ASSESSMENT OF ACUTE TOXICITY, GROWTH PERFORMANCE, HEPATOSOMATIC INDEX AND HISTO-MORPHOMETRY OF *LABEO ROHITA* EXPOSED TO CR+CD MIXTURE

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(Received 25th Sep 2024; accepted 7th Jan 2025)

Abstract. This research work was designed to check the impacts of waterborne chromium (Cr) + cadmium (Cd) mixture on the growth performance, hepatosomatic index and liver histo-morphometry of *Labeo rohita*. In the initial stage of the experiment, the 96-h LC_{50} and lethal concentration of a Cr + Cd mixture were assessed using an acute toxicity bioassay, resulting in values of 18.65 mg/L and 31.22 mg/L, respectively. In the second phase, fish was exposed to 1/3 of LC_{50} value of Cr + Cd-mixture for 8 weeks to assess the growth performance, hepatosomatic index and liver histomorphometry of fish. The Cr + Cd-treated fish showed significant decrease in growth parameters; daily weight gain, daily length gain, specific growth rate, condition factor and feed conversion ratio in comparison to control group during 8 weeks of experimental period. Hepatosomatic index also decreased in Cr + Cd-treated fish but increased in control group. The histomorphological analysis Cr + Cd-treated fish liver showed significantly reduced hepatocyte diameter and increased sinusoid width compared to the control group. Additionally, various histological abnormalities were observed in the liver viz. hemorrhage, irregular shaped hepatocytes (loss of their polygonal shape), enucleated, eccentrically situated nuclei, vacuolization and necrosis were also seen in Cr + Cd-treated fish.
Keywords: *heavy metals, toxicity, biomarker, aquatic pollution, histology*

Introduction

Aquatic contamination due to anthropogenic activities is a serious worldwide issue that draw the attention of researchers from last few decades (Bakshi and Panigrahi, 2018).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_87258740

Water bodies eventually receive various pollutants like heavy metals, fertilizers, plastics, pesticides, toxic chemicals, pharmaceutical wastes, among them heavy metals are non-biodegradable and are among the most harmful contaminants (Azmat et al., 2016). These metals can remain in the aquatic habitat for long time once entered and cause long term biological effects (Gholamhosseini et al., 2021; Shahjahan et al., 2022).

Heavy metals are those metals that have a density above 5 g/cm3 and are toxic even in a small quantity. Common heavy metals include chromium, cadmium, mercury, arsenic, thallium and lead. A few trace elements are also included in this list i.e. zinc, copper and selenium. While each of these substances is necessary for a normal metabolism but they can all become hazardous if their concentrations increase (Fatima et al., 2020).

The most commonly applied aquatic ecotoxicity test for environmental risk assessment is acute toxicity test of fish which is performed according to internationally recognized guidelines (USEPA, 2016) or OECD Test Guideline 203 (OECD, 2019). In acute tests, the test organism (fish) are exposed to any chemical for 96 h and the mortalities/abnormalities are monitored every 24 h interval. The data obtained during test will be used to calculated LC50 concentration of chemical; the concentration which kill the 50% of test organism population. (Oliveira et al., 2009; Russom et al., 1997).

Fish feed and dwell in aquatic habitats, where they are unable to escape the negative effects of toxins, making them more vulnerable to pollution (Saleh and Marie, 2014). Fish expose to heavy metals during ingestion, respiration and ion exchange through skin (Ahmed et al., 2014). Heavy metals from aquatic environment ultimately enter into the food chain where their bioaccumulation and magnification can induce morphological and physiological changes in both aquatic and terrestrial species, as well as humans (Chatta et al., 2016). The effects of increased heavy metal levels on fish are related to the uptake and accumulation of the metal in organism, which causes metal-induced disruptions in the structures and functions of tissues and organs (Aly and Abouelfadl, 2020).

Chromium is a common metal pollutant that enters in aquatic medium through industrial effluents such as textiles, mining, electroplating, medicines, printing, tanneries, stainless steel manufacturing, dyeing and rubber industries (Bakshi and Panigrahi, 2018). Chromium is a well-known necessary micro-mineral that plays a key function in fish nutritional and physiological responses (Liu et al., 2010). Aside from its role in glucose, lipid, and protein metabolism, Cr exhibits particular toxicity characteristics (Aslam and Yousafzai, 2017). Cadmium occurs naturally but it is increasing day by day due to its presence in sludge, insecticides, dental alloys, fungicides, agriculture fertilizers, electroplating, exhaust and motor oil. Cadmium ranks among the most toxic heavy metals and is unnecessary for the survival of living organisms. In recent decades, cadmium contamination in aquatic habitats has significantly increased, resulting in greater bioaccumulation of cadmium in aquatic organisms (Okocha and Adedeji, 2011). The natural levels of chromium Cr and Cd in aquatic systems are influenced by geological features and local environmental conditions. Chromium can be found in rivers and lakes in concentrations ranging from 1-10 $\mu g\ L^{-1}$. According to the EPA, chromium concentrations of 50-100 $\mu g\ L^{-1}$ are acceptable for human and aquatic animal health. (Rashed et al., 2001). Cadmium may cause sublethal effects in fish when Cd concentration exceed from 3 $\mu g\ L^{-1}$ in fresh water (Levit, 2010). However, effluent discharge from industrial products increased these concentrations in the aquatic environment (Abbas and Ali, 2007).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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In fish, growth performance is a characteristic that reflects environmental toxicity, and even low heavy metals concentration have a negative impact on growth and metabolism thus also effect the fish health and lowering its survival rates (Hussain et al., 2010). Exposure to hexavalent chromium may cause diversification of metabolic energy for growth and development into detoxification of chromium toxicity, resulting in fish growth suppression (Kim and Kang., 2016). Cadmium can impact the fish growth (Heydarnejad et al., 2013). Long-term contact to cadmium, especially at concentrations exceeding 3 µg/L in freshwater, may result in sublethal effects such as reduced growth in fish (Levit, 2010).

