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Determination of histological and genotoxic parameters of Nile Tilapia, *Oreochromis niloticus* exposed to lead (Pb)

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ABSTRACT

This study investigates the hazardous effects of lead on the histological and genotoxic parameters of the fish, *Oreochromis niloticus.* Present work was conducted in a series of three steps. In first step, acute toxicity, LC_{50} and lethal lead concentration were measured using Probit analysis method. LC_{50} value and lethal concentration for *O. niloticus* was measured as 77.673 mgL^{-1} and 150.924 mgL^{-1}, respectively. In second step, histological changes were assessed by preparing slides of tissues of the gills, liver and kidney of both control and Pb-stressed *O. niloticus* and examining the respective tissues under the light microscope. The inferences showed significant histological alterations ($p<0.05$) in the gills of Pb-exposed fish including necrosis, edema, vascular congestion, shortening and curling and lifting of the epithelium of secondary lamella in gills. The cellular degeneration and dilation of sinusoids in liver and loss of hemopoietic tissue, necrosis and edema in kidney was observed. Histomorphometry of the liver showed a decrease in diameter of the central vein and hepatocyte along with an increase in width of sinusoids. The histomorphometry of kidney showed an increase in the diameter of renal corpuscle, glomerulus, proximal and distal convoluted tubules. The nuclear anomalies were studied in the RBCs of fish. Non-parametric Mann-Whitney U-test was conducted to compare nuclear abnormalities and the frequency of micronuclei among the control and lead-treated fish groups. Results declared an increased micronucleus, notched and de-shaped nuclei frequency, in RBCs of fish exposed to lead as compared to control group.

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Introduction

Toxicity of heavy metals, their accumulation in living organisms and non-biodegradable nature is substantially contributing to environmental pollution now a day (1, 2). Several aspects were observed as a key factor for contamination of water sources, these includes the dispersion by running off water or wind after being extracted, contaminated sources nearby and oxidation of sulfide minerals being exposed to the climate, gets dissolved in mining site and hence, produce a mine drainage or mine drainage contamination (3,4-6). The intake of heavy metals by aquatic biota takes place by ingesting from the food web; or by absorbing them directly from sediments or the water ecosystem (7). Accumulation of vast quantities of heavy metals takes place in the fish body due to its dominance as leading heterotroph of food chain in water (8), causing the food chain to become biomagnified/bio-accumulated by them (9,10). Chromium, lead, barium, cobalt, selenium, cadmium, vanadium and mercury are metals that organisms do not need even in a low concentration, and are also reported as highly toxic (11). The detection of detrimental consequences caused by such heavy metals can be done by carrying out toxicity tests leading to dose-response relationship formation (12, 13) which in turn may help in the regulation of the discharge of toxic substances into the water reservoirs along with the prediction of chronic and acute effects on aquatic animals (14). Bioaccumulation of heavy metals in different tissues of fishes may lead to harmful effects for instance genotoxics and histological alterations (15, 16). In the aquatic ecosystem, the indictor used for the assessment of water pollution is fish health (17). As bioindicators of underwater contamination, a number of fish have been used (9, 10, 18).

A commercially important fish Tilapia (*Oreochromis niloticus*) can survive harsh environmental conditions as their respiratory needs are less, their disease-resistance is high, high ammonia levels, a wide range of salinities and

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tolerating low oxygen often (19). Tilapia is considered one of the most significant commercial species that with great potential, especially in low-income nations. It has been revealed to play key roles in financial and nutritional support for the poorest rural. So, the present study was designed to assess the impacts of lead on the fish *O. niloticus* (20, 21). Evaluation of acute toxicity (96-hr) LC_{50} and lethal concentration of lead for the fish *O. niloticus* under controlled laboratory conditions was one of the objectives of this study. Assessment of histological changes in gills, liver and kidney tissues of *O. niloticus* caused by lead exposure was the second objective of this study. Genotoxic parameters such as evaluation of micronucleus, notched and de-shaped nucleus formation in erythrocytes of *O. niloticus* due to lead exposure was the third objective of this study.

