu | $0.035^{a} \pm 0.006$ | $1.35^{b} \pm 0.43$ | 1-3 | 1 | 0.5-1.5 |
| Pb | Inconclusive | $0.29 \pm 0.04$ | 0.05 | 0.05 | 0.05 |
| Hg | $0.016^{a} \pm 0.007$ | $0.16^{b} \pm 0.01$ | 0.05 | 0.02 | 0.02 |
| As | 0.016±0.007 | $0.04 \pm 0.001$ | 0.05 | 0.05 | 0 |
| Cd | 0.012±0.006 | $0.31 \pm 0.04$ | 2.00 | 0.01 | 0.01 |

Values in same row with different superscripts are significantly different ($P$<.05)

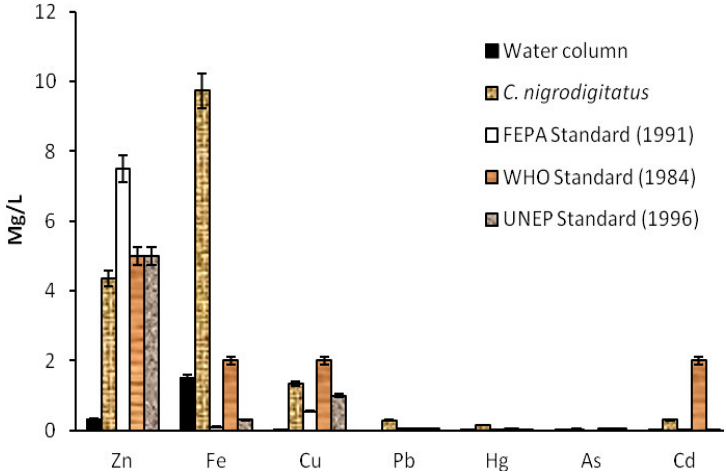


Figure 1. Concentrations of heavy metals in water and *C. nigrodigitatus* fish of Niger River

From Table 1 and Figure 1, it was observed that River Niger at Onitsha was contaminated with the analyzed heavy metals whose concentrations in water were lower than in fish, indicating bioaccumulation of the metals by the fish *C. nigrodigitatus* in which Fe, Pb and Hg, exceeded the recommended standards (UNEP 1996; FEPA 1991; WHO 1984) in aquatic foods. Fabris *et al.* (1994) had reported lower concentrations of Pb, Cd, and Hg in the water of Dervent estuary Australia compared to the higher values recorded in the tissues of the mussel *Mytilus edulis planulasus* from the same estuary. Obasohan and Oronsaye (2000) also reported higher concentrations of Fe, Cu, Zn, and Cd in tissues of *Clarias gariepinus* from Ikpoba River in Benin City, Nigeria compared to the lower values recorded in the same river.

In the present study, the concentration of zinc in the river was lower than the WHO (1984) Standard of 5mg/L

for domestic and aquatic life water, which was similar to the low concentrations reported in Ogba River (Obasohan & Oronsaye 2004) and Olomore waters (Idodo-Umeh 2002) but in contrast to the higher value of 15mg/l for Lagos lagoon (Okoye 1991) and 18.8mg/l for Dilimi River in Jos (Njoku & Keke, 2001). Though the bioaccumulation of zinc in the studied fish was lower than the recommended standards in aquatic foods, it indicated mild input of zinc into the river. Zinc in the river must have probably come from sources such as sewage, refuse dumps, effluent from paper & pulp, batteries, and paint industries located around the river. Zinc is an essential micro-nutrient often associated with enzymes and proteins (Gill *et al.* 1992) but at toxic levels it causes pale and congested gills in *Oreochromis niloticus* (Abad-el-Gawad 1999) and massive intestinal mucus secretions (Hogstrand 2000).

The higher concentrations of Fe in both the river and *C. nigrodigitatus* over the standards indicated that the river was grossly polluted with elevated levels of this metal ion. This could be attributed to high influx of Fe-rich effluents in the River. Iron is a major element in various minerals and rock types and reaches natural water from many sources including leaching and flaking rust from Ferro metal pipes. The elevated Fe concentrations in River Niger is similar to the findings of Fufeyin (1998) who reported 2-7mg/L of Fe in the water of Ikpoba River as well as Ajiwe *et al.* (1999) who reported 8.05mg/L in ground waters in Anambra State. The high Fe levels in fish species of this zone of the Niger River contrasted with the lower concentrations of 2.28µg/l recorded for *Malapterurus electricus*, 11.24µg/l for S*ynodontis clarias*, and 11.24µg/l for *Tilapia mariae* from Ogba River in Benin (Wangboje and Oronsaye, 2001). The high levels of Fe in *C. nigrodigitatus* could be due to the predatory feeding habit of the fish, suggesting metal enrichment at higher trophic levels. Heavy metal enrichment along food chain has been described as a situation where end-consumers have higher metal concentrations than the primary producers and consumers (Forstner & Prosi, 1979).

