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Research Article

Toxicity, Histological, Hepato-Somatic Index and Growth Responses of Cadmium Stressed *Labeo rohita*

Sumaira Fiaz[1*], Huma Naz[1*], Tanveer Ahmed[2*], Iqra Zulfiqar[1], Khalid Mehmood[1], Muhammad Jabbar[1], Syed Qaswar Ali Shah[1], Muhammad Usman[4]

[1]*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan*
[2]*Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan, Pakistan*
[3]*Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan.*

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SF executed the research work. HN planned the research work. TA, IZ, KM and MJ equally contribute in writing article. SQAS and MU worked as member of supervisory committe.

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Acute, Chronic, Toxicity, Metals, FCR, Liver

Abstract | The concentration of heavy metals accelerated every day in aquatic surrounding causing serious health problem in fish and ultimately in human. So, current study was planned to evaluate effects of cadmium on the growth performance, liver histology and hepato-somatic index of *Labeo rohita*. The test was conducted in two phases. In the primary phase, the 96-hr LC_{50} and lethal concentration of water borne cadmium for fish *L. rohita* were determined as 12.38mgL^{-1} and 23.6mgL^{-1}, respectively. In secondary phase, fish was subjected to sub-lethal ($1/3^{rd}$ of LC_{50}) value of cadmium for 60-days. Result showed significant decrease in daily weight gain, daily length gain, condition factor, specific growth rate and feed conversion ratio of Cd-treated fish as compared to control fish. In liver, the diameter of hepatocytes was significantly lesser in Cd-treated fish in comparison with control fish. The width of sinusoids in Cd-treated fish was significantly greater in comparison with control fish. Some other histological disorders were also seen in liver of Cd-treated fish viz. infiltration of sinusoid, dilation and increased hemorrhage in sinusoids, inflammation and vacuolation in hepatocytes and ruptured hepatocytes after 60-days of experimental period. Hepatosomatic index was significantly less in Cd-treated fish than control fish throughout the experimental period. Addressing this problem requires a coordinated effort among governments, industries, and individuals to reduce heavy metal pollution, protect aquatic ecosystems, and safeguard human health.

Novelty Statement | The study is novel for fish farming as it finds out that cadmium has clear negative effects on the growth performance, liver histology and hepato-somatic index of fish.

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Introduction

These days, heavy metals pollution is a worldwide issue due to continuous accumulation and persistence of metallic pollution in aquatic environment (Hyun *et al.*, 2006). In the aquatic biota, significant consideration of heavy metal is due to their amassing tendency and severe toxicity. Both the ecological equilibrium of the receiver environment and the variety of water creatures may be severely impacted by heavy metals (Javed, 2004).

Fish are the only animals that are unable to escape the negative consequences of metal contamination in water

Corresponding authors: Sumaira Fiaz, Huma Naz and Tanveer Ahmed
dr.humanaz98@gmail.com, tanvirahmeduaf@gmail.com

(Farombi *et al.*, 2007; Vosyliene and Jankaite, 2006). Fish are able to quickly absorb dissolved metals, which may be used to determine how polluted an area is. By absorbing and metabolic activities as the storage metals may accumulate in the tissues of fish (Farkas *et al.*, 2002; Shukla *et al.*, 2007). Cadmium is considered among toxic heavy metals. To all living beings, it is a non-essential component. Cadmium contamination of aquatic habitats has expanded dramatically in recent decades, leading to an increase in Cd bioaccumulation in aquatic organisms across the food chain (Okocha and Adedeji, 2011).

Growth is a simple metric that can account for all of the effects within the fish. If it is primarily intended to know that in what way a toxicant will affect fish growth then it is important to know how loss of appetite occurs due to metallic toxicity (Javed and Saeed, 2010). Cadmium may also alter the growth of fish (Heydarnejad *et al.*, 2013). Chronic exposure to Cd may cause sublethal effects in fish such as decreased growth that is more prominent when Cd concentration exceed from 3µgL^{-1} in fresh water (Levit, 2010). Altered behavior and slow growth rate were observed in fish at high dose of dietary cadmium after long time exposure (Nogami *et al.*, 2000).

