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Original article

Determination of toxic effects of lead acetate on different sizes of zebra fish (*Danio rerio*) in soft and hard water

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ABSTRACT

The main objective of the present study is to determine lead toxicity on various sizes of zebra fish in soft and hard water. Lead toxicity analysis was carried out in the laboratory trials for four days at various concentrations for the determination of LC50 value. Zebra fish was grouped into various sizes (G1-G4) and treated with lead for 96 h. Lead induced toxicity and induced various behavioural changes in Zebra fish. In the case of G1 (1.5 ± 0.2 cm length) fish the LC50 values ranged from 27.2 ± 1.8 mg/L to 9.7 ± 1.3 mg/L. In G2 (2.0 ± 0.2 cm length) fish, the LC50 value was 37.01 ± 2.3 mg/L and it reduced as 32.03 ± 3.4 mg/L after 48 h. In G3 group (2.5 ± 0.2 cm length fish), LC50 value was 38.78 ± 2.4 mg/L after 24 h treatment and this value decreased as 33.18 ± 1.1 mg/L after 48 h. After 72 h, LC50 value was 27.2 ± 2.1 mg/L and this value decreased considerably after 96 h (18.3 ± 2.1 mg/L) in G4 (3.0 ± 0.2 cm length) fish. Further, G4 fish was treated with lead in hard water and LC50 value was analyzed. LC50 value was observed as 28.62 ± 2.7 mg/L in hard water and the same fish group (G4) showed 18.3 ± 2.1 mg/L after 96 h. Toxicity analysis revealed that lead is less toxic in hard water than in soft water.

1. Introduction

Heavy metals are highly toxic to the environment, and the main sources are coal burning, mining, agriculture, sewage, domestic and industrial runoff. In aquatic environment, metal toxicity affects fishes and these toxic heavy metals affected ion regulation in fishes. Heavy metal poses severe threat to the aquatic organisms and has various sources to enter aquatic system. Aquatic pollution causes stress to the aquatic organism and induce negative effect. Lead is the one of the metals have no nutritive value and cause pollution in aquatic environment (Vasanthi et al., 2019). These metals easily enter into the food chain and subsequently involved in bioaccumulation process (Azaman et al., 2015). Toxicity of heavy metals to the organism mainly based on the route of exposure, absorbed dose and duration of exposure (Chaurasia et al., 2016a; Kumaresan et al., 2016; Chaurasia et al., 2016b; Kumaresan et al., 2015a). This can lead to many disorders and also cause excessive damage due to oxidative stress mainly induced by the formation of free radicals (Jaishankar et al., 2014). Some heavy metals are very essential for routine physiological function of fish however become toxic when heavy metals accumulate in the muscle and body tissues (Pandey and Madhuri, 2014). Also, elevated level of heavy metals can effectively change its physiological function that leads to mortality in fishes (Avenant-Oldewage and Marx, 2000). The river is mainly contaminated by heavy metals due to the outcome of industrial waste, mining, domestic wastes including battery and agricultural wastes. Contamination of heavy metals in aquatic environment may affect the diversity and food web of aquatic organisms (Varol, 2011). Like other animals, fishes from the pond, lake or river water cannot escape from the harmful effects from these heavy metals (Ravichandran et al., 2017; Arasu et al., 2017a,b,2016; Ravichandran et al., 2016).

