RESEARCH ARTICLE Agricultural Science Digest, Volume 41 Special Issue: 247-251 (April 2021)

Toxic Impact of Zinc Salt ($ZnSO_4$) on Some Haematological Parameters in a Freshwater Mud Eel *Amphipnous cuchia* (Hamilton, 1822)

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10.18805/ag.D-5156

ABSTRACT

Background: Zinc (Zn), a heavy metal present in aquatic environment, has toxic effect on fishes. Present study was to assess toxicity of Zinc salt ($ZnSO_4$) on total erythrocyte count (TEC), haemoglobin (Hb) content (g/dl) and haematocrit value (PCV%) of blood of freshwater mud eel Amphipnous cuchia.

Methods: Live fishes collected from ponds were acclimatized in laboratory. TEC, Hb content and PCV% of blood samples collected after 15, 30, 60 and 90 days, of control fishes and fishes exposed to low (0.5mg/l) and high (1mg/l) concentrations of ZnSO4, were determined by standard methods.

Result: TEC, Hb content and PCV% varied respectively from 2.48 x 10^6/mm^3 to 2.70 x 10^6/mm^3, 12.02 g/dl to 12.30 g/dl and 28.50 % to 33.60 % in fishes in low and from 2.36 x 10^6/mm^3 to 2.60 x 10^6/mm^3, 11.82 g/dl to 12.20 g/dl and 26.20 % to 31.50 % in fishes in high concentration of $ZnSO_4$ solution, showing decreasing trend in all three blood parameters vis-a-vis their control values *viz*. TEC (2.74 x 10^6/mm^3), Hb content (12.60 g/dl) and PCV% (34.50 %), decrease being more prominent in fishes exposed to high than those to low concentration. The depletion in these blood parameters may be attributed to the haemotoxic characteristics of Zinc (Zn).

Key words: *Amphipnous cuchia*, Erythrocyte, Haemoglobin, Haematocrit, Zn salt.

INTRODUCTION

The contamination of freshwater bodies with a wide range of pollutants has become a matter of concern over last few decades. Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and on a variety of aquatic organisms. Van der Oost *et al*. (2003) reported that natural aquatic systems are suffering extensive heavy metal contamination due to heavy metal salts released in domestic, industrial, mining and agricultural effluents and in other man-made activities. Gandhewar and Zade (2019) observed that elevated levels of common heavy metals like Copper (Cu), Lead (Pb), Mercury (Hg), Cadmium (Cd), Nickel (Ni) and Chromium (Cr) in aquatic resources constitute serious threat to aquatic ecosystem as they bioaccumulate in vital organs of fishes and prove highly hazardous to the health of fishes and humans. Hadeel and Ahmed (2019) reported that heavy metal aquatic pollutants like Pb, Cu and Zn, which are not eliminated from aquatic system by natural methods, are considered most toxic to humans, fishes and environment and that the toxic effects of these heavy metals differ in fishes, depending on their age, development and other physiological factors. As fishes cannot escape the detrimental effects of pollutants built up in their food chain in aquatic systems, they are widely used to evaluate the health of aquatic ecosystems. It was reported that heavy metals do bio-accumulate in aquatic biota especially fishes. Obasohan and Equavoen (2008) reported higher accumulation levels of Cu, Manganese (Mn), Zn, Cd, Ni and Pb in freshwater fish *Erpetoichthys calabaricus* than in surrounding waters

in Ogba River, indicating a bioaccumulation in fishes, which exceeded WHO recommended limit also, thereby suggesting that fishes of the river were not suitable for human consumption. Ayotunde *et al*., (2011), also observed during their investigation that heavy metals *viz*. Zn, Cu, Iron (Fe), Cobalt (Co), Pb, Cd and Cr undergo bioaccumulation in tissues of aquatic organisms including fishes, through food ingestion, skin and gills. The studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in their tissues as well as in their blood.

Some of the heavy metals are essential nutrients required for various physiological and biochemical activities of human body but become harmful when present in high

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How to cite this article: Sinha, M.R. and Kumari, S. (2021). Toxic Impact of Zinc Salt ($ZnSO_4$) on Some Haematological Parameters in a Freshwater Mud Eel *Amphipnous cuchia* (Hamilton, 1822). Agricultural Science Digest. 41 (Special Issue): 247-251. DOI: 10.18805/ag.D-5156.

