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Enzymological changes in *Danio rerio* exposed to lead nitrate and water quality assessment

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ABSTRACT

Lead is an industrially significant heavy metal used in explosives, manufacturing paints, batteries, pottery, and other various essential products of daily life. The marine environments and ecosystems are degraded by the constant discharge of heavy metals. Currently, the toxic effects of heavy metals on aquatic organisms are attracting widespread interest, especially in studies linked with industrial contamination. Lead nitrate does not have a role in the human body. So the present experiment is designed to study the toxicological consequences of lead nitrate on enzymological changes in the muscle and gill of zebrafish. In the present experiment, test fish (Danio rerio) were procured and, after acclimatization, categorized into six groups: one control group and two treated groups. Every 24 hours, tissues were extracted from fish taken out of the aquarium to examine the enzyme activity of glycolysis and the Kreb's cycle. The rate of oxidative metabolism decreases due to the toxic stress of lead nitrate in zebrafish. Enzyme activity decreases in the muscle and gill as the concentration of lead nitrate increases. Compared to the muscle, the gill enzyme function is affected due to lead nitrate. Due to toxic stress, the rate of oxidative metabolism slows down in zebrafish, which results in a decrease in the enzymatic activity, such as LDH and SDH. Lead nitrate binds to enzymes, inhibits their function, and alters the structure of enzymes. Due to modifications in the mitochondrial membrane function, the amount of LDH decreases. Lead disrupts carbohydrate metabolism in zebrafish. Lead nitrate affects the water quality and changes physicochemical properties, such as pH, salinity, bicarbonate, and bicarbonate. Slight increase in water pH with positive correlation. Increase in the salinity of water and dissolved oxygen with positive correlation. Bicarbonates in the water decrease with a negative correlation. The result clearly indicates that zebrafish exposed to lead nitrate affect not only zebrafish but also the physicochemical properties of water. Toxic heavy metals alter the activity of enzymes and cause severe harm to various tissues. Therefore, long-term exposure to heavy metals with high concentrations of lead nitrate severely affects the health conditions of fish.

Keywords: *Zebrafish, lead nitrate, LDH, SDH, Tissues, water quality*

Introduction

Nowadays, the effect of heavy metals on aquatic animals is attracting widespread interest, particularly in studies related to industrial contamination [1]. Pollution due to heavy metals in aquatic habitats has become a source of great concern [2]. Different sources produce heavy metal, including natural and anthropogenic sources [3]. Industrial discharges contain pesticides, suspended solids, inorganic, organic, and several compounds of toxic metals [4, 5]. In aquatic environments these pollutants pose detrimental effects on reproduction, growth, physiology, and survival rate of organisms, particularly on fish [6]. The application includes heat stabilizers in nylon and polyester, rodenticides, and coatings of photothermographic paper [7]. Lead enters the aquatic environment from effluent related too industries such as refining, battery manufacturing, smelting, and so on. In addition, an extensive range of chemicals are used in aquaculture that frequently consists of heavy metals [8]. Lead-induced oxidative damage is one of the mechanisms through which lead exposure causes toxicity in organisms as well as fish [9]. Acute heavy metal toxicity alters enzyme activities which frequently reflect organ or cell damage in a particular organ [10]. Fish have the capability to bio-magnify and bioaccumulate Pb so good indicator are fish for such pollution [11]. The effects of Pb pollution vary in relation to fish physiological status (size and sex), aquatic environment (temperature, hardness, pH, and dissolved oxygen), Pb concentration, and exposure period [12]. The aquatic habitat can become polluted by heavy metals, particularly lead, which increase a wide range of biochemical and physiological disorders in fish as a result of bioaccumulation in various organ [13, 14]. Zebrafish is a small freshwater tropical fish spotted in the north India and the eastern Ganges rivers. Zebrafish exhibit similar circadian rhythms and toxicity tolerances as mammalian models. Additionally, they have the capability to regenerate tissue of photoreceptor cells, the heart, spinal cord, and retinal neurons. The blood, muscle, eyes, and kidney share several characteristics with the tissue of humans [15]. So, the present experiment is designed to study the toxicological consequences of lead nitrate on enzymological changes in the muscle and gill of zebrafish and physicochemical properties of the treated water of zebrafish exposed to lead nitrate.

