



METALS MIXTURE (ZINC+LEAD) INDUCED CHANGES IN MOVEMENT PATTERNS OF *CIRRHINA MRIGALA*

HAROON S¹, ABDULLAH S¹, NAZ H^{*2}, ABBAS K¹, AHMED T³

¹Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad

²Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

³Department of Life Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim

ARTICLE INFORMATION

Article History:

Received : 30th November 2020

Accepted: 9th December 2020

Published online: 11th January 2021

Author's contribution:

All authors contribute equally.

Key words:

Fish, metal mixture, behavior, toxicity

ABSTRACT

The widespread use of metals for industrial and non-agricultural purposes has resulted in the presence of their residues in various environmental matrices. Metal contamination of surface waters has been well documented worldwide and constitutes a major issue that gives rise to concerns at local, regional, national and global scales. Metals have great impact on the behavioral parameters of fish. Therefore, in present research work was designed to evaluate the behavioral changes in fish, *Cirrhina mrigala* exposed to 96-hr LC₅₀ concentration of zinc(Zn)+lead(Pb) of mixture for 4 days. Results showed that the exposure of Zn+Pb mixture increased the jumping, equilibrium status, opercula movement, fin movement, convulsion, hyperactivity and surfacing frequency of fish as compared to control group. It was also concluded that metals exposure altered the behavior of fish.

1. INTRODUCTION

Heavy metals are present naturally in the environment; however, as a consequence of industrial, agricultural, and anthropogenic activities levels of them are increasing rapidly. Heavy metals at high concentrations can cause hazardous effects to many aquatic organisms by changing genetic, metabolic, physiological, biochemical, and behavioral parameters (Atli and Canli, 2007; Ramesh et al., 2009). Fish are most susceptible to the adverse impacts of pollutants and provide as an excellent implement for understanding the health condition of aquatic ecosystem (Lushchak, 2008 2011). Fish can uptake heavy metals from the adjacent environment and add them in its different organs and tissues resultant into biomagnifications in the food chain make the fish an important marker of metallic ions pollution (Abdullah et al., 2007).

Essential heavy metals like zinc, chromium, copper, nickel, molybdenum, iron and cobalt take part an important role in numerous biological processes. However, the necessary metals, when set up at higher concentration can cause toxic effects on the organisms (Sivaperumal et al., 2007). Zinc (Zn) is one of the most important essential trace elements involved in animal growth and the most widely used metal cofactor of many enzymes involved in protein, nucleic acid, carbohydrate, and lipid metabolism (Carpene et al. 2003; Sun et al. 2005). Zinc in certain concentration is desirable for fish growth but its over accumulation is hazardous to exposed fish (Murugan et al. 2008). Zinc is one of the most common contaminants in aquatic systems and is associated with urban runoff, soil erosion, industrial discharges, pharmaceuticals, pesticides and a variety of other activities and sources (Schmitt 2004; Bowen et al. 2006).

*Corresponding Author: dr.humanaz98@gmail.com

Copyright 2017 University of Sindh Journal of Animal Sciences

The danger of Zn is aggravated by its almost indefinite persistence in the environment because it cannot be destroyed biologically and is only transformed from one oxidation state or organic complex to another (Everall *et al.* 1989). Lead is extremely toxic metal as it is reported to be accountable for death or sub-lethal changes in reproduction, enlargement and act of the fish (Ramsdorf *et al.*, 2009). Lead is one of the most injurious contaminants of aquatic environment. The chief sources of lead contamination are removal and smelting of lead ores, industrial effluents, fertilizers, pesticides and community sewage wastes (Needleman, 2006). Lead may penetrate into the fish body throughout different routes i.e., skin, gills and respiratory region (Olaifa *et al.*, 2004). Once immersed, lead becomes dispersed in the liver, kidney, gills, heart, gonads with the blood of fish (Astdar, 2005). The studies on fish behaviors provide a lots of knowledge and information because, any behavior alteration can be related to physiological biomarker in aquatic species (Amiard-Triquet, 2009). For example, the monitoring of behavioral response becomes an imminent option to environmental change, disease, stress and the presence of toxic compound in water, which most of this condition initiates the variation of fish behavior (Almazan-Rueda *et al.*, 2004; Gerhardt, 2007).

