Histopathological biomarking changes in the internal organs of Tilapia (Oreochromis niloticus) and catfish (Clarias gariepinus) exposed to heavy metals contamination from Dandaru pond, Ibadan, Nigeria

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ABSTRACT

The study assessed histopathological alterations in the organs of Oreochromis niloticus (Nile tilapia) and Clarias gariepinus (Catfish) harvested from Dandaru Pond, Ibadan, Nigeria. The histopathological effects were evaluated through biomarkings and heavy metals measurements of the fish organs and tissue. Results showed the kidney with signs of coagulated necrosis of the renal tubules. In the liver, there were hepatocytes which appeared foamy and contained large-sized vacuoles. The gills appeared with some congestion in their capillaries in addition to numerous bluish staining walled-off structures. There was severe thinning of the myocardial fibres in the fish heart. The chambers were moderately congested. The fish organs contained 0.06–0.90 µg/g lead. The Pb concentrations and those of manganese and iron exceeded the WHO permissible limit in fish. It is conclusive that the bioaccumulated heavy metals in the tissue and fish organs consequently impaired their histopathological condition, and thus affect fish health status.

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1. Introduction

Metal contamination of aquatic ecosystem has long been recognized as a serious pollution problem to aquatic life. Some of the major contaminants in the water bodies are pesticides, pharmaceutical products, heavy metals, polyaromatic hydrocarbon (PAHs) and others. The heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and diversity of aquatic organisms [1,2]. Most heavy metals released into the terrestrial environment find their way into the aquatic environment as a result of direct input, atmospheric deposition, flooding and erosion. Therefore, aquatic animals may be exposed to elevated levels of heavy metals due to their wide use for anthropogenic purposes by man [3,4]. In aquatic environments, however, heavy metals pose a major threat to the fauna resources especially fishes in such aquatic bodies [5]. Heavy metals are non-biodegradable and once they enter water bodies, bio-concentration occurs in the fish organs by means of metabolic and bio-sorption processes [6]. Heavy metals such as cadmium, lead, copper and more specifically mercury are potentially harmful to most aquatic organisms even at very low concentrations and have been reported as hazardous pollutants able to accumulate along the food chain with severe risk for animal and human health. The presence of pollutants has been associated with decreased fertility and other reproductive abnormalities in mammals, birds, fish and shellfish and also alters their immune function. Fish accumulate toxic chemicals such as heavy metals directly from water and the diet taken in the environment; contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in water, sediment and food [7]. Heavy metals are normal constituents of marine environment that occur as a result of pollution principally due to the discharge of untreated wastes into water bodies by many industries. Bioaccumulation of heavy metals in tissues of marine organisms has been identified as an indirect measure of the abundance and availability of metals in the marine environment. Monitoring contamination in fish tissue serves as an important function of early warning indicator of sediment contamination or related water quality problems. It also enables appropriate actions that will protect public health and the environment [8]. Gill tissue is an organ that has a large surface area; it separates blood from water in fish and it is very susceptible to changes in concentrations of the variables...
(heavy metals, temperature, pH, etc.) in the environment. These variables affect the structural integrity of the gill and cause morphological changes for this reason; gills are good indicators of water pollution [9]. It is estimated that fish can act as front-line indicators of suspected aquatic pollutants such as metals [8]. Fish may absorb dissolved elements and heavy metals from surrounding water and food, which may accumulate significant concentrations of metals even in waters in which those metals are below the limit of detection in routine water samples [10]. Transportation of metals in fish occurs through the blood where the ions are usually bound to proteins. The metals are brought into contact with organs and tissues of the fish and consequently accumulated to a different extent in different organs and tissue of the fish. Man is exposed to lead by consuming fish contaminated with lead. Heavy metals like mercury and cadmium are known to accumulate in aquatic organisms and cause reproduction impairment and hepatic dysfunction in human life [11].

