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Research article

Biophysicochemical changes in Nile tilapia, *Oreochromis niloticus* exposed to $ZnSO_4.7H_2O$ and $ZnCl_2$ metal toxicant

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Keywords: LC_{50}; acute toxicity test, biophysicochemical changes, zinc sulphate, zinc chloride, *Oreochromis niloticus.*

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Abstract

During the present investigation, the LC_{50} values and lethal concentrations of $ZnCl_2$ and $ZnSO_4.7H_2O$ for *Oreochromis niloticus* were determined. The LC_{50} and lethal concentrations for each metal salts were computed by using Probit analysis method at 95% confidence interval. The 24, 48, 72-hr LC_{50} and lethal concentrations of Nile tilapia for zinc chloride were found to be 50.27 mg/L, 37.05 mg/L and 17.99 mg/L and for zinc sulphate were 68.73 mg/L, 51.71 mg/L and 44.62 mg/L in respective time intervals. The tolerance limits of fish for both zinc chloride and zinc sulphate varied significantly in terms of time and lethal concentrations. However, fish were significantly tolerant to zinc sulphate than that of zinc chloride. With the increase in metallic ion concentration of the water, the level conductivity and TDS (Total Dissolved Solids) increased, while that of dissolved oxygen decreased constantly. The reverse order of dissolved oxygen with concentration indicating that oxygen consumption by the fish decreases under metallic ion stress. The present study also assessed the proximate composition, including protein, lipid, moisture, dry ash, organic contents of *Oreochromis niloticus* exposed to $ZnCl_2$ and $ZnSO_4.7H_2O$. This showed a significant decrease in protein, lipid, dry ash content and increase in moisture and organic content.

Introduction

Fish plays an important role in providing protein, essential vitamins, minerals, and fatty acids for Bangladeshi people. Fish account for about 70% of the animal protein intake with annual fish consumption of about 14 kg per person [1]. Bangladesh enjoys a sub-tropical climate and vast areas of shallow water provide ideal conditions for fish production. To meet the soaring demand for food, there is a huge potential of exotic fish tilapia (*Oreochromis niloticus*) farming in Bangladesh. With increasing popularity among consumers, tilapias have become the world's second most important cultured fish after carps. In recent years, a considerable number of farmers are involved in tilapia culture in Bangladesh due to its profitability. Market conditions largely determine the profitability of tilapia farming. The market for tilapia is associated with strong demand, driven by continued increases in population. Mainly due to population growth there is a growing gap between supply and demand of fish in markets in Bangladesh. Narrowing the gap not only requires increasing production of fish but also improvements of all aspects of marketing, distribution systems and quality of fish. Fish are relatively sensitive to changes in their surrounding environment. Fish quality therefore reflects and gives a good indication of the health status of a specific aquatic ecosystem.

Capacity of the water body to support aquatic life as well as its suitability for other uses depends on many factors among which are trace element concentrations. Some metals such as manganese, zinc, copper, and nickel, when present in trace concentrations are important for the physiological functions of living tissue and regulate many biochemical processes of fish. Generally, trace amount of metals is always present in freshwaters from the weathering of rocks and soils and other natural mechanisms. Contamination of heavy metals in aquatic environment is increasing globally and describes one of the most critical environmental risks [2]. Some metals when discharged into natural waters at increased concentration in sewage, industrial effluent or from mining and refining operations can have severe toxicological effects on aquatic environment and humans [3]. Generally, acute toxicity is usually from a sudden or unexpected exposure to a relatively high concentration of chemicals in a short period of exposure, consequently, acute effects symptoms can appear after exposure [4]. The level of heavy metal in fish tissues is influenced by biotic, abiotic, and environmental factors that include fish species, habitat, fish age, concentration of metal in water, exposure period, water temperature, pH in water, dissolved oxygen (DO) concentration, conductivity, water salinity and other physiological conditions [5-10].