Studies on fish's histopathology can help determine how toxicants affect the fish (Ahmed *et al.*, 2015; Pimpao *et al.*, 2007), and has been used widely in both field studies and in laboratory as a biomarker in the assessment of fish health (Thophon *et al.*, 2003). For critical function of body such as accumulation and biotransformation of chemicals in fish, liver is considered a vital organ of body (Gernhofer *et al.*, 2001). In heavy metal pollution, liver has been known as the storage site of cadmium (Gbem *et al.*, 2001). Cadmium can cause observed effects such as physiological dysfunction and morphological disruption in exposed organ (Satarug *et al.*, 2010; Huo *et al.*, 2017).

The hepatosomatic index (HSI) is utilized in fisheries research as a measure of the liver's ability to store and release energy reserves. Hepatosomatic index is the percentage of weight of fish liver to total weight of fish that offers information on the fish's health and water quality (Pandit and Gupta, 2019; Dane and Sisman, 2020). In field and laboratory trials, the HSI has proven to be effective. The HSI had only been utilized by a few fish toxicologists as a fish health biomarker in connection to pollution (Javed and Usmani, 2017). Therefore, this study was designed to estimate the growth performance, liver histology and hepatosomatic index of *L. rohita* exposed to Cd.

Materials and Methods

Fish collection and acclimatization

The 150 days old *Labeo rohita* was collected from Fisheries Research and Training Complex, Bahawalpur. Fish was brought to Fisheries Research Laboratory at Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan in plastic bags and handled with proper care. Fish was adjusted to laboratory conditions for 14 days before starting a trial. Water medium was refreshed every 24 h to eliminate feeding debris and fecal matter.

Acute toxicity test

The 96-h LC_{50} and lethal concentration of cadmium for *L. rohita* were checked by carrying out the acute toxicity bioassay tests. Stock solutions (1000 mg/L) of Cd were prepared by mixing $Cdcl_2$ in 1L distilled water. Cadmium concentration in each aquarium was gradually increased by 0, 2.5, 7.5, 12.5, 17.5 and 20mgL^{-1}. The LC_{50} was the Cd concentration at which 50% of fishes were died and the concentration at which 100% fishes were dead was lethal concentration.

Growth determination

After determination of 96-h $LC_{50,}$ fish was exposed to cadmium for 60 days at 1/3rd of LC_{50} value. Just before exposure, the length and weight of *L. rohita* were measured. To measure the growth, daily weight gain (DWG), daily length gain (DLG), condition factor, specific growth rate (SGR) and feed conversion ratio (FCR) was measured on a weekly basis throughout the experimental period.

$$\text{Daily weight gain} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Day}}$$

$$\text{Daily length gain} = \frac{\text{Final length} - \text{Initial length}}{\text{Day}}$$

$$\text{Specific growth rate} = \frac{(\text{In Final Weight} - \text{In Initial Weight})}{\text{Expermintal period in day}} \times 100$$

$$\text{Condition factor} = \frac{\text{Weight (g)}}{\text{Length(cm)}^3} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Quantity of feed given}}{\text{Weight gain}}$$

Liver histology

Liver tissues were isolated from both experimental and control fish. Samples were preserved in a 10 percent neutral-buffering formalin solution then dehydrated and followed by paraffin wax embedding. For histological analysis, the 5µm thick slices were cut, stained with hematoxylin-eosin, and observed under a light microscope. as described by Bancroft *et al.* (2013). The diameter of the hepatocytes (50 hepatocytes per slide) and width of sinusoids (20 sinusoids per slide) were measured at random (Kasperk *et al.*, 1994).

