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# CLINICAL ALTERATIONS CAUSED BY LEAD EXPOSURE IN COMMON CARP

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**Abstract**
The current study investigated the toxicological effects of lead acetate trihydrate on bioaccumulation, hematology and histopathological changes in the gills and muscles of common carp (*Cyprinus carpio*). The fishes were divided into seven groups and were treated with different concentrations of lead. Lead bioaccumulation was analyzed in gills and muscles with the help of atomic absorption. Gill tissue absorbed the maximum concentration of lead, followed by muscles. After 24 hours, decrease in the concentration of Hb (65.13±16.81) and RBCs (0.52±0.137) and a maximum increase in the concentration of lymphocytes (91±21.30) with a dose of 30mg /l lead was observed. **Results** showed that in every experiment, the amount of lead in the gills increased gradually with the increase of the doses, as 259.83±58.4 with a dose of 5mg/I, 276.9±71.1 using dose of 10mg/l, 284.3±70.1 treated with 15mg/l, 299.63± 76.80 deal with 20mg/l , 310.26± 79.61 by 25mg and 331.4± 85.42 with a dose of 30mg . A maximum decrease in the concentration of Hb (65.13±16.81) and RBCs (0.52±0.137) and increase in the concentration of lymphocytes (91±21.30) with a dose of 30mg /l was observed from 24 hours exposure. Our findings suggest that common carp exposed to lead develop. histological alterations such as epithelial lifting, interlamellar spaces, gill bridging, curling filaments, swelling, fusion and cell necrosis, irregular and inflammatory cells were observed in gill tissues, while inflammation,degenaration and necrosis of muscle fibers, edema of muscle bundles, and lesions were observed in muscle tissues.

**Keywords:** Lead, Toxicity, Common carp, Bioaccumulation, Hematology, Histopathological Lead toxicity in Common Carp

## 1. Introduction

Fish is a nutritious food item that contains high-quality, readily digested animal protein. ( Murtaza et al.,2020) . It accounted for 17% of the animal protein in total protein consumption by the global population in 2015. With a balanced amino acid composition, it is the best source of protein and a great source of essential amino acids as well as polyunsaturated omega ω-3 (EPA and DHA) and ω-6 (ALA) fatty acids (Afridi et al., 2019; Kamran et al., 2023).It guards against cardiovascular disorders, stroke, cancer, age-related molecular degeneration, and control of optical function (Kamran et al., 2020; Yaqub et al., 2021).

Environmental pollution is a worldwide problem across the globe and has adverse impacts on human health (Fereidoun et al., 2007). Over the last few decades, there is an increase in global concern over public health due to increase in environmental pollution (Kimani, 2007). Comparatively, human exposure to environmental pollutants is more intense nowadays to early days when life on earth came

into existence (Schell et al., 2006).

Excessive concentrations of pollutants due to municipal wastes and burning of fossil fuels cause maximum damage to humans, animals, and plants including tropical rainforests, as well as the wider environment (Nriagu and Pacyna,1988). Metals as an important environmental pollutant refer to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration. Metals are widely distributed in aquatic bodies and are considered as essential in trace amount for normal biological activities of aquatic fauna (Ahsan et al., 2014).

Non-essential heavy metal like Lead (Pb) have no known essential role in living organisms; exhibit extreme toxicity even at very low (metal) exposure level and have been regarded as the main threat to all forms of life including human health (Eisler, 1985; Jarup, 2003). The nonessential components of lead may cause nephrotoxicity, neurotoxicity, decrease growth rate, survival, metabolisms, development and several adverse health effects (Rahman et al., 2012; Yılmaz et al., 2010). Metals that accumulate in higher concentration cause harmful effects on the blood and organs of the aquatic organism by reacting with enzymes, deoxyribonucleic acid, ribonucleic acid, and cellular proteins (Akahori et al., 1999).

Fish is an important part of the human diet because of its high nutritional quality (Sioen et al., 2007). However, nonessential trace elements in the edible tissues of fish have been detected due to their bioaccumulation in organism (Burger and Gochfeld, 2005; Zhang and Wang, 2012) . However, fish are relatively situated at the top of the aquatic food chain; therefore, they normally can accumulate heavy metals from food, water and sediments (Yilmaz et al, 2007; Zhao et al, 2012).

The content of toxic heavy metals in fish can counteract their beneficial effects; several adverse effects of heavy metals to human health have been known for long time (Castro and Mendez, 2008). This may include serious threats like renal failure, liver damage, cardiovascular diseases and even death (Busaidi et al, 2011; Rahman et al., 2012).The excessive uptake of essential and nonessential metals ends in accumulation in various tissues.Metals in higher concentration change the biological activities of the fish (Canli and Atli, 2003). Consumption of such metal-contaminated fishes by human can cause serious health issues (Kamaruzzaman et al., 2011). Metals deteriorate the ecological balance of the aquatic environment (Vinodhini and Narayanan, 2008; Mastan, 2014) because fish are at the end of the aquatic tropic level and they have a higher tendency to accumulate metals in their body (Mastan ,2014 ).

In the aquatic system, they diffuse radially and fish often being on the top of the aquatic food chain are more susceptible to the hazardous effects as compared to terrestrial vertebrates and therefore is critical to investigate and monitor its bioaccumulation pattern (Rauf, 2009; Kousar and Javed, 2014). Heavy metals undergo metabolic activation that provokes a cellular change in the affected fish. The tissue lesions and apoptosis arise from bioaccumulation, infections, diseases and parasites stimulate necrotic alterations in the fish with an inflammatory defensive reaction (Roganovic – Zafirova *et al.*, 2003). It is imperative that histological biomarkers are the indicators of pollutants in the overall health of the entire population in the ecosystem (Velkova – Jordanoska and Kostoski, 2005). The freshwater fish, common carp (*Cyprinus carpio* L.) is of great commercial importance because it is the most common fish widely consumed worldwide. Therefore, it can be a good model to study the responses to various environmental contaminations. The investigation of histological changes in organs of fish is an accurate way to assess the effects of xenobiotics compounds in experimental studies. The aim of the present study was to investigate heavy metal bioaccumulation, alteration in histopathological and hematological indices in different organs like gills and muscles of common carp exposed to different concentrations of heavy metals.

*Cyprinus carpio* is distributed worldwide and is an economically important fish in tropical and subtropical regions of Asia and Pacific. Because of its high value throughout Southeast and East Asia, this species frequently adopted as an animal model for toxicological testing to determine the toxicity of chemicals in the aquatic environment (Wang et al., 2011). The aim of the present study was to investigate lead bioaccumulation, hematological and histopathological alteration in different organs such as gills and muscles of common carp exposed to different concentrations of lead

**Chemicals**

Lead acetate trihydrate (Pb(C2H3O2)2.3(H2O) was obtained from Sigma-Aldrich and Merck (Germany). A stock solution of lead nitrate was prepared and diluted to appropriate concentrations for the treatment.

## 2. Materials and Methods

**Fish and Acclimatization**

**2.1.** ***Cyprinus carpio*** (Linnaeus 1758) is an exotic fish to India, generally known as scale carp. It is an extensively cultivated species and is commercially important. *C. carpio* is voraciously omnivorous and grows fast on artificial fish feed. Hence it is considered as one of the important species for pisciculture. In the present study, we selected *C. carpio* with an average weight of 70g ± 0.88 g. Prior to employ in bioassay, fishes were kept in 200 L aquaria to be acclimatized to laboratory conditions for two weeks. Commercial foods were used to feed the fish daily during the study period.