Prolonged exposure to water pollutants even in a very low concentrations has been reported to induce morphological, histological and biochemical alterations in the organs and tissues of fish which may critically influence fish quality when consumed by man [4,12], and possibly fish survival in large water bodies. Histopathological changes have been widely used as biomarkers in the evaluation of health status of fish exposed to contaminant, both in the laboratory and field studies. One of the greatest advantages of using histopathological biomarkers in environmental monitoring is the ability to allow the examination of specific target organs including gills, kidney, and liver that are responsible for vital functions such as excretion, respiration and biotransformation of xenobiotics in the fish. Furthermore, the alterations found in these organs are normally easier to identify than functional ones and serve as warning signs of damage to animal health [13].

Histopathological investigations have been proved to be a sensitive tool to detect direct toxic effects of chemical compounds within target organs of fish in the laboratory experiments [5,14] as well as field investigations. Histopathological investigations have the capacity to differentiate between organ lesions induced by diseases and lesions caused by other environmental factors as the case may be [15]. Histopathological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs such as gills, liver, and gonads [16]. Histopathological biomarkers offer many advantages in the context of detecting long-term injury to aquatic life [17] and are arguably the easiest chronic biomarkers to apply in the field of aquatic biology [18]. Biomarkers of contaminant exposure in fish species are important indicator if we are to maintain a viable fishery and a safe product for human consumption and health. Given the relatively low level but chronic nature of most environmental pollutants, biomarkers should receive most attention in aquatic biology study. Our previous study on two fish species, tilapia (Oreochromis niloticus) and catfish (Clarias gariepinus), harvested from the Danadaru Pond showed considerable level of contamination of fishes by heavy metals bioaccumulation [19]. Hence, this study assessed histopathological changes caused by the accumulated heavy metals in the internal organs of Nile tilapia (Oreochromis niloticus) and catfish (Clarias gariepinus).

2. Materials and methods
2.1. Study area
The water and fish (Tilapia, Oreochromis niloticus and catfish, Clarias gariepinus) samples were harvested from Danadaru pond (longitude, 7°24'16 N; latitude, 3°53'56 E), 205 m above sea level. The pond was selected for the study due to potential threat in the area. Danadaru pond is located near University Teaching Hospital, Mokola area, secretariat road, Ibadan, Nigeria. The pond is under the management of Oyo State Ministry of Fisheries and Forest Resources, Ibadan. It was formerly used in rearing fish but now seriously impaired by anthropogenic activities of man. The Danadaru pond receives wastewater discharges from University College Hospital, Ibadan and domestic wastes from other sources. Control fish samples were purchased from a local market in the outskirts of Ibadan city for comparative study on histopathology of fish organs and tissues.

2.2. Analysis of water samples
The water sample was digested with 10 ml of the nitric acid, filtered into the borosilicate container and analyzed using Atomic Absorption Spectrophotometer (Perkin-Elmer Analyst 300). All analyses were carried out in triplicate.

2.3. Analysis of fish samples
One gram of each fish sample was weighed into the pre-cleaned borosilicate 250 ml capacity and digested with concentrated HCl/HNO₃ (3:1). The mixture was filtered into 250 ml volumetric capacity borosilicate container. The filtrate was made up to the mark with de-ionized water. The digested fish samples were analyzed for the heavy metals which included Pb, Co, Hg, Fe, Mn and Zn. Analysis of each metal was carried out in triplicate per fish sample.
2.4. Histopathological investigations

Tissue specimens from the gills, kidney, heart and the liver were fixed in 10% buffered neutral formalin, dehydrated, embedded and sectioned at 4 µm, then stained with haematoxylin-eosin (HE) and observed under the light microscope.

2.5. Statistical analysis

Data were analyzed using Analysis of variance (ANOVA). Statistical data processing was accomplished by a computer programme “SPSS for Windows XP” and it included computation of arithmetic mean, standard deviation and correlation analysis.

3. Results and discussion

3.1. Histological alterations

Results of the present study revealed that Nile tilapia (Oreochromis niloticus) and Catfish (Clarias gariepinus) from the pond showed histopathological changes in the liver, kidney, heart and gills. This could be due to heavy metals and other domestic wastes which enter the pond. The histopathological changes in the internal organs of both fish are in agreement with those observed by many investigators on aquatic research [20–24] who have studied the effects of different pollutants on fish organs in such aquatic environment [25].

