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Laboratory attempt to evaluate the short term toxicity of micronutrient (ZN) to the fresh water fish, *Rasbora daniconius* HAM

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Abstract
Man made activities like mining, industrial discharge, sewage; sludge disposal, fertilizers and pesticides applications have been the major culprit for elevated level of copper, Lead, Mercury and various heavy metals in all the natural ecosystems. The metals which find their way in to commercial industrial application posses certain biocidal properties. Metal being immutable, cannot be destroyed or transform in to other metal and hence, once it is mobilized in to the environment, it total amount remain same. However, it can be converted in to one or the other salt and enter in to different organisms. Among the non-essential elements exposure, lead and zinc are of particular importance. Although a certain fraction of the amount absorbed is excreted, lead and zinc tends to accumulate in important organs of body. There is a narrow tolerable region of these metals. Hence, once accumulated these metals disturb the well being of the human body. Although, it is known that many metals are toxic to aquatic organisms. Knowledge regarding their exact actions and the level at which they become harmful is far from complete. Therefore, an attempt has been made to study the effects of Zn on common edible fresh water fish, *Rasbora daniconius*.

Keywords: zinc, *Rasbora daniconius*, LC_{50}, behavior response

Introduction

The aquatic environment in recent time is witnessing an unprecedented in pour of various kinds of biocides in alarming quantities and sources of such release are too much to be mentioned.

Rapid industrialization and consequent discharge of effluent in to water system made heavy metal a major pollutant of aquatic ecosystem. The common toxic metal found in industrial effluents are cadmium, chromium, nickel, mercury, lead, copper, zinc etc. are non-essential elements and are not required by animal. Pollution by toxic heavy metals has been widely studied and documented topic because of the disastrous effects and disease that are produced in man after consumption of contaminated food like fish, shellfish and crustaceans. Since, toxic heavy metal are not required by animals, therefore, any accumulation of these metals is a burden on the organism and is likely to prove as a source of toxicity. Contamination of the aquatic environment with the cadmium is a matter of concern, because this heavy metal can enter the food chain and as a result of bioaccumulation can cause health problem in human (Friedberg *et al*. 1973 and Piscator, 1980 [5, 12].

Many chemical substances are known to deteriorate the quality of water and important among them are heavy metal salts. In the biological studies, the term heavy metals and metals are used in broad sense. The term heavy metal includes all the elements beyond the calcium in the periodic table of elements. However, an attempt has been made to classify metal ions on the basis of an empirical thermodynamic data, namely the trends in the magnitude of equilibrium constant that describe the formation of metal ions (Ligand complex) (Evert Nieboer and David H. S. Richardson, 1980) [4]. It has been observed that the metals of the same group of periodic table, having similar physiochemical properties such as ionic radius and electronic configuration, having similar toxicological properties.

Most of the ionic forms of metal are toxic to living organisms at higher concentration while some metal ions are toxic at low concentration and very few are toxic even in trace quantities, such as mercury and lead. Presence of high toxic metal ions in natural water is a subject of serious concern, when such water resources are used for drinking purposes by human and living organisms. Prolonged use of metallic contaminated water resources for drinking purposes is dangerous for public health.

Toxicity is an inherent capacity of a metal to affect any biological activity. A metal is regarded to be toxic if it impairs growth, reproduction and metabolism of an organism, when supplied above certain concentration. According to Wood (1974), metals are classified on the basis of their toxicities into three categories:

- Non-critical e.g. - Sodium, Potassium, calcium, magnesium
- Toxic but very rare or very insoluble e.g. Rare metal
- Lastly very toxic, soluble and relatively accessible e.g. Selenium, Arsenic, Zinc, Copper, Lead, Mercury, Cobalt, Nickel.

The heavy metals are the common constituents of the earth crust and so all the process of mining, refining, extracting, synthesis and their use in industries and for domestic purpose result in environmental contamination. Many heavy metals and their salts have important applications in various industries and so their presence in the effluent is not surprising. The current information regarding similarities or differences between the effects of attributable to single metal and those when these metal are combined is far from complete (Lloyd, 1961, Burton, 1972, Anderson and Morel, 1978) [11, 2].