The impact of these heavy metals and other various pollutants, on fishes can be tested by toxicity analysis, which is mainly used to evaluate and to detect the toxic effects of heavy metals on aquatic

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organisms (Fu et al., 2013). Fishes are commonly applied to validate the nature of aquatic ecosystems because heavy metals and other pollutants involved in food chain mechanism and are highly responsible for severe impacts and mortality in the aquatic ecosystem. Fishes get trace elements through food, gill, or through body surface (Arockiaraj et al., 2015a; Palanisamy et al., 2015; Arockiaraj et al., 2015b; Chaurasia et al., 2015; Kumaresan et al., 2015a,b; Rao et al., 2015). Heavy metals such as, Zn, Cu Mn, Co and Cr are very important for the growing organisms, however As, Pb, Cd and Hg are not required for aquatic organisms for their metabolism (Sathyamoorthi et al., 2019;Kumaresan et al., 2019; Sathyamoorthi et al., 2018; Ravichandran et al., 2018; Sathyamoorthi et al., 2017). These heavy metals at higher concentrations in the aquatic environment cause hazardous and highly toxic. Once heavy metals enter into the aquatic environment, these heavy metals are absorbed on solid surface, and suspended in water or absorbed by fauna. Also, animal tissues accumulate heavy metals from the environment. Heavy metals such as cobalt is a highly toxic substance, fertilizers are the importance source of cobalt contamination in the running water and cause heavy risk to the environment (Mansouri et al., 2011, 2012).

Lead causes severe impact to fishes and cause mortality at lethal concentrations and at sub-lethal concentrations cause impotency, behaviour and growth performance changes (Afshan et al., 2014). In aquatic environment Pb^{2+} is highly stable form of lead and has been accumulated in fish organs such as, liver, gills, kidney, scales, muscles and skin. Exposure of lead in the aquatic environment causes mortality, growth inhibition properties, and abnormalities in the muscle and changes in reproductive performance (Srivastav et al., 2013). The contaminated water by heavy metals easily entered into the food chain in freshwater ecosystem. Based on absorption and excretion pattern, bioaccumulation rate differs. Many factors such as, chemical and physical including pH of the environment directly influence bioaccumulation in various fish tissues. Heavy metals affect reproduction, cause mortality, physiological functions and also affect growth rate and feed conversion rate in fishes (Abascal et al., 2007). In aquatic environment, heavy metals enter into the fish body through body surface, digestive system and gills. However, accumulation of heavy metals through body surface is limited. The objective of the present analysis is to determine lead toxicity on various sizes of zebra fish in soft and hard water.

2. Materials and methods

2.1. Experimental fish

In our study, the experimental animal, Zebrafish (*Danio rerio*) was collected from the aquarium and stocked in a rectangular glass aquarium tank (75 × 60 × 35 cm, 20 L capacity) for about two weeks. About 14 h light and 10 h dark cycle was maintained throughout the experimental period. The aquarium tank was connected with air compressor and ensured uninterrupted aeration. Initially 250 animals were collected from the aquarium and maintained. Zebra fish was grouped into experimental and control. The fish was graded based on the length. A total of four experimental groups were maintained strictly based on the length, viz, 1.5 ± 0.2 cm (G1), 2.0 ± 0.2 cm (G2), 2.5 ± 0.2 cm (G3) and 3.0 ± 0.2 cm (G4), respectively. In each group, twenty two experimental animals were incorporated. Control and experimental animals were fed with brine shrimp (*Artemia*), tubifexworm and pellet feed daily.

2.2. Chemicals

For toxicity analysis lead acetate was purchased from Sigma, U.S.A and stock was prepared in double distilled water. All other reagents, chemical used were analytical grade (Himedia, Mumbi, India).

2.3. Toxicity analysis in soft water

Lead toxicity analysis was carried out in the laboratory trial in the above conditions for four days. Lead acetate was prepared at eight different concentrations, 5, 10, 15, 20, 25, 30, 35 and 40 mg/L. Heavy metal stock was prepared in double distilled water. Three experiment analyseswere performed and an average was considered for this study. Exchange of water was performed daily and fresh metal was added daily. Finally, mortality rate of fish was determined after 24–96 h. Neurotoxicity was also monitored during experimental trials. Dead animals were separated from the tank and stored at −20 °C up to the completion of all experiments.

