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LEAD ACCUMULATION EFFECTS ON LIVER AND GILL TISSUES OF *RUTILUS RUTILUS CASPIUS*

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ABSTRACT

Lead (Pb) is the most destructive environmental pollutants with extremely undesirable impacts on tissues of living beings. The present study investigates the physiological and morphological changes in liver and gill tissues after being exposed to Pb in different time periods and different concentrations. The specimens have been placed in aquaria containing 0.1, 0.2 and 0.4mg l⁻¹ of Pb at different exposure times (46, 96, 144, and 168 hrs). Then, to specify the histopathological effects, the fishes have been taken from the experimental tanks and their gill and liver tissues have been sampled. As the findings show, histopathological changes in liver have entailed sinusoidal extension, vacuolization, hyperemia and hemorrhage, nucleus picnosis, hepatocyte necrosis, and hemosiderin accumulation of melano macrophage cells. Cell acidification, lymphocyte invasion, and focal necrosis have also been examined. Further, hyperemia, filaments and secondary lamellae, mucosal accumulation, telangiectasia, dysplasia pavement cells metaplasia into mucosal cells and gill mucosa, and distal hyperplasia of secondary lamellae destroy gill tissues. Moreover, increasing the concentrations of the contamination leads to intensifying damages because the minor histopathology occurs at 0.1mg l⁻¹ of Pb while by increasing the exposure time, levels of 0.1, 0.2 and 0.4mg l⁻¹ of Pb reveal major histopathological effects.

Keywords: *Pb, Rutilusrutiluscaspius, Liver Tissue, Gill Tissue*

INTRODUCTION

Detection of the metal pollutants such as lead and their serious effects in physiological mechanisms are considered as more important than toxicological problems.

Typically, industrial wastes discharged into the river basins and seas contain various Pb-compounds. Such water pollutant compounds cause different physiological problems in fishes` body and their death at a specific concentration.

The methodologies studying the metal exposure-induced alterations in tissues of aquatic organisms are treated as the most efficient way of specifying the level of aquatic environmental pollution and their biological effects. Currently, thousands of chemicals in fresh and salt water organisms are discharged into their habitats leading to harmful effects.

Due to many detrimental effects in aquatic ecosystems, it is highly necessary to investigate the impact levels of Pb on aquatic ecosystems. Pb is followed by undesirable effects on ecosystem characteristics including reproduction rate of the existing organisms, their distribution pattern and survival. Great amount of Pb characterized by mutagenic and carcinogenic impacts is entered into aquatic ecosystems through different ways.

Histopathological study refers to examining the environmental impact of contaminants on fish. Different types of contaminants cause specific histopathology in fish organs. These histopathologies can be used as bio-indicator to determine the presence of the contaminant in the natural ecosystems. Many researchers have investigated histopathological effects of Pb in different fish organs like liver, kidney, gills, olfactory epithelium, and spleen in the exposure of the pollutant. The present work has attempted to examine the effects of Pb in gill and liver tissues of *Rutilus*.

Mohammadzadeh *et al.*, (2009) investigated the responses to aluminum exposure to reveal sub lethal concentration of aluminum sulfate as a contaminant at acidic pH on gill tissues of *Rutilus*. Based on the

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study, they observed histopathological changes such as hypertrophy, hyperplasia, increased mucosal cells, hyperemia, hemorrhage, amorism, inflammation, and tissue necrosis.

Again, Mohammadzadeh *et al.*, (2009) examined histopathological changes in 20 fish specimens (1.5 to 2g) from Caspian Sea coastal areas after treating by aqueous mercury solution at different levels (0 50, 100 and 350ppb).

The observed histopathological changes in gills included acute necrosis and hyperplasia; vacuolization of hepatic cells, and glomerulus broadening in kidney tissue.

Arellano *et al.*, (1999) also investigated histopathological changes in liver and gill tissues of Sole Senegalese subjected to the copper solution.