Hepato-somatic index (HSI)

To examine the health state of fish, HSI was calculated. By using the method of (Rajaguru, 1992) HSI was calculated by following formula:

$$HSI = \frac{\text{liver weight}}{\text{total weight}} \times 100$$

Statistical analyses

Probit analysis was implemented to find out the 96-h

LC_{50} and lethal concentration of cadmium for *L. rohita*. Data of growth performance and hepatosomatic index was analyzed statistically by One-way analysis of variance (ANOVA) through software program Statistix 8.1 version. While for histological examination of liver Tukey test was used through software program SPSS to check the effect of treatment. Histological observations were made on Optika microscope and image J software was used for histomorphometry.

Results and Discussion

Acute toxicity

The 96-h LC_{50} and lethal concentration of *L. rohita* were determined through acute toxicity bioassay as 12.38mgL^{-1} and 23.6mgL^{-1}, respectively. The lower and upper limits for LC_{50} were 9.95 and 14.48, respectively. The lower and upper limits for lethal value were 20.26 and 31.01, respectively. Figure 1 shows the fix mortality against cadmium metal concentration. Figure 2 shows the probability plot for 96-h LC_{50} and lethal value of Cd for *L. rohita*.

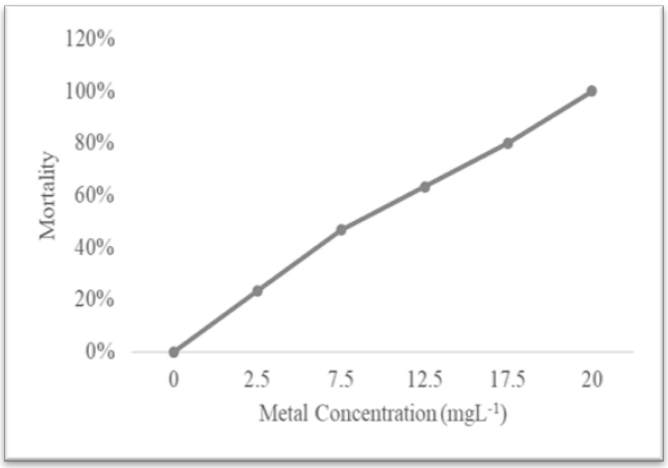

Figure 1: Mortality of *L. rohita* during acute toxicity bioassay for Cd.

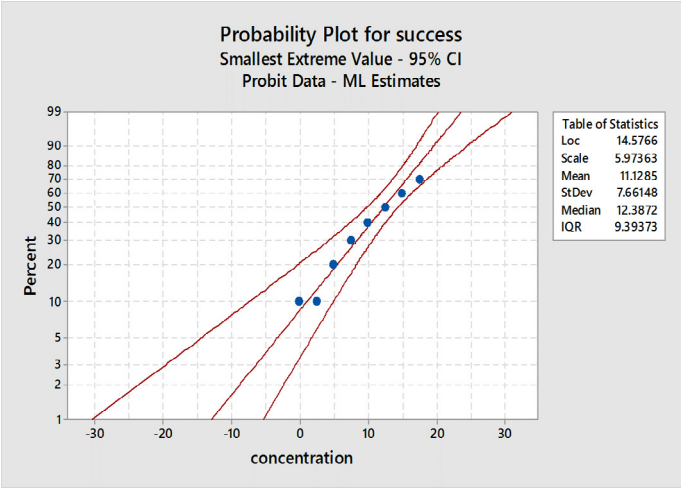

Figure 2: Probability plot for 96-hr LC_{50} and lethal value of Cd for *L. rohita*.

Growth performance

The growth performance of both the treated and control fish group fluctuate significantly during the trial period of 60 days. All the growth parameters daily weight gain, daily length gain (standard length, fork length and total length), condition factor, feed conservation ratio and specific growth rate were significantly lesser in Cd-treated fish as compared to control fish.

Table 1: Growth performance of control and Cd treated *L. rohita*.