Among heavy metals, zinc is an essential trace element in living organisms, being involved in the nucleic acid synthesis and occurs in many enzymes. The common sources of it are galvanized iron work, zinc chloride used in plumbing and paints containing zinc. Zinc is non-toxic and is an essential metal that is an important constituent of cells. In addition, several enzymes depend on zinc as a cofactor. Although, small quantities of zinc are required for the normal development and metabolism but if its level exceeds the physiological requirements, it can act as a toxicant [11-13]. This results in general enfeeblement, retardation of growth and may bring about metabolic and pathological changes in various organs in fish. Several trends are evident that (1) freshwater fish are more sensitive to zinc than marine species; (2) embryos and larvae are the most sensitive developmental stages; (3) effects are lethal or sub-lethal for most species in the range 50-235 µg Zn/L and at 4.9-9.8 µg Zn/L for the brown trout (*Salmotrutta*); and (4) behavioral modifications, such as avoidance, occur at concentrations as low as 5.6 µg Zn/L. Signs of zinc poisoning in fish included hyperactivity followed by sluggishness before death, fish swam at the surface, were lethargic and uncoordinated, showed hemorrhaging at gills and base of fins, shed scales, and had extensive body and gill mucous [14]. Many factors modify the lethal properties of zinc to fish. Zinc is more toxic under conditions of comparatively low dissolved oxygen concentrations, high sodium concentrations, decreased loadings of organic complexing agents and low pH. Zinc is most toxic to yearlings of brown trout in soft water at pH 4-6 and pH 8-9; toxicity at alkaline pH is attributed to the formation of $ZnOH^+$, $Zn(OH)_2$, and $ZnCO_3$ in both hard and soft water suggesting increased entrapment of metal precipitates within mucous and epithelial layers of the gill [15]. Acute zinc poisoning in fish is generally attributed to blockade of gas exchange across the gills, causing hypoxia at the tissue level. Tissue hypoxia in fish is a major physiological change before death once the gas exchange process at the gills is no longer sufficient to meet its oxygen requirements. Cardio-respiratory responses to zinc in the spangled perch (*Leiopotherapon unicolor*) are like those induced by hypoxia; zinc-poisoned perch had damaged gill epithelia, resulting in impaired gas exchange and lowered oxygen tension in arterial blood. Acute exposures to high lethal concentrations of zinc also caused histopathology of epithelia lining the oral capacity [16].

The culture of Tilapia is one of the fastest aquacultural growth with an average annual growth rate of 13.4% in the world and recent past in Bangladesh. In the present study, target samples are hybrid tilapia, which has become popular through a collaborative program with world fish center on Genetic Improvement of Farmed Tilapias as the GIFT. The *Oreocromis niloticus* tilapia has been selected due to the following reasons: high productivity, significant improvements in growth rate in successive generations, as well as remarkable survival rates, which became a poor man’s protein source, it is easily available and is a hardy fish species. Therefore, this study has been undertaken on tilapia fish because it appears to have great economic and ecological importance in Bangladesh. Young organisms are often more sensitive to toxicants than is adults viz., the early stages are more sensitive than the later stages [18]. For this reason, the use of early life stages, such as fish fingerlings is required for all tests.

In the present study, the acute toxicity effect of $ZnSO_4.7H_2O$ and $ZnCl_2$ on Nile tilapia, *Oreochromis niloticus* was investigated. The specific objectives of this research were to the determination of the LC_{50} value of $ZnSO_4.7H_2O$ and $ZnCl_2$ in *Oreochromis niloticus* at different concentration. Excess zinc accumulation by the body of the fish sample and to observe the change in total protein, lipid, moisture, dry ash content in fish body as well as the variation of physicochemical parameters of experimental water with different concentration along with exposure time.

Experimental

Method and materials

Study area and period

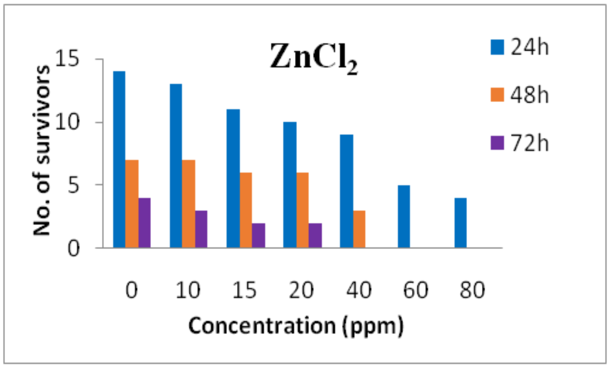
The experiment was carried out in the Animal House Complex of the Department of Zoology, University of Dhaka, located at the animal garden of the Curzon Hall campus. Analytical and other laboratory works were carried out at the laboratory of the Department of Chemistry, Centre for Advanced Research in Sciences (CARS) and Department of Zoology of University of Dhaka.