Fish behavior represents the fish physiological response towards the environmental factor. Moreover, the coordination of fish behavior related to the ecology can be easily observed even if it can quantified (Scott and Sloman, 2004) and at the same times effecting the consequences of metal toxicity upon the concentration and species, including size (Vosyliene *et al.*, 2003; Hussain *et al.*, 2011). For instance, the existence of metal ion in the environment mediation increased the mucus like secretion from gill, excessive excretion, anorexia and also the fin movement (Ezeonyejiaku *et al.*, 2011). Therefore, present works was designed to see the behavioral changes in *Cirrhina mrigala* due metals mixture exposure.

2. MATERIALS AND METHODS

The experiment was conducted with *Cirrhina mrigala* at Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. Acute exposure of zinc (Zn) and lead (Pb) mixture was given to *C. mrigala* for 4-days. Prior to experiment, fish fingerlings were kept in laboratory conditions for two weeks for acclimatization. After acclimation period, fish were transferred to 70-liter glass aquarium for oxidative stress studies. Ten fish were kept in each aquarium.

The water temperature (30°C), pH (7.0) and total hardness (200 mg L⁻¹) were kept constant throughout the study period. Continuous air was supplied to the test and control mediums with an air pump through capillary system. Chemically extra pure compounds of zinc and lead were used to prepare stock solutions of desired dilution. The LC₅₀ (96 hr) value of Zn+Pb mixture for *C. mrigala* was computed as 71.55±0.36 (Naz and Javed, 2012). The fish was observed after every 2 hours for 4-days.

Behavioral Study

During Zn+Pb mixture exposure, the behavioral parameters of fish viz. jumping, equilibrium status, opercula movement, fin movement, convulsion, hyperactivity and surfacing frequency were studied.

Data Presentation

Data is presented in the form of Normal (0), Mild (+), Medium(++), High(+++).

3. RESULTS AND DISCUSSION

In present study behavioral changes of *C. mrigala* exposed to Zn+Pb mixture were observed (Table 1). During first three days shows the jumping, increased opercula and fin movement, surfacing frequency, convulsion and hyperactivity. The equilibrium status was abnormal. Fish *C. mrigala* exposed to Fe+Ni+Pb+Zn mixture showed increased hyperactivity, swimming rate, mucous secretion and fin movement with increasing the concentration of mixture (Naz *et al.*, 2018). According to Eissa *et al.* (2009) locomotion parameters are sensitive endpoints and useful biomarkers in behavioral studies of freshwater toxicity. Fish exposed to Zn showed sluggish movement, rapid operculum movement and loss of equilibrium (Abdel-Gawad *et al.*, 2011). Visualized the behavioral changes in fish, Nile tilapia (*O. niloticus*) and catfish in response to copper sulphate (50, 60, 70, 80 100 and 120 mg/l). *O. niloticus* showed more severe effects, as compared to catfish. Avoidance behavior was observed by unsteady swimming pattern with jerky moments. Fish were observed to be suspended in vertical position with tail pointing downwards. Finally fishes sank in the bottom and became motionless (Bhat *et al.*, 2012). The abnormal behavior shown by fish may be due to abnormal level of neurotransmitters. These changes occur much earlier than mortality (Little and Finger, 1990). Jumping to and fro signify the avoidance reaction of the fishes to the toxicants. Fish avoid the area containing chemical so mostly fishes remain in the corners of the tank. The increase in surfacing and gulping of air from surface water after toxicant exposure could be an attempt of the animal escape from the toxicant and to avoid breathing in the contaminated water. Secretion of excessive mucus is

probably due to irritation of the skin due to direct contact with the toxicant. Mucus forms a layer between the body and toxicant to minimize irritating effect (Rao, 2006) and also inhibit the diffusion of oxygen during gaseous exchange (Kumar et al., 2015). Preference of upper layer may be due to respiratory stress in the exposed groups (Katja et al., 2005). Lateral swimming and loss of equilibrium is probably due to the impairment of the nervous system (Sinha and Kumar, 1992). Ultimately, fish sank into the tank bottom with a least operculum activity showing failure to fight with stress and ultimately the fish died. Inappropriate behavioral responses to environmental and physiological stimuli due to the toxic effect of aquatic contaminants can have severe implications for survival (Weber and Spieler, 1994).