The hepatosomatic index (HSI), which is the ratio of a fish's liver weight to its total weight, provides insights into the fish's health and the quality of the water (Dane and Sisman, 2020). This index indicates the organism's metabolic status and stress levels caused by metal exposure, where use of energy reserves beyond a certain limit can lead to a decrease in HSI (Verma and Prakash, 2019). Both field and laboratory trials have demonstrated the effectiveness of HSI. However, only a few toxicologists have used HSI as a biomarker for fish health (Javed and Usmani, 2017).

Histopathological studies also used stress biomarker in fish (Yancheva et al., 2016). It is helpful for assessing the health of fish exposed to pollutants and provides a group of biomarkers that enable the examination of certain target organs (AL-Taee et al., 2020). Long-term exposure to pollutants in the fish body may result in irreversible alterations in the histology of the liver (Galus et al., 2013). Therefore, during the investigation of heavy metal toxicity, fish liver has the highest popularity (Sabullah et al., 2015).

The most widely culturable Indian major carp of South Asia is *Labeo rohita* commonly known as "rohu" which belongs to carp family. The fish rohu is commercially important fish due to its high market demand (Ashaf-Ud-Doulah et al., 2021; Shahjahan et al., 2021). According to FAO (2022), globally, 1.67 million tons of rohu is produced annually. In natural aquatic environment, the density of these fish species have declined due to exposure of fish to a variety of toxicants like metals (Ameer et al., 2013).

Numerous preventive methods (Hasan et al., 2016) and natural remedies (Keesstra et al., 2018) are being used to limit the heavy metals discharge into the water system, yet the heavy metals even persist in the aquatic environment (Khound and Bhattacharyya, 2017). Therefore, this study was aimed to assess the growth performance, hepatosomatic index and liver histomorphometry of *L. rohita* exposed to Cr + Cd mixture.

Materials and methods

The 150 days old freshwater fish *Labeo rohita* commonly known as rohu was chosen as an experimental animal and collected from the Fisheries Research and Training Complex, Bahawalpur, Pakistan. The experiment was conducted at Fisheries Research Laboratory at Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan. Two months' chronic waterborne exposure of chromium (Cr) and cadmium (Cd) mixture was given to *L. rohita* to check its effects on growth, hepatosomatic index and liver histomorphometry of fish.

After being collected, the fish were acclimated to laboratory conditions for two weeks. They were fed standard feed in the morning and evening, with a maintained cycle of 12 h of light and 12 h of darkness. After this, fishes were moved to glass aquarium (50 L). The parameters of water that were kept constant throughout the experiment are temperature (28°C), pH (7.5) and total hardness (200 mg L^{-1}). Methods of A. P. H. A. (1998) followed

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for the calculation of variable parameters viz. magnesium, calcium, potassium, sodium, total ammonia, carbon dioxide and dissolved oxygen.

Acute toxicity

After acclimatization, an acute toxicity bioassay was used to determine the 96-hr LC_{50} and lethal value of Cr + Cd mixture for *L. rohita* by following the standard guidelines of APHA (2017) and OECD (2019). This experiment was performed with three replicates. Ten fish were kept in each aquarium. The pure chloride salts of chromium and cadmium were used to prepare the stock solutions (1000 ppm) of each metal separately. The mixture of Cr + Cd was prepared by mixing of stock solution (1:1). Different mixture concentrations (0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 mg L^{-1}) were given to the fish. At 24, 48, 72, and 96-hr after the start of exposure, mortality was measured and dead fish was removed promptly. The LC50 value represents the concentration of the Cr + Cd mixture at which 50% of the fish died, while the lethal concentration is the amount at which 100% of the fish were dead.

Chronic toxicity

After determination of LC_{50}, two months chronic trail was performed at 1/3 of LC_{50} value of Cr + Cd mixture (6.22 mg L^{-1}) to check the growth, hepatosomatic index and liver histology of exposed *Labeo rohita*. The control fish was kept in clean water (0 treatment).