### 2.2. Experimental Design

Acclimatized vverage sized fish were transferred to glass aquaria for lead induced toxici ty. were divided into seven group each containing 5 fishes in which six group were treated with lead induced toxicity while control was exempted from this toxicity. The treated group were exposed lead acetate for 1, 7, 14, 30, 45, and 60 day. For experimentation, a sample size of 5 fish from stock population was shifted to both control and test aquaria, having 50 L water. The experiment was conducted in semi-static conditions, following The Organization for Economic Co-operation and Development (OECD, 1994) Guideline Number 203. Sub lethal concentration of lead acetate trihydrate ($C_4H_{12}O_7Pb.\ 3H_2O$) was 30, 25, 20, 15, 10, and 5mg$L^{-1}$ respectively. Control group was run simultaneously for each experimental group having 5 fish.

After the stipulated time, Three fishes from each group were sacrificed randomly including the untreated control aquaria were anaesthetized by MS 222 powder which were mixed with water in a beaker and fish head was held in the beaker for few minutes. Fish were dissected manually, and the required tissues were removed, weighted, and were stored at −20°C for further analysis.

### 2.3. Hematological Parameters.

For the examination of the hematological parameter, blood was drawn from the caudal vein just behind the anal pore of common carp through a sterile heparinized syringe (5cc) having half the size of the needle. After collection, the blood samples were transferred to ethylenediaminetetraacetic acid (EDTA) tubes to prevent blood clotting. The blood was gently shaken in EDTA tubes so that the blood gets mixed with the anticoagulation drops in the EDTA tube to prevent blood coagulation, and then, the blood samples were observed through hematological analyzer.

### 2.4. Lead analysis.

#### 2.4.1. Tissue Digestion

Frozen tissue samples of gills, intestine, muscle and skin were thawed, and blotted in blotting paper. Known weights of gill, intestine, muscle and skin of each fish were shifted to 250 ml volumetric flasks for digestion. Samples were digested according to methods described by Van Loon (1980) and Du Preez and steryn (1992), with slight modification at the time of digestion , 5 ml nitric acid(55%) and 1 ml perchloric acid (70%) were added to each flask and kept safely overnight. Next day a second dose of 5 ml nitric acid (55%) and 4 ml perchloric acid (70%) was added to each flask. The flasks were then placed on hot plate and allowed to digest at 200 to 250ºC until a transparent and clear solution was obtained. Dense white fumes from the flasks after brown fumes were an indication of completion of the process of digestion.

Samples after digestion were cooled and diluted to 10 ml with distilled water by proper rinsing of the digestion flasks. Samples were stored in properly washed glass bottles until the metal concentration could be determined. Atomic absorption spectrophotometer (Spectra AA-10) was used to determine the concentration of $Pb^{2+}$ in the tissue sample of each fish. A range of analytical standards for lead

was prepared from Merck stock solutions. Standard curves were prepared and calibrated against the standard curves to know the concentration of lead present in samples.

### 2.5. Histological Studies.

After the fish dissection, portions of tissues (gills and muscles) were preserved in 10% formalin for histological studies. The Bernet et al., (1999) procedure was adopted for the preparation of tissues for histopathological examinations. The preserved tissues were processed in various grades of ethanol, cleared in xylene, and impregnated with wax (mp; 72°C). Five-micron-thick sections were cut using a rotary microtome (Accu-Cut® SRMTM 200 Sakura). Tissue sections were stained with hematoxylin and eosin (H&E). Stained slides were observed and photographed under a high-resolution microscope (Olympus DP71, U-CMAD3 Japan) fitted with a digital camera (Bell et al., 1999).

### 2.6. Statistical Analysis

All the statistical analyses were performed using student *t*-test. Data are presented as mean ± standard error (±S.E). *P*-values of<0.05 were considered significant.

## 3. Results

### 3.1. Bioaccumulation.

During day1 exposure, maximum concentration of lead was accumulated in gill tissue that was 331.4±85.4 µg/g. Similarly, in muscle tissue, lead was accumulated in maximum concentration that was 121.5±31.24 µg/g respectively, as shown in Table 3. Likewise, treatment of fish for day 7 indicated the maximum accumulation of lead in gill tissue that was 310.2±79.6 µg/g followed by 299.6±76.8, 284.3±70.1, 276.9±71.1 and 259.83±58µg/g for 14,30,45 and 60 days respectively. Similarly, in muscle tissue, lead was accumulated in maximum concentration that was 121.5±31.24 µ/g for 24h followed by 110.56±27.7, 104.13±25.7, 98.53±25.1, 96.33±24.7 and 91.76±23.4 µg/g for 7,14,30,45 and 60 days respectively, as shown in Table 3. Therefore, the present study demostrates that gill and muscle tissues showed maximum affiliation towards lead accumulation depends on the lead dose present in water. As in control group the lead amount is negligible.

### 3.2. Hematology

Lead toxicity reduces the concentration of hemoglobin (65.13±16.81***) and RBC,s (0.52±0.137***) to the maximum extent over 24h exposure. Similarly, WBC, s and monocytes also end with increase in their concentration against various doses of lead, but the maximum increase was noticed against 60 days exposure as the dose was minimum, this is because the body is releasing more of these cells to fight at the start of infection and inflmation. At 24 hours exposure the decreased counts of WBCs and monocytes with severe sepsis is caused by excessive netosis (cell death), as the lead dose was 30mg/l which is comparatively high than other treated groups.The increase in the WBC count can be correlated with an increase in antibody production, while lymphocyte against the toxic media of lead ends with increase in concentration and the maximum increase was noticed at chronic 60 days exposure as shown in Table 2. Increase in the number of white blood cells and lymphocytes are a normal reaction to a toxicant, which demonstrates the protective responses of immune system under toxic conditions Monocytes showed an increase in the concentration against various doses of lead except for 24 hours exposure as shown in Table 1.

3.3. Histopathology. Various pathological alterations against lead toxicity have been observed as shown in Figures 1 and 2.

## 4. Discussion

Metals accumulation in fish tissues depends on the exposure concentration and time as well as other factors such as temperature, age, interaction with other metals, water chemistry and metabolic activity of the fish (Heath 1995).The current study was conducted which aimed at analyzing the accumulative concentration of heavy metals such as lead to scrutinizing the effect of such accumulation on hematological parameters, gill and muscle tissues of common carp after exposing the fish for 1, 7, 14, 30,

45 and 60 days against lead concentration for specific time periods. Gills are the first target of water-borne pollutants and heavily prone to accumulation of heavy metals due to the constant contact with the external environment and the main place for the uptake of heavy metals (Ruiz-Picos and E. L´opez, 2012). The extremely branched morphology of gill tissues and the movement of water through it result in maximum accumulation of heavy metal (Ruiz-Picos and E. L´opez, 2012).The heavy metals do not contact directly with the muscle tissues, as compared to gill tissues which are completely exposed to an aquatic environment which correlates with the present study (Frances *et al*, 1998). Intake of pesticides and toxic metals via gills ends in accumulation of these toxic chemicals in gills, thus damaging the gills'lamella which in turn affects the ion exchange mechanism during osmoregulation (Mallatt,1985) . It has been reported that the accumulation of heavy metals in gills is because of its thinnest epithelium among all the organs of the body through which metals can easily pass (Yousafzai and Shakoori, 2006).

It has been reported that that heavy metals accumulated maximum in the gills of *Labeo dyocheilus* and *Wallago attu* (Wilson and Taylor, 2010; Yousafzai, 1992). In addition to it, other studies have also reported that higher concentration of heavy metals is a usual trend of bioaccumulation in different fishes mostly in gills such as Channa punctatus (Yousafzai, 1992) and *Wallago attu* (Bhatnagar, 1992) In gill and muscle tissues of fishes, all the heavy metals absorb in different quantities according to the availability of heavy metals (Zirong and Shijun, 2007). Lead accumulation in *Anabas testudineus* showed usual differences with a high degree of organ specificity after 30 days of exposure to a sub lethal concentration of lead (Camusso, 1995). Other studies found that the lowest concentration of Pb was detected in the muscle, skin, and gill tissues (Mary, 2015). Another study indicated that concentrations of heavy metals on wild fish were higher in skin samples than in the muscle tissues (Altinok and Murseli, 2007).