Materials and Methods

Experimental design

The fish *Oreochromis niloticus* was taken from the Fisheries Research and Training Complex, Bahawalpur and were brought to the lab of the Cholistan University of Veterinary and Animal Sciences, Bahawalpur. Fish were kept in glass aquariums. The fish were kept in environmentally controlled laboratory i.e., with controlled temperature, humidity and light. The fish were acclimatized for two weeks. After the acclimation period, fish were transferred to 70-liter glass aquariums. Ten fish were kept in each aquarium.

Acute toxicity test

Lead was dissolved in distilled water, for static bioassays. The 10 fish were used per concentration per replicate for the acute bioassay tests. In 100 L capacity test tanks, lead concentrations ranged from 15, 30, 45, 60, 75, 90, 105, 120 and 135 mg/L. Keeping all other conditions constant, the control group was kept in normal water without the addition of lead. Before and during the tests, water quality parameters, pH, temperature and salinity were periodically calculated in the aquaria. For a duration of 96-hr, all experiments were carried out. After every 12-hr interval, dead fish were counted and were removed immediately from the aquaria. Total mortality rate was determined after 96-hr period. Acute toxicity test was carried out according to Hotos and Vlahos (22). For the assessment of acute toxicity of lead for Nile Tilapia, Probit Analysis was performed @ 95% confidence interval (C.I) by Minitab software. Various physico-chemical parameters were measured by following the methods described in APHA (14).

Histological examination

Dissection of fish was done after the LC_{50} value assessment. The gill, liver and kidney tissues were sampled from lead-exposed and control fish groups for the histological study. Bancroft et al. (23) used the Paraffin-embedding technique to examine samples under light microscope

Histomorphometry of Gills

A light microscope (OPTIKA® Italy) having camera and linked to computer, was used to study the slides. In histomorphometry, for the determination of vascular congestion, epithelial lifting, necrosis, fusion, curling and shortening of the secondary lamella, a commercial program (Prog Res®4083.B3 Capture Prog Camera Control Software) was used.

Histomorphometry of liver

To measure the diameter of the central vein, 20 central veins were measured randomly from each group. Measurement of horizontal (l1) and vertical (l2) axis length was recorded and a mean (l1 +l2)/2 was obtained for representation of the diameter of the central vein (22). With image analysis software, the width of hundred sinusoids per sample was measured. Measurement of sinusoids with visible ends only was made.

Histomorphometry of kidney

A light microscope (OPTIKA® Italy) having camera and a link with computer was used to study the slides. Pixel Pro Software was used to determine the collecting duct, glomerulus density, proximal convoluted tubules, cortex thickness, corpuscle diameter, distal convoluted tubules, and corpuscle density, for the histomorphometry of kidney.

Genotoxicity

Preparation and analysis of slides

A drop of blood was smeared directly on slides from the caudal vein of fish and then dried in the air. With 10% Giemsa stain solution, slides were stained for 8 minutes, after being fixed in methanol for 10 minutes. Using an Olympus Provis (Germany; Hamburg) fluorescence microscope, the frequency of erythrocytes with micronucleus was determined at 1250X magnification. For each specimen, an examination of total of 1000 erythrocytes with intact cellular and the nuclear membrane was done.

Analysis of micronuclei

Without knowledge of the origin of samples, Blind scoring of micronuclei was performed on slides being coded in order to minimize technical changes. Cells that had intact cellular and nuclear membrane were scored. A nonrefractory particle, ovoid or round in shape comprising structure and color similar to chromatin and the main nucleus having a diameter of either 1⁄3–1⁄50 (for fish), found being detached from it was given the name as micronucleus. Generally, MN should possess the same or lower than that the color intensity of main nuclei. Particles having higher intensity than that of main nuclei were not recorded as MN. The frequency of induction of micronuclei was assessed at a magnification of 1200x. Assessment of erythrocytes with intact nuclear abnormalities was made by following the procedure of Barsiene *et al.* (25).

Statistical Analyses

The Probit Analysis method for LC_{50} (25) was used to assess the acute toxicity and lethal concentration of lead using Minitab at a confidence interval of 95%. T-test was used to subject the data obtained from histological studies at the $p<0.05$ level of significance using IBM SPSS Statistics Version 22. The non-parametric Mann-Whitney U-test was performed to compare the frequency of micronuclei and other nuclear abnormalities between control and lead-treated fish groups (27). The data obtained were subjected to One-way ANOVA and Tukey's test was applied for multiple comparisons between control and exposed fish.