The copper concentrations observed in the River compared well with values reported for Ogba River (Obasohan & Oronsaye, 2004), Ikpoba River (Fufeyin, 1998) and Olomoro waters (Idodo-Umeh, 2002) but were below WHO Standard of 1mg/L for aquatic food. The Cu bioaccumulation in the fish studied was however lower than Cu value (3.69mg/L) reported in fresh water fish from Delta (Kakulu & Osibanjo1987) and concentrations found in *O. niloticus* from Ogba River (Obasohan & Oronsaye 2004). Elevated concentration of Cu in fish binds to amino and sulphydryl groups of enzymes thus blocking the pathways for enzyme activity (Hutton 1987). It also antagonizes calcium accumulation by inhibiting its uptake thereby enhancing the removal of Calcium which is indispensable in bone formation in vertebrates including fish (Okonkwo & Eboatu, 1998).

The concentrations of Pb in the fish studied was found to be higher than the WHO Standard of 0.05mg/L in aquatic foods, thus indicating that Niger River at Onitsha was already experiencing some significant impairment in relation to Pb ion. The implication is that consuming fish from the River could lead to Pb-induced health problems. Lead is a cumulative poison and a potent enzyme inhibitor which is easily incorporated into enzyme structures. Pb inhibits the synthesis of haeme in organisms and thus interferes with the effective utilization of iron (Hulton 1987; Ademoroti 1995). Elevated concentrations of Pb cause cytological degenerations in fish organs as well as heart, liver and kidney dysfunction in man (Oronsaye 1997; Benoff *et al*. 2000). Lead in the River must have come chiefly from automobile and power generating plants' exhaust pipes as well as lead deposits in soils which were washed into the river by runoffs during the rains.

The low concentration of mercury in the fish species was not significantly higher than the recommended standard of 0.05mg/L. Ajiwe *et al*. (2002) also reported low bioaccumulation of Hg in fishes from the River Niger. It was observed that the concentrations of cadmium in the fish species were below the WHO Standard of 2mg/L but higher than those of UNEP (1996) and FEPA (1991) in aquatic foods. This finding is similar to the concentrations reported for *Brycinus chaperi* and *C. nigrodigitatus* from Ikpoba River (Fufeyin 1998).

Obasohan & Oronsaye (2004) also reported low concentrations of Cd in *O. niloticus* from Ogba River. Idodo-Umeh (2002) reported 0.12mg/L of Cd in the inland fishes of Olomoro water. The likely sources of Cd into the Niger River include effluents from industries and use of Cd-containing fertilizers in the near-by crop farms (Okonkwo & Eboatu, 1998).

Plates 1 & 2 are photomicrographs showing alterations in the histology of the liver tissues of *C. nigrodigitatus* while those in the kidney tissue of *C. nigrodigitatus* are shown in Plate 3. It was not possible to attribute every histopathological change observed in liver and kidneys of the fish to a particular heavy metal since parasitic infections and other chemical pollutants may also cause similar alterations (Nosakhare *et al*. 2013). However an attempt was made here to discuss the cause and effect relationships between chemical pollution and specific tissue and organ conditions in fish.

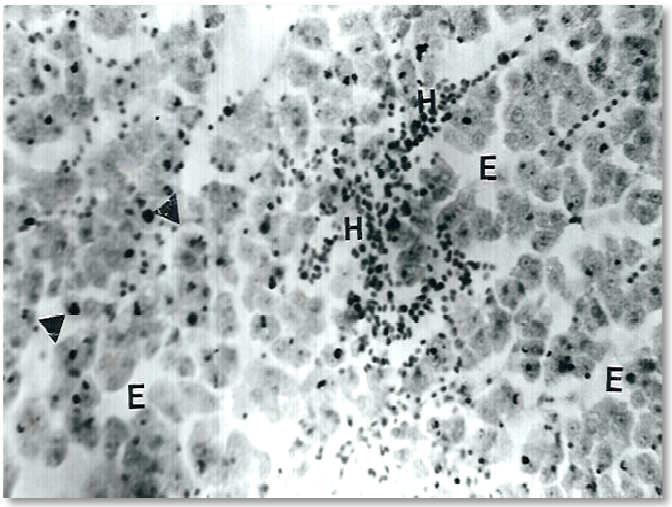


Plate 1. Photomicrograph of section of Liver of *Chrysichthys nigrodigitatus.* Note extensive hyperaemia [H], oedematous sinusoids [E], and hepatocytes in apoptosis with pyknotic nuclei [►]. H&E stain x 320.

