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Toxicity of zinc and the efficacy of antidotes on *Labeo rohita*, using atomic absorption spectroscopy

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ABSTRACT

Zinc is an essential heavy metal for human diet and it plays a significant role in metabolic process. The accumulation of zinc in different organs of Labeo rohita clearly shows that the accumulation directly proportional to the exposure period. In this work an attempt has been made to study the acute toxicity of heavy metal zinc and the effect of antidotes D-Penicillamine and Ethylene Diamine Tetra Acetic acid (EDTA) on the selected organs of the fresh water fingerlings of Labeo rohita using Atomic Absorption Spectroscopy. The concentration patterns in the organs of the fingerlings shows that liver is the major site to metal binding and muscle tissue accumulate least metal concentrations. It has also been observed using absorption spectroscopy that the administration of chelating agent D-Penicillamine reduces the zinc concentration in all tissues more effectively than the administration of the chelating agent EDTA.

Introduction

Today environmental pollution has become not only a national but also an international problem. Developed and developing countries which are progressing rapidly in the field of agriculture, technology and industries are continuously releasing various kinds of harmful substances into the biosphere and thereby causing a severe threat to the environment (APHA 1995, Abbasi et al 1998). Pollution due to heavy metals is a major ecological concern due to its impact on human health through the food chain and its high persistence in the environment (Lantzy et al 1979, Piechalak et al 2002). Heavy metal pollution is a threat to human health, animals, plants and the planet itself, and is mainly caused by industrialization and its consequences (Viarengo1989). Zinc is one of the most important heavy metal involved in animal growth and widely used metal cofactor of enzymes involved in protein, nucleic acid, carbohydrate and lipid metabolism that support life (Carpene et al 2003, Sun et al 2005). However, zinc becomes toxic when elevated concentrations are introduced into the environment (Eisler et al 1993). It 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 et al 2004, Lizabeth Bowen et al 2006).

Fishes are good bioaccumulators and they accumulate metals many times more than found in the ambient medium (Balaji et al 1991, Neduka et al 2006). As a secondary consumer fish in the food chain has equal importance as it forms the major food for the higher tropic levels and is the best biological indicator of pollutant in the aquatic system. The heavy metals accumulated in fish organs become toxic when accumulation reaches substantially high levels. The contamination of metals in fish indirectly affects the human beings (Dhawan 2000). For this reason, the utility of fish for assessing environmental conditions in aquatic ecosystems has gained prominence in recent years (Adeniyi et al 2008). The objective of this study were three fold: (1) to evaluate the LC_{50} of zinc sulphate, zinc carbonate and zinc chloride in fresh water fingerlings Labeo rohita for 96hrs and find out which one is more toxic; (2) to assess the bioaccumulation of heavy metal zinc on the selected organs of fresh water fingerlings Labeo rohita; (3) to study the efficacy of chelating agents, D-Penicillamine (DPA) and Ethylene Diamine Tetra Acetic acid (EDTA) in reducing the body burden of zinc and from the Labeo rohita. The concentration of metal accumulations was measured by using Atomic Absorption Spectrometer.

Procurement of fish and toxicity testing

The fish *Labeo rohita* is highly sensitive to various toxicants and is commercially and nutritionally important. The fingerlings of *Labeo rohita* were collected from Govt: fish farm at Puthur, Nagapttinam district, 10km away from the Annamalai University campus. The fingerlings (average length 5±1cm and average weight 10±1gm) being brought into the laboratory was placed in a glass trough equipped with continuous air supply. To avoid overcrowding 25 numbers of fingerlings were kept in each trough. Under laboratory conditions, the fingerlings were acclimatized for a period of one week in the experimental trough. The fish were fed with oil free groundnut cakes (Himalaya Company, India), which had no detectable amounts of zinc content. However, feeding was stopped 3 days prior to the commencement of the toxicity test to keep the animals more or less in the same metabolic state. In toxicity testing the test organism is exposed to the chemical indirectly by mixing the chemical into the water in which the animal lives thus producing test concentration. A screening test was first conducted to avoid delay and save time and effort, and in the definitive test a narrow

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range of concentrations of the test chemicals selected. A record is made of the number of fish alive, overturned and dead in each concentration. From this definitive test LC_{50} value for 96hr can be calculated by probit analysis method.