The software used for statistical analysis was Statistix 8.1 Version.

Results

Acute toxicity

Test fish *Oreochromis niloticus* was exposed to heavy metal lead (Pb) as lead chloride ($PbCl_2$) for 96-hrs. The percent mortality corresponding to each concentration has been displayed in Figure 1. The 96-hr LC_{50} and lethal concentration of lead for *O. niloticus* were found to be 77.673 mgL^{-1} and 150.924 mgL^{-1}, respectively. No mortality was observed over 96 hours in the control group fishes.

Histological alterations

The histological alterations were observed in the gills, liver and kidney of *O. niloticus*. The alterations occurring in the lead-treated groups were compared to the control group with no histological change. The data obtained from the histological analysis is given in Table 1.

Gills

The results showed significant histological degenerations in the gills of *O. niloticus* exposed to lead as shown in Table 1. Figure 2 [A] Showed the normal gill showing primary and secondary lamella of normal gill tissue of *O. niloticus*. Figure 2 [B] and [C] showed the significant changes in the gills as vascular congestion and curling of secondary lamella and necrosis, respectively after lead exposure. The fusion of secondary lamella, shortening of secondary lamella and decrease in inter-lamellar space and lifting of epithelia of secondary lamella were observed in Figure 2 [C] and [D], respectively.

Liver

According to the statistical analysis, the diameter of the central vein in the liver of lead-treated group of *O. niloticus* was decreased (Figure 3B) compared to the control group (Figure 3A) and was found to be statistically significant. Hepatocyte diameter was decreased in the treated group compared to the control due to the shrinkage of cells in lead-treated fish with a significance level at $p<0.05$

Table 1. The intensity and severity of histological alterations in the gills, liver and kidney of fish.

Parameters	Control	Treated
Gills		
Vascular Congestion	-	++
Epithelial Lifting	-	++
Fusion of Secondary Lamella	-	++
Curling of Secondary Lamella	-	+++
Necrosis	-	+
Shortening of Secondary Lamella	-	+++
Liver		
Cellular Degeneration	-	++
Dilation in sinusoids	-	++
Kidney		
Loss of Hemopoietic Tissue	-	++
Necrosis	-	+
Edema	-	++

Normal (-), Mild (+), Moderate (++), Severe (+++).

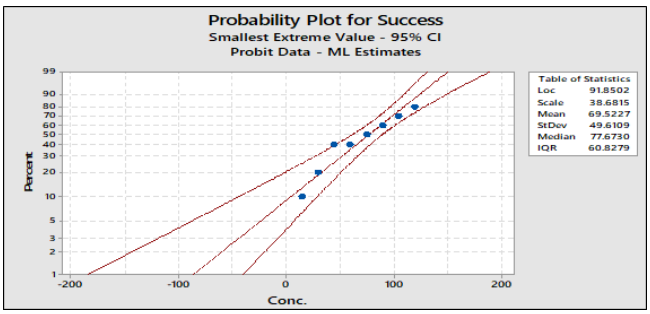

Figure 1. Probability graph for 96-hour LC_{50} and lethal concentration.

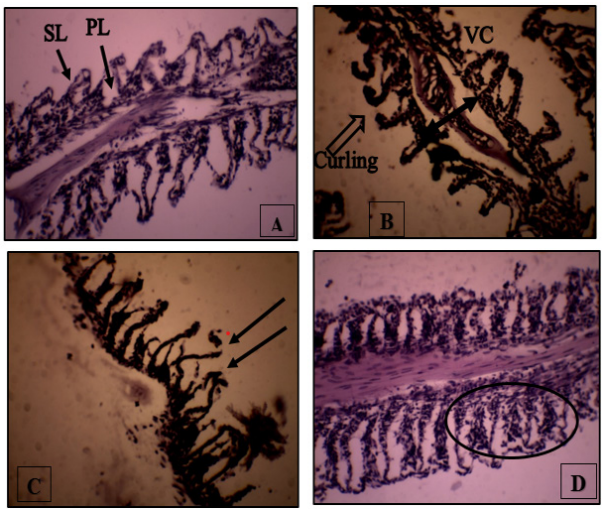

Figure 2. Histology of gills of control and Pb-treated *O. niloticus* [A] Normal gill showing primary and secondary lamella [B] Vascular congestion (VC) and secondary lamella curling [C] necrosis in the secondary lamella [D] Fusion of secondary lamella.