Submitted: 19-02-2020 **Accepted:** 08-07-2020 **Online:** 10-11-2020

quantity. It has been reported that heavy metals do bioaccumulate in living organisms and human body through various processes. Therefore the impact of heavy metals on physiology and metabolism of living organisms is being evaluated by many investigators *viz*. Damien *et al*., (2004), Farombi *et al*., (2007), Jezierska *et al*., (2009), Dirican *et al*., (2015) and Authman *et al*., (2015). Malik *et al*., (2016) have observed significant decrease in body weight as well as increase in AST, ALT and ALP in blood plasma in major carp due to heavy metal Ni toxicity and concluded that Ni acts as a hepatotoxic and endocrine disruptor. Kumar *et al*., (2017) were of the view that fish blood is highly susceptible to aquatic pollutants and as such is a useful bio-indicator to assess the toxic effect of various pollutants. As such, the present research project was taken up with an aim to investigate the toxic impact of heavy metal Zinc on the haematology of *Amphipnous cuchia* and a commonly used Zinc salt, Zinc Sulphate ($ZnSO_4$) was selected.

MATERIALS AND METHODS

In the present investigation a freshwater mud eel *Amphipnous cuchia* (Hamilton, 1822) was taken as the experimental animal. It is an omnivorous, air breathing fish belonging to the family - Synbranchidae, Order - Synbranchiformes.

Live fishes were collected from local fishermen of Patna town and adjacent localities during the month of April and May of the research period year 2017 – 18 for the present research project. The live fishes were brought to the Zoology department laboratory, Magadh Mahila College, Patna University, Patna, Bihar in polythene bags containing the pond waters. The fishes were disinfected by washing properly with dilute $KMnO_4$ and then transferred to many large aquaria (90 cm x 60 cm x 45 cm). The fishes were left for acclimatization to laboratory conditions for ten days. During this period, water of each aquarium was changed on alternate days to prevent fungal growth.

Healthy acclimatized fishes of 25 ± 4 cm body size were then selected and kept as stock for the experiments. Only 8 to 10 fishes were kept in each aquarium throughout the experiment. Tap water of similar quality from in-house college borewell was used throughout the experiment. The fishes were fed with pieces of goat's liver and fish feed available in the local market, twice daily *ad-libitum*. Fishes were divided into two groups, group I served as control and group II as experimental one.

In the present investigation acclimatized fishes from group II were divided into two groups and were exposed to $ZnSO_4$ solutions of two different concentrations prepared in two separate aquariums each containing ten litres of water by dissolving 0.5 mg/l $ZnSO_4$ in aquarium which is marked as low concentration aquarium and 1 mg/l $ZnSO_4$ in aquarium which is marked as high concentration aquarium. The sub lethal concentration of the $ZnSO_4$ was selected based on static bio-assay method as per APHA (2005). The treatment of the experimental fishes continued for ninety days and sampling was done fortnightly up to 30 days and thereafter every 30 days. The treated fishes were collected with the help of hand net and water from the body was soaked with the help of a tissue paper. The blood was collected as per the method suggested by Nagpure *et al*., (2007) with the help of a sterilized syringe and collected in an EDTA coated vial.

The blood parameters *viz*. TEC, Hb content and PCV% were determined by employing standard methods as described by Dacie and Lewis (2006). Along with the experimental fishes, blood from control group I fishes were also collected and the above referred blood parameters were determined for making a comparison with the values obtained from the experimental fishes to evaluate the impact of the toxic effect of $ZnSO_4$ solution. All experiments were performed in triplicate groups.

RESULTS and DISCUSSION

In the present study, TEC in experimental fishes exposed to low concentration of $ZnSO_4$ shows a range of 2.48 ± 1.02 x $10^6/mm^3$ to 2.70 ± 0.65 x $10^6/mm^3$, while in higher concentration TEC varies from 2.36 ± 1.56 x $10^6/mm^3$ to 2.60 ± 0.94 x $10^6/mm^3$. The Hb content exhibits a range of 12.02 ± 1.20 g/dl to 12.30 ± 1.33 g/dl and 11.82 ± 1.04 g/dl to 12.20 ± 1.15 g/dl respectively in lower and higher concentrations of $ZnSO_4$. The PCV% was found in the range of 28.50 ± 0.26 % to 33.60 ± 0.56 % and 26.20 ± 1.04 % to 31.50 ± 1.12 % respectively in lower and higher concentrations of $ZnSO_4$. All the three blood parameters investigated in present study show a decreasing trend right from the start of the treatment in both concentrations of $ZnSO_4$ when compared with their respective control values. The control values of TEC, Hb content and PCV% recorded at the start of experiment were 2.74 ± 1.03 x $10^6/mm^3$, 12.60 ± 1.98 g/dl and 34.50 ± 1.56 % respectively. The values were statistically found significant at $p < 0.01$ level. (Table 1 and Fig 1, 2 and 3).