Acclimation of fish

Fish fingerlings that were used for this experiment were of size 3-4 cm in length and 2-3 g in weight. Fish fingerlings were collected from the Pioneer Fish Seed Hatchery, Chandpur, Bangladesh. In each test, all organisms were approximately the same age and taken from the same source in order to minimize the diversity of response to experimental materials [19]. Fingerlings were acclimatized in the fish tanks for one week prior to the experiment. The tanks were well cleaned and during that period water exchanges were done at every two days. Tanks were carefully handled by covering with the net to ensure fish safety and proper reservation. The water was continuously aerated with oil free air to maintain the dissolved oxygen relatively constant. Fish were fed with crumbled feed once in 24 hours. Feeding was maintained by giving the food 5% of its total body weight. Feeding was stopped during the last 24-hours of adaptations and throughout acute toxicity tests.

Test toxicants

$ZnCl_2$ and $ZnSO_4.7H_2O$ analytical grade were used for the preparation of different concentration of toxicant solution. The fish were exposed to various concentrations (0, 10, 15, 20, 40, 60 and 80 mg/L) of zinc chloride and zinc sulphate (0, 20, 40, 60, 65, 70 and 80 mg/L) for a period of 24h, 48h, 72h exposure and dead fish were removed immediately from the test media. To find out the survival time in each concentration of $ZnCl_2$ and $ZnSO_4.7H_2O$ observations were recorded (Figure 1). The procedure used here is consistent with Olaifa *et al.* and El-Naga *et al.* [20, 21].



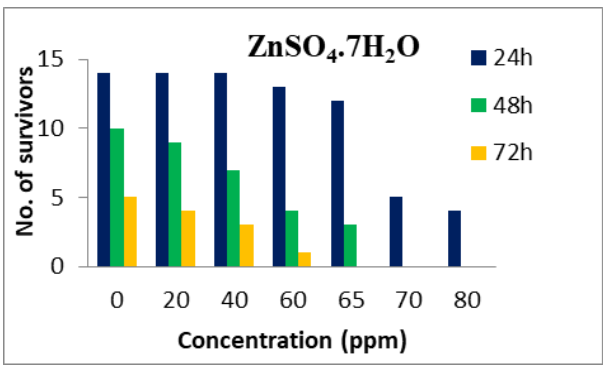


Figure 1. Number of survival of fish exposed to different concentration of $ZnCl_2$ and $ZnSO_4.7H_2O$ at 24h, 48h and 72h.

Physicochemical parameters

The physicochemical characteristics of test water were measured before and during the experimental trial. The temperature was measured by an N-filled mercury thermometer in °C. Electrical conductivity meter was used to measure the capacity of ions in an aqueous solution to carry electrical current. As the ranges in aqueous solutions are usually small, the basic units of measurements are milli Siemens/cm (mS/cm) and micro Siemens /cm (μS/cm). TDS tester was used to measure total dissolved solids in water. The values are usually read in parts per million (ppm). pH was measured using a waterproof digital combo pH meter. Dissolved oxygen concentration was measured using a digital dissolved oxygen meter.

Determination of zinc

For the determination of zinc accumulation, the fish sample was digested separately by using a mixture of HNO_3 and $HClO_4$ in 2:1 ratio. The procedure used by Prester *et al.* was followed to prepare sample [22]. The digested solution was analyzed by atomic absorption spectroscopy (AAS) (Varian AA 240).

