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Research Article

Acute Toxicity, Catalase Activity and Histological Examination of Pb+Cd Mixture Stressed Tilapia

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Authors' Contributions
MR executed the research work. HN did supervision, and manuscript review and editing. TA and MU performed formal analysis. TA, SA and SQAS helped in writing the draft. MA and NZ helped in lab work. NZ did histological observation. MU did software and visualization.

Keywords
Lead, Cadmium, Intestine, Brain, Enzyme, *O. niloticus*

Abstract | The study was conducted to examine the acute toxicity, catalase activity and histological of cadmium (Cd)+ lead(Pb) mixture stressed tilapia. The fish, tilapia was exposed to different concentration of Cd+Pb to find the acute toxicity for 96-hr. The 96-hrs LC_{50} and lethal value with a 95% confidence interval were calculated as 180.35 and 347.81 mg/L, respectively through Probit analysis approach. After acute toxicity test, effects of Cd+Pb mixture on catalase (CAT) activity and histology were also observed. The findings of this investigation revealed that Cd+Pb -stressed *O. niloticus* had reduced CAT activity in all organs viz. liver, gills, intestine and brain as compared to control. The results of histology showed the changes in brain and intestine of Cd+Pb-stressed tilapia. In brain of stressed fish severe damage including vacuoles destruction, necrosis, edema, granular layer degeneration was noted. In intestine, widening of intestinal lumen, curling of intestinal villi, mucous cells degeneration, and necrosis in villi was reported.

Novelty Statement | This Study expanded the knowledge on the effect of metal (Pb) toxicity by establishing a connection between exposure and tissue level responses, thus contributing to an in-depth evaluation of the toxic impact of Pb on fish. Findings of this research highlight the role of fish as bioindicators of lead pollution in water ecosystems.

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Introduction

Heavy metals (HMs) are naturally occurring environmental components and are thought to be potential aquatic contaminants (Afshan *et al.*, 2014; Lacerda *et al.*, 2020). While some heavy metals serve

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as catalytic and structural elements of enzymes and proteins, they are necessary for organic frameworks in the human body. The heavy metals such as Zn, Cu, and Ni are the few metals which may be harmful when safe amount is surpassed. Even in small amounts, other metals including Cd, Pb, and Cr are dangerous pollutants (Gall *et al*., 2015; Zheng *et al*., 2015). HMs comprise the main pollutants in environment because of their carcinogenicity, perseverance, teratogenicity, mutagenicity and capacity to bioaccumulate in food chains (Wang *et al*., 2005; Yang *et al*., 2017; Karimi *et al*., 2020; Zhao *et al*., 2020).

HMs is entered into fish bodies by direct assimilation from water through their skins and gills, or by ingestion of sullied food (Ayyat *et al*., 2020). Subsequently, exposure to Microplastics (MPs) can promote to various poisonous impacts, e.g., endocrine disruption, intestinal loss, immune response, oxidative pressure and ultimately reduced growth and survival (Greven, 2016; Liu *et al*., 2019; Montero de Espinosa *et al*., 2017). Similarly, lot of synthetic antibiotics are utilized in fish farms, animal husbandry, and medical therapy (Abbas *et al*., 2022). The discharge of these compounds into surface water effect the biological creature in the ecosystem.

Lead, cadmium, and mercury are examples of HMs that can be extremely hazardous and have no biological or useful functions in living things (Ekeanyanwu *et al*., 2015). Even at trace polluted water is the significant reason for bioaccumulation of metals in the different crucial organs of the freshwater fish (Afshan *et al*., 2014). The World Health Organisation (WHO) has released guidelines that state the ideal amounts of Pb and Cd are 0.01 and 0.003 mg/l, respectively (Mahboob *et al*., 2020).