2.4. Toxicity analysis in hard water

The approximate $CaCO_3$ hard water level of tap water was 20 mg/L. To this tap water Ca $(NO_3)2$ was added to increase the hardness of water (upto250 mg/L). Lead was prepared at various concentrations in double distilled water. The concentration of lead was maintained as described previously. Zebra fish (3.0 cm long) was introduced into the glass aquarium which was previously acclimatized and fed with live and pellet feed. LC50 value was determined for 24–96 h.

3. Results

3.1. Lead toxicity induces bahavioral changes in Zebra fish

Lead induced toxicity cause various behavioural changes in Zebra fish. The changes like loss of equilibrium, erratic swimming was observed during the study period. The experimental animal resting at the bottom of the glass aquarium aggregated at a corner of the tank and frequently swims at the surface of the tank. The heavy metal treated fish lost control, hyper secretion of mucus from the body, heavy breathing with strong opercula movement. In the control fish, behavioural response is normal.

3.2. Lead toxicity based on the size of the fish

Zebra fish was grouped into various sizes and lead acetate was prepared at five different concentrations. In the case of G1 fish the LC50 values ranged from 27.2 ± 1.8 mg/L to 9.7 ± 1.3 mg/L. After 96 h incubation, LC50 value was 9.7 ± 1.3 mg/L. In G2 fish, the LC50 value was 37.01 ± 2.3 mg/L and it reduced as 32.03 ± 3.4 m g/L. After 72 h, LC50 value was 27.1 ± 4.1 mg/L and it reduced considerably after 96 h (17.8 ± 2.3 mg/L). In G3 fish, LC50 value was 38.78 ± 2.4 mg/L after 24 h treatment and this value decreased as 33.18 ± 1.1 mg/L after 48 h. After 72 h, LC50 value was 27.2 ± 2.1 mg/L and decreased considerably after 96 h (18.3 ± 2.1 mg/L). In G4 fish, LC50 value was found to be 18.62 mg/L after 4 days incubation in soft water (Fig. 1).

3.3. Lead toxicity in hard water

The average LC50 value for tested hard water for Zebra fish is described in Fig. 2. The survival rate of Zebra fish was found to be high in hard water than soft water. The LC 50 values were, 48.91 ± 2.2, 37.28 ± 2.8, 32.13 ± 3.1 and 28.62 ± 2.7 mg/L after 24, 48, 72 and 96 h, respectively (Fig. 2).

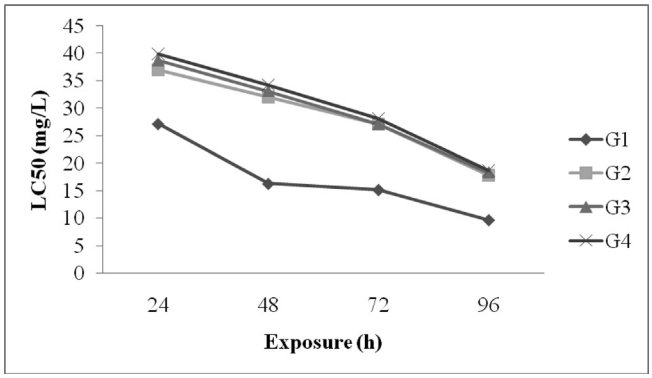

Fig. 1. LC50 value for Zebra fish exposed to lead at various experimental groups. G1 = 1.5 ± 0.2 cm length fish, G2 = 2.0 ± 0.2 cm length fish, G3 = 2.5 ± 0.2 cm length fish and G4 = 3.0 ± 0.2 cm length fish.