Zinc ranks fourth among metal of the world in annual consumption, being surpassed only by steel, aluminum and copper. This metal though widely used, is not obvious in its application. It is exceedingly versatile and useful and is essential to modern living. For example, the automobile industry account for almost one third of U.S. slab zinc consumption. Other important uses include its role as a major alloying ingredient in brass, as a protective coating of steel, and as a chemical compound in rubber and paints.

Zinc is a bluish white metal with an atomic weight of 65.37, Density-7.13g cm^3 at 25°C, Melting point -419.6°C and boiling point -90.7°C. Zinc is chemically active and alloys readily combined with other metals, these properties are utilized industrially in preparing a large number of Zinc containing alloys and compounds. The relatively high position of Zinc in the electromotive series largely account for its extensive use to protect ions and steel products against corrosion.

Zinc was first to be an essential nutrients for mammals more than 40 years ago. As with many other traces elements later shown to be essential, inherent Zinc metabolism in man focused largely on its toxic properties; it was considered that the ubiquity of Zinc in the environment made human deficiencies unlikely, however, in 1976, Lei *et al*, suggested that the syndrome of dwarfism and hypogonadism seen in adolescent mice, might be due to Zinc deficiency. Since, then there have been reports of Zinc deficiency occurring in groups of people in widely differing circumstances and in many different countries. Thus, although Zinc is ubiquitous in the environment, so too appear to be human Zinc deficiency.

Both lead and zinc are constituents of earth crust. They are of common occurrence in rivers, lakes and other water bodies. Because of their frequent discharge in water bodies, their environment persistence, their toxic effects on aquatic life and their ability to be incorporated into the food chain, both lead and zinc causes serious pollution problems (Daterao, M.S. 1990) [3]. The principle sources of zinc contamination are waste sludge's from petroleum refineries, waste sludge's from manufacture of alkyl lead compound, waste solvent; paint sludge's from manufacture of lead acetate batteries, solvent & waste water from printing ink production.

Zinc and its compounds are toxic. Zinc is a key element in our health & life style. It has been used by human since immemorial and today finds myriad application in our industrial society. Zinc is essential for normal activity of DNA polymerase enzyme, protein synthesis, and thus play vital role in the healthy development of many life forms. Excessive amount of zinc, however, may be toxic, especially to the aquatic biota.

The present study is undertaken to evaluate the effects of zinc toxicity on fresh water fish, *Rasbora daniconius*.

Materials and Methods

The static bioassay method standardized by the APHA (American Public Health Association, 1975) [1] was employed. The test fish regularly brought from Jota compound nallah, situated at chavindra, Bhiwandi, District-Thane. The aquaria were washed thoroughly with 0.1% solution of $KMnO_4$ before the test fish. Aged tap water used to maintain the fish and water in maintenance tank was two liters or more per gram of fish. It was constantly aerated and 90% of water was changed every alternate day. During the day, fish were exposed to diffused light coming through the window. Necessary precautions were taken to keep the aquarium tank away from serious mechanical or visual disturbances. During this period, the fish were regularly fed with live tubifex worms, but feeding was stopped for 2 days prior to experiment. The fish measuring 3.2 to 3.4 cm in length and 0.48 to 0.50 gms in weight were used in the experiment. The fish allowed acclimatizing to laboratory condition in this way for a period of one week at room temperature of 28 ± 2°C. If in any batch, mortality exceeds 5% during acclimatization, that entire batch of fish was discarded. Static continuous flow through system for 96hrs. was adopted for the entire bioassay.

Acute or lethal toxicity test were conducted in the laboratory by exposing healthy stock fish to various concentrations of zinc salts for different intervals.

The stock solution of zinc was prepared by dissolving 2.083 gms of analytical grade zinc chloride (pure) in one liter of distilled water. The required concentration was obtained by adding the stock solution to the dilution water.

The tap water was stored in big reservoir for few days and the chlorine free tap water thus obtained was used as dilution water for the entire test.

The physicochemical characteristics of water such as temperature, P^H, etc. were checked regularly during the test period.