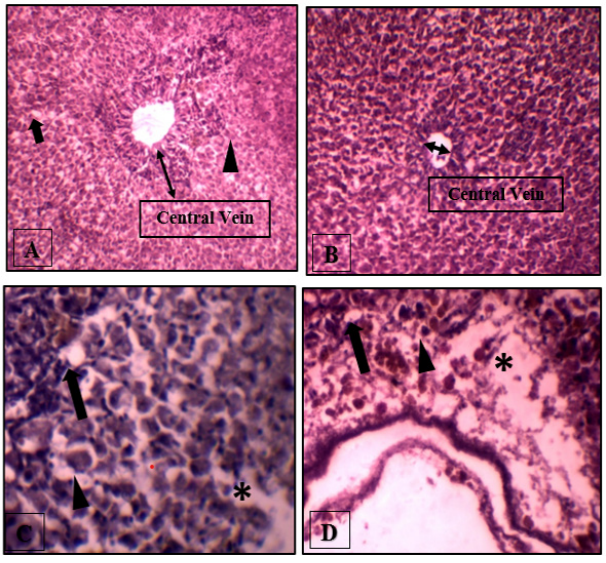

Figure 3. Histology of liver of control and Pb-treated *O. niloticus* [A] Normal liver structure showing normal central vein (double arrow), hepatocyte (arrow head) and sinusoid (arrow) of liver of control group [B] Decrease in central vein diameter [C] Dilated sinusoids (sinusoids), shrunken hepatocytes (arrow head) and necrosis (*) [D] Dilated sinusoids (sinusoids), shrunken hepatocytes (arrow head) and necrosis (*)

(Figure 3C, D). However, the sinusoid width increased due to the shrinkage of hepatocytes in lead treated liver of fish as compared to control with $p<0.05$ as shown in Figure 3C, D. The changes observed in the lead-treated liver

were dilation, sinusoid congestion, and cellular degeneration (Table 1).

Kidney

There was a significant increase in glomerular diameter of the lead-treated kidney (Figure 4B) as compared to control group (Figure 4 A; Table 1). The corpuscle diameter also increased in lead treated group as compared to the control group (Figure 4B; Table 1). The diameter of the proximal convoluted tubule of lead-treated kidney also increased compared to the control group (Figure 4 B; Table 1). The same trend was observed in the diameter of the distal convoluted tubule of the lead-treated group compared to the control group of *O. niloticus* (Figure 4B; Table 1). A significant histological change was observed in the kidney as loss of hemopoietic tissue, necrosis and edema (Figure 4 C).

Genotoxicity

Figure 5 showed the [A] normal RBC of control *O. niloticus* and [B] micronuclei [C] notched nuclei [D] de-shaped nuclei in RBCs of Pb-treated *O. niloticus.* The results of this study showed that the frequency of micronuclei, de-shaped and notched nuclei increased in lead-treated fish compared to control groups after 2 and 4-days period. An increased number of micronuclei, de-shaped and notched nuclei were observed in RBCs of fish *O. niloticus*, as compared to control groups. In graph, the frequency on the y-axis exhibits the variation in frequency of genotoxic parameters against the time period of 2 and 4 days on x-axis (Figure 6-8).

Micronuclei

The frequency of micronuclei was increased in Pb-treated groups compared to the control groups in *O. niloticus*. As shown in Figure 6, the frequency of micronuclei after 2 days was increased as compared to control group after 2 days. The increase in frequency of micronuclei after 4 days in treated groups was higher as compared to control group. Results revealed that the frequency of micronuclei after 2 days was higher than the frequency of micronuclei after 4 days Pb-exposed *O. niloticus*. Figure 6 shows micronuclei in RBCs of Pb-treated fish compared to RBCs of the control group.