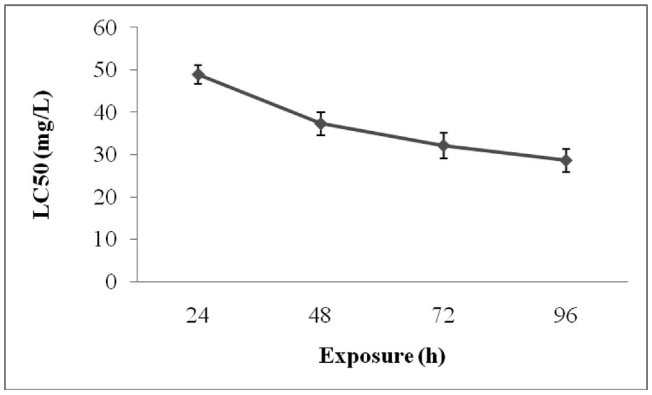

Fig. 2. Influence of hard water on toxicity for Zebra fish exposed to lead for G4 (3.0 ± 0.2 cm length) fish.

4. Discussion

In this study, lead was exposed to Zebra fish at various concentrations and at various sizes (G1 – G4) to analyze the impact of lead towards size. Initially behavioural changes were observed in the experimental animal. Large fishes (G4) were less susceptible and small sized fishes (G1) were largely affected by lead toxicity. Experimental fishes lost equilibrium and rested at the bottom of the tank. Some fishes were found near the corner of the tank and erratic swimming was also noticed. It could be noted that heavy metals contamination is very common in aquatic environment. Heavy metals are mainly considered as hazardous material to the aquatic system because of high toxicity, ability to bioaccumulate by living organisms (Pandey and Madhuri, 2014). Hardness and pH of the aquatic environment greatly affected the availability of heavy metals. Heavy metals affect locomotion of fishes in various ways, including avoidance, homing, alters sensory perception and reduce swimming performance (Prashanth et al., 2011). Lead also causes severe health problems in humans. Lead accumulates in the blood, bones and muscles and fat. Young children and newborns are highly susceptible to lead even at very low concentrations (Gurer-Orhan et al., 2004).

In this study, lead caused toxicity to zebra fish at various concentrations. At higher exposure time, severe effect was registered. Behaviour changes were observed during the study due to neruotoxic effect. In aquatic ecosystem, heavy metal contamination results from geologic weathering, atmospheric deposition, industrial, domestic and municipal waste products also wastewater plants generate various heavy metals (Bauvais et al., 2015; Demirak et al., 2006). High concentrations of heavy metals in the aquatic environment pose serious threat to living organisms. Importantly, fishes may accumulate large quantity of heavy metals and cause acute and chronic diseased to humans beings (Al-Yousuf et al., 2000). Metals are non degradable matters and can accumulate in the particular environment and cause various health issues. In fishes, accumulation of heavy metals was mainly through gastrointestinal tract and gills (van der Oost et al., 2003). Fishes are mainly confined in a particular environment and are highly vulnerable to metal toxicity. Toxicity in an aquatic environment is mainly used to validate the toxicological properties of environmental contaminants on aquatic organisms. It is very important to explore the presence of water borne heavy metals on tolerance of the highly sensitive organisms such as fish. In natural environment, the reported lead concentration in the surface water was 0.02 μg/L and lead rarely exceeds at this value. High exposure of lead in the aquatic organism critically causes alteration in blood, generative damage, alteration in nerve cells in aquatic organisms, including fishes (Kalay et al., 1999; McCoy et al., 1995).

The results obtained from the toxicity analysis of lead for Zebra fish reveals that the mortality of the fish dramatically increased with exposure time and increasing lead concentrations. In a study, Ullah et al. (2016) observed lead toxicity to *Oreochromis niloticus*. It was subjected to toxicity analysis and 96 h LC50 value of lead nitrate was found to be 44 mg/L. This value was found to be higher than our finding, shows Zebra fish is highly sensitivity than *Oreochromis niloticus*. Batool and Javed (2015) observed lead toxicity on *Labeorohita*, *Cirrhina mrigala* and *Catla catla*. The LC50 value was found to be 36.72 ± 0.37 mg/L, 40.54 ± 0.32 mg/L and 31.25 ± 0.22 mg/L, respectively. In a study Ferrer et al. (2006) used crab, *Chasmagnathus granulate* in its early stage to determine heavy metal toxicity and the reported ranges of LC50 values among the tested heavy metals such as, Zn, Cu and Pb. Chinni and Yallapragda (2000) used the heavy metals such as, Cu, Cd, Zn and Pb to determine heavy metal toxicity on post larvae (PL) of *Penaeus indicus*. The findings revealed that copper pose serious threat to the PL stage of *P. indicus* than other tested metals. However, the toxicity value may differ between fish, experimental procedure, water pH, age of fish, sex and temperature of the aquatic environment.

