Effects Of Sublethal Concentrations Of Zinc (II) Sulphate Heptahydrate ($Znso_4.7H_2O$) On Blood Glucose Level Of Freshwater Fish Heteropneustes Fossilis

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Abstract: Laboratory study was undertaken to evaluate glucose level changes resulting from the exposure of a freshwater fish, *Heteropneustes fossilis* at different concentration of Zinc (II) Sulphate Heptahydrate ($ZnSO_4.7H_2O$) in water for three days. A group of ten fish were subjected to serial dilutions of the stock solution of $ZnSO_4$ ranging from 25ppm to 500ppm in twelve large plastic bowls of 25 liter capacity by the semi-static (renewal) method. At the end of the exposure period, blood samples were taken from the control and experimental fish. Blood glucose level was monitored. The study showed that the values of blood glucose have a positive correlation with concentrations of this metal. The glucose levels were found higher in the exposed fish when compared to the control. In conclusion, the changes observed indicate that glucose level can be used as an indicator of zinc related stress in fish.

Key words: Zinc (II) Sulphate Heptahydrate ($ZnSO_4.7H_2O$), *Heteropneustes fossilis*, toxicity, Blood glucose level.

INTRODUCTION

Among the various toxic pollutants, heavy metals are particularly severe in their action due to tendency of bio-magnification in the food chain. The global heavy metal pollution of water is a major environmental problem. With the advent of agricultural and industrial revolution, most of the water sources are becoming contaminated [1]. Industrial discharges containing toxic and hazardous substances, including heavy metals [2] [3] contribute tremendously to the pollution of aquatic ecosystem. Zinc, an essential element, is one of the most common heavy metal pollutants. It is an essential element acting as structural component and having specific properties indispensable for life [4]. The danger of zinc is aggravated by its almost indefinite persistence in the environment because it cannot be destroyed biologically but are only transformed from oxidation state or organic complex to another. Zn is a potential toxicant to fish [5], which causes disturbances of acid-base and ionoregulation, disruption of gill tissue [6]. At high concentrations, zinc exerts adverse effect in fish causing structural damage, which affects the growth, development and survival of fish [7]. Zinc accumulates in the gills of fish and this indicates a depressive effect on tissue respiration leading to death by hypoxia [8]. Zinc pollution also induces changes in ventilatory and heart physiology [9]. Sublethal levels of zinc have been known to adversely affect hatchability, survival and haematological parameters of fish [10], [11], reported that zinc could cause sub-acute effects that change fish behaviors. Such observed behaviors include lack of balance since most fins are motionless in the affected fish, agitated swimming, air gulping, periods of quiescence and death. Thus, it can be said that, accumulation of it has attained a serious dimension. Zn in certain concentration is desirable for the growth of freshwater animals but its over accumulation is hazardous to exposed fish as well as to those who consume them directly or indirectly through food chain. Since Zinc is a significant heavy metal so, this study aims to know the cute level of concentration on blood glucose.

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MATERIALS AND METHODS

Healthy specimens of *Heteropneustes fossilis* were obtained from a local fish farm to the laboratory. The experimental trials were conducted in a laboratory bioassay system. The system consisted of 12 plastic aquariums with twenty–five liters capacity each and acclimatized for 14 days to the laboratory conditions. During this time they were provided with artificial feed and ground shrimps obtained locally to avoid possible effects of starvation. Fish of both sexes were used without discrimination. The length of the fish varied from 12.2 to 13.9cm and the weight 8.6 -13.44 g .The mean of both length and weight are shown in Table 1. In this experiment, stock solution of the test metal compound $ZnSO_4.7H2O$, Zinc(II) Sulphate Heptahydrate (Analytical grade), molecular weight 287.54 g/mol was used.

Table 1: The length and weight of the fish.

Parameters	Range	Mean
Length (cm)	12.2 -13.9	13.05
Weight (g)	8.6 -13.44	11.02

The fish were divided into 2 groups of 12 fish each and treated as follows: Group 1 (control) was exposed to tap water only; while Groups 2 was exposed to 25ppm to 500ppm $ZnSO_4$. The test was performed by the semi-static (renewal) method. The parameters of the diluting water

used to reach the desired concentration of test chemical were determined by standard methods and are presented in Table 2. The exposure period lasted 96 hours, after which blood samples were taken from the control and experimental fish. The blood samples were taken by puncturing the caudal vessels, using EDTA (ethylenediaminetetraacetate) as anticoagulant and then centrifuged at 3500 rpm for 10 min to obtain serum samples for the analysis of blood glucose. The glucose levels in the serum samples were analyzed using 'One tech ultra^{TM}-2' device. To use it, 10µl serum samples were added to sticks and inserted to the machine. The reading was recorded every time.