Estimation of LC_{50} for $ZnSO_4$

A wide range of concentrations 10, 20, 30, 40, 50, 60ppm of $ZnSO_4$ solution was prepared. 10 fingerlings were introduced in each glass trough containing 50 liters of water with required amount of metal. The screening test was continued to assess the concentration at which all the fingerlings survived for 96hrs and likewise the concentration at which most of the fishes died simultaneously. The result showed that toxicity effect of $ZnSO_4$ on *Labeo rohita* fell in the range of concentration of the metal from 20ppm to 40ppm and beyond 40ppm of zinc sulphate concentration all the test fishes died. The 96h LC_{50} estimated using probit analysis as described by Finney (1971). Thus 96hrs of LC_{50} estimated in the study of $ZnSO_4$ was about 28.5ppm of the toxicant.

Estimation of LC_{50} for $ZnCO_3$

A wide range of concentrations 2500, 3000, 3500, 4000, 4500, 5000ppm of $ZnCO_3$ solution was prepared. Then 10 fingerlings were introduced in each glass trough containing 50 liters of water with required amount of metal. The screening test was continued to assess the concentration at which all the fingerlings survived for 96hrs and likewise the concentration at which most of the fishes died simultaneously. The result showed that toxicity effect of $ZnCO_3$ on Labeo rohita fell in the range of concentration of the metal from 4000 ppm to 5000 ppm and beyond 5000 ppm of zinc carbonate concentration all the test fishes died. The 96hrs of LC_{50} estimated in the exploration of $ZnCO_3$ is about 4280 ppm of the toxicant.

Estimation of LC_{50} for $ZnCl_2$

Similar to the above two, a wide range of concentrations 78, 80, 82, 84, 86, 88, 90ppm of $ZnCl_2$ solution was prepared. Then 10 fingerlings were introduced in each glass trough containing 15 liters of water with required amount of metal.

The screening test was continued to assess the concentration at which all the fingerlings survived for 96hrs and likewise the concentration at which most of the fishes died simultaneously. The result showed that toxicity effect of $ZnCl_2$ on Labeo rohita fell in the range of concentration of the metal from 78 ppm to 88 ppm and beyond 88 ppm of zinc chloride concentration all the test fishes died. The 96hrs of LC_{50} estimated in the study of $ZnCl_2$ was about 82 ppm of the toxicant. The observed of mortality of test species is shown in the Table 1.

From the observations we found that zinc sulphate was more toxicant than other two zinc compounds namely zinc carbonate and zinc chloride. Thus zinc sulphate was selected for the toxicity study, which is very soluble in water.

The physicochemical characteristic of test water (pH: 7.5 ± 0.2; alkalinity: 212 ± 12 mg/L; hardness: 320 ± 15 mg/L as CaCO3; calcium: 50 ± 6.5 mg/L; magnesium: 18 ± 1.5 mg/L) were measured daily according to American Public Health Association (2005) and maintained throughout the study.

Experimental technique

Experimental design for studying the effect of antidotes

The AnalaR grade $ZnSO_4$ obtained from SD Fine Chemical, Bangalore, India, was used without further purification. The chelating agents DPA and EDTA were obtained from Sigma Aldrich Company, Bangalore, India. A stock solution of zinc sulphate was prepared by dissolving 2.4693g in 1000 ml of double distilled water. All the working solutions were prepared by diluting the stock solution with distilled water. The fish were exposed to 9.8ppm of zinc sulphate (1/3rd of LC_{50}). The test specimens were divided into five groups; each group consists of 30 fishes in 50 liter plastic trough equipped with continuous air supply. The experimental design for the study is shown in the table 2.

The test water was changed daily at 7am. by slowly siphoning the water from each container along with the waste feed and fecal material. The containers were refilled and redosed with the metal toxicant. Metal analysis of water was carried out periodically and kept with 95% of the required concentration throughout the study.

Preparation of the sample specimens

At the end of the experimental periods, the fishes were sacrificed and organs like gill, liver, kidney, muscle, bone and brain were collected and stored in plastic bags at -80^{0}C until used. The organs were dried in a lyophilizer for 12hr to remove the water content in the samples.

The samples were then ground in an agate mortar and pestle and made into a fine powder. The powdered samples were digested with concentrated nitric acid and perchloric acid mixture (Topping 1973) in the ratio 3:1, respectively.

The estimation of zinc concentration in the powdered tissue samples of fish was made using a double beam ELICO SL176 Atomic Absorption Spectrometer at wavelength of 217 (accuracy = 0.008 ppm; the detection limit determined by the blank solution was 0.1 ppm).

Results and discussion

Exposure of acute concentration of zinc for 14days resulted in a zinc accumulation profile in the order Liver > Gill > Kidney > Brain > Bone > Muscle. The accumulation of zinc in various tissues of the control and treated fish exposed to acute concentrations were presented in the table 3. Table 4 represents the accumulation of zinc in various organs of *Labeo rohita* after treated with antidotes D-Penicillamine, EDTA and fresh water for 7 days.