The kidney is one of the first internal organs to be affected by contaminants in any aquatic environment [26]. Figure 2 reveals the photomicrographs of the kidney in this study. The normal structure of the kidney is shown in Figure 2(a) (the control sample). In contrast to Figure 2(a), there were histopathological alterations in the kidney of both fish species shown in Figures 2(b–d). A moderate coagulative necrosis of the renal tubules is revealed in Figure 2(b). While the haematopoietic compartment is normal, there is marked tubular dissolution (Figure 2(c)). In addition, a marked tubular dissolution and normal glomeruli are also displayed in Figure 2(d). Exposure to metals frequently causes alterations in the tubules and glomeruli [26,27].

The photomicrographs of the gills are shown in Figure 3. The normal aspect of the gills is shown in Figure 3(a). However, the photomicrographs in Figures 3(b,c) show that the primary lamellae are swollen with hyperplastic conditional signs. Both fish species from Dandaru pond showed moderate congestion in their capillaries, as compared with the normal histopathological structures of the gills. Similar study and effect on fish gills has been reported elsewhere [28]. There are numerous staining walled-off structures which possibly look like a cysts or mucinous cells that have been lobulated together to form a cyst (Figure 3(d)). The lamellae are thinner and slightly distinct as shown in Figure 3(e,f).

Figure 4 shows the normal histopathological structures of the heart through the photomicrograph of the heart of the fish species in this study. In comparison with the normal structure of the heart (Figure 4(a)), there is severe thinning of the myocardial fibres (Figure 4(b,c)). The chambers are moderately congested and the cardiomyocyte has undergone moderate vacuolar degeneration (Figure 4(d,e)). It is observed that there is a severe thinning of the myocardial fibres as shown in Figures 4(f,g), in addition to marked thinning of the myocardial fibres (Figure 4(h,i)).

Liver of fish is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver, thus exposing it to a high degree of infection than other organs [29]. Figure 5 shows the histopathological structures of the liver of the studied fish species. Figure 5(a) is the normal structure of the liver. The hepatocytes, which have a foamy appearance due to the presence of cytoplasmic vacuoles showed histopathological alteration in the liver (Figure 5(b)). This is in addition to slight dissociation of the hepatic cords. In Figure 5(c), there are large-sized cytoplasmic vacuoles within the hepatocytes and multifocal single-cell necrosis of hepatocytes. There are very large-sized cytoplasmic vacuoles within the hepatocytes which appear to be more pronounced around the portal areas (Figure 5(d)). These vacuoles compress the nuclei in some hepatocytes. These findings are significant as liver is considered the organ of detoxification, excretion, and binding proteins such as metallothionein (MTs).

3.2. Heavy metals contaminations and histopathological alterations in the fish species

Tables 1 and 3 show results of the heavy metals measured in the various organs of O. niloticus and C. gariepinus harvested from the Dandaru pond. Mercury was not detected in any of their internal organs. Results for the control samples for the two fish species are presented in Table 2 and 4, which show the toxic heavy metals, Pb and Hg, were not detected in the internal organs of the control fish samples. For the test samples, the results of other heavy metals present in the fish organs ranged as follows: Mn (2.12–5.13 µg/g), Co (3.65–5.74 µg/g), Zn (0.22–0.75 µg/g), Fe (37.90–63.30 µg/g), Pb (0.06–0.10 µg/g). The results obtained for O. niloticus used as control samples were: Mn (0.16–0.16 µg/g), Co (0.04–0.31 µg/g), Zn (0.10–0.13 µg/g), Fe (15.00–48.29 µg/g) [19]. The results revealed that the kidney had the highest concentration accumulation of the metals. The result obtained for heavy metal analysis of the gill, liver and kidney of C. gariepinus from Dandaru pond ranges as follows: Mn (0.50–1.39), Co (0.28–3.54), Zn (0.20–0.69), Fe (30.50–57.30), Pb (0.60–0.90) and Hg which was not detected. The results obtained for C. gariepinus used as control samples were Mn (0.09–0.13), Co (0.08–0.27), Zn (0.10–0.13), Fe (15.00–48.29) [19].
In aquatic ecosystems, heavy metals are taken up through different tissues of the fish at different levels [30,31]. Various biotic and abiotic factors control metal bioaccumulation in fish tissues such as feeding habits, life style, fish age, gender, body mass, and physiologic conditions, as well as water temperature, pH value, and dissolved oxygen concentration [32,33].