Aquatic species are extremely sensitive to heavy metal (cadmium), which is also a non-essential nutrient. Cadmium accumulates in fish bodies and causes clinical abnormalities such as gill damage, skeletal deformities and changes in various internal organs especially intestine, liver and kidney (Santosh *et al*., 2018). Fish inhale Cd from the water via its gills or digestive system and dispersed in various tissue and organ, each of which has a varied Cd level (Asagba *et al*., 2008; Squadrone *et al*., 2013).

Human health is negatively impacted by lead's toxicity. The health risks associated with ingesting lead are significant (Hussan *et al*., 2016; WHO, 2011). The effects of exposure depend on the dose, exposure time, the mode of exposure, personal habits, traits, and presence of other chemicals (Bujjamma and Padmavathi, 2018). Heavy metals such as cadmium+lead (Cd + Pb) are common in the environment and quite dangerous (Demey *et al*., 2018; Jamali *et al*., 2016).

Fish, such as the Nile tilapia, *Oreochromis niloticus*, play a vital role in the aquatic food web and are frequently utilised as biomarkers in toxicological investigations. When they consume those organisms which contaminated with heavy metals, negative health impacts occur (Al-Bairuty *et al*., 2013; Clearwater *et al*., 2002; Intamat *et al*., 2016; Mustafa *et al*., 2012). Single or several tissues can be analyzed to track the impact of toxicants in specific parts of certain organs, as well as tissue degradation in other places, in order to identify the observed alternations. Histopathology is employed for causation, diagnosis and illness prevention, and now regarded as one of the most accurate and valuable tool for toxicological effects study (Reddy and Rawat, 2013). Heavy metals disturb the fish internal organs so badly including intestine and brain (Naz *et al*., 2021).

Enzymatic antioxidant system was activated to defend living organisms from these shocks (Jaishankar *et al*., 2014). Essential metals (Cu, Zn, and Fe) can potentially have negative health effects from overdosing as compare to non-essential heavy metals (Cd and Pb), which can demonstrate high toxicity even in tiny concentrations. Reason behind high attention to heavy metals is not only threats to organisms of water bodies but also due to human health issues from these pollutants (Opaluwa *et al*., 2012; Bawuro *et al*., 2018; Ali *et al*., 2019). Therefore, work was designed to evaluate to the lethal concentration, antioxidant enzyme and histological alternation in intestine and brain of *O. niloticus* exposed to of Cd+Pb.

Materials and Methods

Experimental design

The fish tilapia (*Oreochromis niloticus)* was taken from Fisheries Research and Training Complex, Bahawalpur and were brought to the lab of Cholistan University of Veterinary and Animal, Bahawalpur. Fish were kept in glass aquariums. The fish were housed in a lab with an environment that was controlled. Fish were acclimatized for 14 days. In each aquarium, ten fish were housed. Throughout the duration of the examination, the water's pH (7.0), total hardness (200 mg L^{-1}) and temperature (30°C) remained unchanged. With the aid of a capillary system and an air pump, regular air was provided to entire of the test and control mediums.

Determination of physico-chemical parameters

Digital meters WTW inolab were used to test pH and electrical conductivity, while electronic meters HANNA HI-9146 were used to detect water temperature and dissolved oxygen. However, measurements of total ammonia, calcium, CO_2, hardness and magnesium were made as well.

Acute toxicity test

The chloride salts of both cadmium+lead (Cd+Pb) were mixed in distilled water, separately to make solution for static bioassays. In different concentrations of Cd+Pb by the ratio of 1:1. The test organisms (fish) were randomly distributed. For the acute bioassay experiments, ten fish were employed per concentration and per replication. In 100 L capacity test aquarium, Cd+Pb concentrations were administered 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 255, 270, 285 and 300 mg/L. Keeping all other conditions constant, the control group was held in plain water without the addition of Cd+Pb. All experiments were conducted for 96-hr duration. The aggregate of dead fish was taken account of after every 12-hr interval and their immediate removal from the aquaria was made sure. At the end of 96-hr period, mortality rate was determined.