Some pilot tests were performed using two or more concentrations of each toxicant separately. This helped in determining the concentration to be selected for final test. The range of concentration selected was such that they resulted in 0 to 100% mortality of the fish tested. After obtaining partial or complete results another set of experiment was conducted to increase the precision of results. Control test with '0' toxicant concentration was simultaneously performed exactly under analogous condition.

Range finding bioassay test was carried out to decide the test concentrations to be selected. Based on range finding bioassay test, 5 different concentrations ranging from 2PPM to 10 PPM were selected for the statics bioassay test. Mortality was recorded after every 24 hrs. and the dead animals were removed from the tank. The data was subjected to statistical treatment as per the method suggested by Litch-field and Wilcoxon ((1949) [10]. The probit analysis was carried out. LC_{50} values were calculated for the period of 24, 48, 72 & 96hrs.

The bioassay tests were continued for 4 days. The tests were started in the morning and observation on general behavior and symptoms of intoxication were made during the day. The percentage of surviving and dead fish was noted at the end of 24 hrs for 4 days. The fish were considered dead when there is no respiratory and other movement and evoked no response to gentle prodding with glass rod. Dead fish were removed and examined for morphological changes. During the toxicity study the behavioral changes in intoxicated fish were also critically noted and compared with control.

Result and Discussion

Acute toxicity is based on the percentage mortality of the fish population as a quantitative biological response to acute or lethal action of Zinc. The effects of heavy metal on different organisms differ with the dose and duration of exposure to heavy metal. In the present investigation, the % mortality was found to be increased in increased exposure period. The increase was maximum in higher concentration of Zinc. The recorded LC_{50} values are for 24 hrs. is 12.2 mg/liter, for 48 hrs. is 9.8 mg/liter, for 72 hrs. is 8.3 mg/liter and for 96 hrs. is 6.2 mg/liter.

The Zinc LC_{50} values for different acute exposure period of the fish under investigation differ considerably from those recorded for other species of fish. The literature on lethal toxicity on Zinc to fish cited earlier by other investigator reveals that Zinc toxicity to fish varies from species to species and under changing environmental condition. In our experiment on Zinc toxicity, considerable quantities of white salts were seen to be accumulated in the experimental tank containing higher concentration of Zinc in mg/liter, giving milky appearance to dilution water. These were probably insoluble Zinc carbomates, hydroxides, phosphates etc. It is likely that the formation of these salts could reduce the Zinc concentration below the lethal level (Lam *et al*, 1976) [8]. The Zinc toxicity to any fish therefore depends on the dissolved fraction of Zinc and not the total amount of Zinc present in dilution water.

In the present study on the fish, *Rasbora daniconius* the first toxic symptoms of Zinc was an increased respiratory rate as indicated by rapid operculum movement in treated fish. After about 24 hrs. of exposure, hypersensitivity, erratic swimming and muscular spasm were reported noticed. Similar symptoms were reported by Haider (1964) [6] & Holcombe *et al*, in rainbow trout exposed to Zinc. During the subsequent period of toxicity test, the fish became very lethargic and frequent body tremors were reported suggesting neurological disorder. In the present study, as the exposure period increased, the treated fish were often seen near the surface of water gulping the atmospheric air. Finally, they sank to the bottom of the tank and showed a loss of equilibrium. The poisoned fish were seen lying on one side of the body for a sufficient long period of time before death. These observations on the fish of the present study exposed to Zinc are in agreement with those made by Haider (1964) [6] & Holcombe *et al*, (1976) [7] in rainbow trout exposed to Zinc. In the present study, often in Zinc exposed fish, prior to death a copious mucous secretion was seen exuding from the operculum opening and mouth.

Table 1: Physico-chemical parameters of water used for toxicological study

Temperature	28°C
PH	7.5
DO	6mg|Litre
Free Chlorine	Nil
Total Acidity	3.2mg/Litre
Total Alkalinity	45 mg/Litre
Total Hardness as $CaCO_3$	32 mg/Litre
Length of fish	3.2 ± 3.54cm
Weight of Fish	0.48 ± 0.50gm

Conclusion

From the present finding it can be concluded that Zinc is highly toxic element and causes severe mortality amongst fishes and other aquatic organism at low concentration which is an ultimate effects of any toxicant. However, further investigation is required to monitor its effects on other physiological parameters of the fish to increase the precision of results.

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