The two fish species *C. gariepinus* and *O. niloticus* analyzed for heavy metals accumulation showed that *O. niloticus* accumulated more of the heavy and trace metals compared to *C. gariepinus*. The reason for the higher concentration of heavy metals accumulation in the *O. niloticus* could be attributed to the planktivorous feeding habit of *O. niloticus*, according to [34], plants bioaccumulate and concentrate heavy metals from the environment and could be picked up by the cichlids through feeding. There have been various reports on the adverse effects of heavy metal contamination on fish health, which include histopathological alterations in their internal organs [35].

Mn is known to be an essential element in animals. Severe skeletal and reproductive abnormalities have
been associated with the deficiency of Mn in mammals. Mn detected in all the fish samples was analyzed from the polluted pond. The highest value of Mn was detected in the kidney of *O. niloticus* (5.13 µg/g) while the lowest was found in the liver of *C. gariepinus* (0.50 µg/g). For the control sample of both *O. niloticus* and *C. gariepinus* the values ranged from 0.09 to 0.16 µg/g which were lower than the values detected in fish from polluted pond. In all of the organs of fish analyzed except the liver of *C. gariepinus* the values obtained were higher than the World Health Organization (WHO) limits of 0.50 µg/g. The estimated level could pose a threat to fish especially due to long-time exposure. The same trend was reported by [36], where all organs had Mn values higher than the permissible limits.

Zn, an essential element, is one of the most common heavy metal pollutants and at higher concentrations, it produces adverse effects in fish through structural damages, which affects the growth, development and survival of fish [37]. The highest value of Zn in the organs of the fishes from the polluted pond is found in the kidney of *O. niloticus* (5.74 µg/g) while the lowest value of Zn was found in the gills of *C. gariepinus* (0.28 µg/g). The values detected from the control samples of both fishes where quite lower compared to those of the polluted pond.
Figure 5. Photomicrograph of the liver. Normal structure of the liver (a); foamy appearance in the hepatocytes (b); multifocal single-cell necrosis of hepatocytes (c); large-sized cytoplasmic vacuoles within the hepatocytes (d).

Table 1. Heavy metals measured in different organs of Nile tilapia (Oreochromis niloticus) from Dandaru pond, Nigeria.

| Metals (μg/g) | Gill | Liver | Kidney |
|--------------|------|-------|--------|
| Mn           | 2.12 ± 0.89<sup>a</sup> | 4.23 ± 0.56<sup>b</sup> | 5.13 ± 0.12<sup>b</sup> |
| Co           | 3.65 ± 0.71<sup>a</sup> | 4.50 ± 0.88<sup>b</sup> | 5.74 ± 0.99<sup>b</sup> |
| Zn           | 0.22 ± 0.11<sup>a</sup> | 0.48 ± 0.22<sup>b</sup> | 0.75 ± 0.21<sup>b</sup> |
| Fe           | 37.9 ± 1.54<sup>a</sup> | 53.4 ± 1.30<sup>b</sup> | 63.3 ± 1.28<sup>b</sup> |
| Pb           | 0.10 ± 0.01<sup>a</sup> | 0.08 ± 0.01<sup>a</sup> | 0.06 ± 0.01<sup>a</sup> |
| Hg           | ND   | ND    | ND     |

Notes: The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P < 0.05 (Source: [19]).

ponds. The value of Zn in all the organs of both fishes was lower than the World Health Organization (WHO) limits of 5.0 µg/g. The high values obtained for O. niloticus may be attributed to the scales on its body which definitely plays a significant role in the accumulation of the metals [38]. It was observed that the scale of Tilapia had the highest concentration of Zn than other tissues of the body and this result is again in agreement with the findings of previous investigator [39].

The concentration of Fe in the organs of O. niloticus and C. gariepinus in the polluted pond ranged from 30.50 to 63.3 µg/g. The highest concentration was recorded in the kidney of O. niloticus, and even the lowest value of Fe exceeded the WHO limit of 0.30 µg/g in the organs of both fishes. The high concentration of Fe could be majorly associated with the bio-accumulation of this metal from the effluent coming directly from the hospital and from other anthropogenic sources [40]. However, Fe is required in the diet for the prevention of anaemia which is common in low-income countries.