Materials and Methods

Experimental fish

This study was carried out in the Department of Zoology during the months of July-August 2024at Veeranari Chakali Ilamma Women's University.

In the present study, zebrafish of both sexes were procured (weight (350–500 g) and length (4–4.5 cm)) from a local seller and carefully transported to the lab. For each group, the aquarium was filled with 6 L of dechlorinated water. A total of 30 fish were procured and monitored throughout the period of the experiment. Before introducing toxicants, fish were acclimatized for 14 days. All exposure takes place at 26-29°C with a 14:12 (light: dark) photoperiod. Continuous aeration in the aquarium was provided and maintained during day and night to prevent hypoxic conditions in theenvironment. Aquariums were covered using monofilament netting to prevent fish from jumping out of the aquarium. Zebrafish were starved for one day before the initiation of the experiment. Fish were fed commercial fish food (crude protein: 25%, crude fiber: 8%, crude fat: 2%, and moisture: 10%). The toxicant used in the experiment was lead nitrate. Dead fish were taken out of the aquarium to avoid organic deterioration and oxygen reduction. Zebra fish were categorized into 3 groups, with 8 fish in each group in 3 separate aquariums for a period of 96 hours. In an aquarium, water was renewed every 24 hours, and fresh metal was added to clean out residual fish food, defecation, or dead fish.

The toxicant used in the experiment was lead nitrate. The lead nitrate toxicity experiment was carried out for 4 days in the lab. Acclimatized fish were categorized into 3 groups: 1 control and 3 experimental groups. Each group accommodates 8 fish. Zebrafish were categorized into 3 groups, except the control group of fish; all other groups of tested fish were exposed to 20, 30 mg/L of lead nitrate concentration for a period of 96 hours. For the preparation of lead nitrate stock solution, double-distilled water was used. Different lead nitrate concentrations were made freshly in distilled water before mixing with the aquarium water. Lead nitrate is dissolved in distilled water and thoroughly mixed by stirring to prepare a heavy metal stock solution.

Estimation of enzyme activities and Physicochemical analysis of water

After a 24-hour exposure period, fish from each group were sacrificed to isolate the muscle and gill, including fish from the control group. Every 24 hours, fish were sacrificed for enzymological parameters that were estimated. Precautions were taken at the time of fish handling to prevent stress. Zebrafish asphyxiated on ice after asphyxiation muscle and gills were isolated. The LDH enzyme activity was estimated by using the Nachlas method [16]. The SDH enzyme activity was estimated by using the Nachlas method [17].

The primary reason for analysing water quality is to evaluate whether the water is suitable for its intended use or not. Analysis of pH, salinity, bicarbonate, and dissolved oxygen of water samples of different doses (20, 30 mg/L of lead nitrate) was done using standard methods (APHA) [18].

Statistical analysis

The data obtained is represented as mean and standard deviations. The results obtained from this experiment were statistically analysed using one-way ANOVA. to determine the correlation between concentration and mortality.

Results

Results indicate thatthere is a decrease in the activity of LDH (Fig 1) in muscle and gill exposed to 20, 30 mg/L of lead nitrate. Enzyme activity is affected byan increase in the concentration of

lead nitrate, and long-term exposure also resulted in a decrease in the activity of LDH. Compared to muscle the enzyme activity is affected in gill.The activity of SDH in the muscle and gill of zebrafish exposed to 20 and 30 mg/L of lead nitrate. Depicted in Fig-2 indicates that compared to the control, SDH activity decreased in the muscle and gill. There is a slight increase in the pH of water with 20 and 30 mg/L of lead nitrate; there is a positive correlation between pH and concentration of lead nitrate compared to the control group, and it has a positive correlation. (Fig 3)The salinity of the water after 96 hours exposed to lead nitrateincreased compared to the control group.(Fig4)Bicarbonate in water decreased with an increase in the concentration of lead nitrate, and it has a negative correlation. (Fig-5)In the case of dissolved oxygen, it increases in water with an increase in the lead nitrate concentration with a positive correlation.(Fig-6)