Parameters	Control	Cd treated
Daily weight gain	1.23±0.68[a]	0.03±0.04[b]
Standard length gain	0.04±0.00[a]	0.006±0.00[b]
Fork length gain	0.03±0.00[a]	0.006±0.005[b]
Total length gain	0.04±0.009[a]	0.01±0.012[b]
Specific growth rate	1.95±1.61[a]	0.02±0.07[b]
Condition factor	2.53±0.43[a]	1.43±0.03[b]
Feed conservation ratio	1.23±0.09[a]	-0.02±0.22[b]

Means with similar letters in a single row are statistically similar at $p<0.05$.

Histology of liver

The histomorphometric parameters, diameter of hepatocytes in Cd-treated fish was significantly less in comparison with control fish (Table 2, Figures 3-4). The width of sinusoids in Cd-treated fish was significantly greater in comparison with control fish (Table 2, Figures 5-6). Some other histological disorders *viz*. infiltration in sinusoids, congestion in sinusoids, increased dilation in sinusoids (Figure 7) vacuolation in hepatocytes, inflammation in hepatocytes and abnormal shape of hepatocytes (Figure 8).

Table 2: Morphological parameters of control and Cd-treated *L. rohita*.

Parameters	Control	Cd-treated
Diameter of hepatocytes	10.95±0.18[a]	8.64±0.23[b]
Width of sinusoids	5.91±0.41[b]	8.44±0.04[a]

MEAN± SEM: In the row, superscripts show values are significantly different ($p< 0.05$).

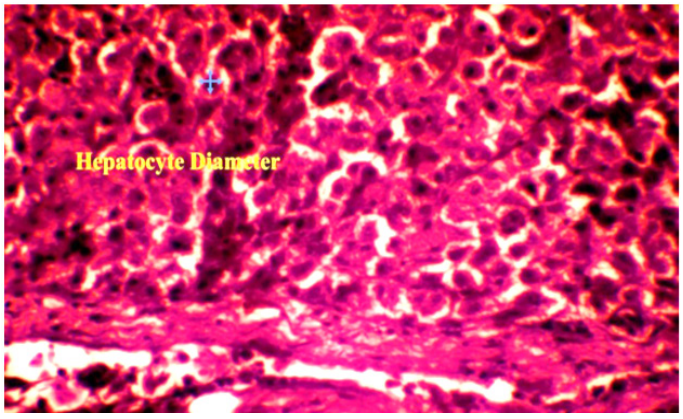

Figure 3: Diameter of Hepatocytes of control *L. rohita*.

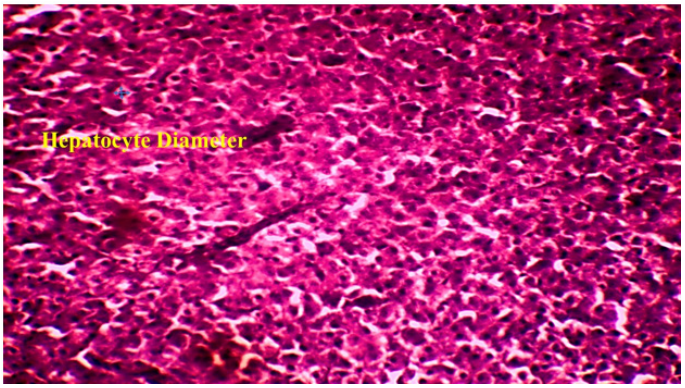

Figure 4: Diameter of Hepatocytes of Cd-treated *L. rohita*.