The use of haematological techniques in fish culture has growing importance for toxicological research, enironmental inspecting and fish health conditions. Pb significantly changes the blood parameters of fish (Shah, 2006; Tawari, 2008; Stanley et al., 2010). In this study WBC,s lymphocytes and monocytes also end with increase in their concentration against various doses of lead. The increase in the WBC count can be correlated with an increase in antibody production, which helps in survival and recovery of the fish exposed to sublethal concentrations of Pb (Joshi and Tsai, 2002) .The results of the present study are in good agreement with earlier work that reported a decrease in RBC count, haemoglobin content of freshwater fish exposed to toxicants (Blahova et al., 2014). Decline in erythrocyte and hemoglobin concentration was observed against lead in comparison with the control group in the present findings which is in correlation with Santos and Hall (1990) that sub lethal concentrations of lead exposure have resulted in hemolytic anemia due to break down of red blood cells along with reduction in red blood cells and Hb . Our results were also in the agreement with (Gross *et al*., 1975) which demonstrated that decrease in erythrocyte count and hemoglobin concentration may perhaps be due to the blocking of genes in bone marrow or can be due to impairment in intestinal membrane to absorb enough amount of iron or may be hypoxic conditions and destruction of hematopoiesis that have been induced by exposure of specimen to selected toxicants. Similarly Reduction of Hb and RBC accompanied by a compensatory response (increased hematopoietic rate) in lead-intoxicated rainbow trout (Ahmed et al., 2022). A severe microcytic anemic state was reported in Barbus conchonius exposed to 47.4 µg/l of lead (decrease in RBC, Hb) (Tulaby et al., 2020) .

Similar results were reported in (Vutukuru, 2005) that decrease in hemoglobin content occurs due to lead and causes alteration in synthesis of hemoglobin. Deficiency in hemoglobin and erythrocyte count of all exposed fish may be due to hemolysis or may be due to inhibition of enzymes necessary for hemoglobin synthesis. Monocytes have an important role in phagocytosis and also in ingestion of large particle such as necrotic cellular debris, large microorganism as well as effective against the toxicant environment (Jiraung *et al*., 2007). Similarly, in the present study, lead exposure caused maximum decrease in monocytes at 24 h to cope with the stress environment. Increase in lymphocyte counts against lead at 24 h was observed in the present study which is in correlation with the results documented by (Witeska et al., 2010) who reported that fish species are susceptible to the deleterious effects of heavy metals as reflected in the blood changes such as lymphocytosis, anemia, and

eosinophilia. The heavy metal damage is an important factor in many pathological and toxicological processes (Hemalatha and Banerjee, 1997).

In this study observed changes in gills such as hyperplasia, lamellar fusion, epithelial necrosis, and edema were generally attributed to the toxic effects of lead. Similar alterations in the gills have also been reported in the fish exposed to metal Pb (Ayoola and Alajabo, 2012). Gills are good indicators of aquatic pollution (Munshi et al., 1996). The gills of lead nitrate treated fish showed degener- ative changes in gill filaments and secondary lamellae. These results are also in agreement with Hadi and Alwan (2012) who reported similar changes in gills of fresh water fish, *Tilapia zillii*, exposed to aluminium. According to Nath and Banerjee (Nath and Banerjee,1995) in muscle tissues, different histological alterations such as necrosis of muscle fibers, swelling, degeneration of muscle fibers, edema of muscle fibers, enlarged lesions in the epidermis of muscle tissue, inflammation, and zig-zag of muscle fibers were noticed at 7 days against 6.83 ppm concentration of lead.

## 5. Conclusion

Lead toxicity was documented in common carp (Cyprinus carpio) through examination of gill and muscle tissues, as well as haematological indicators. When exposed to lead, the concentrations of Hb, RBCs decreased, nevertheless the concentration of WBCs, monocytes and lymphocytes increased in ascending order after exposure. Edema like gill tissues absorbed the highest possible concentration of lead at 24 hours exposure as the lead dose was maximum. After gills ,muscles absorbed lead over 24 hours of exposure. The inflammation and necrosis of muscle fibres and in gill tissues were maxi-mum on day 1 and decreased gradually till day 60. Histological changes such as epithelial raising, interlamellar gaps, curling filaments, swelling and fusion of cells, irregular cells, death of epithelial cells, and cellular necrosis and degeneration were seen in muscle fibres.

**Table 1: Hematological indices in the blood of *Cyprinus carpio* exposed to lead for 1,7 and 14 days under toxic conditions.**

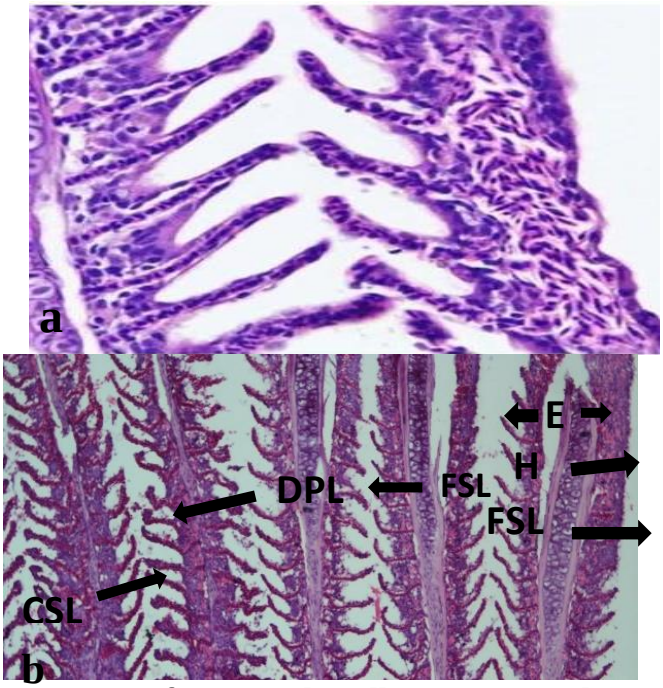
| Lead exposed fish groups | Control (n=3) Mean±S.E | day 1 Treated(n=30) Mean±S.E | day 7 Treated(n=3) Mean±S.E | day 14 Treated(n=3) Mean±S.E |
|---|---|---|---|---|
| RBCs | 1.43±0.370 | 0.52±0.137*** | 0.62±0.160* | 0.69±0.178* |
| Hemoglobin | 71.7±18.50 | 65.13±16.81*** | 66.13±17.07* | 67.1±17.31* |
| WBCs | 32.4±8.359 | 32.16±8.304*** | 32.41±8.359*** | 33.56±8.638*** |
| Lymphocyts | 68.6±17.71 | 69.66±17.93*** | 71.66±18.44* | 74±18.93** |
| Monocytes | 9.633±2.484 | 11.33±2.878*** | 10.66±2.707*** | 11.66±2.964*** |

**Table 2: Hematological indices in the blood of *Cyprinus carpio* exposed to lead for 30,45 and 60 days under chronic conditions.**