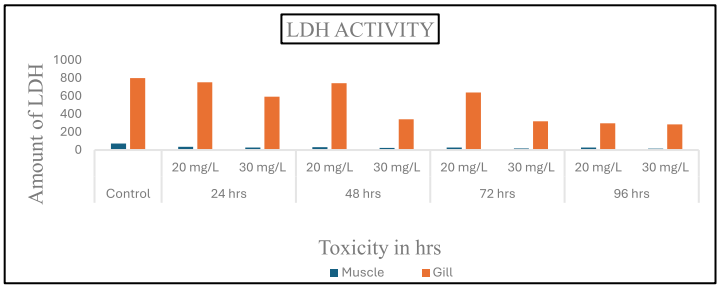

Fig-1 LDH activity (µ moles of formazone formed/mg protein/hour in the muscle and gills of control and experimental group (mean values, p-0.987123)

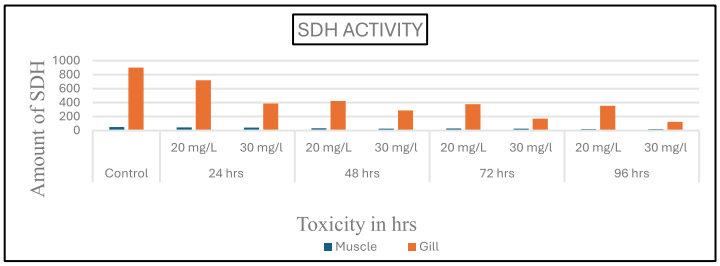

Fig-2 The SDH activity (µ moles of formazone formed/mg protein/hour) in the muscle and gills of control and experimental group (Mean values, p-0.930378)

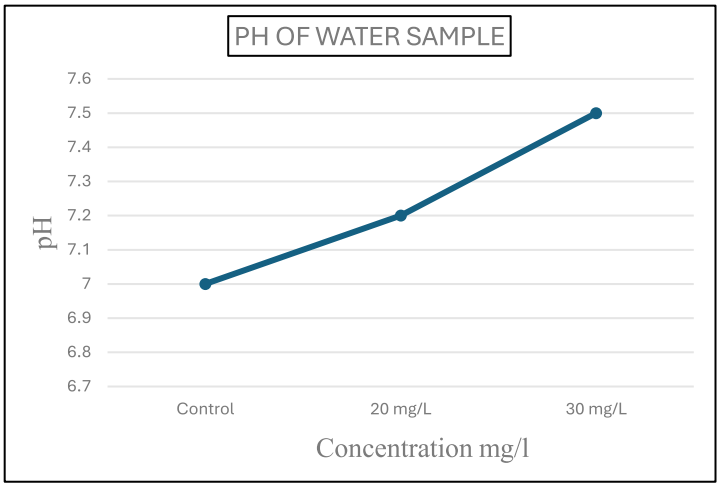

Fig-3 pH of water sample control group after 96 hours treatment of zebrafish with different conc. Of lead nitrate