In our study, exposure of lead caused severe impact on small Zebra fish than larger one after 96 h exposure at sub – lethal level. It was previously reported that, long-term exposure of heavy metals to the fishes at low doses did not show any significant lose externally, moreover, the heavy metals critically affect the reproductive behaviour of fishes by reduced reproductive organ and the fish population (Kime, 1995). Previous studies reported nuclear degeneration, severe pituitary damage and reduced egg hatching rates due to metal toxicity (Popek et al., 2006). Lead has been well known to cause haematological, neurological, circulatory, reproductive and histochemical changes (Rout and Naik, 2000). The toxic heavy metals significantly affect metabolic and physiological functions, reproductive functions, growth rate and cause mortality to fishes (Woodward et al., 1994). The LC50 value of fish was 9.7 ± 1.2 mg/L in small fish which was found to be less than large fish. In a study, LC50 value for lead on *Labeo rohita* was analyzed ant it was ranged between 27.2 mg/l and 32.70 ± 2.23 mg/l (Javid et al., 2007; Abdullah et al., 2007).

The present findings show that lead is highly toxic to Zebra fish in both hard and soft water. In our experiment increased survival rate was registered at higher concentrations of hard water. After 96 h, the LC50 value was 18.62 ± 4.5 mg/L in soft water; however this value increased as 28.62 ± 4.5 mg/L in hard water. The increased LC50 value was reported previously with various heavy metals. The reduction of lead toxicity in hard water was mainly due to the formation of insoluble complexes in hard water. Pascoe et al. (1986) used rainbow trout to analyze cadmium toxi-

city after 96 h. After 96 h, LC50 value was 1.3 mg Cd/L in soft water, whereas, the LC50 value was 2.6 mg Cd/L in hard water. In hard water, heavy metal uptake significantly decreased. Jaishankar et al. (2014) stated that metal toxicity depends upon the duration of exposure, the route of exposure and adsorbed dose. Heavy metals lead to many disorders and can also results in severe damage due to severe oxidative stress. Rathore and Khangarot (2003) studied the influence of metal concentration and water hardness in *Tubifex tubifex* Muller. The LC50 value was reportedly higher in hard water than soft water in the case of zinc, nickel, mercury, manganese, lead, iron, copper chromium, cobalt and cadmium. These tested heavy metals enhanced mucus production and induced autonomy in the caudal region. In a study, Mansouri and Baramaki (2011) studied the influence of pH and water hardness on acute toxicity of mercury on *Capoetafusca borhan*. In this freshwater fish, the LC50 value was 0.180 mg/L in hard water, whereas, it was found to be low in soft water (0.118 mg/L). These findings revealed increased heavy metal toxicity in hard water than in soft water. Other toxic metal such as copper and zinc also reported as highly toxic to the organism in soft water than hard water (Ebrahimpour et al., 2010).

5. Conclusion

Lead is highly toxic to Zebra fish and caused behaviour changes at various concentrations. This behavioural response was mainly due the ill effect in nervous system cause by lead. After 24 h of exposure, LC50 value was found to be high and it decreased after 96 h of treatment. The increased exposure increases lead absorption through gill, skin and digestive system. In the present finding soft water showed high toxicity than hard water. In hard water, lead absorption may be less through body surface, gill and digestive systems. The reduction of lead toxicity in hard water was mainly due to the formation of insoluble complexes in hard water.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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