Pb is a toxic heavy metal. It was detected in the liver, gills and kidney of both fish samples (Tilapia, Oreochromis niloticus and catfish, Clarias gariepinus), though the metal could not be detected in their respective control samples. The highest values of Pb in the

Table 2. Heavy metals measured in different organs of the control sample of Nile tilapia (Oreochromis niloticus).

| Metals (μg/g) | Gill | Liver | Kidney |
|--------------|------|-------|--------|
| Mn           | 0.16 ± 0.13<sup>a</sup> | 0.16 ± 0.14<sup>a</sup> | 0.16 ± 0.10<sup>a</sup> |
| Co           | 0.20 ± 0.12<sup>a</sup> | 0.31 ± 0.02<sup>a</sup> | 0.04 ± 0.01<sup>c</sup> |
| Zn           | 0.10 ± 0.02<sup>a</sup> | 0.12 ± 0.02<sup>a</sup> | 0.14 ± 0.13<sup>a</sup> |
| Fe           | 32.8 ± 1.63<sup>a</sup> | 40.3 ± 1.09<sup>b</sup> | 55.5 ± 1.12<sup>c</sup> |
| Pb           | ND   | ND    | ND     |
| Hg           | ND   | ND    | ND     |

Notes: The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P < 0.05 (Source: [19]).
internal organs of the fish samples from the pond were 0.10 µg/g measured in the gills of O. niloticus and 0.90 µg/g measured in the liver of C. gariepinus. Pb was not detected in the internal organs of the control sample. The values of Pb in the various organs of O. niloticus and C. gariepinus were above the WHO limit of 0.01 µg/g. Previous investigators [36] have reported similar finding of high concentration of lead in the gills of O. niloticus. The high lead concentration measured in these internal organs would, almost certainly, contribute to the histopathological alteration found in the fish organs.

According to Tables 1–4, results of the heavy metals analyzed in different organs of Oreochromis niloticus show that the kidneys accumulate more heavy metals than the liver which in turn bioaccumulates more than the gills. The high concentrations of the analyzed metals in the body tissues investigated could be related to their heavy presence in the Dandaru pond and the storage role played by the investigated tissues of the fishes.

From this study, the relatively high bioaccumulation of the heavy metals, certainly, affected the fish species. This resulted in their internal having serious pathological alterations and histological distortions as shown in Figures 1–5. This, consequently, would result in the inhibition of metabolic processes and haematological changes, thus resulting in decline in immunity, fertility and survival of the fish species in the aquatic environment [38].

4. Conclusion

The result indicates that the heavy metal contamination in the Dandaru pond resulted in cumulative effects in the biological/biochemical system of Oreochromis niloticus (Nile tilapia) and Clarias gariepinus (Catfish) as shown in this study. This could definitely hinder the growth and development of the aquatic lives in the freshwater habitat and, thus, pose serious danger to consumers on the other hand. Hence a scientific method of detoxification is essential to improve the health status of these aquaculture important fish species in any stressed environmental conditions. However, the high concentrations of the analyzed metals in the whole body-tissues investigated could be due to the storage role played by these tissues. It can be conclusively deduced from this study that histopathology is a useful biomarker tool for environmental management process, and therefore assist in the bio-monitoring process of aquatic ecosystems. Histopathology, therefore, helps to assess the potential for human exposure to environmental pollutants and for predicting the associated human risks of consumption of food from aquatic sources. The alterations that occurred in fish from the polluted pond can possibly appear in human if such fish is consumed.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Farombi E, Adelowo O, Ajimoko Y. Biomarkers of oxidative stress and heavy metal levels as indicator of environmental pollution in African catfish (Clarias gariepinus) from Nigeria Ogun River. Int J Environ Res Public Health. 2007;4:158–165.