Ranbhare and Bakare (2012) have observed appreciable decline in the biochemical profiles such as total glycogen, total lipids and total protein content of experimental fishes, due to toxic effects of heavy metals present in Krishna River at Karad leading to decrease in productivity of the fish population. Afshan *et al*., (2014) have reported that heavy metals present in aquatic media enter the fish body by three possible ways *viz*. through digestive track, gills and body surface, the latter two being most significant for direct uptake of heavy metals and its toxicity may affect their growth rates, physiological function, mortality and reproduction. Bawuro *et al*., (2018) reported accumulation of heavy metals *viz*. Zn, Pb, Cd and Cu in liver, gills and flesh of fishes. They observed that fishes exhibit interspecific variation in heavy metal accumulation, the accumulation pattern being Zn > Cu > Pb > Cd. They also found that accumulation was more in liver than in other organs, a view also supported by Gorar *et al*., (2012). The higher level of bioaccumulation of metals in liver may be linked to its functioning like detoxification

Table 1: The values of different blood parameters quantitatively analyzed in control and experimental fish *Amphipnous cuchia* after exposure to low and high concentration of Zn salt ($ZnSO_4$) for different duration.

Duration of Exposure (days)	TEC ($x10^6/mm^3$)			Haemoglobin content (g/dl)			Haematocrit Value (%)		
	Control Value	Value in low conc.	Value in high conc.	Control value	Value in low conc.	Value in high conc.	Control value	Value in low conc.	Value in high conc.
15	2.74±1.03	2.70±0.65	2.60±0.94	12.60±1.98	12.30±1.33	12.20±1.15	34.50±1.56	33.60±0.56	31.50±1.12
30		2.65±1.24	2.51±0.96		12.20±1.06	12.18±0.98		31.20±1.06	30.20±1.16
60		2.53±0.86	2.42±1.08		12.19±1.36	11.96±0.76		30.40±1.15	29.50±1.09
90		2.48±1.02	2.36±1.56		12.02±1.20	11.82±1.04		28.50±0.26	26.20±1.04

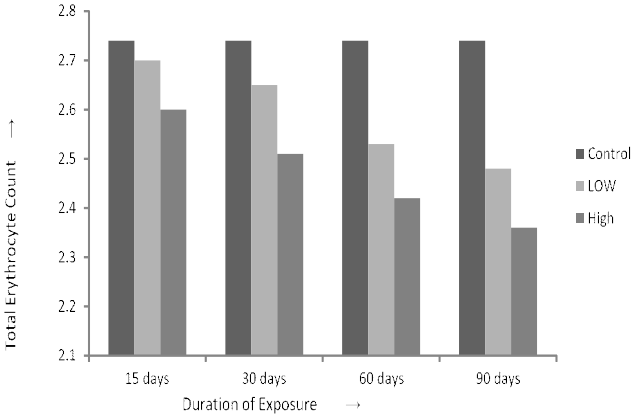

Fig 1: Comparative study of changes in Total Erythrocyte Count (TEC nos x $10^6/mm^3$) of blood after exposure to low and high concentration of Zn salt ($ZnSO_4$) for different duration in *Amphipnous cuchia* .

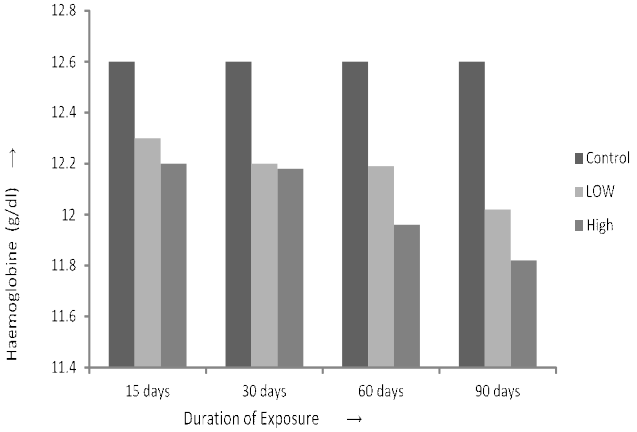

Fig 2: Comparative study of changes in Haemoglobine content (g/dl) of blood after exposure to low and high concentration of Zn salt ($ZnSO_{4)}$ for different duration in *Amphipnous cuchia* .