Growth performance

The growth trial was conducted with three replicates. Ten (n = 10) fish were placed in each of the aquariums having 100 L water capacity, and exposed to sub-lethal concentrations of the metal mixture for eight weeks. The whole experiment was conducted with three replicates. To maintain the sub-lethal concentration of the metal mixture, the exposure media were partially replenished at regular intervals. Commercial feed was given to satiate the fish two times a day. The growth parameters were monitored weekly basis. Throughout the 8-week chronic exposure to the metal mixture, growth parameters such as daily weight gain (DWG), daily length gain (DLG), specific growth rate (SGR%), condition factor (CF), and feed conversion ratio (FCR) were calculated weekly using the following formulas:

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

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

$$\text{Specific Growth Rate} = \frac{(\ln \text{ Final weight} - \ln \text{ Initial weight})}{\text{Experimental period in days}} \times 100$$

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

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

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Hepatosomatic index (HSI)

For HSI index, 20 fish were kept in a separate aquarium with same sub-lethal concentration. Hepatosomatic index was calculated on weekly basis. To calculate the HSI fish (n = 2) was dissected and the liver was removed and weighed by digital weight scale. The index was calculated by dividing liver weight by total weight of fish. The formula for HSI is as under:

$$\text{Hepatosomatic index} = \frac{\text{Liver weight}}{\text{Total fish weight}} \times 100$$

Liver histology

Liver samples from both the control and exposed fish were preserved in a 10% neutral-buffered formalin solution, then dehydrated and embedded in paraffin wax. Sections 5 µm thick were cut, stained with hematoxylin and eosin, and examined under light microscopy for histological analysis, following the method described by Bancroft, Floyd, and Suvarna (2013). The diameters of 20 randomly selected hepatocytes per slide were measured. Length of horizontal axis (l_1) and vertical axis (l_2) will be measured and mean $(l_1 + l_2)/2$ were taken to represent hepatocyte diameter (Kasperk et al., 1994). Both the diameter of hepatocytes and width of sinusoids having visible ends were measured as described by Gokcimen et al. (2007). The histological alterations were examined according to Mishra and Mohanty (2008). The frequency of these changes is classified into four stages like no abnormalities [0% damage, (–)], mild abnormalities [10% damage (+)], moderate abnormalities [10% to 50% damage (++)] and severe abnormalities [greater than 50% damage (+++)].

Statistical analyses

The data obtained were then subjected for the estimation of 96 h LC50 value for the heavy metal of interest, following probit analysis method suggested by Finney (1971). Data obtained from growth performance and hepatosomatic index was analyzed by using One-way ANOVA followed by t-test. Data were statistical analyses through software program Statistix 8.1 version. The data obtained from liver histology was analyzed by Tukey's HSD test and was subjected to statistical analyses through software program SPSS to determine the effects of treatments. Image J software program was used for histo-morphometry analyses of liver.

Results

Acute toxicity

In the acute toxicity bioassay, the 96-h LC_{50} and lethal concentration of the Cr + Cd mixture for *L. rohita* were found to be 18.65 mg/L and 31.22 mg/L, respectively (*Figs. 1* and *2*). The LC50's lower and upper limits were 16.05 mg/L and 20.86 mg/L, while the lethal concentration's lower and upper limits were 27.93 mg/L and 37.07 mg/L.

Growth performance

Throughout the 8-week chronic trial, the growth performance of the treated fish differed significantly from that of the control fish. Growth parameters such as daily weight gain,

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daily length gain, specific growth rate, condition factor, and feed conversion ratio were significantly lower in the Cr + Cd-treated fish, whereas the control fish exhibited better growth across all these parameters. The daily weight gain in treated fish was 0.04 ± 0.08 g while in control fish it was 0.57 ± 0.33 g. The daily length gain (standard length) in treated fish was 0.01 ± 0.01 cm while in control fish it was 0.04 ± 0.02 cm. The daily length gain (fork length) in treated fish was 0.008 ± 0.009 cm while in control fish it was 0.04 ± 0.02 cm. The daily length gain (total length) in treated fish was 0.01 ± 0.009 cm while in control fish it was 0.04 ± 0.02 cm. The specific growth rate in treated fish was 0.07 ± 0.15 while in control fish it was 1.12 ± 0.46. Condition factor in treated fish was 1.49 ± 0.03 while in control fish it was 1.90 ± 0.10. The feed conversion ratio in treated fish was 0.09 ± 0.22 while in control fish it was 1.73 ± 0.06 (*Table 1*).