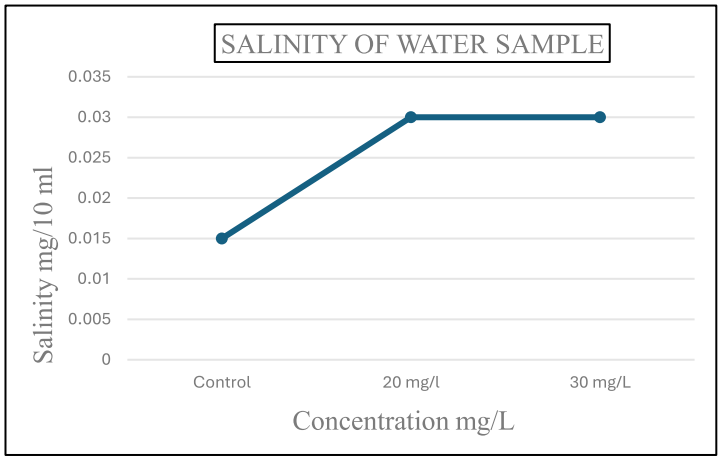

Fig-4 Salinity of water sample control group after 96 hours treatment of zebrafish with different conc. of lead nitrate

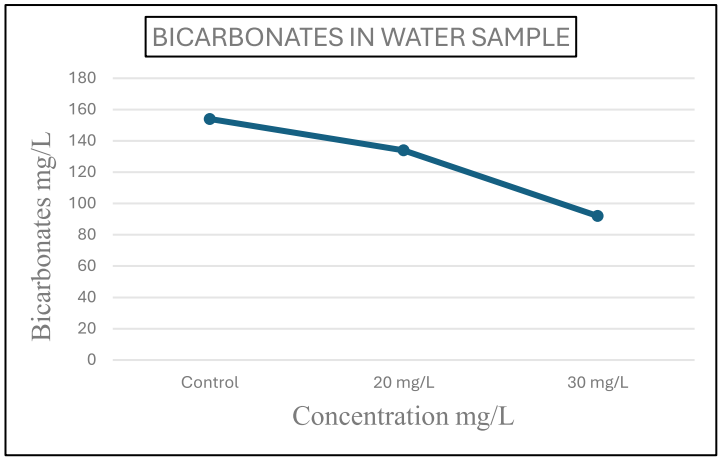

Fig-5 Bicarbonates in water sample after 96 hours treatment of zebrafish with different conc. Of lead nitrate

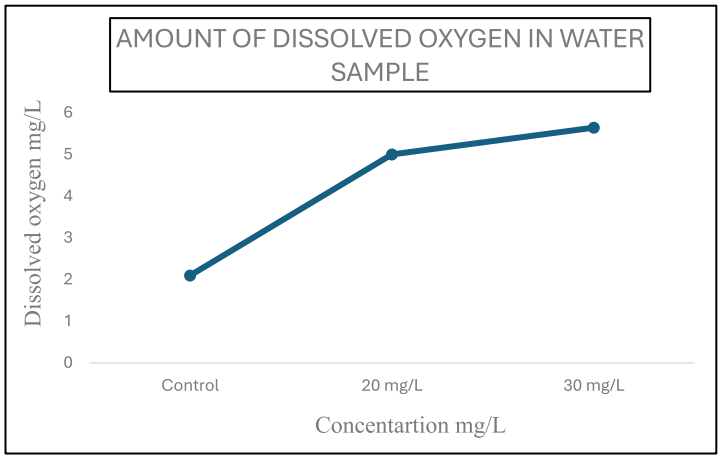

Fig-6 Dissolved oxygen in water sample control and after treatment of zebrafish with different conc. of lead and correlation

Discussion

Lead has high affinity to SH groups and suppresses SH-dependent enzymes, which are necessary for biological function. Secondly, lead acts in a similar way like calcium and inhibits the action of calcium in substantial regions like the intracellular second messenger system and oxidative phosphorylation in mitochondria [19]. Heavy metals generate reactive oxygen species [20]. These species induce oxidative injury by different mechanisms. Heavy metals are accumulated by aquatic organisms in their tissue via absorption along the lungs, intestinal walls, liver, and gill layers to higher levels than those of the surrounding environment [21].