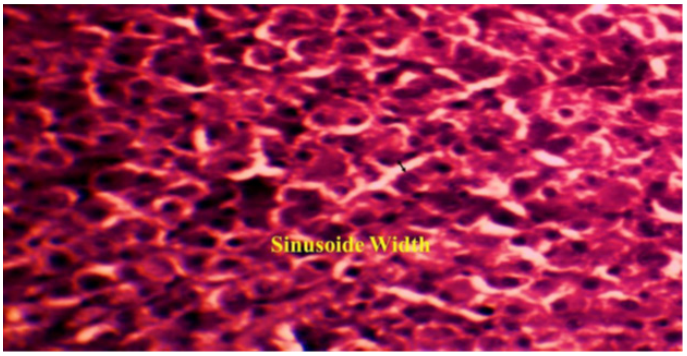

Figure 5: Width of sinusoids of control *L. rohita*.

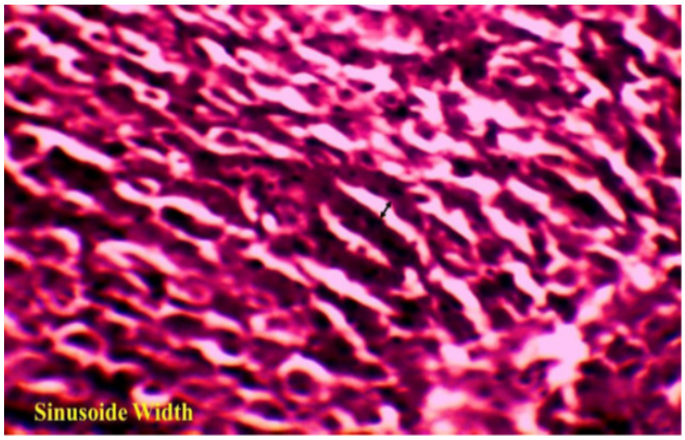

Figure 6: Width of sinusoids of Cd-treated *L. rohita*.

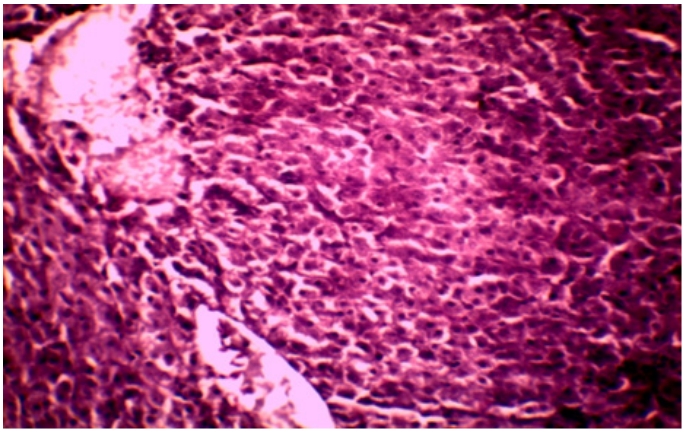

Figure 7: Infiltration of sinusoid, dilated sinusoid and increased hemorrhage in sinusoids in Cd-treated *L. rohita*.

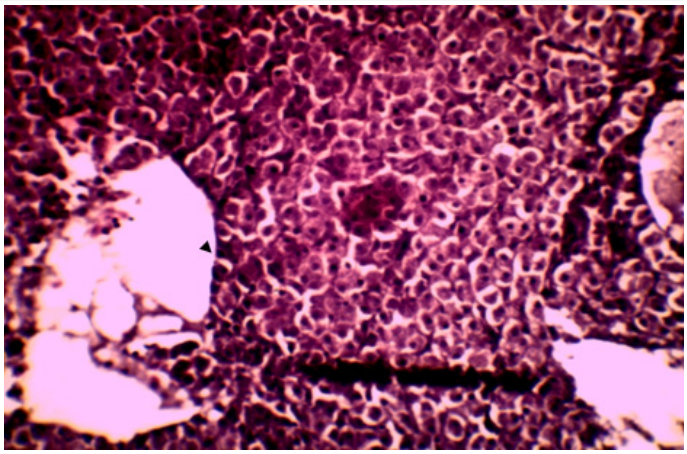

Figure 8: Inflammation, vacuolation in hepatocytes and ruptured hepatocytes in Cd-treated *L. rohita*.