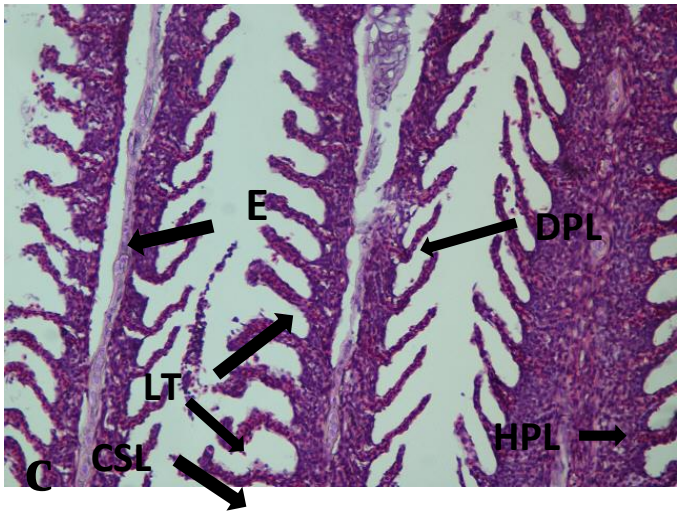
| Lead exposed fish groups | Control (n=3) Mean±S.E | day 30 Treated(n=3) Mean±S.E | day 45 Treated(n=3) Mean±S.E | day 60 Treated(n=3) Mean±S.E |
|---|---|---|---|---|
| RBCs | 1.43±0.370 | 0.76±0.198* | 1.40±0.375 | 1.31 ±0.340*** |
| Hemoglobin | 71.7±18.50 | 68.23±17.61*** | 68.86±17.78*** | 69.78±17.95** |
| WBCs | 32.4±8.359 | 33.23±8.577*** | 33.53±8.641 | 34.23±8.83 |
| Lymphocyts | 68.6±17.71 | 76.66±19.57 | 87±19.77 | 91±21.30*** |
| Monocytes | 9.633±2.484 | 11.66±2.964** | 12.6±3.221 | 14.33±3.651 |

**Table 3: Accumulative concentration of copper, lead, and chromium in gill and muscle tissues observed after 1, 7, 14, 30, 45 and 60 days of treatment (unit: µg/g).**

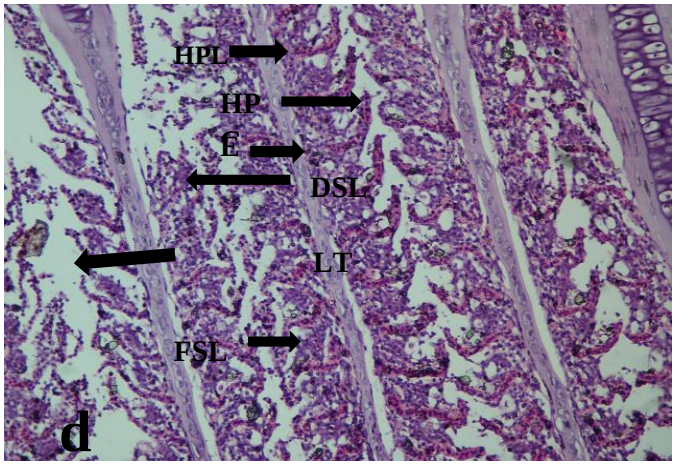
| Lead exposed groups | Control (n=3) Mean±S.E | Day1 treated (n=3) Mean± S.E | Day7 treated (n=3) Mean±S.E | Day14 treated (n=3) Mean± S.E | Day30 treated (n=3) Mean± S.E | Day45 treated (n=3) Mean ±S.E | Day 60 treated (n=3) Mean ±S.E |
|---|---|---|---|---|---|---|---|
| **Gills** | **209.5±53.2** | **331.4±85.4*** | **310.2±79.6*** | **299.6±76.8*** | **284.3±70.1***** | **276.9±71.1*** | **59.83±58.4***** |
| **Muscles** | **82.4±21.15** | **121.5±31.24*** | **110.56±27.7**** | **04.13±25.7***** | **98.53±25.1***** | **96.33±24.7**** | **91.76±23.4***** |



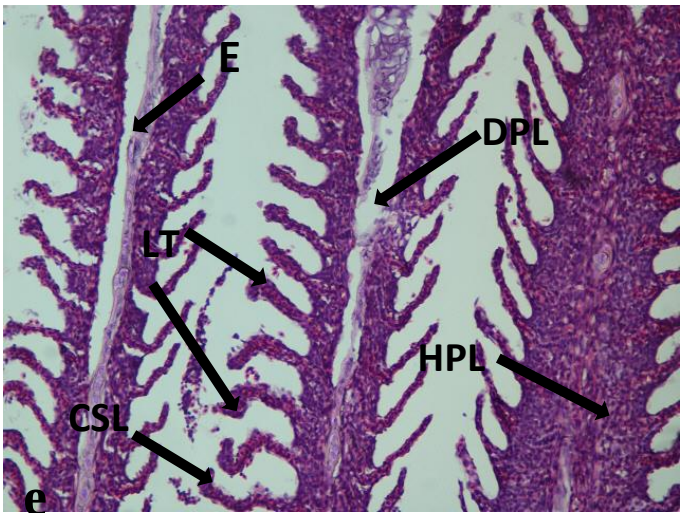

**Gills: Degeneration of primary lamellae (DPL), Curling of secondary lamellae (CSL) Edema (E), Hyperplasia (H), Fusion of secondary lamellae (FSL).**



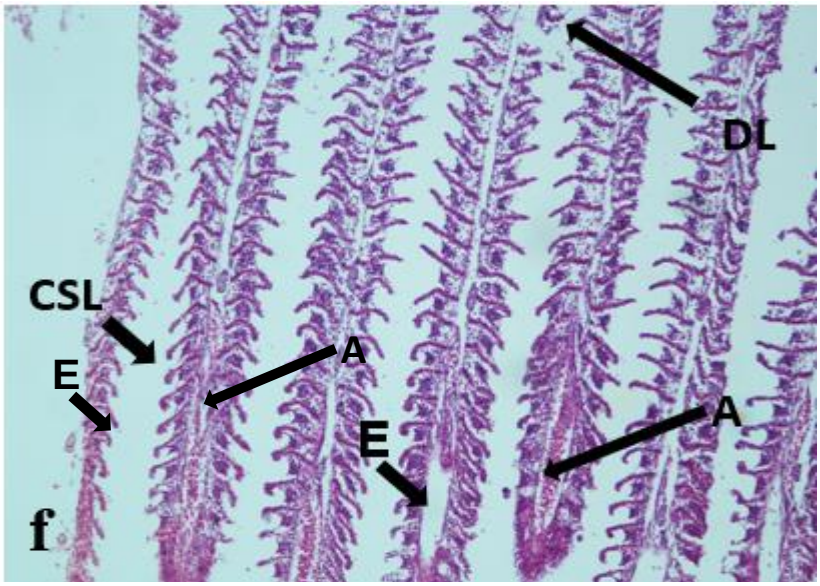

**Gills: Edema(E), Lamellar telangiectasias (LT), Curling of secondary lamellae (CSL), Degeneration of primary lamellae (DPL), Hypertrophy of primary lamellae (HPL)**



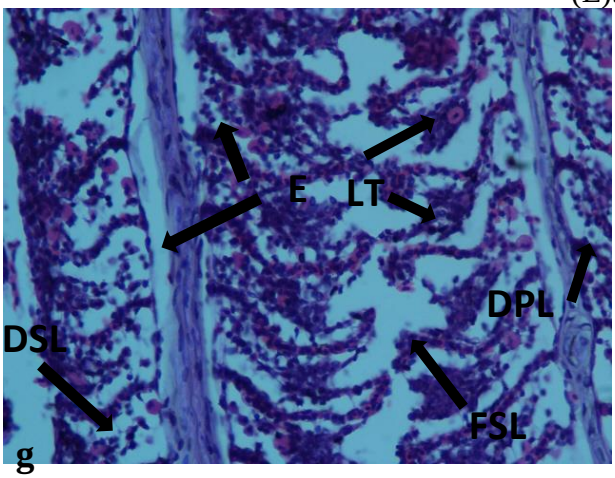
**Degeneration of secondary lamellae (DSL), Lamellar telangiectasias (LT),Fusion of secondary lamellae (FSL), Edema (E), Hypertrophy of pillar cells (HPC), Hypertrophy of primary lamellae (HPL).**