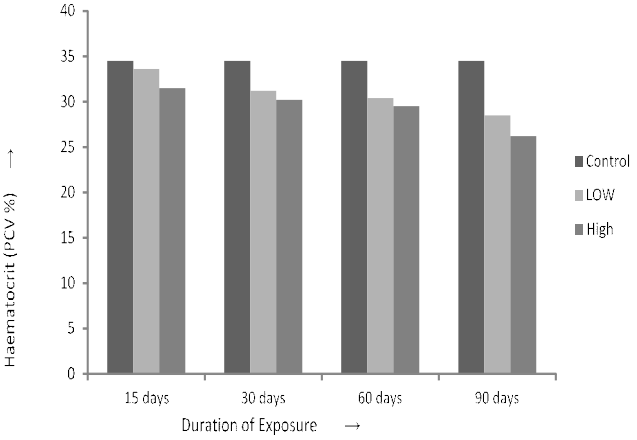

Fig 3: Comparative study of changes in Haematocrit (PCV %) values of blood after exposure to low and high concentration of Zn salt ($ZnSO_4$) for different duration in *Amphipnous cuchia*.

and redistribution (Dural *et al*., 2007). On the other hand Witeska and Kosciuk (2003) during their investigation came to conclusion that heavy metals such as Cd, Cr, Ni and Pb might alter the properties of Hb by decreasing their affinity towards oxygen binding capacity rendering the erythrocytes more fragile and permeable, which probably results in cell swelling, deformation and damage. Influence of Cd on differential blood count of *Anguilla anguilla* has been reported by Gill and Epple (1993). Significant decrease in the haematocrit level of blood induced by heavy metals in freshwater common carp *Labeo rohita* after an exposure period of 1, 8, 16 and 32 days was observed by Vinodhini and Naryana (2009), which is in agreement with the observations of Vutkuru (2005) who reported a significant decrease in TEC, Hb content and PCV% in freshwater fishes exposed to heavy metals. Shalaby (2001) also observed depletion in the values of blood parameters in Nile tilapia *Oreochromis niloticus* due to heavy metal toxicity. Afifi *et al*., (2016) have observed that prolonged exposure to high doses of Zn nanoparticles probably induces neurotoxicity resulting in neurodegenerative and apopotic lesion in brain of fishes which may cause behavioural changes, a view also supported by Valdiglesias *et al*., (2013). Kumar *et al*., (2017) reported that exposure of *Clarias batrachus* to sub-lethal concentration of Cd and Pb resulted in significant decrease ($p < 0.05$) in TEC, Hb content and PCV%, while there was a slight increase in case of Cu treatment. Some morphological changes in RBC like poikilocytosis or anisocytosis in the blood of *Clarias batrachus* on exposure to sub-lethal concentrations of Cd (7.5 ppm), Pb (3.6 ppm) and Cu (0.5 ppm) was also reported by them. However, Serezli *et al*., (2011) observed that TEC in *Salmo coruhensis* exposed to high concentration of Pb salt shows a significant depletion only after 48 hrs of treatment though a rise was noticed in TEC during short term treatment. Whereas, Chidiebere *et al*.,(2019) reported that fishes were seen swimming to the surface frequently with their opercula and mouths moving rapidly when exposed to different concentrations of Cu and Zn salts and concluded that this is an indication that toxic effects of the heavy metals caused the depletion of Oxygen content of the medium.

The scholars were of the view that the result of the present study is in conformity with the observations of the above referred investigators and the haemotoxic characteristics of heavy metal Zinc may be responsible for the significant depletion in the blood parameters.

CONCLUSION

The haematological parameters measured react reasonably to high Zinc concentration when treated for longer duration. As a consequence of the present study, which shows that heavy metals induce significant anomalies to the blood parameters, these blood parameters appear to be a good indicator of toxicants.

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