He observed that compared to the control specimens, there were more oil droplets in liver tissue specimens of the fish after 7 day exposure to So_4Cu at $100\mu\text{g l}^{-1}$ as. Also, partial disruption in microvillus, endothelial and sinusoid were detected in treated liver specimens. As it was revealed by these works, there were histopathological changes even at concentrations below the sub lethal levels of heavy metals in aqueous environment.

Dolin (2005) studied histopathological effects of mercury methyl on newly-hatched fries of carp in Australia.

In this study, fries were subjected to mercury methyl at specific condition *in vitro*. The research findings confirmed the intense toxicity of mercury and its negative influence on protein production, growth level and mitosis cell divisions.

Moreover, Adams and Hinton (1997) concluded that bioaccumulation of cadmium in tissues of *Oreochromis niloticus* leads to the pathological changes in organs like liver, brain, nervous system, gills and skeletal systems.

As reported by other studies done by Saeedi Saravi *et al.*, (2009) on bioaccumulation of heavy metals in body organs of Cyprians carpio, metals were accumulated in gills and liver according to the following order: $\text{Cd} > \text{Pb} > \text{Ni}$. Bioaccumulation of lead and cadmium showed significant increase in carp organs.

MATERIALS AND METHODS

Methodology

The specimen includes 200 fishes provided from Ghare-Soo Fishery Research Station (Torkman Port in Caspian Sea Coasts).

The specimen was transferred to the laboratory, and then kept in aquaria equipped with fresh water for 7 days for adaptation.

Fishes were exposed to different concentration of Pb (0.1, 0.2, 0.4mg l^{-1}) and underwent a control treatment. The stress level in fishes was minimized by controlling all considerations.

The fishes were sampled for further examination from each aquarium after each time interval (48, 96, 144 and 168 hrs).

Liver and gill tissues were separated as soon as removing fishes at the end of each time interval. Next, the tissue specimens were fixed in the tubes of 10% formalin solution. 24 hrs later, the specimens were rinsed with fresh water and then kept in alcohol to be prepared for passaging; and the tissues were put into a passaging apparatus for dehydrating, clearing and impregnation. Then, the specimens were exposed to embedding and the tissues were cut into $6\mu\text{m}$ slices using a microtome (LEICA RM 2255). Finally, the specimens were investigated under a light microscope.

RESULTS AND DISCUSSION

As it was seen, the damages to gill and liver cells were intensified by increasing the concentrations of lead nitrate solution so that the least damage was occurred in the concentration of 0/1 mg/l. That is, the longer the exposure time was, the more detrimental effect would be observed.

As it was observed, there was no disturbance in control tissue specimens during 168 hrs exposure time. As shown in Figure 1, Nuclei were also distributed regularly in the tissue and sinusoid distance was normal.

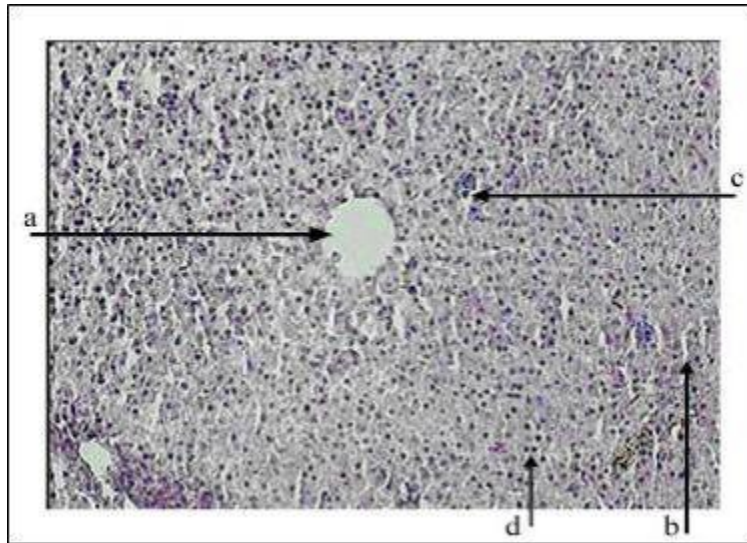


Figure (1): Microscopic Image from Liver Tissues in Control Samples (400x) A- Lobular Central Vein B-Sinusoid C-Nucleus D-Hepatocyte