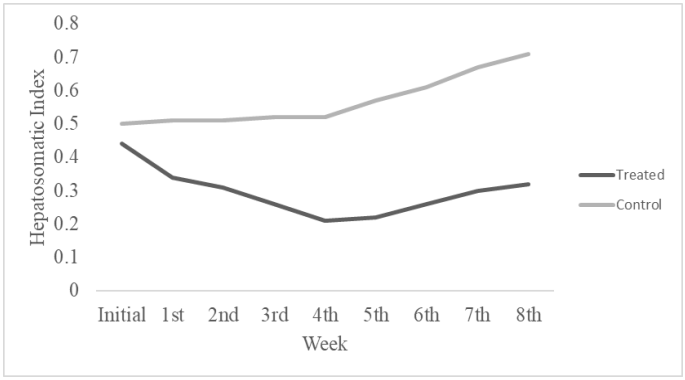

Figure 9: Hepato-somatic index of control and Cd-treated fish.

Hepato-somatic index (HSI)

Throughout the 60-days trial period, the HSI of both the treated and control fish fluctuated significantly. The HSI of Cd-treated fish firstly decreased then slightly increase. The HSI of control fish increased throughout experimental period. Figure 4 shows HSI of Cd-treated and control fish.

Acute toxicity

Acute toxicity tests are used to assess the hazards of chemical pollutants to organisms. The LC_{50} value is the concentration of a toxic substance at which 50% of the exposed population died and lethal value (LC_{100}) is the concentration of a toxic substance at which 100% of the exposed population died. Through acute toxicity test, the 96 h LC_{50} and lethal value of Cr for fish *L. rohita* were calculated as 12.38 and 23.6mgL^{-1}, respectively. According to the findings of Bekmezeci (2010), the acute toxicity of metals fluctuates greatly amongst different fish species. At the same dosage, a fish that is more susceptible to one metal may exhibit greater resistance to another metal. This can happen even if the two metals are in the same environment. Previous studies showed 96-h LC50 of cadmium for aquatic species liable on the type of species and metal. Yalsuyi *et al.* (2017) calculated 96-h LC50 of cadmium for *Carassius auratus* and *Cyprinus carpio* as

9.202 and 8.845 mgL^{-1}, respectively. Younis *et al.* (2013) stated that the 96-hr LC_{50} value of $CdCl_2$ for *Oreochromis niloticusis* as 14.8 mgL^{-1}. Rani *et al.* (2015) determined the 96-hr LC_{50} value of Cd to *Puntius ticto* as 26 mgL^{-1}.

Growth performance

Growth is a direct expression of the influence that metal has on the fish since growth incorporates all of the stimuli within the body (Azmat and Javed, 2011). In this study, fish growth parameters viz. daily weight gain, daily length gain, specific growth rate, condition factor, and feed conversion ratio of Cd-treated fish were significantly lesser in comparison with control fish. The findings of the present study were justified by Hansen *et al.* (2002) who concluded that growth reduction in fish occurred as a consequence of behavioral and physiological pressures during long-term exposure to heavy metals. Rahman *et al.* (2018) concluded that Cd stress decreased *O. niloticus* growth. Naz *et al.* (2013) concluded that chronic heavy metals exposure to *L. rohita, C. catla*, *H. molitrix*, *C. mrigala*, and *C. idella* gave significantly lower average weights and fork and total lengths in comparison to unstressed fish. They also concluded that condition factor was significantly better in unstressed fish species. According to (Javed, 2012) fish health and growth were adversely affected by metal exposure at sub-lethal concentrations. Verma and Prakash (2019) concluded that there is a significant decrease in fish appetite due to heavy metal toxicity that resulted into decrease in feed conversion efficiency and condition factor of fish. Abdel-Hakim *et al.* (2016) showed a substantial decrease in WG, SGR, FCR, and survival rate of *O. niloticus* after chronic exposure to Hg, Cu, Cd, and Pb. Rahman *et al.* (2018) investigated dropped growth rate of *O. niloticus's* when exposed to cadmium stress. Fish health and growth are negatively impacted by metal exposure at sub-lethal levels, according to (Javed, 2012).