4. CONCLUSION

The present study concluded that the exposure of metals mixture altered the behavior of fish.

5. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interests regarding the publication of this article.

REFERENCES

- [1] Abdel-Gawad CK, Nadia M, Hassanein MA, Bassem SM (2011) Evaluation of DNA Damage in Fish and Aquatic Insects Induced By Environmental Pollutants in River Nile. *World Appl. Sci. J.* 2011:1085-1090.
- [2] Abdullah S, Javed M, Javid A (2007). Studies on acute toxicity of metals to the fish (*Labeo rohita*). *Int. J. Agric. Bio.* 2: 333-337.
- [3] Almazán-Rueda P, Schrama JW, Verreth JAJ (2004). Behavioural responses under different feeding methods and light regimes of the African catfish (*Clarias gariepinus*) juveniles. *Aquaculture*, 231: 347-359.
- [4] Amiard-Triquet, C (2009). Behavioural disturbances: The missing link between suborganismal and supra-organismal responses to stress? Prospects based on aquatic research. *Hum. Ecol. Risk Assess.* 15: 87-110.
- [5] Atli G, Canli M (2007). Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. *Comp. Biochem. Physiol.* 145: 282-287.
- [6] ATSDR (Agency for Toxic substances and Disease Registry), 2005. Draft toxicological

- profile for lead. Atlanta: US Department of Health and Human Services, 54: 102-225.
- [7] Bhat IA, Bhat BA, Vishwakarma S, Verma A, Saxena G (2012). Acute Toxicity and Behavioural Responses of *Labeo rohita* (Hamilton) to a Biopesticide "NEEM-ON". *Current World Environment.* 2012; 7:175-178.
 - [8] Bowen L, Werner I, Johnson ML (2006). Physiological and behavioral effects of zinc and temperature on coho salmon (*Oncorhynchus kisutch*). *Hydrobiol.* 559:161-168
 - [9] Carpena E, Andreani G, Monari M, Kindt M, Isani G (2003). Biochemical changes during post-larval growth in white muscle of Gilthead Sea Bream (*Sparus aurata*) fed Zinc fortified diets. *Vet. Res. Commun.* 27:215-218
 - [10] Eissa, B.L., Ossana NA, Ferrari L, Salibian A (2009). Quantitative behavioral parameters as toxicity biomarkers: Fish responses to waterborne cadmium. *Arch Environ. Contam. Toxicol.* 58:1032-9.
 - [11] Everall NC, MacFarlane NAA, Sedgwick RW (1989). The interactions of water hardness and pH with the acute toxicity of zinc to the brown trout, *Salmo trutta* L. *J Fish Biol.* 35: 27-36.
 - [12] Ezeonyejiaku CD, Obiakor MO and Ezenwelu CO (2011). Toxicity of Copper Sulphate and behavioral locomotor response of tilapia (*Oreochromis niloticus*) and Catfish (*Clarias Gariepinus*) Spices. *Online J. Anim. Feed Res.* 1: 130-134.
 - [13] Gerhardt A (2007). Aquatic behavioral ecotoxicology prospects and limitations. *Hum. Ecol. Risk Assess.* 13: 481-491.
 - [14] Hussain SM, Javed M, Javid A, Javid T, Hussain N (2011). Growth responses of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* during chronic exposure of iron. *Pak. J. of Agric. Sci.* 48: 225-230.
 - [15] Katja S, Georg BOS, Stephan P, Christian EWS (2005). Impact of PCB mixture (Aroclor 1254) and TBT and a mixture of both on swimming behavior, body growth and enzymatic biotransformation activities (GST) of young carp (*Cyprinus carpio*). *Aquat. Toxicol.* 71:49-59.
 - [16] Kumar, M., Kumar, P., Devi, S. 2015. Toxicity of copper sulphate on behavioral parameters and respiratory surveillance in freshwater catfish, *Clarias batrachus* (Lin.) *Research Journal of Chemical and Environment Sciences.* 3(1):22-23.