[2] Javed M, Ahmad M, Usmani N, et al. Multiple biomarker responses (serum biochemistry, oxidative stress, genotoxicity and histopathology) in Channa punctatus

| Table 3. Heavy metals measured in different organs of Catfish (Clarias gariepinus) from Dandaru pond, Nigeria. |
|-------------------------------------------------------------|
| Internal organs | Mn     | Co     | Zn     | Fe    | Pb    | Hg |
| Liver           | 0.50 ± 0.18bc | 2.72 ± 0.11c | 0.20 ± 0.01ab | 30.50 ± 0.72a | 0.90 ± 0.01c | ND |
| Gills           | 1.12 ± 0.01bc | 0.28 ± 0.06a  | 0.28 ± 0.06a  | 42.5 ± 1.04b  | 0.06 ± 0.01a  | ND |
| Kidney          | 1.39 ± 0.08bc | 3.54 ± 0.90d  | 0.69 ± 0.01bc | 57.3 ± 1.25c  | 0.06 ± 0.01a  | ND |

Notes: The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P < 0.05 (Source: [19]).

| Table 4. Heavy metals measured in different organs of the control sample of Catfish Clarias gariepinus |
|-------------------------------------------------------------|
| Internal organs | Mn     | Co     | Zn     | Fe    | Pb    | Hg |
| Liver           | 0.13 ± 0.01b  | 0.27 ± 0.08c | 0.11 ± 0.01a  | 15.0 ± 0.84c | ND   | ND |
| Gills           | 0.09 ± 0.01a  | 0.08 ± 0.01a  | 0.10 ± 0.01a  | 26.4 ± 1.03b  | ND   | ND |
| Kidney          | ND       | ND     | 0.13 ± 0.10d | 48.29 ± 1.15c | ND   | ND |

Notes: The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P < 0.05 (Source: [19]).
exposed to heavy metal loaded waste water. Sci Rep Nat. 2017;7:1675.

[3] Kalay M, Canli M. Elimination of essential (Cu, Zn) and non-essential (Cd, Pb) metals from tissues of a fresh water fish, Tilapia zillii. Turk J Zool. 2000;24:429–436.

[4] Javed M, Usmani N. Accumulation of heavy metals and human health risk assessment via the consumption of freshwater fish Mastacembelus armatus inhabiting, thermal power plant effluent loaded canal. SpringerPlus. 2016;5:776.

[5] Fatima M, Usmani N, Hossain MM. Heavy metal in aquatic ecosystem emphasizing its effect on tissue bioaccumulation and histopathology: a review. J Environ Sci Technol. 2014;7:1–15.

[6] Wicklund-Glyn A. Cd and Zn kinetics fish: studies on water-borne Cd and Zn turnover and intracellular distribution in minnows, Phoxinus phoxinus. Pharmacol Toxicol. 1991;69:485–491.

[7] Osman A, Wuerzt S, Mekkawy I, et al. Lead induced malfunctions in embryos of the African catfish Clarias gariepinus (Burchell, 1822). Environ Toxicol. 2007;22(4):375–389.

[8] Mansour S, Sidky M. Ecotoxicological studies 6: the first comparative study between Lake Qarun and Wadi El-Rayyan wetland (Egypt) with respect to contamination of their major components. Food Chem 2003;82:181–189.

[9] Elahee K, Bhagwant S. Hematological and gill histopathological parameters of three tropical fish species from a polluted lagoon on the West coast of Mauritius. Ecotoxicol Environ Saf. 2007;68(3):361–371.

[10] Fatma A, Nahed S. Distribution of some heavy metals in tissues of Oreochromis niloticus Tilapia zillii and Clarías lazera from Aabu za’baal lakes and their impacts on some biochemical parameters and on the histological structures of some organs. Egyptian J Aquat Biol. 2005;4:1110–1113.

[11] Mansour S, Sidky M. Ecological studies, 3: heavy metals contaminating water and fish from Fayoum Governorate, Egypt. J Food Chem. 2002;78(1):15–22.

[12] Kaoud H, El Dahshan A. Bioaccumulation and histopathological alterations of the heavy metals in Oreochromis niloticus fish. Nat Sci. 2010;8(4):147–156.

[13] Gernhofer M, Pawet M, Schramm M, et al. Ultrastructural alterations induced in gill epithelium of African catfish, Clarias gariepinus, exposed to copper sulphate. Asian J Exp Biol Sci. 2011;2:278–282.