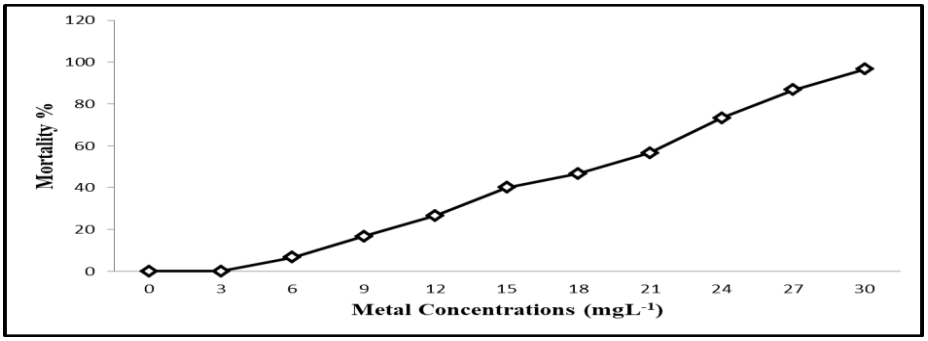

Figure 1. *Mortality of L. rohita during acute toxicity bioassay for Cr + Cd mixture*

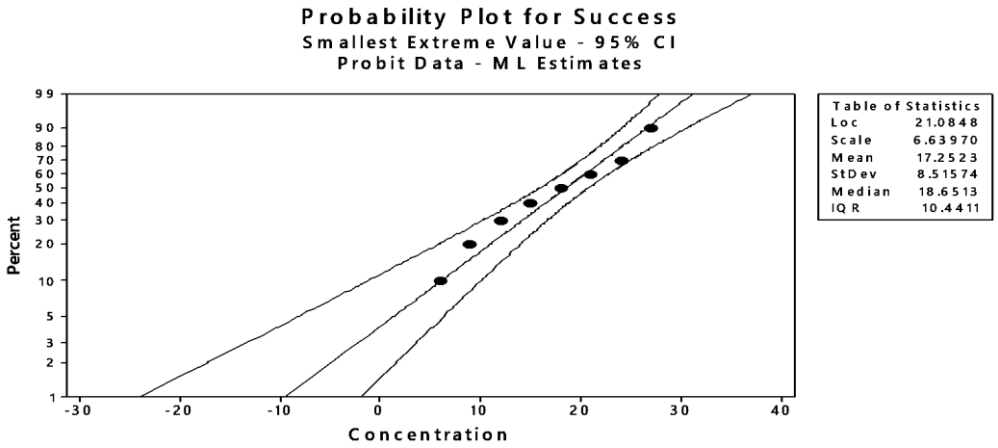

Figure 2. *The 96-h LC_{50} and lethal value of Cr + Cd mixture for L. rohita*

Table 1. *Growth performance of control and Cr + Cd-treated L. rohita*

Parameters	Control	Cr + Cd
Daily weight gain	0.57 ± 0.33^{a}	0.04 ± 0.08^{b}
Daily length gain (standard length)	0.04 ± 0.02^{a}	0.01 ± 0.01^{b}
Daily length gain (fork length)	0.04 ± 0.02^{a}	0.008 ± 0.009^{b}
Daily length gain (total length)	0.04 ± 0.02^{a}	0.01 ± 0.009^{b}
Specific growth rate	1.12 ± 0.46^{a}	0.07 ± 0.15^{b}
Condition factor	1.90 ± 0.10^{a}	1.49 ± 0.03^{b}
Feed conversion ratio	1.73 ± 0.06^{a}	0.09 ± 0.22^{b}

MEAN ± SD of 8-weeks readings of fish (n = 15). In the row, superscripts show values are significantly different ($p < 0.05$)

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Hepatosomatic index (HSI)

Hepatosomatic index of Cr + Cd-treated fish was significantly lesser in comparison to control group and also decreased in treated fish and increased in control group throughout experimental period (*Fig. 3*).

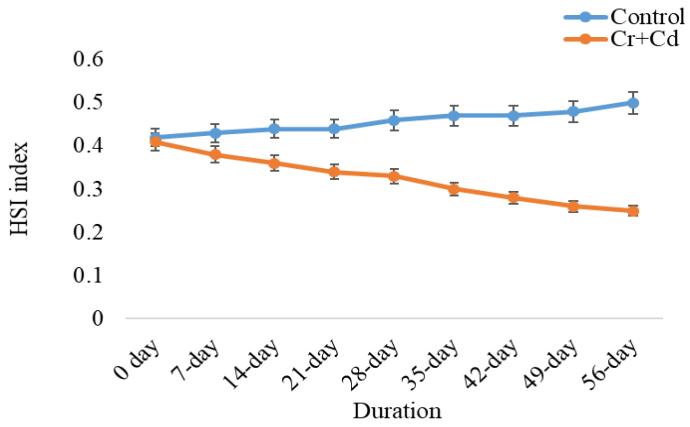

Figure 3. *Hepatosomatic index of Cr + Cd-treated L. rohita. The figure shows as the exposure duration increase the HIS was decreased*

Liver histology

The histomorphometric parameters revealed that the diameter of hepatocytes in Cr + Cd-treated fish was significantly smaller compared to the control fish, while the width of sinusoids was significantly larger in the treated fish (*Fig. 4*). Additionally, several histological abnormalities were observed in the Cr + Cd-treated fish, including hemorrhage (bleeding in the liver), irregularly shaped hepatocytes (loss of their polygonal shape), enucleated hepatocytes (cells without a nucleus), eccentrically located nuclei (nuclei not centrally positioned), vacuolation (formation of vacuoles), and necrosis (death of hepatocytes) (*Fig. 5*) while the control fish showed normal histology (*Table 2*).