According to the obtained results, the changes in liver tissues of treated specimens can be reported as follows:

- (1) Sinusoidal projection and hyperemia (Figure 2a, b, c)
- (2) Atrophy and necrosis of liver cells
- (3) Inflammation of liver tissue (lymphocyte invasion and inflammatory cells)
- (4) Hemosiderin deposition

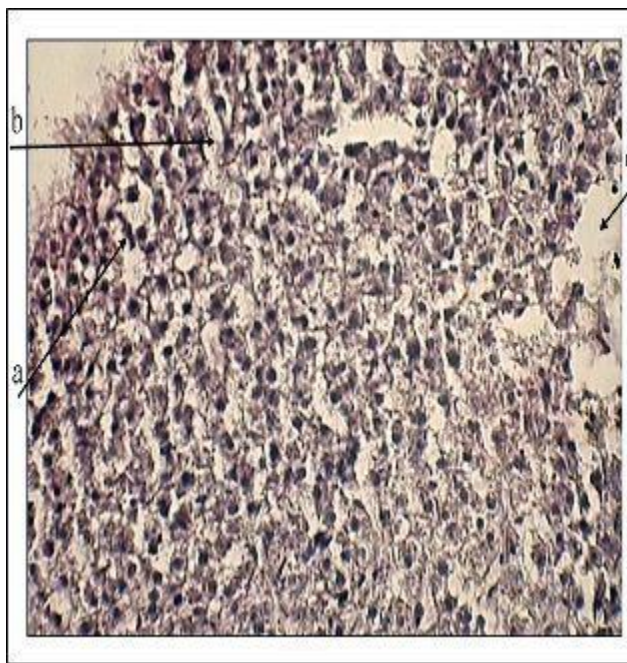


Figure 2(a): Liver Tissues of the Treated Samples by Pb Concentration of 0.4 Mg L⁻¹ during 48 Hrs Exposure Time (40x) A-Necrosis B- Sinusoidal Projection C- Focal Necrosis

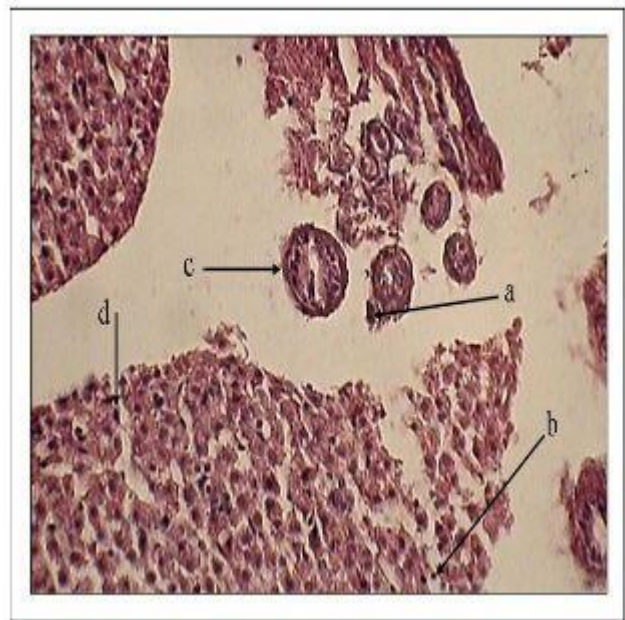


Figure 2(b): Liver Tissues of Treated Samples by Pb Concentration of 0.4 Mg L⁻¹ during 168 Hrs (40 Xs)