Assay of catalase (CAT)

CAT activity was determined according to Chance and Mehaly (1977) by determining its ability to decreased hydrogen peroxide at 240 nm.

Histological examination

After a 96-hrs exposure period, tiny sections of intestine and brain were fixed in 10% formalin, dehydrate and cleaned in xylene. Light microscopy was used to analyze and take pictures of fixed tissues that had been implanted in paraffin wax and separated at a thickness of 5µm. The paraffin-embedding technique was applied for light microscopic investigation of brain and intestinal samples (Bancroft *et al*., 2013).

Statistical analyses

The collected data was examined by practicing the proper statistical techniques (Steel *et al*., 1997). Graphs were drawn in Microsoft Excel. Probit Analysis technique was used to calculate lethal concentration of Cd+Pb and LC_{50} for Nile tilapia with confidence limit of 95% by software Minitab. ANOVA was applied on enzymatic data. The histological observation was made on microscope.

Results and Discussion

Acute toxicity

The 96-hr LC_{50} and lethal concentrations of Cd+Pb for fish tilapia were determined as 180.349 and 347.806 mg/L, respectively (Figure 1). Even at low amount, non-essential elements like Cd+Pb are extremely hazardous (Fernandes *et al*., 2008). The most grounded connections frequently take place in binary mixtures, and as the number of mixture components increases, the impact of the interactions may become less significant Liu *et al*. (2015). The relative potency and affinity of toxicants, the amounts and ratios of dissolved metals, and the background solution's composition all affect how toxic metal combinations react (Balistrieri and Mebane, 2014). Almeida *et al*. (2002) reported the 96-hr LC_{50} value of Cd was18.58 mg/L for the same species as used in the present study. Ullah *et al*. (2016) described the LC_{50} of Pb for *O. niloticus* was 44 mgL^{-1}. Utami *et al*. (2018) described the LC_{50} value of Pb for *O. niloticus* as 324.38 ppm. Faheem *et al*. (2012) described the LC_{50} of Pb for *O. niloticus* as 35.848 mgL^{-1}

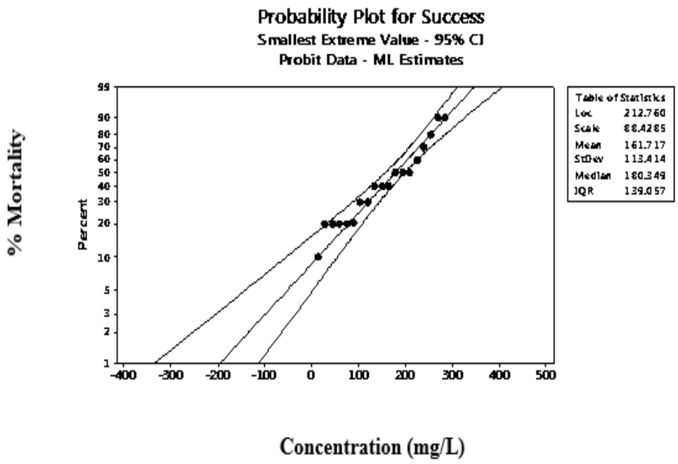

Figure 1: Graph of probabilities for lethal concentration (mg/L) and 96-hrs LC_{50} of Pb+Cd mixture for tilapia.

Catalase activity

In present study catalase activity was measured in brain, intestine, gills and liver of fish, tilapia after acute exposure of binary metal mixture Cd+Pb. The findings of the current investigation revealed that Cd+Pb stressed *O. niloticus* had reduced CAT activity in all organs as compared to control. Statistical data showed a substantial difference between the Cd+Pb treated *O. niloticus* CAT activity and the control (Figure 2). The metal ions can binds to SH groups of proteins (catalase) and inhibit the activity which increases the production of radicals O-2 and H_2O_2 (Atli and Canli, 2010). The response of antioxidant enzymes against metals is associated with multiple factors including exposed organ, pollutant nature and exposure time (Atli and Canli, 2010).