Determination of variation of some biological parameters

The mass of the fish tissue was recorded before and after the heating in an oven using aluminum foil at a temperature of 110 °C to measure the moisture content. Protein content in fish tissue was determined by Kjeldahl method demonstrated by Mendham *et al.* [23]. After extraction, the lipid content was determined by following the procedure used by Bligh and Dyer rapid method of total lipid extraction and purification [24]. The dry ash content was measured by heating a known amount of dry fish in an open flame using a crucible and then cooled and weighed. Heating was continued until constant weight was obtained varying from 500 to 600 °C. Subtraction of dry ash content from the taken weight of dry sample measures the organic contents of the fish tissue.

Statistical analysis

Probit analyses method was used to calculate the LC_{50} and lethal value for both zinc chloride and zinc sulphate at 95% confidence interval [25, 26]. In order to find the relationships among physicochemical parameters, correlation and regression parameters, correlation and regression analyses were performed. The statistical difference among various parameters, defined for the study was studied by using EPA probit analysis programme (version 1.5) obtained from www.epa.org.

Results and discussion

In the present study, fishes showed normal behavior in control groups but severity in different responses were observed with the increase in zinc concentrations and passage of time. The control group showed the normal behavior during the completely experimental periods. When exposed to the lowest concentration of the metal, fishes showed normal responses as the control along with staying motionless in-group at the bottom. When the concentrations of metal increased gradually the neurotoxin effects resulted in the loss of balance, rapid opercular movements, followed by vertical movements. At the lethal concentration, the fish started to swim spirally with sudden jerks. Finally, the fish started to settle at the bottom of the test chamber. The appearance of jerks, in this case, is in accordance with Findings of Fukuto [27]. Beauvais *et al.* [28] and Scholz *et al.* [29] reported that behavioral study is one of the important parameter in the assessment of toxicity of metals in fish. In the present study, the abnormal behavior includes loss of balance, staying motionless in a group at the bottom, lying laterally at the bottom, swimming in a spiral fashion with jerks, revolving in water, opened mouth and rapid opercular movements. Results were in agreement with other studies reported by Sarikaya and Yilmaz [30] and Selvi *et al.* [31]. The cause of death owing to lethal action of metals may be due to damage of the respiratory surface over the gill and damage of body surface. Similar reasons have been given by Nagbhushanam *et al.* [32] and Rajeshkumar and Munuswamy in fish tissues [33].

It is necessary to study the relationship and effects of heavy metal salt on physiochemical parameters of water. It was observed during the entire study period that dissolved oxygen showed an inverse relationship with metal concentrations. This showed that at high concentrations of heavy metals, oxygen consumption by fish increased significantly. Toxicity of metals was also greatly influenced by the hardness of water during the present investigation and found that hardness of water decreases with increasing metal concentrations which indicate that the toxicity of metals decreases significantly with increasing water hardness (Figure 2). We observed an inverse relationship between water pH and concentrations of zinc salts (Figure 2). Increases in water temperature can enhance the uptake of metals by the aquatic organisms.

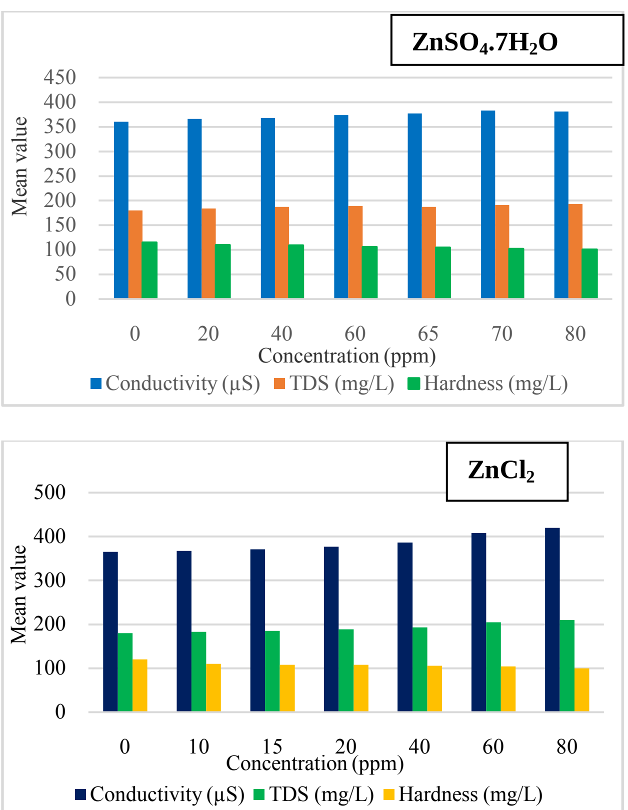


Figure 2. Variation of water quality parameter with different concentration for $ZnSO_4.7H_2O$ and $ZnCl_2$ at 24h, 48h and 72h.