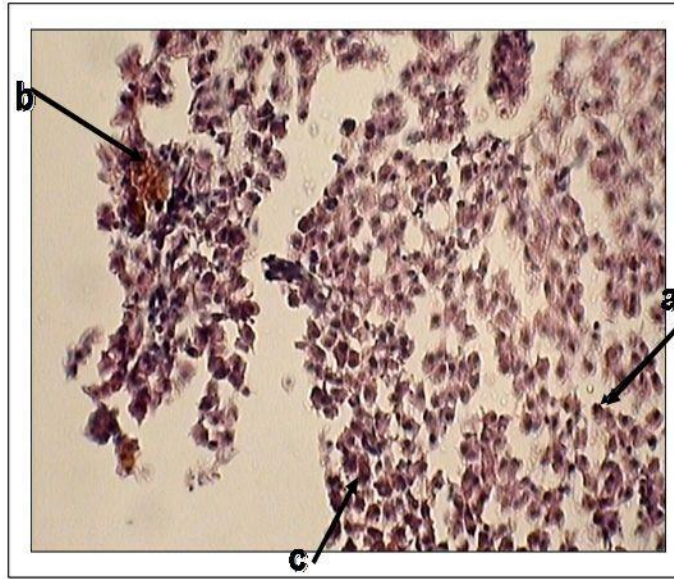


Figure 2(C): Liver Tissues of the Treated Samples by Pb Concentration of 0.4 Mg L⁻¹ during 168 Hrs (40x) A-Necrosis B- Hemosiderin Deposition C-Sinusoidal Attachment

As Figure 3 presents, no disturbance was seen in the results of changes in gill tissues of control samples during 168 hrs exposure time.



Figure 3: Microscopic Image of Gill Tissues in Control Samples at Normal Condition (10 Xs)

Shown in the following figures, the results of changes in liver tissues of treated samples Edema are as follows:

- 2) Hypertrophy and hyperplasia
- 3) Cell necrosis
- 4) Hyperemia
- 5) Hemorrhage and mucous accumulation
- 6) Fusion
- 7) EGCs (Eosinophilic Granular Cells)
- 8) Dysplasia and Metaplasia
- 9) Telangiectasia (Figure 4a, b, c, and d & Figure 5a, b)

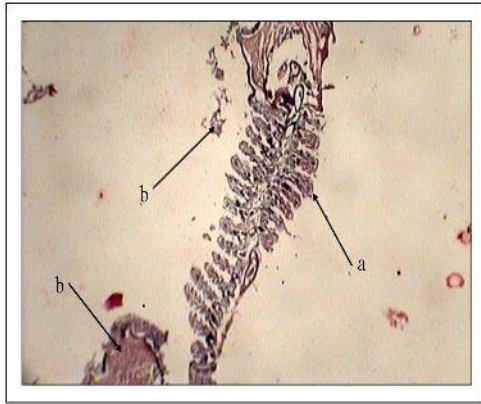


Figure 4a: Gill Tissues of the Treated Samples by 0.2 Mg L^{-1} of Pb Solution during 96 Hrs of Exposure A- Club-Shaped Of Distal Membrane B- Hemorrhage (40 Xs)

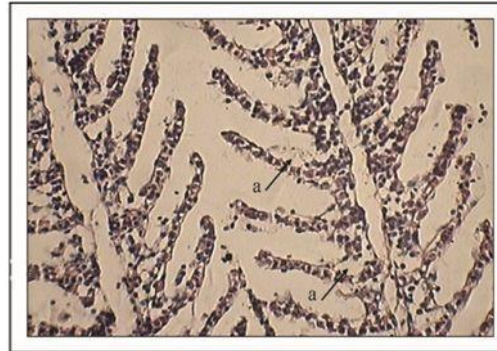


Figure 4(B): Gill Tissues of Treated Samples by 0.4 Mg L^{-1} during 168 Hrs of Exposure A- Edema (40 Xs)

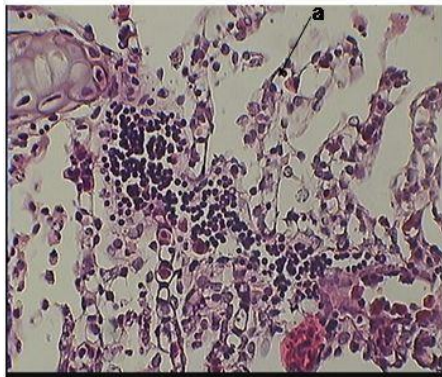


Figure 4(c): Gill Tissues of the Treated Samples by 0.4 mg l^{-1} during 148 hrs of Exposure a- Mucosal Cells (100 xs)