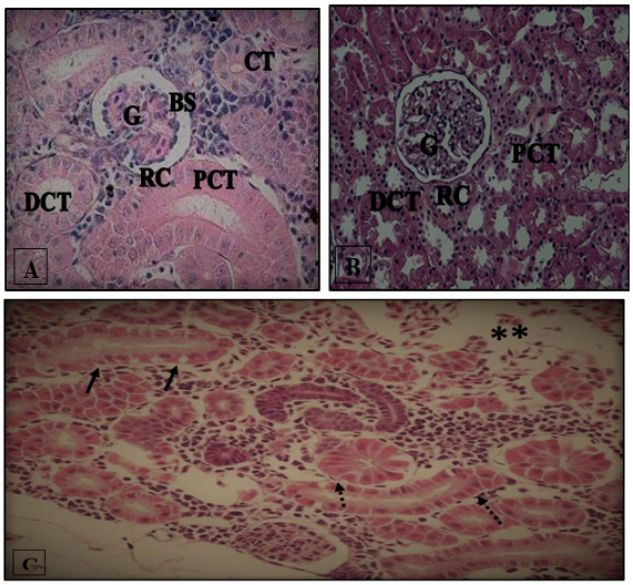

Figure 4. Histology of kidney of *O. niloticus* [A] glomerulus (G), renal corpuscle (RC) proximal (PCT), distal (DCT) and collecting ducts (CT) [B] shrinked glomerulus (G), renal corpuscle (RC) proximal (PCT), distal (DCT) and collecting ducts (CT) of kidney of Pb-exposed *O. niloticus* [C] Loss of hemopoietic tissue (*), necrosis (dotted arrow) and edema (arrow) in the Pb-treated kidney of *O. niloticus.*

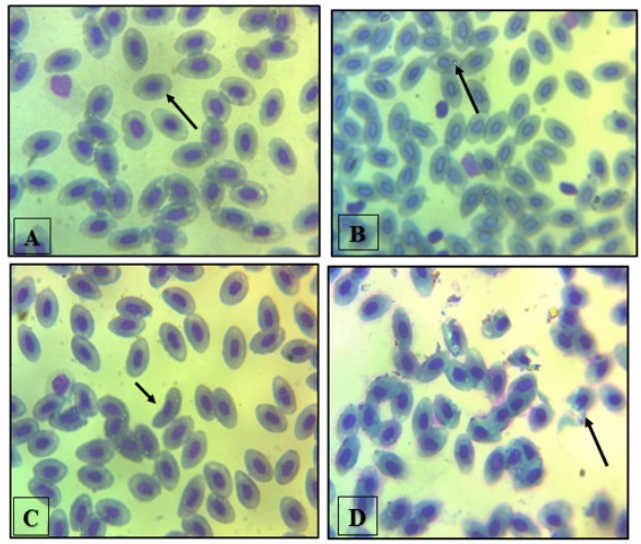

Figure 5. [A] Normal RBC (arrow) of control *O. niloticus* [B] Micronuclei [C] Notched nuclei [D] De-shaped nuclei in RBCs of Pb-treated *O. niloticus.*

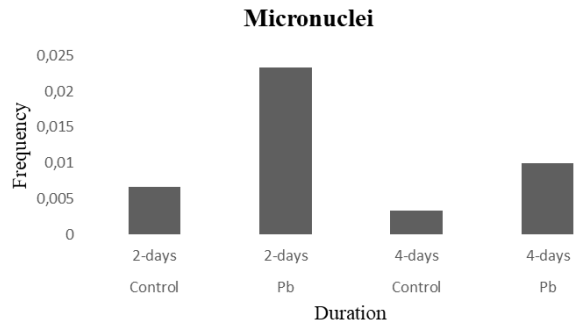

Figure 6. Frequency of Micronucleus in RBCs of *O. niloticus.*

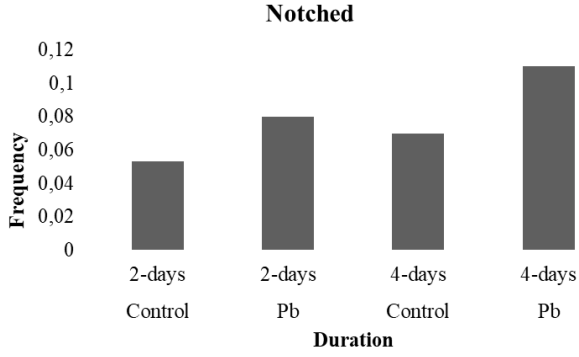

Figure 7. Frequency of Notched nuclei in RBCs of *O. niloticus.*

Notched

The frequency of notched nuclei was increased in Pb-treated groups compared to the control group in *O. niloticus*. As shown in Figure 7, the frequency of notched nuclei after 2 days was higher as compared to the control group. The increase in notched frequency after 4 days of treatment was higher in Pb-treated groups as compared to con-