[14] Olajo E, Olurin K, Mbaka G, et al. Histopathology of the gill and liver tissues of the African catfish Clarias gariepinus exposed to lead. Afr J Biotechnol. 2005;4(1):117–122.

[15] Velcheva I, Tomora E, Arnadovda D, et al. Morphological investigation on gills and liver of freshwater fish from Dam Lake Studon Kladenets. Bulg J Agric Sci. 2010;16:364–368.

[16] Javed M, Ahmad M, Usmani N, et al. Studies on biomarkers of oxidative stress and associated genotoxicity and histopathology in fish (Channa punctatus) from heavy metal polluted canal. Chemosphere. 2016;151:210–219.

[17] Thophon S, Kruatrachue M, Upathan E, et al. Histopathological alterations of white seabass, Lates calcarifer in acute and subchronic cadmium exposure. Environ Pollut. 2003;121:307–320.

[18] Camargo MMP, Martinez CBR. Histopathology of gills, kidney and liver of a neotropical fish caged in an urban stream. Neotrop Ichthyol. 2007;5(3):327–336.

[19] Javed M, Usmani N, Ahmad M, et al. Studies on the oxidative stress and gill histopathology in Channa punctatus of the canal receiving heavy metal loaded effluent of Kasimpur Thermal Power Plant. Environ Monit Assess. 2015;187:4179.

[20] Health AC. Water pollution and fish physiology. 2nd ed. Boca Raton: Lewis Publishers; 1995. pp. 125–140.

[21] Dural M, Göksu L, Özak A, et al. Bioaccumulation of some heavy metals in different tissues of Dicentrarchus Labrax L, 1758, Sparus aurata L, 1758 and Mugil cephalus L, 1758 from the Çamlık Lagoon of the eastern cost of Mediterranean(Turkey). Environ Monitor Assess. 2014;118:65–74.

[22] Yilmaz F, Özdemir N, Demirak A, et al. Heavy metal levels in two fish species Leuciscus cephalus and Lepomis gibbosus. Food Chem 2007;100(2):830–835.

[23] Kumaruzzaman B, Ong M, Rina S. Concentration of Zn, Cu and Pb in some selected marine fishes of the Pahang coastal waters, Malaysia. Am J Appl Sci. 2010;7(3):309–314.

[24] Fernandes C, Fontainhas Fernandes A, Peixoto F, Salgado M, Reis M, Almeida J. Histopathological alterations of white seabass, Lates calcarifer after sublethal exposure to methylated. Environ Pollut. 1996;92:329–341.

[25] Hinton D, Lauren D. Integrative histopathological approaches to detecting effects of environmental stresses on fishes. Am Fish Soc Sym. 1990;8:51–66.

[26] Hinton D. Cells, cellular responses and their markers on chronic toxicity of fishes. In: D Malins, G Ostrander, editors. Aquatic toxicology: Molecular, biochemical and cellular perspectives. Boca Raton: Lewis Publishers; 1994. p. 207–239.
[36] Joseph C, Saiwa M, Bashir S, et al. Bioaccumulation of heavy metals in fish samples from river Benue in Vinikilang, Adamawa State, Nigeria. Am J Anal Chem. 2012;3:727–736.

[37] Afshan S, Ali S, Ameen U, et al. Effect of different heavy metal pollution on fish. Res J Chem Environ Sci. 2014;2(1):74–79.

[38] Taiwo I, Ipinmoroti M, Olopade OA, et al. The presence of some heavy metals in the sediments, water and Sarotherodon galilaeus in Ilo-Idimu River, Ogun State, Nigeria. Niger J Anim Prod. 2014;41(2):219–225.

[39] Mastan S. Heavy metals concentration in various tissues of two freshwater fishes, Labeo rohita and Channa striatus. Afr J Environ Sci Technol. 2014;8(2):166–170.

[40] Olowu R, Ayejuoyo O, Adewuyi G, et al. Determination of heavy metals in fish tissues, water and sediment from Epe and Badagry Lagoons, Lagos, Nigeria. J Chem 2010;7(1):215–221.

[41] Adeniyi AA, Yusuf KA. Determination of heavy metals in fish tissues, water and bottom sediments from Epe and Badagry Lagoons, Lagos, Nigeria. Environ Monitor Assess. 2007;37:451–458.