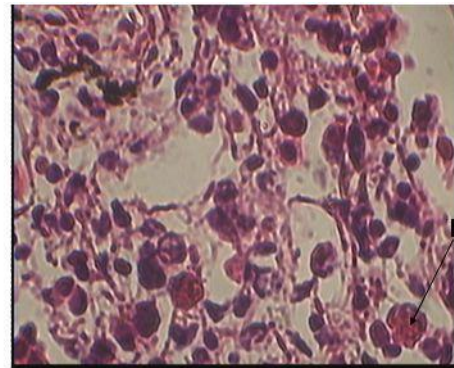


Figure 4(d): Gill Tissues of the Treated Samples by 0.4 mg l^{-1} during 148 hrs of Exposure a- Mucosal Cells (100 xs) b-EGCs (100X)

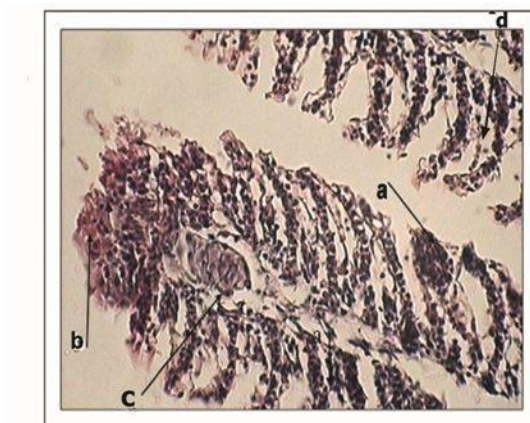


Figure 5(a): Gill Tissues of The Treated Samples by 0.4 Mg L^{-1} during 168 Hrs of Exposure A- Telangiectasia B- Mucous C- Chondrocytes (40 Xs) D-Edema



Figure 5(B) -Gill Tissues of the Treated Samples By 0.4 Mg L^{-1} during 168 Hrs of Exposure A- Hyperplasia and Hypertrophy B- Necrosis C- Broad Hemorrhage and the Invasion of Inflammatory Cells (10 Xs)

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Conclusion

Environmental pollution as well as metal pollution as its important part is one of the serious global problem. Industrial developments also lead to ecosystem pollution and contaminant distribution (Diagomanolin *et al.*, 2004). Certainly, lead contamination causes destructive and morphological changes. As confirmed by the findings of the present study, Pb concentration and exposure time are considered as the main factors affecting histopathological changes. Distinguishable changes were observed after 48 hrs by histopathological examinations of treated fish gill and liver tissues by 0.1 ppm of Pb content at 21°C. Also, damages were increased in treatments containing 0.4 and 0.2 ppm of Pb.

Based on the research findings, hyperemia of filament and secondary lamellae can be observable by the separation/isolation of the pavement cells from the basal membrane. Additionally, hypertrophy (swollen pavement cells), hyperplasia (increased number of pavement cells) fills the free space between two secondary lamellae, attaching together- called as fusion- disturbing oxygen exchange. Further, symptoms of hyperemia hemorrhage club-shaping of gill lamellae at distal membrane, the formation of Eosinophilic Granular Cells (EGCs) at distal membrane of secondary lamellae were detected demonstrating metal exposure-induced cells, telangiectasia splasia and metaphase of the pavement cells and their transformation into mucosal cells, pillar and chloride cell necrosis, vacuolization, granular cytoplasm inflammation of epithelial cells in filaments, lymphocyte invasion and cellular inflammation.

As reported by Saeedi Saravi *et al.*, (2009) investigating common carp fish subjected to a great amount of cobalt, cells in the middle section of each filament were destroyed in an unrecognized way. The surface edges of these cells were considerably deformed and revealed an increase of the cellular mucus. As concluded, the mucosa secretion is increased by the increase of toxicant concentration. The finding of the this study is consistent with the finding of the present paper indicating that the increased mucosal secretion from filament surfaces in *Rutilus* fishes was detectable, in particular after 168 hrs exposure to 0.4 mg l⁻¹.