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

Parameters	Control group	Cr + Cd-treated group
Diameter of hepatocytes	11.03 ± 0.21^{a}	8.15 ± 0.07^{b}
Width of sinusoids	5.37 ± 0.08^{b}	7.50 ± 0.03^{a}

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

Discussion

Trace metals play an important role in animal life; however excessive levels in water can be poisonous and have negative consequences for fish (Jabeen and Javed, 2011). Heavy metal pollution has become a major issue for environmentalists (Praveena, 2013). Metal mixtures are more harmful than single metals. The toxicity of metal mixtures is dependent on the concentration, composition and period of exposure on the fish (Vosyliene and Jankaite, 2006). Chromium is a common metal pollutant that enters in aquatic medium through industrial effluents and has serious health consequences (Ahmed et al., 2013). Cadmium is a hazardous metal that has no biological function and is very harmful to aquatic organisms (Van Dyk et al., 2007).

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Acute toxicity tests evaluate the risks posed by chemical pollutants to organisms. The LC_{50} value indicates the concentration of a toxic substance at which 50% of the exposed population dies, while the LC_{100} (lethal value) represents the concentration at which 100% of the population dies. During the acute toxicity bioassay, the 96-h LC_{50} and lethal values of the Cr + Cd mixture for *L. rohita* were calculated to be 18.65 mg/L and 31.22 mg/L, respectively. Latif et al. (2012) determined the 96 h LC_{50} value of $CdCl_2$ for *L. rohita* as 22.92 mg L^{-1}. Younis et al. (2013) reported that the 96 h LC_{50} value of $CdCl_2$ for *Oreochromis niloticusis* as 14.8 mg L^{-1}. Ahmed et al. (2013) reported that the 96-hr LC_{50} value of Cr for *Heteropneustes fossilis* was 35.72 mg L^{-1}. Johnson and Radhakrishnan (2015) determined the 96-hr LC_{50} value of Cr for *Clarius batrachus* as 36.65 mg L^{-1}. Rani et al. (2015) reported the 96-hr LC_{50} value of Cd to *Puntius ticto* as 26 mg L^{-1}.