trol group after 4 days. On comparing the micronucleus frequency of the treated group after 2- and 4-day lead exposure it was clear that the notched frequency after 4 days treatment with lead was significantly higher than that of the frequency of the treated group after 2 days of exposure of lead to fish. Figure 7 shows notched nuclei in RBCs of Pb-treated fish compared to RBCs of the control group.

De-shaped

The frequency of de-shaped nuclei was significantly higher in the treated group after 2- and 4-days exposure to lead in *O. niloticus* than in the control group. Figure 8 depicts that there was an increase in frequency of de-shaped nuclei after lead exposure in treated groups after a time period of 2 days as compared to the control. The same was the case with the treated group after 4 days as the frequency of de-shaped nuclei was also increased compared to the control group. However, there was a slight difference in the increased frequency of de-shaped nuclei in treated groups after 2- and 4-days Pb-exposure as seen in Figure 8.

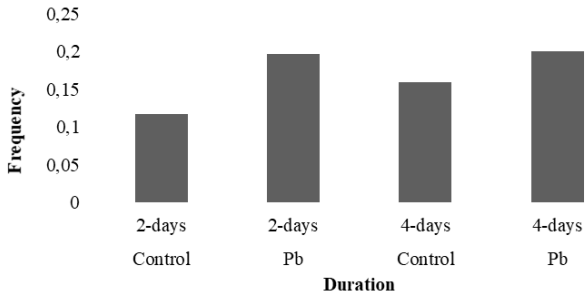

Figure 8. Frequency of De-shaped in RBCs of *O. niloticus.*

Discussion

Acute toxicity

The inferences showed the 96-hr LC_{50} value of lead for *O. niloticus* 77.673 mgL^{-1}. Babatunde and Idris (28) measured the acute toxicity value as 50.12 mgL^{-1} of lead for *Clarias gariepinus*. The LC_{50} value of lead for *Labeo rohita* was determined as 34.20 mgL^{-1} by Singh and Manjeet (29). Bawa-Allah and Saliu (30) measured the acute toxicity value 51.516 mgL^{-1} of lead in juveniles of *C. gariepinus*. Munoz *et al.* (31) determined the 96-hr lethal concentration of lead (Pb) in Silver catfish, *Rhamdia quelen* and was found 108 mgL^{-1}. The 96-hr LC_{50} value was reported as 130.094 mgL^{-1} by Osuala and Bawa-Allah (31) for *O. niloticus* fingerlings. A comparison was made by Abedi *et al.* (33), regarding the LC_{50} value of lead between two fish species, *Cyprinus carpio* and *Pangasius hypophthalmus*. In *Pangasius hypophthalmus*, lethal concentration was found to be 48.06 mgL^{-1} and 77.3 mgL^{-1} for *C. carpio.* Esbaugh *et al.* (34) examined the acute toxicity of lead (Pb) to *Ceriodaphnia dubia*. For both organisms, acute toxicity and found 180 mgL^{-1} and Pimephales promelas.. The value of LC_{50} of lead for *Poecilia reticulata* was 3.79 mgL^{-1}, while the value for copper was found to be 5.6 mgL^{-1} for *Rasbora sumatrana* (35). In *Tench tinca*, the LC_{50} value was 300 mgL^{-1}, for lead and copper (36). Gandhewar *et al.* (37) reported 378 mgL^{-1} LC_{50} value of lead nitrate for *C. batrachus*. The LC_{50} value of lead was reported ranged from 17.43-24.29 mgL^{-1} by Ergonul *et al.* (38) for *C. carpio*, which was lower than our results. The LC_{50} values of $Pb(NO_3)_2$ for *Tympanotonus fuscatus* (Periwinkle) were estimated by Otitoloju (39) and were found to be 370.77 mgL^{-1}. In *O. niloticus*, the 96-hr LC_{50} value for lead came out to be 12.45 mgL^{-1}, which was lower than our studies, as stated by Al-Akel and Shamsi (40), while the LC_{50} value of lead for *C. gariepinus* was assessed as 22.65 mgL^{-1}. Ullah *et al.* (41) reported 44 mgL^{-1} LC_{50} value for *O. niloticus* that was almost in accordance with our findings. The value of LC_{50} for *Heteropneustes fossilis* (Bloch) for the lead was estimated as 925 mgL^{-1} by Parashar and Banerjee (42).