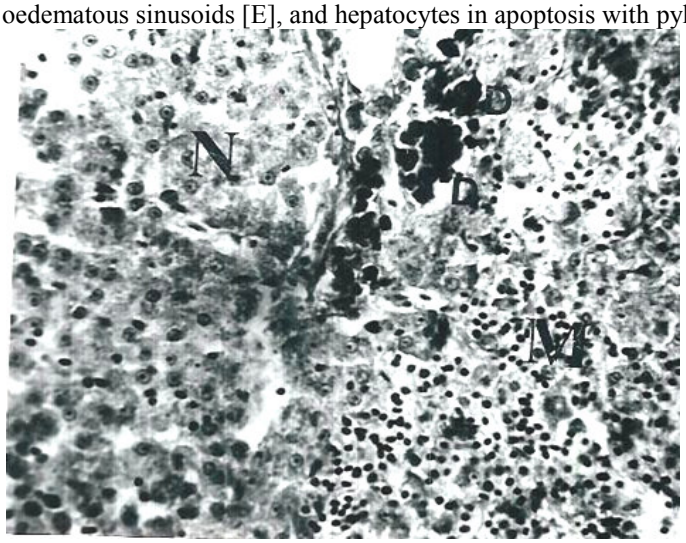


Plate 2. Photomicrograph of section of liver of *Chrysichthys nigrodigitatus* of Niger River. Note the hepatocytes with pyknotic nuclei [N], wide spread necrosis of hepatocytes with mononuclear leucocytes infiltration of the area [M], and pigment deposits [D]. H & E stain x 200.

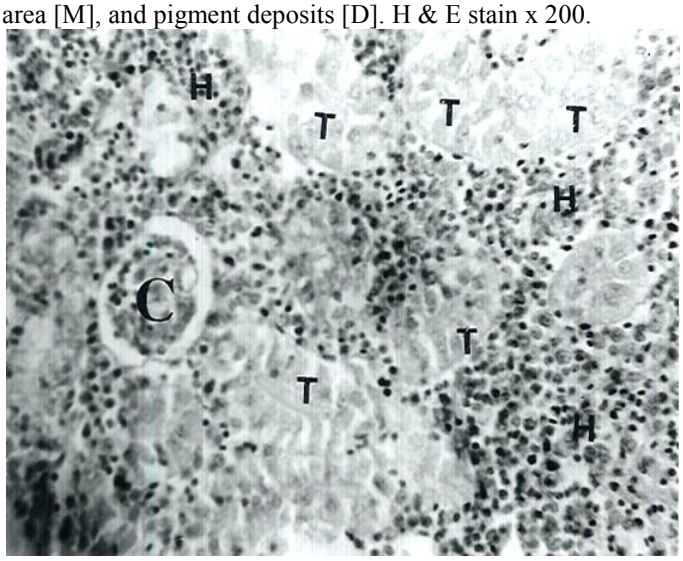


Plate 3. Photomicrograph of section of kidney of *Chrysichthys nigrodigitatus* of River Niger. Note the normal renal corpuscles [C], severe hyperaemia of the interstices [H], and tubular epithelial cells [T] in degeneration and necrosis. H&E stain x 320.

The histopathological alterations in the liver and kidney of *C. nigrodigitatus* were however similar to changes

reported in fishes exposed to sub-lethal concentrations of metals (Crandall & Goodnight 1962; Roberts 1978; Sipple *et al.* 1983; Oronsaye 1989 & 2001; Folorunsho & Oronsaye 1990). The necrotic degeneration of the hepatocytes, vacuolation and aggregation of macrophages observed in the liver sections of the fish were similar to changes reported in the liver of fish exposed to metal from Elbe Estuary (Heidemaria & Peters 1985). The lesions in the liver were more severe than those of the kidney in the fish species, perhaps the toxicant reaching the kidney were the excess that had not been rid off by the gills and liver. Since the liver is a major metabolic centre while the kidney is indispensable in the ionic balance and osmo-regulation in fish, it is therefore evident that these histopathological changes may affect the general well-being of the fishes from the River Niger and may consequently militate against maximum productivity.