**Gills: Edema (E), Lamellar Telangiectasias (LT), Curling of secondary lamellae (CSL), Degeneration of primary lamellae (DPL), Hypertrophy of primary lamellae (HPL).**

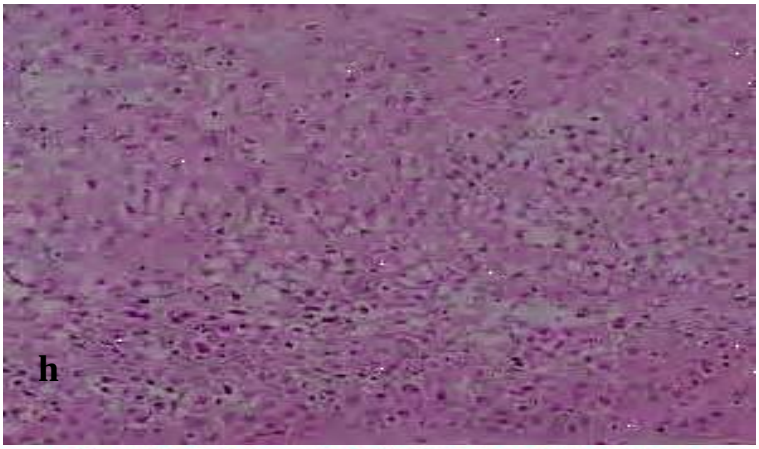


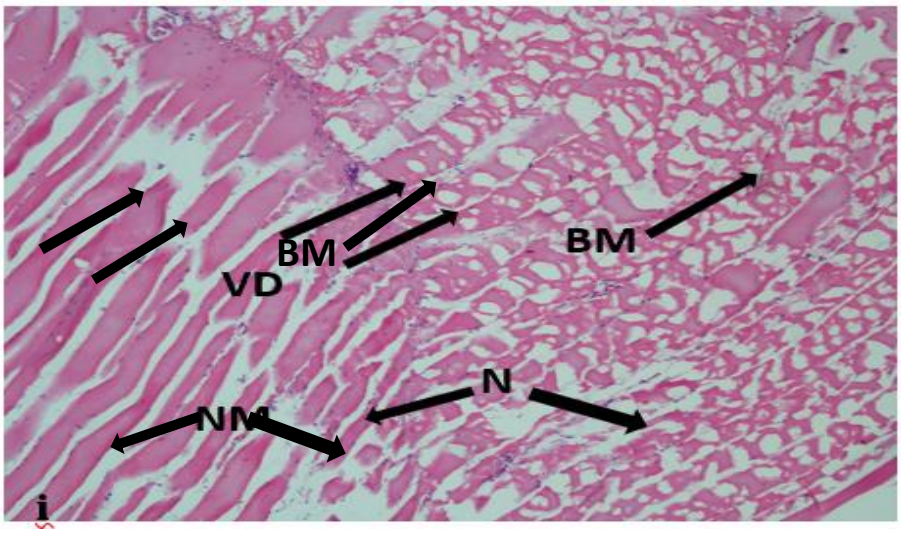

Gills : Curling of secondary lamellae (CSL), Degeneration of lamellae (DL), Aneurysm (A), Edema (E).



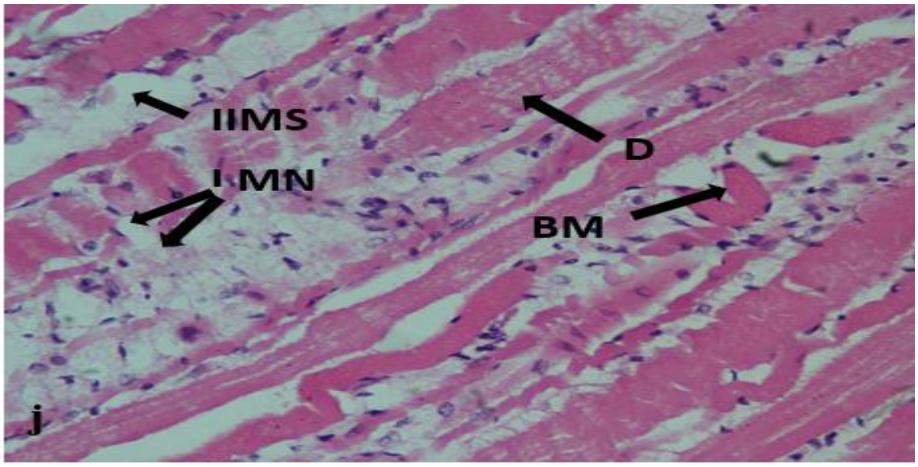
**Gills: Degeneration of secondary lamellae (DSL),Edema (E),Lamellar Talen (LT), Fusion of secondary lamellae (FSL), Degeneration of Primary lamellae (DPL)**

**Fig. 1. Photomicrographs showing histological structures of gills of lead acetate trihydrate treated *C. carpio* fish stained with Hematoxylin and Eosin. (1a) Control gill (1b) 30 mg/l treated gill; (1c) 25 mg/l treated gill (1d) 20 mg/l treated gill; (1e) 15 mg/l treated gill (1f) 10 mg/l treated gill and 5mg /l treated gill**