Histological alterations

Histopathological markers are used to evaluate and identify detrimental effects in fish exposed to pollutants (43). Significant histological alterations were observed in the gills of *O. niloticus* exposed to lead. Vascular congestion, epithelial lifting and fusion of secondary lamella were moderate histological degenerations. Curling of secondary lamella and shortening of secondary lamella were considered severe histological degenerations. Necrosis, alongside these histological degenerations, was found to be mild in gills. Fish exposed to metals have been found to exhibit histological changes to their gills, including the lifting of lamellar epithelium, epithelial hyperplasia and hypertrophy, lamellar telangiectasis, and lamellae fusing (44, 45). Due to gill toxicity, oxygen deprivation is the most frequent cause of cellular deterioration in gill filaments (46). One of the first places where environmental contaminants are exposed is the gill epithelium, which exhibits histological changes such as epithelial lifting, epithelial hypertrophy, hyperplasia, telangiectasia, epithelial desquamation, and necrosis as early as 3 hours after exposure (47) which were similar to present studies. Effects of exposure of lead in the morphology of the liver and gills of sculpin were summarized by Jantawongsri *et al.* (48). The gill telangiectasia and liver necrosis were found to be higher in sculpins exposed to lead. Effects caused by lead in liver and gills were evaluated by Hussain *et al.* (49) in Cirrhinus mrigala. Choudhary *et al.* (50) exposed *Pethia ticto* with the sub-lethal concentration of lead nitrate and noted histopathological changes in the gills. These changes showed the damages in tissue vacuole formation, lead deposition, shrinkage of the lamella, fusion of gill lamella tips, and swelling of the gill's epithelial layer. Khalesi *et al.* (51) examined the effects of two non-essential heavy metals: cadmium and lead exposure to *C. carpio* at sub-lethal concentrations. The exposed fish showed fusion of gill lamellae, vessel dilatation, hyperaemia, and hyperplasia of gill epithelial cells. Barbieri *et al.* (52) examined the histopathologic alterations in the gills of *O. niloticus* to determine the possible effects of lead, carbon nanotubes, and lead + carbon nanotubes on their histological integrity. The main alterations observed were epithelial structure, hyperplasia and displacement of epithelial cells, and alterations of the structure and occurrence of aneurysms in the secondary lamella.

Fish liver histology has been suggested by Mobarak *et al.* (53) as a model for exploring the relationships between environmental variables and hepatic architecture and functions. The present study revealed a significant change in histological appearance of the liver of lead-treated fish.

As a result of lead exposure, shortening of the diameter of central vein, shrinkage of hepatocytes and widening of sinusoids was observed. Along with these degenerations, cellular degeneration was also observed in the liver of lead-treated fish. Mustafa *et al.* (54) investigated the histopathological alterations of the gills, liver and kidney in *C. carpio* exposed to lead acetate. The main histopathological changes caused by lead observed in fish gills were edema in the filamentary epithelium, lifting of lamellar epithelia, hyperplasia of the epithelium with fusion of adjacent lamellae and necrosis in primary and secondary lamellae. Sultana *et al.* (55) examined the alteration in liver like aggregation, hypertrophy of hepatocytes, cytoplasmic degeneration, hemolysis between hepatocytes, necrosis and degeneration in lead and copper-stressed *L. rohita*.