Tufail *et al*. (2019) reported the lowest CAT activity in liver of contaminated (pesticides) exposed *Channa striata*. Metals mixtures significantly decrease the CAT and SOD activities in gills, brain and liver of fish (Rehman *et al*., 2021). According to Usman *et al*. (2020) pollutants has ability to induce a significant reduction in liver, brain and gills, CAT level of *C. idella*. Rana *et al*. (2018) also noted the similar results in gills of *C. striata*. Reduction in CAT level in liver, brain and gills of *C. striata* due Pb+Ni was noted by Arshad *et al*. (2018). Metals mixture (Fe+Ni+Pb+Zn) exposure to *Cirrhinus mrigala* showed a reduction in liver, gills, and brain CAT activity (Naz *et al*., 2018).

Catalase is an essential cell reinforcement defense part shields fish from oxidative pressure by changing the hydrogen peroxide into water and oxygen (Atli and Canli, 2007). Hansen (2006) revealed the reaction of multiple

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antioxidant enzymes, including CAT in three populations of *Salmo trutta* exposed to metals in their natural habitat. They discovered that the kidney and liver of metal-exposed trout had higher enzyme activity than reference trout. Similarly, after Cd exposures, CAT activity rose in the liver and kidney of *O. mossambicus* (Basha and Rani, 2003) showing a prospective shift towards a detoxifying component following Cd exposure.

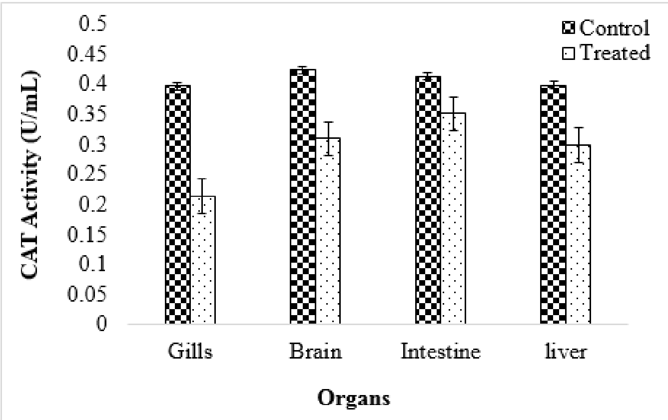

Figure 2: Activity of catalase in organs of fish, tilapia exposed to Cd+Pb mixture.

Intestine histology

In present study, Cd+Pb - exposure to tilapia showed severe damages to intestine histology as compared to control group (Figure 3) at a very high extent. The damages include complete villi structure damages with distortion of villi boundaries there is no prominent cellular bodies with nuclei seen, necrosis in mucosa and hemorrhage in submucosa noticed. The decay of structures in the hepatic tissues and in the gut of fish when injected with Cd (Younis *et al.*, 2013). Bioaccumulation of sub-lethal elements like Cd+Pb mixture resulted in tissue harm which promoted the weakened behavior and impaired physiology of the stressed living being (Patnaik *et al.*, 2011). Epithelium of columnar cells damage badly and complete destruction of blood vessels, lymphocytes observed. Some villi shows tips facing lumen damage which revealed that, the damage starting from tips to the basal layer. There are no goblet cells seen after metal exposure. Dilation in lamina propria occurred. Our findings are in accordance with Younis *et al.* (2013), same damages in intestine of tilapia after exposure of cadmium was noted. Liu *et al.* (2020) reported villi structure damages in big head carp after lead exposure. Metal buildup in fish can result in a variety of adverse outcomes, including changes in enzyme activity and organ damage (Al-kshab and Yehya, 2021).

It is a truth that mainly consumption of heavy metals happens via gills but it also take place through intestine as reported by Mohamed (2008), Gills are the first organs to face external pollutants but intestine has a lot of finger like projections which increases its surface area (Osborne *et al.*, 2015). For absorptive function, intestinal villi supply a large epithelial surface area (Hosoyamada and Sakai, 2005). Lead stress has been shown to increase zebrafish intestinal pathogens, disrupt the physical barrier of the gut in previous research and induce goblet cell mucin secretion (Xia *et al.*, 2018).