The other morphological changes mostly observed in stress-exposed fish specimens were abnormal morphologic change of epithelial cells. Such finding has been implicated by dysplasia and metaplasia of the pavement cells (epithelial cells) to the mucosal cells in the present research.

Basically, it is important to examine morphological changes of fish gill tissues at various stressful conditions to discover the relation between these toxicant-induced changes and fish metabolism. The present study investigated the blockage of cell-irrigators, efferent capillaries, injuries of cell membrane, imbalance of ionic stability, disrupted cellular respiration, injuries to ATPs enzymatic activity, disturbance in cellular phosphorylation, ATP production, as well as disturbances in the synthesis of constructive and enzymatic proteins. The mentioned mechanisms with bimolecular effects in lead and consequently intercellular biochemical changes lead to the cellular morphological changes.

Investigating the toxic effects of cobalt on common carp gills revealed that mucosal secretion and the number of mucosal cells on gill to surface were increased by the increase of the cobalt level. Additionally, hypertrophic of the pavement cells and secondary lamellae, thickening (clubbing) of secondary lamellae, intensive fusion in secondary lamellae along with hyperemia, capillary projection and hemorrhage were also detectable in common carp gills. Notably, gill damages also involved hyperplasia of epithelial cells in primary and secondary lamellae. Higher cobalt level intensified hyperplasia of lamellae attached capillaries to each other which are placed among hyperplasia pavement leading to hypoxia and disturbances in blood circulation in the fish gills and consequently the increase of blood pressure. Finally, cellular hypertrophy and increased concentration of mucous reducing moco polysaccharides characteristics of mucous led to the focal fusion of secondary lamellae.

The findings of the present research investigating the lead accumulation in liver tissue of *Rutilus* and its detrimental effects are consistent with previously reported results. The recently done studies have confirmed the use of liver tissue as an indicator to evaluate heavy metal pollution, in particular at higher levels in aqueous ecosystems. For instance, Olojoo *et al.*, (2005) examining histopathological effects of lead in *Clarias* reported destructions in liver and gill tissues at different concentrations of heavy metals.

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In another study done by Staniskiene (2006) exploring spatial distribution of heavy metals throughout the whole body of fresh water fish, liver showed the highest level of metals accumulation.

Demtrioveraldola *et al.*, (2006) also studied liver histopathology induced by increasing mercury level in freshwater fishes taken from the natural environment in northeastern parts of Spain. They observed the most histopathological effects of mercury in liver tissues.

In the present study, the results of the symptoms on liver tissues of *Rutilus* revealed hemorrhage at all necrosis stages, necrosis (integrated distribution of cytoplasm in necrosis cells, small-sized nucleus surrounded by concentrated chromatin), atrophy (decreased cell size against its normal size), hyperemia (in small veins and hepatocyte sinusoids), lymphocyte invasion (the presence of inflammatory cells), haemosidrin deposition in melano macrophage cells around biliary ducts, increased sinusoid distance, enhanced nuclear density, and focal necrosis. This finding is consistent with the conclusion driven by Mohammadzadeh Barani *et al.*, (2009) examining mercury influences on liver and kidney tissues of *Hoplismalabaricus* revealing much more injuries like as necrosis, atrophy and hemorrhage. It is also consistent with the finding of Yannick *et al.*, (2008) studying Mercury-exposed Zebra fishes showed muscular injuries including necrosis and atrophy.

Park (2006) also investigated the toxicant effects of Zn and Cd on liver tissue of a fresh water fish in South Africa. He observed similar histopathological changes in metal-exposed liver specimens including necrosis of liver hepatocyte cells, hyperemia and hemorrhage (increased blood density in capillaries), the presence of lymphocyte cells (inflammatory cells), and vacuolization of hepatocyte cells.

Saeedi Saravi *et al.*, (2009) also compared the bioaccumulation of Cd, Cu, and Pb in muscular and liver tissues of the healthy and ill fish specimens. The obtained results showed a significant relation between metal accumulation and histopathological status of the fish.

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