Acute toxicity tests were performed with *Oreochromis niloticus,* to determine the LC_{50} and lethal concentrations of $ZnSO_4.7H_2O$ and $ZnCl_2$ to the fish. Many studies have been conducted to test the tolerance limit of *Tilapia nilotica* against heavy metals. However, very little data is available on the acute toxicity of zinc salts to *Oreochromis niloticus.* The comparative 24, 48, and 72 hours LC_{50} values were observed for $ZnSO_4.7H_2O$ and $ZnCl_2$ are recorded in table 1.

Table 1. Comparative LC_{50} values for fishes treated with toxicant.

Toxicant	LC_{50} of 24h (mg/L)	LC_{50} of 48h (mg/L)	LC_{50} of 72h (mg/L)
$ZnCl_2$	50.27	37.05	17.99
Zn^{2+}	24.01	17.76	8.62
$ZnSO_4.7H_2O$	68.73	51.71	44.62
Zn^{2+}	15.63	11.76	10.15

During the study period, it was observed that fish showed more sensitivity toward $ZnCl_2$ than ZnSO4.7H2O (Figure 3). The LC_{50} values for $ZnSO_4.7H_2O$ at 24h, 48h and 72h are 68.73, 51.71 and 44.62 mg/L respectively and for $ZnCl_2$ 50.27, 37.05 and 17.99 mg/L respectively. It was also observed that LC_{50} values for both toxicants decrease with the increased time of exposure. This indicates that a low amount of $ZnSO_4.7H_2O$ and $ZnCl_2$ have a potential to be a big threat to the freshwater ecosystem and other animals if they are exposed for a long period of time.

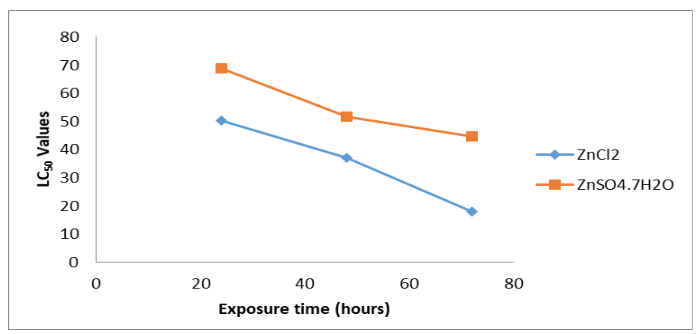


Figure 3. Comparison of LC_{50} values of zinc chloride and zinc sulphate at 24h, 48h and 72h.

The accumulation of zinc in the fish body for both toxicants analyzed by using Atomic Absorption Spectrometer (AAS). It has been found that the accumulation of $ZnSO_4.7H_2O$ and $ZnCl_2$ is maximum at the 48 hours (Figure 4). The amount is found to decrease at 72 hours and this may be because of the prolonged exposure of fish to a toxicant reduce the amount by excreting out through its excretory system.

Percentage of protein contents in the fish sample at different LC_{50} values for $ZnSO_4.7H_2O$ and $ZnCl_2$ were calculated at 24h, 48h and 72h. The results illustrated in graph (Figure 5), indicate that protein content in treated groups is lower than the fish used in control condition. Decline in protein level in other groups suggests the toxic stress in metabolic process. Depletion in tissue proteins in fish exposed to various toxicants has also been reported by Hameed *et al.* [17]. From the above observations, it can be suggested that heavy metals in aquatic ecosystem will change the metabolism of body. A graphical representation of the variation of lipid content, shows lipid decreases significantly than the fish used in control condition (Figure 5). The decrease in lipid content suggests that for the absence of toxic element at control condition the lipid have not been channelized to mitigate the toxic stress but for the other group zinc toxicity in fish suppressed lipid metabolism as a result fish were severely stresses and weak. Variation of dry ash content *in Oreochromis niloticas* for both toxicant at different exposure time's LC_{50} values also maintained the same trend as protein and lipid content.