Accumulation of zinc and the efficacy of D-Penicillamine, EDTA and metal free water on the liver, gill, kidney, brain, bone and muscle tissue of Labeo rohita is shown in the figure1, 2 and 3.

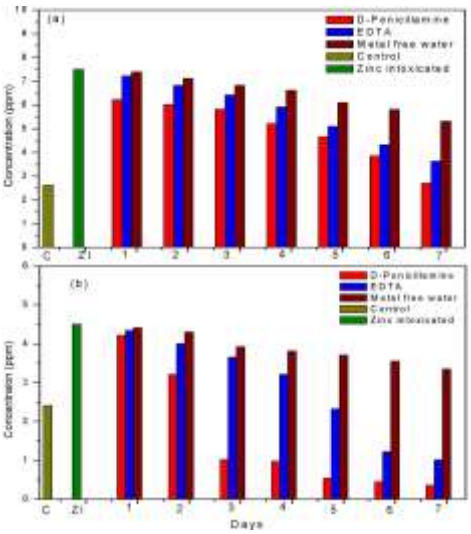

Figure1.Accumulation of zinc and the efficacy of D-Penicillamine, EDTA and metal free water on the (a) liver, (b) gill tissues of Labeo rohita

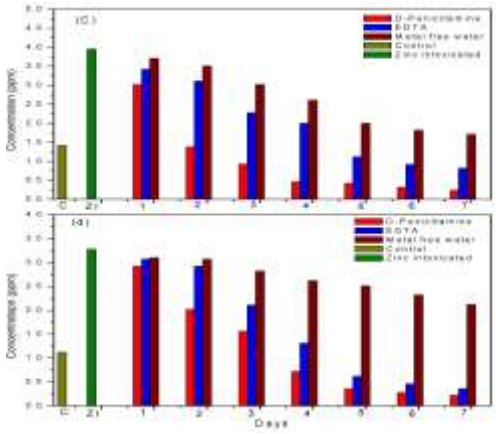

Figure2.Accumulation of zinc and the efficacy of D-Penicillamine, EDTA and metal free water on the (c) kidney, (d) brain tissues of Labeo rohita

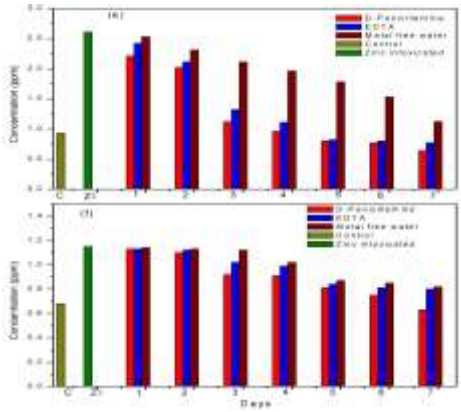

Figure3.Accumulation of zinc and the efficacy of D-Penicillamine, EDTA and metal free water on the (e) bone and (f) muscle tissues of Labeo rohita

In this study the maximum accumulation of zinc was found in liver (7.501ppm). The treatment of chelating agent D-Penicillamine reduces the concentrations of zinc very effectively than EDTA. The metal binding protein metallothionein is most important in the accumulation of metals. The liver is the main target organ for homeostasis in fish, for cleaning the blood substance entering the circulation from the gastro-intestinal tract passing through the liver before reaching the systemic circulation. Therefore the liver removes the toxicants from the blood, bio transforms them into bile and prevent their distribution to other parts of the body. Hence the liver, which is the major producer of metal binding proteins, shows higher concentrations of the heavy metal zinc. The tissues like gill, kidney and brain accumulate significant amount of heavy metal and the administration of chelation agent D Penicillamine effectively removes zinc than EDTA from these tissues of *Labeo rohita*. Kidney is considered as the primary target organ of accumulation for heavy metals, because of the strong irrigation and in relation to the function of excretion. During the excretion process, the excess amount of zinc ions is rapidly eliminated from the body through the kidney mainly due to the detoxification mechanism. Hence the toxic substance present in the blood is delivered to kidney in large quantities. Subsequently, kidney is a major target organ of acute zinc exposure. Bone and Muscle tissues of *Labeo rohita* accumulate least amount of zinc and the exploit of D-Penicillamine was more efficient for eliminating zinc from the bone and muscle tissue of the *Labeo rohita* fingerlings.