In*Cirrhinusmrigala*, the activity of LDH is inhibited in the liver, gill, and muscle due to the rate of glycolysis.[22]. The surface of fish gill is negatively charged, and as a result, it provides the site for the binding of positively charged metal [23]. Active tissue is not muscle for bioaccumulation of metals, and like other tissue, this lowest potential could be as a result of the lowest level of protein binding in the muscle [24]. LDH activity of the kidney gill and intestine in *Carassius auratus gibelio* decreased when exposed to copper [25]. Alterations in the function of the mitochondrial membrane result in suppression of LDH activity. [26] .Similarly, *Clarias gariepinus*, the African catfish, of early ontogenetic stages, on exposure to lead nitrate, LDH activity declined [27]. The targets of toxicants are enzymes, which cause alterations in their activities depending on tissue and period of exposure [22]. Cd and Pb individual exposure decreased SOD and LDH activity [28]. Decreased rate of glycolysis indicates an important stress; stimulation of the enzyme may predict an increased dependence on anaerobic metabolism of carbohydrates [25]. Electron transfer to molecular oxygen is hindered, which results in the inhibition of the activity of SDH, shifting the aerobic metabolism to anaerobiosis [29]. It agrees with our results: *Oreochromis niloticus*, when exposed to cadmium, has decreased LDH activity [26]. According to [30], the formation of a complex inhibits the function of the enzyme or the metabolism inhibition process [26]; the interaction between LDH and heavy metal is direct [31]. Nevertheless, the primary sources of lead, like lead batteries, the smelting industry, crystal and ceramic mining, undoubtedly contribute to the lead-induced deleterious effects on the people and environment [32]. Decrease in SDH activity in *Channa striatus*, representing impaired oxidative metabolism to meet total energy consumption, and it depends on anaerobic glycolysis [33]. [34] have observed a significant suppression of SDH activity in *Labeorohita* to meet total energy demands. [35] has reported that in *Oreochromis mossambicus*, the activity of SDH declined on exposure to sublethal concentrations of lead. They stated that shift in metabolism from aerobic to anaerobic. Although few other environmental factors in the fish pH, O2 concentration, water temperature, hardness, alkalinity, and dissolved oxygen may affect and play a key role in heavy metal toxicity [36]. *Danio rerio*, when exposed to lead nitrate in both hard water and soft water, has higher toxicity in soft water than hard water because through the gills, body surface, and digestive system, lead absorption is less. In hard water, the formation of insoluble complexes reduces lead toxicity [37]. The physicochemical properties of water change due to not only the addition of heavy metals but also the discharge of faecal matter, metabolic waste, and unconsumed food [38]. Our results are similar to those of [38]; exposure of *Cyprinus carpio* to lead nitrate changes the physiochemical properties of water, such as pH increase and dissolved oxygen increases in water. In the body of fish, the concentration of heavy metal is based on the feeding habitat and food, physicochemical properties of water, trophic status, animal metabolic rate, and degree of toxicity of heavy metals [39].

Conclusion

The explosion of human population, urbanization, and technological developments has introduced pollution into aquatic habitats. Among them, pollution of aquatic environments due to heavy metals is dominant. Some heavy metals are essential in minimal quantities.

Many chemicals, along with heavy metals, are used excessively in various sectors like agricultural, industrial, and domestic activities. On earth, the lives of species are unimaginable without water, which is essential. On the other hand, due to particulate matter and dissolved pollutants, water is no longer natural. The industrial waste produced is discharged into water, causing severe harm and, in certain cases, mortality of aquatic organisms. A toxicity test is used to investigate the impact of heavy metals on aquatic creatures; it has been conducted in a controlled environment. Due to toxic stress, there is a decrease in the activity of LDH and SDH in zebrafish. Our results evidenced that enzyme activity may be a sensitive indicator for aquatic toxicology. Even small changes in the water quality impact fish health. Comparable studies can be used to evaluate human exposure dose and amount of chemical residue that are permissible in aquatic environments. From the present work, it has been concluded that heavy metals affect the enzymological functions and physiochemical properties of water. The survival of fish depends on the quality of water. With reference to this, further studies involve interpreting the accurate toxicity of lead nitrate in humans.

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