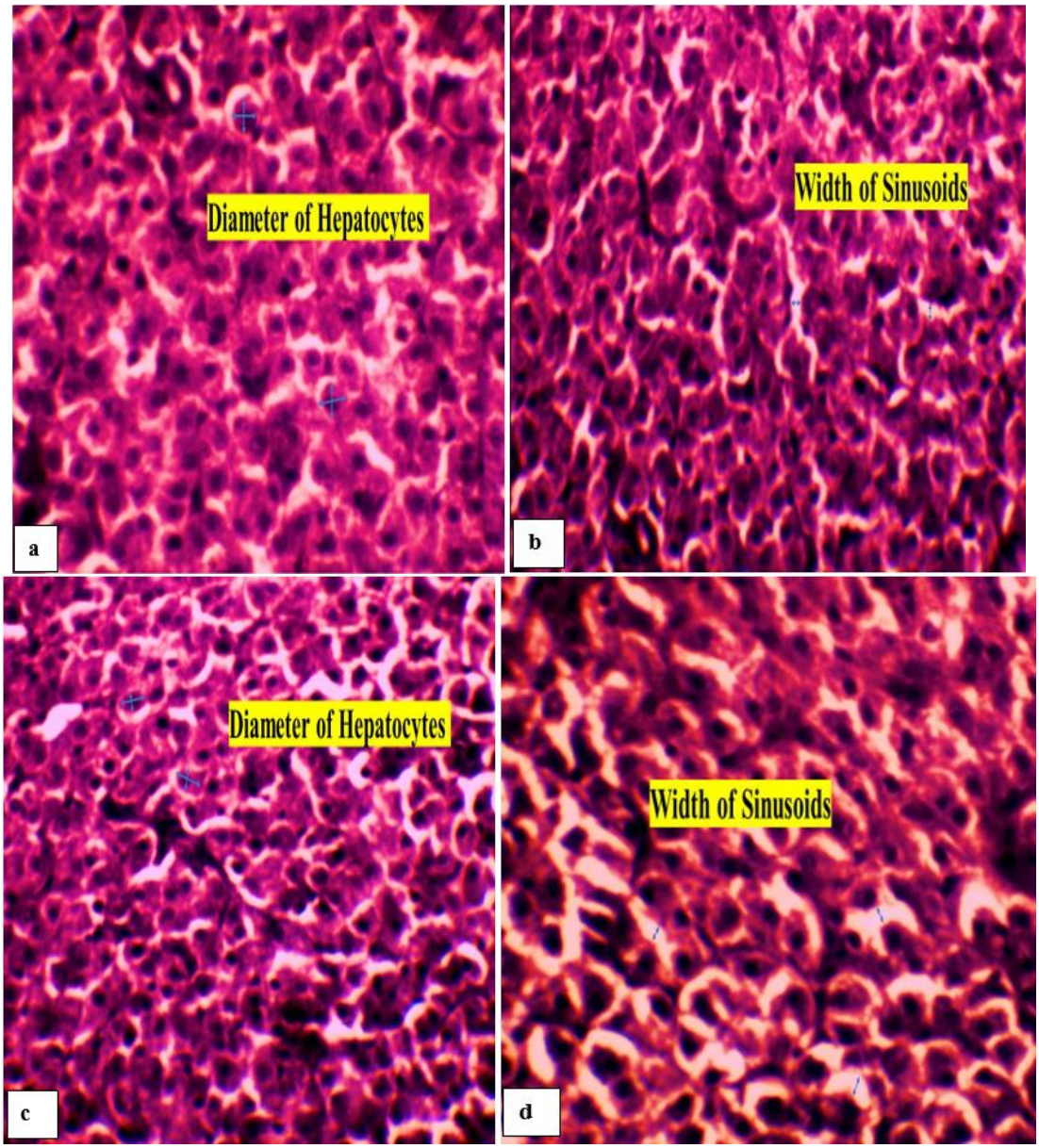

Figure 4. *Histo-morphometric (µm) analysis of liver (a) the diameter of hepatocytes of control fish (b) width of sinusoids of control fish (c) the diameter of hepatocytes of Cr + Cd-treated fish (d) width of Sinusoids of Cr + Cd-treated fish*

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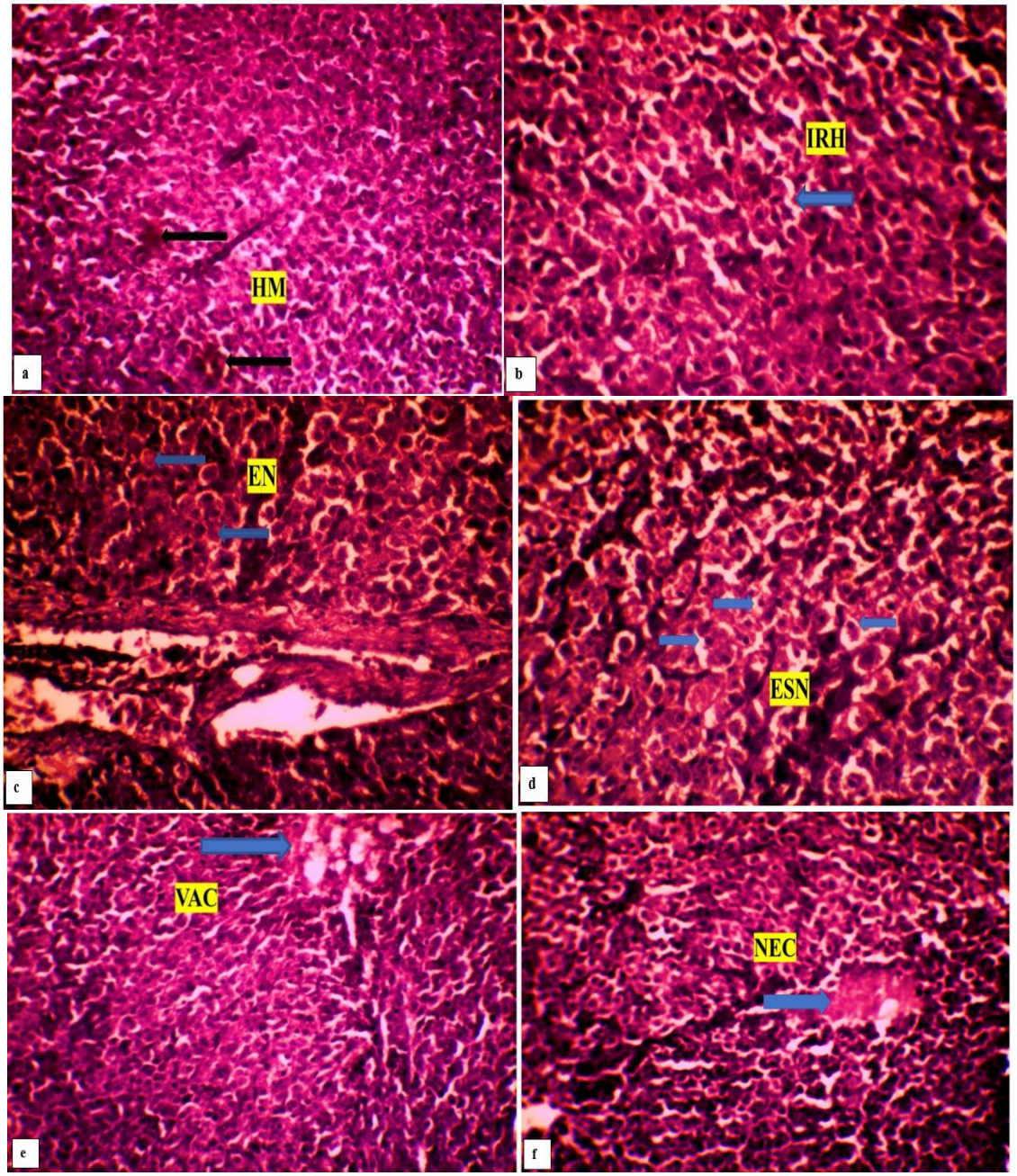