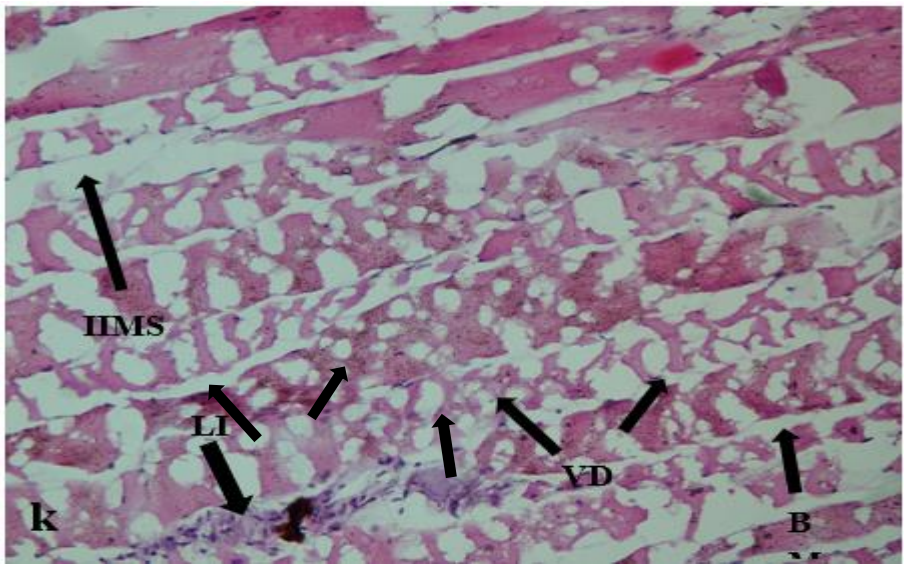


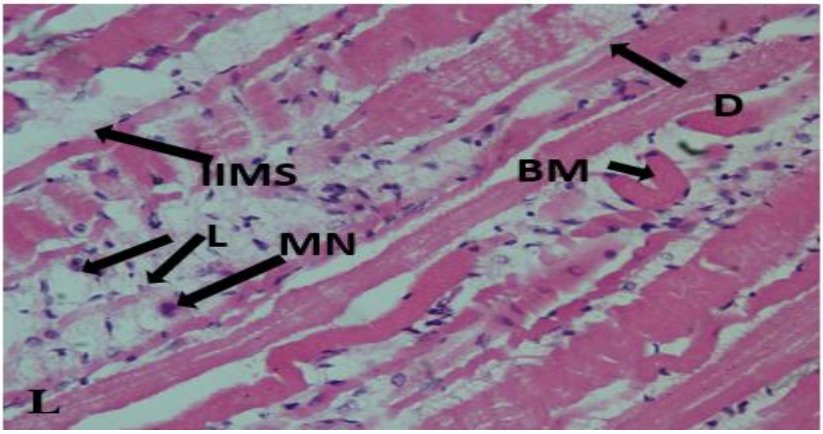

**Muscle: Normal myofibrils (NM), Vacuolar degeneration (VD),Necrosis (N), Break myofibrils (BM)**



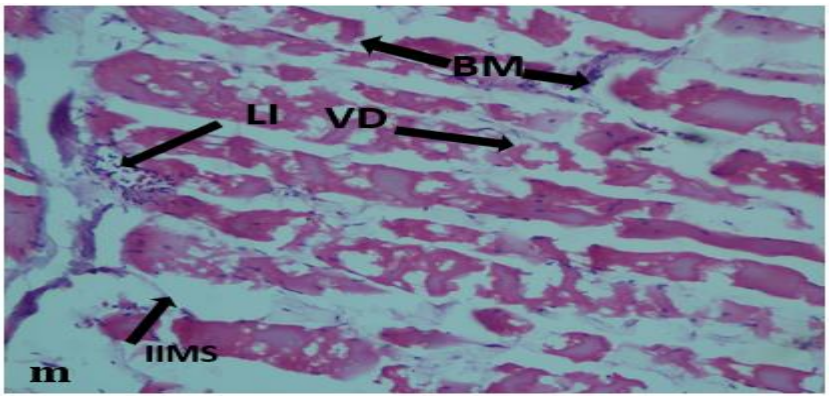

**Muscle: Increase inter myofibril space (IIMS), Leukocyte myofibril necrosis (LMN), degeneration (D), break myofibril (BM).**



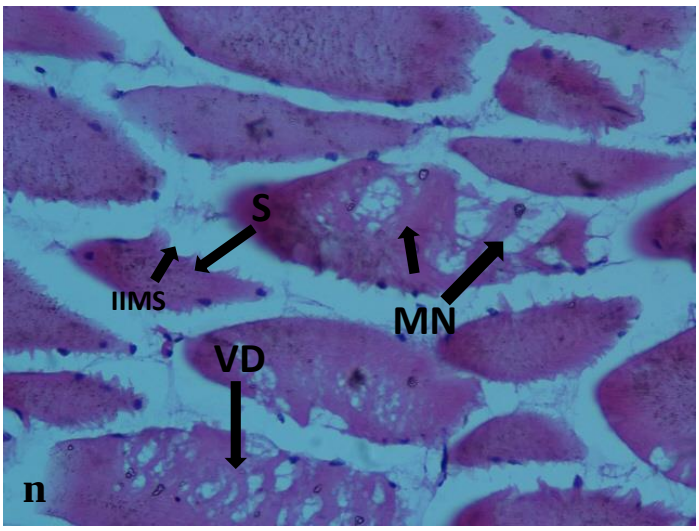

**Muscle: Increase intermyofibrillar space (IIMS), Leukocytic infiltration (LI), Vacuolar degeneration (VD), Break myofibril (BM).**




**Muscle: Increase inter myofibril space (IIMS), Leukocyte Myofibril necrosis (MN), Degeneration (D), Break myofibril (BM),**




**Muscle: Leukocytic infiltration (IL), Vacuolar degeneration (VD), Increase inter myofibril space (IIMS),Break myofibril (BM).**




**Muscle: Increase inter myofibril space (IIMS), Vacuolar degeneration (VD), Sloughing (S), Myofibril necrosis (MN)**

**Fig.2. Photomicrographs showing histological structures of gills of lead acetate trihydrate treated *C. carpio* fish stained with Hematoxylin and Eosin. (2h) Control muscle (2i) 30 mg/l treated muscle ; (2j) 25 mg/l treated muscle (2k) 20 mg/l treated muscle; (2L) 15 mg/l treated muscle (2m) 10 mg/l treated muscle and (2n) 5mg /l treated muscle.**

## 6. References


1. AFRIDI AJ, ZUBERI A, YOUSAFZAI AM, KAMRAN M, Ullah .(2019). Hemp (Marijuana) reverted copper-induced toxic efects on the essential fatty acid profle of Labeo rohita and Cirrhinus mrigala. Mol Biol Rep 46:391–401
2. AHMED, I., ZAKIYA, A., AND FAZIO, F., 2022. Effects of aquatic heavy metal intoxication on the level of hematocrit and hemoglobin in fishes: a review. Frontiers in Environmental Science, vol. 10, 919204..
3. AHSAN KHAN, A. K., YOUSAFZAI, A. M., MUHAMMAD LATIF, M. L., ATIQ-UR-REHMAN, A. U. R., QAISAR KHAN, Q. K., AHMAD ZAIB, A. Z. and ABDUL AZIZ, A. A. (2014). Analysis of selected water quality parameters and heavy metals of Indus River at Beka Swabi, Khyber Pakhtunkhwa, Pakistan.
4. AKAHORI, A., GABRYELAK, T., JÓZWIAK, Z., and GONDKO, R. (1999). Zinc-induced damage to carp (Cyprinus carpio L.) erythrocytes in vitro. *iubmb life*, *47*(1), 89-98.
5. AL BAKHEET, S. A., ATTAFI, I. M., MAAYAH, Z. H., ABD-ALLAH, A. R., ASIRI, Y. A., and KORASHY, H. M., 2013. Effect of long-term human exposure to environmental heavy metals on the expression of detoxification and DNA repair genes. Environmental pollution, vol. 181, pp. 226-232.
6. AL-BUSAIDI, M., YESUDHASON, P., AL-MUGHAIRI, S., AL-RAHBI, W. A. K., AL-HARTHY, K. S., AL-MAZROOEI, N. A., and AL-HABSI, S. H. (2011). Toxic metals in commercial marine fish in Oman with reference to national and international standards. *Chemosphere*, *85*(1), 67-73.
7. ALTINOK, N., AND MURSELI, H. 2007. International database on human capital quality. Economics Letters, 96(2), 237-244.N.M. Roy, S. DeWolf, A. Schutt, A. Wright, L. Steele, Neural alterations from lead exposure in zebrafish, Neurotoxicol. Teratol. 46 (2014) 40-48.
8. ALVES, L. C., and WOOD, C. M. 2006. The chronic effects of dietary lead in freshwater juvenile rainbow trout (Oncorhynchus mykiss) fed elevated calcium diets. Aquatic toxicology, vol. 78(3), pp. 217-232.
9. AN LOON, J.C., 1980. Analyttical atomic absorption spectroscopy. Selected methods. Academic press, New York, USA. pp.337.