Ajmaal *et al.* (2024) also reported the significant increase in villus height, width and muscularis mucosa and the significant decrease in the crypt depth and tunica mucosa in intestinal tissues of Cd-exposed fish.

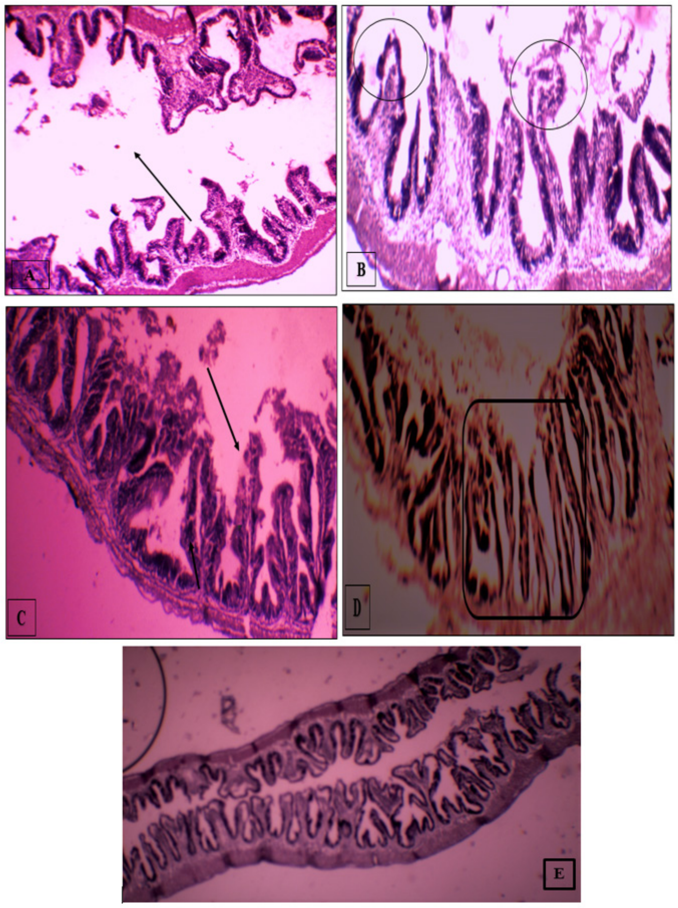

Figure 3: Histological alterations in intestine (A) Widening of intestinal lumen (Arrow); (B) Curling of intestinal villi (Shown by circle) (C) Mucous cells degeneration (Arrow) (D) Necrosis in villi (Shown by rectangle) (E) Control.

Brain histology

After acute exposure of Cd+Pb mixture to tilapia, the brain shows several histological lesions hemorrhage, congestion, necrosis, edema, vacuolar degeneration in the granular layer (Figure 4) as compared to control group (Figure 5). Al-kshab and Yehya (2021) reported the similar damages (necrosis and vacuolization) in *G. affinis* after exposure of lead chloride. The Cd+Pb mixture showed the neuronal cell degeneration, vacuolization, expanding of pyramidal cells and dystrophic changes were trademark highlights saw in the treatment of brain (Patnaik *et al.*, 2011). Vacuolization in tissue might be

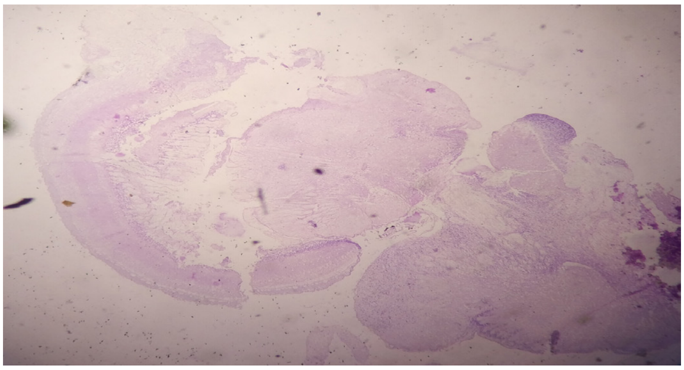

Figure 4: Tilapia brain normal histology of after exposure of Cd+Pb.