Fish exposed to different metal toxicants show numerous histopathological changes which may sometimes lead to death (Carpenter, 1927, Abel, 1989, Oronsaye, 1989, Oluah and Amalu 1998, Obano and Oronsaye 2001). The liver of teleost fish is susceptible to toxic and metabolic disturbances and Roberts (1978) reported acute and extensive necrosis, generalized swelling and pyknosis of hepatocyte nuclei with cytoplasmic vacuolation on liver cells of fish exposed to metal toxicity. Increase in plasma enzyme activity is probably due to damage of the liver. In their studies, Michael *et al* (1987) correlated elevation activity of alanin aminotransferase in the plasma of *Clarias gariepinus* to the degree of liver damage when exposed to nitrite ion. Oluah (1999) further reported that plasma aspartate aminotransferase activity on *Clarias albopunctatus* exposed to sub-lethal concentrations of mercury increased above normal level and caused imbalance in the physiological and biochemical processes of the fish.

Oronsaye (1989) in his study on the histopathological changes in the kidneys and gills of *Gasterosteus aculeatus* exposed to dissolved cadmium in hard water, reported disintegration of the apical regions of the columnar cells that lined the kidney tubules, but prior to exposure to cadmium, these granular masses were confined to the hematopoietic cells. In this study, tissue vacuolation and dark granular masses were also seen in the kidney tubules. The large numbers of these granular masses in the lumina of the tubules and collecting ducts observed in this study suggested that these granular masses were being excreted. Sippel *et al.* (1983) also reported vacuolation and dark granular masses in columnar cells of the kidney tubules in *Salmo gairdneri* exposed to sub-lethal concentration of lead (120µg/L) for thirty weeks. Since normal kidney tubules in teleost fish function to modify the glomerular filtrate by re-absorption or secretion of inorganic ions, it may therefore be assumed that the granular masses in the tubule lamina observed in this study were complexes containing metals and that the fish were apparently excreting the metals.

**Conclusion**

From trends in the hydrological variables of the Niger River during the dry and wet seasons of the study, it could be inferred that the integrated impact of precipitation (rainfall), input of surface runoffs and industrial effluents played an overriding role in determining the absolute levels and temporal patterns in the water quality attributes of the Niger River. The bioaccumulation status of some of the analyzed heavy metals in the fish species studied depicted the Niger River as experiencing significant impairment. Histopathological alterations evident in the liver and kidney tissues of the fish studied implied gradual manifestation of morbid conditions. Therefore prolonged exposure of these fish to chemical pollutants may lead to increased morbidity and mortality, hence decrease in productivity. The consumption of metal-contaminated fish and other aquatic foods may pose serious health risks to fish consumers. In view of the ever-increasing importance of fish as a source of high quality animal protein in Nigeria, it is necessary to make a comprehensive assessment of the hazards posed by these metals in other water bodies in the country.

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**References**

Abel, A. (1989), Reactions of channel catfish to piggery effluents, *Oceans* **4**, 1314-1317.

Abad-El-Gawad, A.M. (1999). Histopathological studies on the liver and Gills of *Oreochromis nitoticus* exposed to different concentrations of lead acetate and zinc sulphate, *J. Egypt. Ger. Sec. Zool.* **30** (c), 13-22.

Ademoroti, C.M.A. (1995). Pollution by heavy metals. *In Environmental Chemistry and Toxicology.* Foludex Press Ltd Ibadan, Nigeria. pp.171-186.