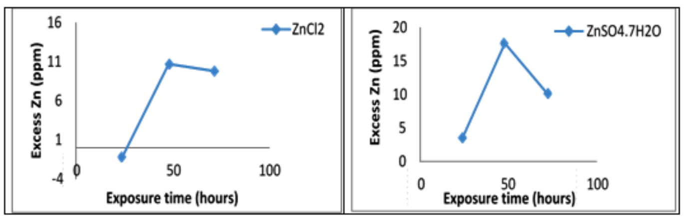


Figure 4. Variation of excess Zn accumulation along with different exposure time for $ZnCl_2$ and $ZnSO_4.7H_2O$ by AAS.

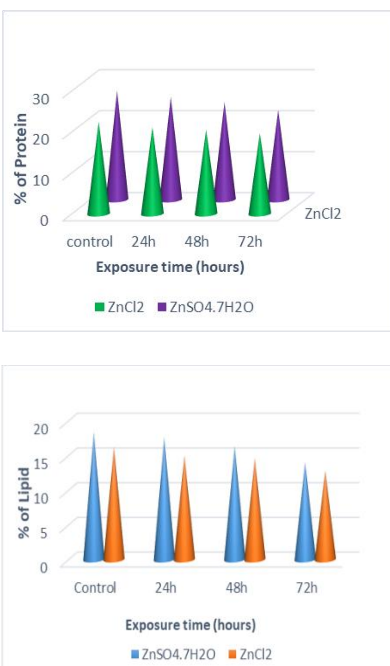


Figure 5. Percentage of lipid and protein content in $ZnCl_2$ and $ZnSO_4.7H_2O$ at different LC_{50} values.

The table 2 shows that the dry ash content is found to increase with increased toxicity is due to increase of inorganic residue indicate the accumulation of zinc in fish tissue. From the table 2 it is found that there is a high amount of organic present in toxic fish. As the concentration of both toxicants increases the organic content increases with the passage of time. As shown in the table the moisture content at LC_{50} concentration increases for 24, 48 and 72 hours than control. This might be due to exposure of the toxic level of zinc and due to increase urge to release the toxicant by biological ways. As a result, the fish become weak and excess fluid is accumulated in the body.

Table 2. Variation of organic, dry ash and moisture content in different LC_{50} values of the toxicants.

Bio-assay test	LC_{50} values	Toxicants $ZnCl_2$	$ZnSO_4.7H_2O$
Moisture content (%)	Control	71.74	70.83
	24h	72.97	73.22
	48h	73.28	73.30
	72h	76.15	77.74
Dry Ash content (%)	Control	9.53	9.41
	24h	9.18	9.03
	48h	8.82	8.43
	72h	7.92	7.96
Organic content (%)	Control	90.47	90.59
	24h	90.82	90.97
	48h	91.18	91.57
	72h	92.08	92.04

In addition, the increased level of moisture may be due to the kidney failure in fish. There is also a hypothesis that the increased level of moisture is due to absorption of water from the environment. Hence, in the present study the cause for rise in moisture could be due to the subsequent utilization of muscle protein. The increased level of moisture content were observed by Gaikwad SA in *Tilapia mossambica* exposed to Thiodan and PMA [34]. Hence, in the present study this is also the possibility that the cause for rise in moisture could be due to the subsequent utilization of muscle protein.

Conclusion

Metal contamination of aquatic environments has become a serious problem because of increased industrialization. Acute and chronic effects of zinc have been widely described for different aquatic organisms and exposure routes. Zinc accumulates in the fish body and mortality rate increases with zinc salt concentration as well as exposure time. Hence, it is necessary to control the use of heavy metals. It is also recommended that further research should be done on the levels of their heavy metals in the fish to monitor and prevent them from reaching high levels that make them toxic to living organisms.

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