Table 2: The water quality parameters.

Parameters	Range
Dissolved Oxygen	6.75mg/l
Free CO_2	43.6mg/l
pH	6.4
Alkalinity	145ppm
Hardness	100ppm
Nitrate	0.60

RESULTS AND DISCUSSION

To test the toxicity of $ZnSO_4$, two trials were done. It was done in the range of concentration of $ZnSO_4$ within 25 ppm to 500ppm (Table 3). From the Table 3, the cumulative mortality rate was calculated which presents that the fish can be killed by the dose above 50 ppm. Thus the fish were treated with the concentration between 50 ppm to 400 ppm to monitor the glucose level (Table 3). The present study showed gradually increasing concentrations of blood glucose level of the treated fish after (24, 48, 72,) hrs. for different concentration. This increasing is due to glycolsis and this took place as response to stress and transformation to glucose for energy requirement, by fish. This was supported by [12], [13], showed that the increasing of glucose level is due to high secretion of hormones like catecholamines, glucocorticords and that lead to increasing of glycolysis resulting to high glucose level in blood. Fish, like other vertebrates, respond to a stressor by eliciting a generalized physiological response, which is characterized by an increase in stress hormones and consequent changes that help maintaining the animal's normal or homeostatic state [14], [15]. This response includes, for example, increases in plasma cortisol, catecholamines and glucose levels,

Table 3. Range $ZnSO_4.7H_2O$ at 24h, 48h and 72h (Trail, n=100) along with cumulative mortality rate (%) and blood glucose reading (mmol/L)

Conc.of $ZnSO_4$ (ppm)	No. of total fish	No. of dead fish after time (hrs.)			Cumulative Mortality Rate (%) after time (hrs.)			Blood glucose level (mmol/L) after time (hrs.)		
		24h	48h	72h	24h	48h	72h	24h	48h	72h
Control(0)	10	0	0	0	0	0	0	4.35	4.67	4.99
25	10	0	0	0	0	0	0	4.77	4.83	5.19
50	10	0	0	0	0	0	0	5.21	5.32	5.57
75	10	0	0	0	0	0	0	5.10	5.60	6.99
100	10	0	0	1	0	0	10	6.32	6.76	7.19
150	10	1	0	1	10	0	10	6.49	7.78	8.80
200	10	2	2	3	20	20	30	7.98	8.30	8.75
250	10	2	3	4	20	30	40	8.30	8.45	8.98
300	10	3	4	4	30	40	40	8.77	9.28	9.86
350	10	4	6	8	40	60	80	9.10	9.35	9.99
400	10	8	8	9	80	80	90	10.32	10.58	11.32
450	10	10	10	10	100	100	100	-	-	-
500	10	10	10	10	100	100	100	-	-	-

This data is shown graphically to denote the increasing amount of glucose in blood.

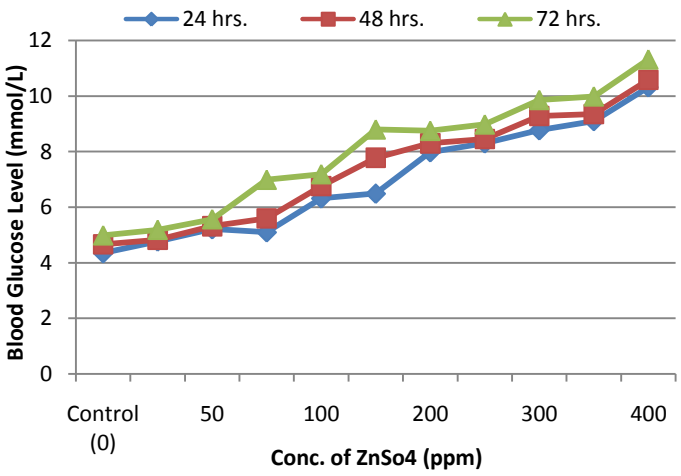

Figure-Changes in Glucose level by $ZnSO_4$

increases in branchial blood flow and increases in muscular activity [16]. A change of plasma glucose was observed in this study. It was found to be insignificant at low concentrations of zinc. Zinc is known to be an essential element of plants and animals. However at high concentrations, it exerts adverse effects by accruing structural damage, which affects the growth, development and survival of the fish [7]. Thereby blood glucose has been employed as an indicator to environmental stress [17]. In conclusion, the changes in the blood glucose indicate that they can be used as indicators of zinc related stress in fish on exposure to elevated zinc levels in the water.