In Tilapia fish exposed to heavy metals, Udotong (56) noted comparable outcomes (Fe, Pb and Cu). The excessive buildup of fat in the cytoplasm causes the liver of those exposed to toxicants to exhibit vacuolation (57). Increased hepatocyte vacuolization might be thought of as a marker of a degenerative process that implies metabolic damage, presumably brought on by exposure to polluted water (58). Cytoplasmic vacuolation, sinusoid dilation and necrosis were observed after histological analysis in tissues of the liver. Earlier research revealed histological abnormalities in fish subjected to pollutants' effects on the kidney (59). The results of the present study showed that exposure to lead caused the glomeruli and renal corpuscle to shrink in size along with the shortening of proximal and distal convoluted tubules. Along with these, loss of hemopoietic tissue, necrosis and edema were also observed in lead-treated fish. The presence of harmful pollutants in the glomeruli filtrate is often linked to abnormal histopathological alterations in kidney tissue (60).

Genotoxicity

Histopathological and physiological investigations make up most of the recent research on metal toxicity in fish. There is still a lack of information on these species' population decline and habitat degradation, as well as possible cyto-genotoxic effects of metals and other genotoxic chemicals (61). The kind of pollutant and fish species exposed to that pollutant has the greatest impact on genotoxic damage (62). The results of our study depicted that the frequency of micronuclei in lead-treated groups increased as compared to the control of fish *O. niloticus*. Micronucleus frequency was higher after 2 days as compared to 4 days. The frequency of D-shaped and notched nuclei increased in the lead-treated group as compared to control groups for *O. niloticus* and was higher after 4 days as compared to 2 days. Fish have sensitive blood and gill tissues, which are recognized as helpful indicators for toxicity evaluation (63). Fish exposed to cytotoxic and/or genotoxic chemicals for 1 to 5 days have micronucleated erythrocytes (64). The term "micronucleus" refers to tiny, spherical, dark-stained formations that are otherwise similar to the cell nucleus in appearance and contain complete or partial chromosomes that have not been merged into the daughter nucleus during cell division (65). Delmond et al. (66) evaluated genotoxic effects influenced by lead exposure in *Astyanax serratus* and reported the damage to blood and change the nuclear morphology of erythrocytes and necrosis induction. Hamed *et al.* (67) revealed a significant increase in the micronuclei by examining the genotoxic effects of lead nitrate in *C. gariepinus*. Additionally, it has been shown that exposed gills and red blood cells of *L. rohita* have higher rates of micronuclei development, greater DNA damage, and higher percentages of tail DNA (68) similar to our results. At 96 hours after exposure, erythrocytes of the three freshwater fish species showed similar strong genotoxic effects of LC_{50} lead and LC_{50} copper (69). According to Cavas (70), erythrocytes, gills, and fin cells of *Carassius auratus* strongly promoted the development of MN when exposed to lead acetate at dosages ranging from 0.01 to 0.1 mgL^{-1} for 2 to 6 days, which were similar to our results. This is comparable to experiments done by Ozkan *et al.* (71), which showed a considerable induction of micronuclei in *O. niloticus* peripheral blood after exposure to sub-lethal levels of $CdCl_2$ for 10 days. Additionally, they stated that MN induction was concentration- and time-dependent. In the erythrocytes of *O. niloticus* treated to a single dose of $CdCl_2$, Bakar *et al.* (72) detected a substantial time-dependent rise in MN and observed for 24, 48, 72, and 96 hrs that is similar to our findings. According to research by Pietrapiana *et al.* (73), heavy metal contamination causes micronuclei more often to form in fish erythrocytes.

In conclusion, lead toxicity to *O. niloticus* cause histopathological changes and genotoxicity, leading to abnormalities and mortality.

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Author Contributions

All authors contributed to the study conception and design. Huma Naz and Tanveer Ahmed designed the idea and research layout. Sadeema Maryam conducted different experiments/lab work. Muhammad Usman and Muhammad Umar Ijaz assisted in experiments handling and results interpretation. Syed Qaswar Ali Shah and Adnan Ahmad Qazi technically monitor the experiments. Sadeema Maryam and Huma Naz wrote the Manuscript. Awatif Omran, Yasmene F. Alanazi, Basharat Ali, Habib Ali, Abdulrahman Alasmari and Kambiz Heidary critically revised the manuscript.

Conflict of interest

There is no potential conflict of interest to declare.

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