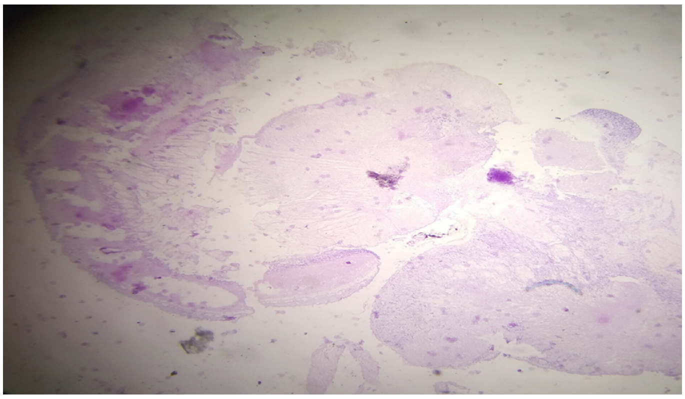

Figure 5: Damages in tilapia brain including lesions, hemorrhage, congestion, necrosis, edema and vacoular degeneration.

the after effect of glycolysis prompting mitochondrial and microsomal dysfunctions (Loganathan *et al*., 2006). The extreme necrosis of neuronal cells in the cerebrum demonstrating the loss of nissl substances. Lead acts straightforwardly on the cerebral vasculatures including blood-cerebrum obstruction and causes cerebral edema. After exposure to lead, glial nodules, malacia, and brain haemorrhage cause organism mortality in *C. gariepinus* (Adeyemo, 2008). Results of Al-Sawafi *et al*. (2017) are also similar to our findings that zebrafish show several abnormalities and gliosis and destruction after exposure of cadmium. Vacuolization occurs after disturbance of biological reactions in *C. carpio* after exposure of lead and cadmium (Patnaik *et al*., 2011). Lead, a highly neurotoxic substance that affects distinct functional parts of the brain in humans and fish and is known to interact at the molecular and cellular levels inside the body. When lead interacts with the brain then each region answers contrarily (Saha *et al*., 2016). Sabullah *et al*. (2020) reported the cell death in *C. gariepinus* after exposure of zinc sulphate following detachments around neurons. The brain of Pb-stressed *O. niloticus* showed the hemorrhage, vacuolation, inflammation, degeneration and atrophy (Rajamanickam and Devadason, 2021). Toxicant (Imidacloprid) induced neural disordered like neuronophagia, vocuolation and complete neural loss in *Clarias gariepinus was noted by* Rahman *et al*. (2023). Naz *et al*. (2023) also observed the degenerative alterations like neuron necrosis, ctoplasmic vacuolization, intracellular edema and congestion in brain tissue of $CuSO_4$ exposed *Labeo rohita*. The histopathological analyses showed significant brain damage characterized by glial scar formation and ventricular enlargement in Pb and As- exposed Zebrafish (Liu *et al*., 2024).

Conclusion

This study concluded that heavy metals are highly toxic to aquatic animals like fish. Because these pollutants can alter the antioxidant enzymes activity and also cause histological changes in fish. Heavy have a number of negative impacts on fish in many ways, which is very detrimental to the production of sustainable aquaculture. These biomarkers can be used to detect the pollution of aquatic bodies. Further research is needed to develop the some new suitable, easy and most convenient approaches to minimize the metals pollution in aquatic environment.

Declarations

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IRB approval

This study was approved by the Institutional Ethical Review Committee of Cholistan University of Veterinary and Animal Sciences, Bahawalpur.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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