Conclusion

Heavy metal discharges to aquatic environment are of great concern due to their toxicity and accumulative behavior. Zinc is an essential trace element required for different physiological functions and plays important role in cellular metabolism. However, it becomes toxic when elevated concentrations are introduced into the environment. Here we made an attempt to study the accumulation of zinc and the efficacy of antidotes D-Penicillamine and EDTA on selected organs of fresh water fingerling *Labeo rohita*. Zinc sulphate, zinc carbonate and zinc chloride solutions had used for the toxicity study, among this zinc sulphate is more toxic than other two compounds. Exposure of acute concentration of zinc for 14days resulted in a zinc accumulation profile in the order Liver > Gill > Kidney > Brain > Bone > Muscle. It has also been observed that the administration of the chelating agent D-Penicillamine reduces the zinc concentration in all the tissues effectively than the administration of the chelating agent EDTA.

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Table1. Observed mortality of *Labeo rohita* exposed to different zinc compounds

Zinc Compound	Concentration (ppm)	Death of fishes in hrs				% of Mortality
		24	48	72	96	
$ZnSO_4$	26	0	0	1	1	20
	28	0	1	1	2	40
	30	1	1	2	2	60
	32	1	1	2	3	70
	34	2	2	2	3	90
	36	2	3	4	1	100
$ZnCO_3$	4000	0	0	1	2	30
	4200	0	1	1	2	40
	4400	1	1	2	3	70
	4600	1	2	2	3	80
	4800	2	3	3	2	100
$ZnCl_2$	78	0	0	1	1	20
	80	0	1	1	2	40
	82	0	1	2	2	50
	84	1	1	2	3	70
	86	1	2	2	3	80
	88	2	2	3	3	100

Table2. Experimental design of the study

Group 1	Fingerlings treated with metal free water and treated as control.
Group 2	Fingerlings treated with 9.5ppm of $ZnSO_4$ for 14 days.
Group 3	Fingerlings treated with 9.5ppm of $ZnSO_4$ for 14 days. After14days $ZnSO_4$ was withdrawn and fingerlings were again exposed to D-Penicillamine for another 7 days
Group 4	Fingerlings treated with 9.5 ppm of $ZnSO_4$ for 14 days. After14days $ZnSO_4$ was withdrawn and fingerlings were again exposed to EDTA for another 7 days.
Group 5	Fingerlings treated with 9.5ppm of $ZnSO_4$ for 14 days. After 14days $ZnSO_4$ was withdrawn and fingerlings were again exposed to metal free water for another 7 days.

Table3. Accumulation of zinc in *Labeo rohita* exposed to acute concentration.

Tissue	**Control**	**Zinc Exposed**
Liver	2.62	7.50
Gill	2.41	4.50
Kidney	1.42	3.95
Brain	1.12	3.28
Bone	0.93	2.61
Muscle	0.68	1.15

Table 4. Accumulation of zinc in various organs *Labeo rohita* after treated with the antidotes D-Penicillamine, EDTA and metal free water.

Antidotes	Organ	Zinc concentration on various periods						
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
D- Penicillamine	Liver	6.23	6.02	5.82	5.21	4.65	3.86	2.70
	Gill	4.22	3.21	1.02	0.98	0.54	0.45	0.36
	Kidney	3.02	1.38	0.93	0.46	0.42	0.32	0.24
	Brain	2.92	2.02	1.56	0.72	0.36	0.28	0.22
	Bone	2.21	2.02	1.12	0.96	0.80	0.77	0.64
	Muscle	1.13	1.10	0.92	0.91	0.81	0.75	0.63
EDTA	Liver	7.22	6.82	6.42	5.92	5.10	4.32	3.62
	Gill	4.34	4.00	3.65	3.21	2.32	1.22	1.01
	Kidney	3.42	3.11	2.28	2.00	1.12	0.92	0.82
	Brain	3.07	2.92	2.11	1.31	0.62	0.46	0.36
	Bone	2.42	2.11	1.32	1.11	0.82	0.80	0.77
	Muscle	1.13	1.12	1.02	0.99	0.84	0.81	0.80
Metal free water	Liver	7.40	7.12	6.82	6.62	6.11	5.81	5.32
	Gill	4.41	4.30	3.92	3.81	3.71	3.55	3.35
	Kidney	3.71	3.51	3.02	2.61	2.00	1 .82	1.71
	Brain	3.10	3.07	2.82	2.62	2.51	2.32	2.12
	Bone	2.53	2.31	2.11	1.96	1.78	1.53	1.12
	Muscle	1.14	1.13	1.12	1.02	0.87	0.85	0.82