Ajiwe, V.I.E., Njoku, O.O. & Onochie, C.C. (1999). Ground water pollution in Anambra State, Nigeria: Policies and Remedies, *J. App. Sci.,* **2**(3), 238- 252.
Ajiwe, V.I.E., Ezeirim, S.O., Ajibola, V.O. & Onochie, C.C. (2002). Heavy metal pollution on coastal fishes off the coast of River Niger, Nigeria, *J. App. Sci.* **5**(2), 2836-2843.
Benoff, S., Jacob, A. & Hurley, I.R. (2000). Male fertility and environmental exposure to lead and cadmium. *Human Reprod. Update* **6**, 107-121.
Carpenter, K.E. (1927). The lethal action of sub-lethal metabolic salts on fishes, *Bri. J. Expt. Biol.* **4**, 484-486.
Crandall, A.C. & Goodnight, C.J. (1962). Effects of sub lethal concentrations of several toxicants on growth of common guppy, *Lebintes reticulates. Limol. Oceanography* **7***,* 233-234.
Drury, R.A.B. & Wallington, E.A. (1976). *Carletons Histological Techniques.* 4th ed. Oxford Univ. Press, Ely house London. W.I. 88-100.
Fabris, J.A., Richardson, B.J., Sullivan, J.F. & Brown, F.C. (1994). Estimation of Cadmium, Lead and Mercury concentrations of Estuarine Waters using the Mussel *Mytilus edulis planulasus, Inter J Envir Toxicology* **9**, 183-192.
FEPA (1991). Federal Environmental Protection Agency, Nigeria: Guidelines for Aquatic life.
Folorunsho, B. & Oronsaye, J.A.O. (1990). The toxicity of cadmium to C*larias angularis* in soft water. *Nig. J. App. Sci.* **8***,* 85-92.
Forstner, U. & Prosi, F. (1979). *Heavy metal pollution in freshwater ecosystem*. In Ravera, O. (ed) Biological aspects of freshwater pollution*.* Published for the Commission of the European Communities.
Frank, A., Galgan, V., Toss, A., Olsson, M., Patterson, L.R. & Bignert, A. (1992). Metal concentrations in seals from Swedish waters, *Ambio.* **21**(8), 529-538.
Fufeyin, P.T. (1998). Heavy metal levels in some dominant fish of Ikpoba Reservoir, Benin City Nigeria, *Env. Res.* **2**(2), 61-69.
Gill, T.S., Bianchi, C.P. & Epple, A. (1992). Trace metal – copper and zinc. Adaptation of organ-system of American eel-*Anguilla rostrata* to external concentrations, *Comp. Biochem. Physiol.* **102**(13): 361-371.
Heidemaria, K. & Peters, N. (1985). Pathological conditions in the liver of ruffle- *Gymnocephallus cernua* (L) from Elbe Estuary, *J. of Fish. Diseases* **8***,* 13-24.
Hogstrand, C. (2000). Anion linked zinc transport. Presented at the Society of Experimental Biology Annual Meeting, Exeter, U.K., March 20.
Hutton, M. (1987). *Human Health Concerns of Lead, Mercury and Arsenic.* In Huschinson, J. C. & Meama, K. M. (Eds): Lead, mercury and cadmium in the environment, Wiley Scope, 85 – 94.
Idodo-Umeh, G. (2002). Pollution assessment of Olomoro water bodies using physical, chemical and biological indices. *Ph.D. thesis.* University of Benin, Benin. Nigeria. 485 pp.
Kakulu, S.E. & Osibajo, O. (1987). Trace metal content of fish and shell fishers of the Niger Delta area of Nigeria, *Environ. Int*. **13**, 247-251.
Michael, M.J., Hilmy, A.M., El-Dormaty, N.A. & Wershana, K. (1987). Serum transaminase activity and histopathological changes in *Clarias lazera* chronically exposed to nitrite, *Camp. Biochem. Physiol.* **86***,* 255-262.
Negin, S. & Mehdi, Z. (2012). Using of Fish Pathological Alterations to Assess Aquatic Pollution: A Review, *World Journal of Fish and Marine Sciences* **4**(3), 223-231.
Njoku, D.C. & Keke, L.R. (2001). *Evaluation of safe water pollution in Dilimi River, Jos due to mine effluents. Need for quality control measures*. Proceedings of the Sixteenth Annual Conference and International Workshop of Nigerian Association of Aquatic Science.
Nosakhare Osazee Erhunmwunse., Alohan, F.I., Alex Enuneku., Isioma Tongo., Martins Ainerua. & Stefano Idugboe. (2013). Pathological alterations in the liver of post-juvenile African catfish (*Clarias gariepinus*) exposed to sub-lethal concentrations of the herbicide Glyphosate, *Journal of Natural Sciences Research* **3**(15), 87-91.
Nsofor, C.I. & Aguiwo, J.N. (2005). The bioaccumulation of Zinc, Iron, Copper, Lead and Cadmium by some fish species from the River Niger, Anambra State, Nigeria, *Envi Stud Res J* **5**, 122-129.
Nsofor, C.I., Igwilo, I.O., Ikpeze, O.O., Ikeogu, C.F., Umeoguagu, F.O. & Okonkwo, C.J. (2014). Bioaccumulation of heavy metals in Shellfish *Macrobrachium rosenbergi* in Niger River at Onitsha, Anambra State, Nigeria. *Inter J Agri Biosci.* **3**(1), 38-40.
Nwuba, L.A. & Ikpeze, O.O. (2009). Helminth parasites of the African Catfish (Pisces: Clariidae) from the polluted water environment of the River Niger at Onitsha, *International Journal of Bioscience* 4(2), 30-33.
Nwuba, L.A., Ikpeze, O.O. & Akpaniteaku, R.C. (2009). Helminth parasites of the Snakemouth (*Channa obsscura* Gunta 1861) from the Lower Anambra River Freshwater System, Nigeria, *Journal of Agricultural Research and Policies* **3**(3), 111-114.
Obano, E.E & Oronsaye, J.A. (2001). Histological changes in gills of *Oreochromis niloticus* exposed to copper in soft water, *J. Aqua. Forestry. Fish.* **1***,* 31-34.
Obasohan, E.E. & Oronsaye, J.A. (2000). Heavy metals in water, sediments and some important commercial fish