- [17] Little EE, Finger SE (1990). Swimming behavior as an indicator of sublethal toxicity in fish. *Environmental Toxicology and Chemistry*. 9:13-19.
- [18] Lushchak O, Kubrak VOI, Nykorak MZ, Storey KB, Lushchak VI (2008). The effect of potassium dichromate on free radical processes in goldfish: possible protective role of glutathione *Aquatic Toxicol.* 87: 108-114.
- [19] Needleman H (2004). Lead poisoning. *Annual Review of Medicine*, 55: 209-222.
- [20] Olafia FE, Olaif AK, adelaja A, Owolabi AG (2004). Heavy metal contamination of *Claris gariepinus* from a lake and fish farm in Ibadan, Nigeria. *Afr. J. Biomed. Res.* 7: 145-148.
- [21] Ramesh M, Saravanan M, Kavitha C (2009). Hormonal responses of the fish, *Cyprinus carpio*, to environmental lead exposure – *Afr. J. Biotechnol.* 8(17): 4154-4158.
- [22] Ramsdorf WA, Ferraro MVM, Oliveiraribeiro CA, Costa JRM, Cestari MM (2009). Genotoxic evaluation of different doses of inorganic lead (PbII) in *Hoplias malabaricus*. *Environ. Monit. Assess.* 158: 77-85.
- [23] Rao JV (2006). Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. *Pestic. Biochem. Physiol.* 86:78-84.
- [24] Naz S, Javed M (2012). Acute toxicity of metals mixtures for fish, *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*. *Pak. J. Agri. Sci.* 49: 357-361.
- [25] Schmitt CJ (2004) Concentrations of arsenic, cadmium, copper, lead, selenium, and zinc in fish from the Mississippi river basin, 1995. *Environ. Monit. Assess.* 90: 289-321.
- [26] Scott GR, Sloman KA (2004). The effects of environmental pollutants on complex fish behaviour: Integrating behavioural and physiological indicators of toxicity. *Aquat. Toxicol.*, 68: 369-392.
- [27] Murugan SS, Karuppasamy R, Poongodi K, Puvaneswari S (2008) Bioaccumulation pattern of zinc in freshwater fish *Channa punctatus* (Bloch.) after chronic exposure. *Turk. J. Fish. Aquat. Sci.* 8: 55–59.
- [28] Naz H. Abdullah S, Naz S, Abbas S, Hassan W, Perveen S, Batool M, Shafique L (2018). Comparative assessment of the acute toxicity, behavior and catalase activity in *Cirrhina mrigala* exposed to Fe+Ni+Pb+Zn mixture. *Punjab University Journal of Zoology*, 33(1): 91-97
- [29] Sinha TKP, Kumar K (1992). Acute toxicity of mercuric chloride to *Anabas testudineus* (Bloch). *Environ. Ecol.* 10:720-722.
- [30] Sivaperumal P, Sankar TV, Viswanathan NPG (2007). Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-à-vis international standards. *Food Chem.* 102: 612-620.
- [31] Sun JY, Jing MY, Wang JF, Zi NT, Fu LJ, Lu MQ, Pan L (2005) Effect of zinc on biochemical parameters and changes in related gene expression assessed by cDNA micro arrays in pituitary of growing rats. *Nut* 22:187–196
- [32] Vosyliene MZ, Kazlauskienė N, Svecevičius G (2003). Effect of a heavy metal model mixture on biological parameters of rainbow trout, *Oncorhynchus mykiss*. *Environ. Sci. Pollut. Res.* 10: 103-107.
- [33] Weber DN, Spieler RE (1994). Behavioral mechanisms of metal toxicity in fishes. In: Malins, D.C., Ostrander, G.K. (Eds.), *Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives*. CRC Press, London UK, p. 421-467.

Table1 Movement patterns of *Cirrhina mrigala* under Zn+Pb exposure

Parameter	Control	Day-1	Day-2	Day-3	Day-4
Surfacing frequency	-	+	+++	+++	+
Jumping	-	+	+++	++	+
Fin movement	-	+	+++	++	+
Hyperactivity	-	+	+++	++	+
Equilibrium status	-	+	++	++	+
Opercula movement	-	+	+++	++	+
Convulsion	-	+	++	++	+

Normal (0), Mild (+), Medium (++), High (+++)