CONCLUSION

The exposure of *Heteropneustes fossilis* to water contaminated with ZnSO4 was found to be toxic to the fish. This is worrisome as the water bodies of Bangladesh were found to contain considerably high concentrations of both zinc and sulphide ions [18]. Though this contamination may have very severe negative consequences for the fish population and in turn may affect the source of animal proteins for human populations, it is relieving to check out the toxicity of this metal ion by elevated blood glucose level. Further studies are required to appreciate better this observed effect of zinc ions with a view to exploiting it in environmental biotechnology, with respect to the remediation of heavy metal polluted fresh water bodies.

REFERENCES

[1]. S. Khare, and S.X. Singh, Histopathological lessons induced by copper sulphate and lead nitrate in the gills of fresh water fish Nandus. *J. Ecotoxicol. Environ. Monit*., 12.,105−111, . 1999.

[2]. T.T. Gbem, J.K. Balogun, F.A. Lawaland, and P.A. Annune, Trace metal accumulation in *Clarias gariepinus* Teugules exposed to sublethal levels of tannery effluent. Sci. *Total Environ.* 271., 1−9, 2001.

[3]. J.D. Woodling S.F. Brinkman and B.J. Horn, Non-uniform accumulation of cadmium and copper in kidneys of wild brown trout *Salmo trutta* populations. Arch. *Environ. Contam. Toxicol.*, 40.,318−385, 2001.

[4]. K.V. Bengari and H. S. Patil, Respiration, liver glycogen and bioaccumulation in *Labeo rohita* exposed to zinc. *Indian J. Comp. Anim. Physiol*., 4.,79−84, 2002.

[5]. N.C. Everall, N.A.A. Macfarlane and R.W. Sedgwick, 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, 1989.

[6]. C. Hogstrand, R.W. Wilson, D. Polgar and C.M. Wood, Effects of zinc on the kinetic of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc. *J. Exp. Biol.*186.,55−73, 1994.

[7]. H. Tuurala and A. Soivio, Structural and circulatory changes in the secondary lamellae of Salmo gaidneri gills to dehydioabietic acid and zinc. *Aquat. Toxicol.*2, 21-29, 1982.

[8]. S. Crespso, B. Flos, and G. Balasch-Alonso, Zinc in the gills of dogfish (*Scychorhinus canicula*) related to experimental aquatic zinc pollution. Comp*. Biochem*. *Physiol*. 63.,261-266.1979

[9]. G.M. Hughes and L. Tort, Cardio-respiratory responses of rainbow trout during recovery from zinc treatment. *Environ. Pollut.* 37., 225-226, 1975.

[10]. P.T. Cardeihac, C.F.Simpson, F.H. White, T.N.P. Thompson and W.E. Carr, Evidence of metal poisoning in acute death of large red dram *Scranops ocellata*. *Bull. Environ. Contam. Toxicol.* 27.,639-644, 1981.

[11]. P.A. Annune, T.T. Lyaniwura, S.O. Ebele and A.A. Olademeji, Effects of sublethal concentrations of zinc on haematological parameters of water (Trewawas). *J. Aquat. Sci*. 9., 1-6, 1994b.

[12]. A.J. Hussein and C. Nadim, Bioaccumulation of zinc and cadmium in freshwater fishes*. Indian J. Fish*., 50(1).,53−65, 2003.

[13]. A. Al-Akel and M . J . K . Shamsi, A comparative study of the toxicity of lead and its impact on the carbohydrate metabolism and some haematological parameters of cichlid fish *Oreochromis niloticus* and cat fish *Clarius garlepinus. Saudi Arabic toxicological environmental chemistry*.74.,9-28, 2000.

[14]. G.K. Iwama, M.M. Vijayan, R.B. Forsyth and P.A. Ackerman, Heat shock proteins and physiological stress in fish. *Am. Zool*. 39., 901-909, . 1999.

[15]. B.A. Barton, Stress in Fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. *Integ. Comp. Biol*. 42., 517-525, 2002.

[16]. B.A. Barton and G.K. Iwama, Physiological changes in fish from stress in aquaculture with emphasis on the response and effects or corticosteriods. Annual Review of Fish Diseases.1., 3-26, 1991.

[17]. E.K. Silbergeld, Blood glucose: A sensitive indicator of environ- mental stress in fish. *Bull. Environ. Contam. Toxicol.* 11., 20-25,1974.

[18]. F.O. Okonkwo, C.E.C.C. Ejike, A.N. Anoka and I.N.E. Onwurah, Toxicity in the catfish, *Heteropneustes fossilis* exposed to sub-lethal concentrations of heavy metals. *Trop. J. Biomed. Allied Sci. Res.;* 5., 500-507, 2010.