10. ATLI, G., and CANLI, M. 2007. Enzymatic responses to metal exposures in a freshwater fish Oreochromis niloticus. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, vol. 145(2), pp.282-287.
11. AYOOLA, S. O., and ALAJABO, O. T. 2012. Acute toxicity and histopathological effects of engine oil on Sarotherodon melanotheron (Black Jaw Tilapia). American-Eurasian Journal of Toxicological Sciences, vol. 4(1), pp. 48-55.
12. BARATA, C., VARO, I., NAVARRO, J. C., ARUN, S., and PORTE, C. (2005). Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran Daphnia magna exposed to redox cycling compounds. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, vol. 140 (2), pp.175-186.
13. BELMADANI, A., TRAMU, G., BETBEDER, A. M., STEYN, P. S., AND CREPPY, E. E. 1998. Regional selectivity to ochratoxin A, distribution and cytotoxicity in rat brain. Archives of toxicology, vol.72, pp.656-662.3.
14. BERNET, D., SCHMIDT, H., MEIER, W., BURKHARDT-HOLM, P., & WAHLI, T. 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. Journal of fish diseases, vol.22 (1), pp.25-34.
15. BHARATH, S. T., DAHIYA, S., SAUNDERS, A., & SRINIVASAN, A. 2011. Lending relationships and loan contract terms. The Review of Financial Studies, vol. 24(4), pp.1141-1203.
16. BLAHOVA J, MODRA H, SEVEIKOVA M, MARSALEK P, ZELNICKOVA L, SKORIC.(2014). Metal Evaluation of biochemical, haematologial and histopathologial responses and recovery ability of common carp (Cyprinus carpio) after exposure to atrazine herbicide. *Biomed Res Intl*. 20(14):1-8.
17. BURGER, J., and GOCHFELD, M. (2005). Heavy metals in commercial fish in New Jersey. *Environmental research*, *99*(3), 403-412.
18. CAMUSSO, M., VIGANO, L.,and BALESTRINI, R., 1995. Bioconcentration of trace metals in rainbow trout: a field study. Ecotoxicology and Environmental Safety, vol.31 (2), pp.133-141.
19. CANLI, M., and ATLI, G. (2003). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environmental pollution*, *121*(1), 129-136.
20. CASTRO-GONZÁLEZ, M. I., and MÉNDEZ-ARMENTA, M. (2008). Heavy metals: Implications associated to fish consumption. *Environmental toxicology and pharmacology*, *26*(3), 263-271.
21. DU PREEZ AND G. STEYN., 1992. "A preliminary investigation of the concentration of selected metals in the tissues and organs of the tigerfish (Hydrocynus vittatus) from the Olifants River, Kruger National Park, South Africa," Water SA, vol. 18, no. 2, pp. 131–136.
22. DURAL, M., GÖKSU, M. Z. L., and ÖZAK, A. A., 2007. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. Food chemistry, vol.102, no.1, pp. 415-421..
23. EISLER, R. (1993). Zinc hazard to fish, wildlife, and invertebrates: a synoptic review. *Contaminant Hazard Reviews*, *10*.
24. ERDORUL, O., and ERBILIR, F., 2007. Heavy metal and trace elements in various fish samples from Sir Dam Lake, Kahramanmaras, Turkey. Environmental monitoring and assessment, vol.130, no. 1-3, pp. 373.
25. EZEMONYE, L. I., ADEBAYO, P. O., ENUNEKU, A. A., TONGO, I., and OGBOMIDA, E., 2019. Potential health risk consequences of heavy metal concentrations in surface water, shrimp (Macrobrachium macrobrachion) and fish (Brycinus longipinnis) from Benin River, Nigeria. Toxicology reports, vol. 6, pp. 1-9.
26. FEREIDOUN, H., NOURDDIN, M. S., RREZA, N. A., MOHSEN, A., AHMAD, R., and POURIA, H. (2007). The effect of long-term exposure to particulate pollution on the lung function of Teheranian and Zanjanian students. *Pakistan Journal of physiology*, *3*(2).
27. FRANCES, J., ALLAN, G. L., and NOWAK, B. F., 1998. The effects of nitrite on the short-term growth of silver perch (Bidyanus bidyanus). Aquaculture, vol.163 (1-2), pp. 63-72.

28. GILL, T. S., and PANT, J. C., 1987. Hematological and pathological effects of chromium toxicosis in the freshwater fish, Barbus conchonius Ham. Water, Air, and Soil Pollution, vol.35, pp.241-250.
29. GITAU, B. M. (2020). occupation health risks associated with garbage recycling at dandora dumpsite, nairobi county, kenya (*doctoral dissertation, school of pure and applied sciences, kenyatta university*).
30. GROSS, S. B., PFITZER, E. A., YEAGER, D. W., and KEHOE, R. A., 1975. Lead in human tissues. Toxicology and Applied Pharmacology, vol.32 (3), pp.638-651.
31. HADI, A. A., and ALWAN, S. F. (2012). Histopathological changes in gills, liver and kidney of fresh water fish, Tilapia zillii, exposed to aluminum. *International Journal of Pharmacy & Life Sciences*, *3*(11).
32. HEATH, A. G. (2018). *Water pollution and fish physiology*. CRC press.
33. HEMALATHA, S., and BANERJEE, T. K., 1997). Histopathological analysis of sublethal toxicity of zinc chloride to the respiratory organs of the airbreathing catfish Heteropneustes fossilis (Bloch). Biological Research, vol. 30, pp.11-22.
34. JÄRUP, L. (2003). Hazards of heavy metal contamination. *British medical bulletin*, *68*(1), 167-182.
35. JIRAUNGKOORSKUL, W., KOSAI, P., SAHAPHONG, S., KIRTPUTRA, P., CHAWLAB, J., and CHARUCHAROEN, S., 2007. Evaluation of micronucleus test's sensitivity in freshwater fish species. Research Journal of Environmental Sciences, vol.1 (2), pp.56-63.
36. JOSHI, S. A., and TSAI, L. W. (2002). Jacobian analysis of limited-DOF parallel manipulators. *J. Mech. Des.*, *124*(2), 254-258.
37. KAMARUZZAMAN, B. Y., RINA, Z., JOHN, B. A., and JALAL, K. C. A. (2011). Heavy metal accumulation in commercially important fishes of South West Malaysian coast. *Research Journal of Environmental Sciences*, *5*(6), 595-602.
38. KAMRAN M, RAZZAQ H, NOORULLAH M, AHMAD M, ZUBERI A (2023) Comparative analysis of genetic diversity, growth performance, disease resistance and expression of growth and immune related genes among fve diferent stocks of Labeo rohita (Hamilton, 1822). Aquaculture 739277:1–13
39. KAMRAN M, YAQUB A, MALKANI N, ANJUM KM, AWAN MN, PAKNEJAD H (2020) Identifcation and phylogenetic analysis of Channa species from riverine system of Pakistan using COI gene as a DNA barcoding marker. J Bioresour Manag 7(2):10
40. KOUSAR, S., and JAVED, M. (2014). Heavy metals toxicity and bioaccumulation patterns in the body organs of four fresh water fish species. *Pak. Vet. J*, *34*(2), 161-164.
41. MALLATT, J., 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Canadian Journal of Fisheries and Aquatic Sciences, vol.42(4), pp.630-648.
42. MARY, S. C. H., BHUVANESWARI, D., and ANANDAN, R., 2015. Biochemical and histopathological studies on lead nitrate induced toxicity in fresh water fish grass carp (Ctenopharyngodon Idella).
43. MASTAN, S. A. (2014). Heavy metals concentration in various tissues of two freshwater fishes, Labeo rohita and Channa striatus. *African Journal of Environmental Science and Technology*, *8*(2), 166-170.
44. MUNSHI, J. S., and DUTTA, H. M. (1996). Fish morphology: Horizon of new research.
45. Murtaza MUH, Zuberi A, Ahmad M, Amir I, Kamran M, Ahmad M (2020) Infuence of early rearing environment on water-borne cortisol and expression of stress-related genes in grass carp (Ctenopharyngodon idella). Mol Biol Rep 47:5051–5060
46. NAKAGAWA, H., NAKAGAWA, K., and SATO, T., 1995. Evaluation of erythrocyte 5-aminolevulinic acid dehydratase activity in the blood of carp Cyprinus carpio as an indicator in fish with water lead pollution. Fisheries science, vol. 61(1), pp. 91-95.
47. NRIAGU, J. O. and PACYNA, J. M. (1988). Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *nature*, *333*(6169), 134-139.