species from Ikpoba River, Nigeria, *J. Appl. Sci. Environ. Mgt.* **4**(2), 61-68.
Okonkwo, E.M. & Eboatu, A.N. (1998). Environmental Pollution and Degradation, Onis Excel Publishing, Zaria, Nigeria 90-93.
Okoye, B.C.O. (1991). Heavy metals and organisms in Lagos Lagoon. Nigeria, *J. Environ. Stud.* **38**, 131-136.
Olojo, E.A.E., Olurin, K.B., Mbaka, G. & Oluwemimo, A.D. (2005). Histopathology of gills and liver tissues of the African catfish *Clarias gariepinus* exposed to lead, *Afr. J. of Biotech.* **4**(1), 117-122.
Oluah, N.S. & Amalu, C.C. (1998). The effect of heavy metals on the plasma alanin aminotransferase activity in the freshwater catfish, *C. albopunctatus, J. Aqua. Sci.* **13**, 5-8.
Oronsaye, J.A.O. (1989). The uptake and concentration of dissolved cadmium by stickleback, *Gasterosteus aculeatus* (L), *J. Ecotoxicol. Environ. Saf.* **14,** 88-96.
Oronsaye, J.A.O. (1997). Ultrastructural changes in the gills of stickleback. *G. aculeatus* (L) in hard water and soft water, *J. Appl. Sc.* **12,** I59-166.
Oronsaye, J.A.O. (2001). Ultrastructural changes in kidneys of stickleback – *G. aculeatus* (L) exposed to dissolved cadmium, *J. Aqua. Sci.* **16**, 53- 56.
Pandy, G.N. & Corney, G.C. (1999). *Environmental Engineering.* Tata-Magraw- Hill Pub. Co. Ltd. New Delhi, India, 23- 36.
Philips, D.J.H. (1981). Selected trace elements and the use of biomotors in subtropical marine ecosystem, *Review of Environmental Cont. Toxicol*, **120**, 105-129.
Oluah, N.S (1999). Plasma Aspartate Aminotransferase activity in the catfish, *Clarias albopunctatus* exposed to sub-lethal zinc and mercury, *Bull. Environ. Contam. Toxicol.* **63**, 343-349.
Roberts, R.J. (1978). The Pathophysiology of Teleosts. *In Fish Pathology*, edited by R.J Roberts, Bailliere Tindal – London Press, 51-91.
Schwaiger, J., Wanke, R., Adam, S., Pawert, M., Honnen, W. & Triebskorn, R. (1997). The use of histopathological indicators to evaluate contaminants-related stress in fish, *Journal of Aquatic Ecosystem, Stress and Recovery* **6,** 75-86.
Sippel, A.J., Allan, G.R. & Hodson, P.V. (1983). Histological and physiological responses of rainbow trout-S*almogairdneri* to sub lethal levels of lead, *Wat. Res.* **17**(9), 1115-1118.
UNEP (1996). United Nations Environmental Protection: Report by Economic Commission for Europe on Aquatic Food, ECE/CHEM. 78 pp.
Vazquez, F.G., Sharma, V.K., Alexander, V.H. & Frausto, C.A. (1995). Metals in some lagoons of Mexico, *Environ. Health. Perspect.* **103**(1), 50-55.
Wangboje, O.M. & Oronsaye, J.A.O. (2001) Bioaccumulation of Iron, lead, mercury, copper, zinc and chromium by fish species from Ogba River, Benin, Nigeria, *Afri. J. Appl.Zool. Environ. Biol.* **3**, 45-49.
WHO (1984). World Health Organization. Guidelines for drinking water quality. Vol 1. Recommendation. Geneva, WHO. 130 pp.

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