Figure 5. *Histological disorders in liver of Cr + Cd-treated fish: (a) Hemorrhage HM (bleeding in liver) (b) Irregular shaped hepatocytes (loss of their polygonal shape) (c) Enucleated (hepatocytes without nucleus) (d) Eccentrically situated nuclei (nucleus not placed in central position of hepatocytes (e) Vacuolation (formation of vacuoles) (f) Necrosis (death of hepatocytes in liver)*

The growth of fish is considered to be a useful indicator of metal stress (Javed, 2012). Mixtures of metals had a more detrimental impact on fish growth rates and behavior compared to individual metals (Naz and Javed, 2013). This study examined fish growth over an 8-week period under chronic exposure to a binary metal mixture (Cr + Cd) compared to a control group. Growth parameters, including daily weight gain, daily length gain, specific growth rate, condition factor, and feed conversion ratio, were

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significantly lower in Cr + Cd-exposed fish than in control fish. These findings align with Hansen et al. (2002), who attributed growth reduction in fish to behavioral and physiological stresses caused by prolonged exposure to heavy metals and their combinations. Physiologically, these metals disrupt essential metabolic processes and induce oxidative stress, compromising energy allocation for growth and development. The interference with endocrine pathways further exacerbates growth inhibition, affecting hormone-regulated processes critical for growth. Behaviorally, fish may exhibit altered feeding patterns and reduced activity levels in contaminated environments, leading to decreased nutrient intake and impaired growth efficiency. Social behaviors and interactions within fish populations can also be disrupted, affecting resource competition and overall growth dynamic. Thus, any notable alteration in feed intake due to stress would manifest as a reduction in fish growth, as demonstrated in the current study (Naz and Javed, 2013). The results of this study also supported by Javed (2015) who stated that heavy metal toxicity significantly affect the feed intake of fish that consequently resulted into lower weight and length gains in fish. Javed (2012) also reported that waterborne heavy metals including Cd exposure to *C. catla*, *L. rohita* and *C. mrigala* caused significantly lesser weight gains compared to control group showed significantly better growth. Naz et al. (2013) discovered that prolonged exposure to heavy metals in *Labeo rohita*, *Catla catla*, *Hypophthalmichthys molitrix*, *Cirrhinus mrigala*, and *Ctenopharyngodon idella* led to a notable decrease in their average weights, fork lengths, and total lengths when compared to fish that were not exposed to such stressors. Additionally, they observed that the condition factor was significantly higher in fish species that were not exposed to stress. Shaheen and Jabeen (2015) reported that exposure of *C. carpio* to Cr lowered its feed consumption and specific growth rate significantly when compared to control group and this drop was linearly proportional to the increase in Cr concentration. Mohamed et al. (2020) concluded that when *O. niloticus* exposed to Cr, it showed a significant decrease in body weight gain, specific growth rate and condition factor as compared to the control group. Ameer et al. (2013) evaluated growth performance of *C. catla* and *L. rohita* under mix exposure of sub-lethal waterborne and dietary metals including Cd by keeping their control groups. They concluded that control fish had significantly better FCR in comparison with treated one.

The hepatosomatic index (HSI) is a useful biomarker for identifying the detrimental impacts of environmental stresses on fish (Sadekarpawar and Parikh, 2013). Fish treated with Cr + Cd had lesser liver weights than fish that were not treated in the current study. Furthermore, over the course of the trial, the HSI of the Cr + Cd-treated fish decreased while that of the control group increased. Verma and Prakash (2019), who suggested that the HSI is a critical measure of metabolic activity and stress brought on by heavy metals, support the results of this study. They proposed that a drop in HSI can result from the greater consumption of energy reserves in reaction to rising demand. Bekmezci (2010) also observed a drop in the HSI of fish exposed to heavy metals, probably as a result of the liver's energy supplies being depleted. Additionally, Singh and Srivastava (2015) discovered that although the control fish displayed an increase in hepatosomatic index (HSI), *H. fossilis's* HSI decreased with extended exposure to heavy metals.