Histology of liver

Fish liver is more vulnerable to aquatic pollutants than other organs because most of pollutants have tendency to accumulate in liver in higher concentration in comparison with other organs (Al-Balawi *et al.*, 2013). The current investigation shows that there are no pathological abnormalities in liver of control fish and it has a normal structure. During chronic trial, diameter of hepatocytes in Cd-treated fish was significantly lesser than control fish. Width of sinusoids in Cd-treated fish was significantly greater than control fish. Some other histological disorders in liver were also seen in Cd-treated fish viz. infiltration of sinusoid, dilated sinusoid and increased hemorrhage in sinusoids, inflammation and vacuolation in hepatocytes and ruptured hepatocytes. Liver is the most affected organ by water pollutants due to its role in detoxification and biotransformation processes, as well as its position and contact with bloodstream. These histological changes are typically linked to the hepatocytes response to toxins (Dyk *et al.*, 2007). Olojo *et al.* (2005) also reported different variations in the liver of *C. gariepinus* including hepatocyte shrinkage and increase of sinusoidal spaces due to heavy metal exposure. Dyk *et al.* (2007) concluded that exposure of cadmium and zinc to *O. mossambicus* showed histological changes in liver included ruptured hepatocytes, inflammation and hepatocyte vacuolation and the degree of these histological alterations was determined by the duration of the exposure period. Prabhahar *et al.* (2012) evaluated the cadmium impact on the liver histology of *C. mrigala*. The findings revealed that the treated fish liver contained ruptured hepatocytes with lost polygonal structure and enlarged vacuoles in the hepatocytes with displaced nuclei. Fatima and Usmani (2013) reported that exposure of fishes (*Channa striatus* and *Heteropneustes fossilis*) to heavy metals including Cr showed various histological alterations in liver including vacuolization and hemorrhages. Alshkarchy *et al.* (2021) investigated the histopathological effects of Zn, Fe and Cu on the liver of the Euphrates River's *C. carpio*. Major histopathological changes in liver were disintegration of the hepatocytes with spaces between the cells and several areas filled with degraded cells from the visceral tissues.

Hepatosomatic index

Hepato-somatic index reflects the metabolic status of the organism as well as the stress situation that has arisen as a result of metal exposure, and the excessive use of energy reserves in response to increased demand may result in a reduction in HSI (Verma and Prakash, 2019). The result of this study shows that hepato-somatic index of Cd exposed fish was significantly less than control fish throughout the experimental period of 60 days. The results of the present study were supported by (Verma and Prakash, 2019) who stated that HSI is the key indicator of metabolic activity and stress condition developed due to heavy metals and the surplus usage of energy assets in response to increase in requirement might cause the reduction in HSI. Bekmezci (2010) also reported that HSI of fish decreased due to heavy metals probably because of depletion of energy reserves in liver. Similarly, Singh and Srivastava (2015) reported that sub lethal exposure of heavy metals to *H. fossilis* caused a reduction of HSI however the control fish showed an increase in HSI. Messaoudi *et al.* (2009) also concluded that when *Salaria basilisca* was treated with cadmium for 14 and 28 days, the liver somatic index drastically reduced.

Conclusions and Recommendations

In this research work, *Labeo rohita* was used to check the effects of cadmium on fish. Cadmium had clear negative effects on the growth performance, liver histology and hepato-somatic index of fish. To mitigate the effects of heavy metals pollution on fish, it is crucial to implement strict regulations and practices to minimize the release of these contaminants into water bodies. Efforts should

focus on reducing industrial emissions, improving waste management systems, and implementing sustainable agricultural practices.

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Conflict of interest

The authors have declared no conflict of interest.

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