48. ORGANIZATION FOR ECONOMIC, OECD Guidelines for the Testing of Chemicals, Organization for Economic, Paris, France, 1994.
49. RAHMAN, M. S., MOLLA, A. H., SAHA, N. and HMAN, A. (2012). Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. *Food chemistry*, *134*(4), 1847-1854.
50. RAHMAN, M. S., MOLLA, A. H., SAHA, N., and RAHMAN, A. (2012). Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. *Food chemistry*, *134*(4), 1847-1854.
51. RAUF, A., JAVED, M. and UBAIDULLAH, M. (2009). heavy metal levels in three major carps (catla catla, labeo rohita and cirrhina mrigala) from the river ravi, pakistan. *Pakistan Veterinary Journal*, *29*(1).
52. ROGANOVIC-ZAFIROVA, D. R., JORDANOVA, M., PANOV, S. and VELKOVA-JORDANOVSKA, L. (2003). Hepatic capillariasis in the Mediterranean barbel (Barbus meridionalis petenyi Heck.) from Lake Ohrid.
53. RUIZ-PICOS, R., and LÓPEZ-LÓPEZ, E., 2012. Gill and liver histopathology in Goodea atripinnis Jordan, related to oxidative stress in Yuriria lake, Mexico. International Journal of Morphology, vol.30 (3), pp.1139-1149.
54. SANTOS, M. A., and HALL, A.,1990. Influence of inorganic lead on the biochemical blood composition of the eel, Anguilla anguilla L. Ecotoxicology and environmental safety, vol.20(1), pp.7-9.
55. SARKAR, T., ALAM, M. M., PARVIN, N., FARDOUS, Z., CHOWDHURY, A. Z., HOSSAIN, S. and BISWAS, N., 2016. Assessment of heavy metals contamination and human health risk in shrimp collected from different farms and rivers at Khulna-Satkhira region, Bangladesh. Toxicology reports, vol. 3, pp.346-350.
56. SCHELL, L. M., GALLO, M. V., DENHAM, M., and RAVENSCROFT, j. (2006). Effects of pollution on human growth and development: an introduction. *Journal of physiological anthropology*, *25*(1), 103-112.
57. SHAH, N., KHAN, A., ALI, R., MARIMUTHU, K., UDDIN, M. N., RIZWAN, M., and KHISROON, M. (2020). Monitoring bioaccumulation (in gills and muscle tissues), hematology, and genotoxic alteration in Ctenopharyngodon idella exposed to selected heavy metals. *BioMed research international*, *2020*.
58. SHAH, S. L., 2006. Hematological parameters in tench Tinca tinca after short term exposure to lead. Journal of Applied Toxicology: An International Journal, vol.26 (3), pp.223-228.
59. SIOEN, I., DE HENAUW, S., VERDONCK, F., VAN THUYNE, N., and VAN CAMP, J. (2007). Development of a nutrient database and distributions for use in a probabilistic risk–benefit analysis of human seafood consumption. *Journal of Food Composition and Analysis*, *20*(8), 662-670.
60. SONG, S. B., XU, Y., and ZHOU, B. S., 2006. Effects of hexachlorobenzene on antioxidant status of liver and brain of common carp (Cyprinus carpio). Chemosphere, vol.65, no.4, pp. 699-706.
61. STANLEY, O. N., and OMEREBELE, U. A., 2010. Changes in the hematological parameters of Clarias gariepinus exposed to lead poisoning. Journal of Fsheries International, vol.5 (4), pp.72-76.
62. STORELLI, M. M., BARONE, G., STORELLI, A., and MARCOTRIGIANO, G. O., 2006. Trace metals in tissues of Mugilids (Mugil auratus, Mugil capito, and Mugil labrosus) from the Mediterranean Sea. Bulletin of environmental contamination and toxicology, vol.77, no.1, pp.43-50.
63. TAWARI-FUFEYIN, P., IGETEI, J., and OKOIDIGUN, M. E., 2008. Changes in the catfish (Clarias gariepinus) exposed to acute cadmium and lead poisoning. Bioscience Research Communications, vol.20 (5), pp.271-276.

64. TULABY DEZFULY, Z., ARAMOON, A., ALISHAHI, M., HALIMI, M., & RAHNAMA, R., 2020. The effects of lead toxicity on the hematological parameters common carp (Cyprinus carpio) at varying salinity levels. Iranian Journal of Toxicology, vol.14, no.1, pp. 1-8.
65. VELKOVA-JORDANOSKA, L. I. D. I. J. A., and KOSTOSKI, G. (2005). Histopathological analysis of liver in fish (Barbus meridionalis petenyi Heckel) in reservoir Trebeništa. *Natura Croatica: Periodicum Musei Historiae Naturalis Croatici*, *14*(2), 147-153.
66. VINODHINI, R., and NARAYANAN, M. (2008). Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp). *International Journal of Environmental Science & Technology*, *5*, 179-182.
67. VUTUKURU, S. S., 2005. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, Labeo rohita. International Journal of environmental Research and Public health, vol. 2, no.3, pp. 456-462.
68. WANG, L., XU, T., LEI, W. W., LIU, D. M., LI, Y. J., XUAN, R. J., and MA, J. J.,2011. Cadmium-induced oxidative stress and apoptotic changes in the testis of freshwater crab, Sinopotamon henanense. PLoS One, vol.6, no.11, e27853.
69. WENDELAAR BONGA, S. E., and LOCK, R. A. C., 1992. Toxicants and osmoregulation in fish.
70. Yaqub A, Awan MN, Kamran M, Majeed I (2021) Evaluation of potential applications of dietary probiotic (Bacillus licheniformis SB3086): efect on growth, digestive enzyme activity, hematological, biochemical, and immune response of Tilapia (Oreochromis mossambicus). Turk J Fish Aquat Sci 22(5):1–12
71. YILMAZ, A. B., SANGÜN, M. K., YAĞLIOĞLU, D., and TURAN, C. (2010). Metals (major, essential to non-essential) composition of the different tissues of three demersal fish species from Iskenderun Bay, Turkey. *Food chemistry*, *123*(2), 410-415.
72. YILMAZ, F., ÖZDEMIR, N., DEMIRAK, A., and TUNA, A. L. (2007). Heavy metal levels in two fish species Leuciscus cephalus and Lepomis gibbosus. *Food chemistry*, *100*(2), 830-835.
73. YOUSAFZAI, A. M. and SHAKOORI, A. R., 2006. Bioaccumulation of chromium, nickle, lead, copper and zinc in the skin of Tor putitora as an indicator of the presence of heavy metal load in River Kabul, Pakistan. Pakistan Journal of zoology, vol.38 ,no.4, pp. 341.
74. YOUSAFZAI, A. M., CHIVERS, D. P., KHAN, A. R., AHMAD, I., and SIRAJ, M., 2010. Comparison of heavy metals burden in two freshwater fishes Wallago attu and Labeo dyocheilus with regard to their feeding habits in natural ecosystem. Pakistan Journal of Zoology, vol. 42, no.5.
75. ZHANG, W., and WANG, W. X. (2012). Large-scale spatial and interspecies differences in trace elements and stable isotopes in marine wild fish from Chinese waters. *Journal of hazardous materials*, *215*, 65-74.
76. ZHAO, S., FENG, C., QUAN, W., CHEN, X., NIU, J., and SHEN, Z. (2012). Role of living environments in the accumulation characteristics of heavy metals in fishes and crabs in the Yangtze River Estuary, China. *Marine pollution bulletin*, *64*(6), 1163-1171.U.S Fish Wildl. Serv. Biol. Rep. 85 (1985) 1-30.
**77.** ZIRONG, X., and SHIJUN, B., 2007. Effects of waterborne Cd exposure on glutathione metabolism in Nile tilapia (Oreochromis niloticus) liver. Ecotoxicology and environmental safety, vol.67, no.1, pp.89-94.