The liver of fish is more susceptible to aquatic pollutants than other organs due to its propensity to absorb these pollutants at higher concentrations (Al-Balawi et al., 2013). Its primary function is to eliminate any impurities or poisons from blood that exits the intestines, which makes it a helpful marker of pollution in aquatic environments (El-Naggar et al., 2009). The hepatocytes that make up the liver are not grouped in distinct

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DOI: http://dx.doi.org/10.15666/aeer/2305_87258740

lobules but rather in branched laminae that are two cells thick, with sinusoids between them. According to Figueiredo-Fernandes et al. (2007), the liver cells exhibit a polygonal form, with a strongly pigmented nucleolus and a central, spherical nucleus. The results of this investigation demonstrate that control fish's livers retain their natural architecture without any pathological aberrations. On the other hand, during the sub-lethal trial, the Cr + Cd-treated fish's sinusoidal width was much wider and their hepatocyte diameter was significantly lower than that of the control fish. These contradictory findings—that treated fish had wider sinusoids and smaller hepatocytes—highlight the intricate physiological reactions to multiple heavy metal exposure. In fish that have been treated, hepatocytes—which are essential for liver function—show a reduced diameter, which may indicate cellular stress or metabolic changes brought on by Cr and Cd. This decrease may be a sign of altered metabolic pathways, compromised cellular integrity, or heightened vulnerability to oxidative damage—all of which are typical reactions to heavy metal toxicity. On the other hand, the wider sinusoids seen in fish treated with Cr + Cd suggest that the liver may undergo circulatory alterations. The expansion of sinusoids may indicate improved blood flow or vascular remodeling in response to metal exposure. Sinusoids are crucial for the exchange of nutrients. These alterations, nevertheless, can potentially indicate inflammation or impaired liver function. Fish treated with Cr + Cd had their livers examined for other histological abnormalities, such as hemorrhage, irregularly formed hepatocytes (loss of polygonal shape), enucleated and eccentrically positioned nuclei, vacuolation, and necrosis. Hemorrhage, which suggests that the toxic effects of chromium and cadmium may have affected the integrity of the vessels. Furthermore, aberrantly shaped hepatocytes were seen, which indicate a disturbance in cellular structure and function. These hepatocytes were distinguished by the absence of their usual polygonal structure. Moreover, the existence of enucleated hepatocytes with nuclei positioned eccentrically suggests that these heavy metals have caused significant cellular stress or damage. Another noteworthy discovery is vacuolation, which denotes the build-up of fats or fluids inside hepatocytes and can damage cells and cause liver disease. Necrosis, or visible indications of tissue damage and cell death as a result of exposure to chromium and cadmium, was also clearly visible. The histological alterations in the liver seen in this investigation were probably caused by a variety of biochemical abnormalities. The liver is one of the organs most impacted by water contaminants because of its roles in detoxification and biotransformation processes, as well as its location and accessibility to the blood supply. Van Dyk et al. (2007) noted that these histological changes are usually linked to the way in which hepatocytes respond to toxicants. Olojo et al. (2005) also reported different changes in the liver of *C. gariepinus*, including hepatocyte shrinkage and increased sinusoidal spaces, as a result of heavy metal exposure. Mishra and Mohanty (2008) also reported different changes in the liver, including an increase in the sinusoidal space of C. punctatus due to exposure to heavy metals. Mahboob et al. (2020) reported that when *O. niloticus* was exposed to heavy metals, the liver displayed a variety of histopathological changes, including sinusoid dilation. Onita et al. (2021) evaluated the severity of histopathological changes in three fish species, *Chondrostoma nasus*, *Barbus barbus*, and *Squalius cephalus* from the Crisul Negru river that were exposed to heavy metals. They came to the conclusion that sinusoid dilatation was among the most frequent histological changes in the liver. Bilal et al. (2011) reported that exposure of catfish to $CdCl_2$ for 30 and 60 days showed various histological changes in liver such as necrosis of hepatic tissue, changes in shape of hepatocytes, enucleation, eccentric position of nuclei and vacuoles development in cell

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cytoplasm. Fatima and Usmani (2013) reported that exposure to heavy metals, including chromium, in fish species such as *Channa striatus* and *Heteropneustes fossilis* resulted in various histological changes in the liver, including tissue vacuolization and hemorrhages. Ahmed et al. (2014) reported that when climbing perch, *Anabas testudineus* exposed to $PbCl_2$ and $CdCl_2$, it showed various histological alteration in liver including irregular shaped hepatocytes and single necrosis.

Conclusion

In this research work, *Labeo rohita* was considered as a biomarker to assess the impacts of heavy metals, chromium and cadmium mixture on fish. Chromium and cadmium mixture had detrimental effects on the growth performance, hepatosomatic index and liver histomorphometry of fish. Therefore, it is recommended that all types of wastewater industrial, sewage, and agricultural should be treated before being discharged into aquatic systems. Additionally, the enforcement of relevant laws and regulations should be prioritized.

Acknowledgments. We thank the Higher Education Commission of Pakistan for funding antinematodal studies under NRPU project No. 5730.The authors would like to acknowledge the Deanship of Graduate Studies and Scientific Research, Taif University, Saudi Arabia, for funding this work.

Conflict of interest. There is no conflict of interest.

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