

Influence of Cu, Zn, Cd and Hg upon ascorbic acid activities in freshwater bivalves *Lamellidens marginalis* and *Lamellidens corrianus*

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ABSTRACT

The bivalves were exposed to LC_0 and LC_{50} values of 96 hrs with concentrations of 182.02 ppm for zinc chloride, 1.62 ppm for copper sulphate, 1.04 ppm for cadmium chloride and 0.687 ppm for mercuric chloride up to 96 hours. In *L. marginalis* monsoon the ascorbic acid content in LC_0 showed more amount in copper followed by cadmium, zinc and mercury. In LC_{50} the more amounts in copper followed by zinc, cadmium and mercury. In winter the LC_0 showed more amount in copper followed by zinc, cadmium and mercury. In LC_{50} the more amounts in copper followed by zinc, cadmium and mercury. In summer the LC_0 showed more amount in copper followed by mercury, cadmium and zinc. In LC_{50} the more amounts in zinc followed by copper, mercury and cadmium. In *L. corrianus* monsoon the ascorbic acid content in LC_0 showed more amount in zinc, followed by copper, cadmium and mercury. In LC_{50} the more amounts in zinc followed by copper, cadmium and mercury. In winter the LC_0 showed more amount in mercury followed by copper, zinc and cadmium. In LC_{50} the more amounts in mercury followed by copper, cadmium and zinc. In summer the LC_0 showed more amount in zinc followed by copper, cadmium and mercury. In LC_{50} the more amounts in zinc followed by copper, cadmium and mercury.

KEYWORDS: Ascorbic acid, heavy metals, *L. marginalis*, *L. corrianus* acute toxicity, seasons.

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I. INTRODUCTION

The assessment of biochemical changes at organism level will help to develop a reliable approach for environmental risk assessment, to predict the early detection and effects of heavy metal water pollution and our understanding of organism response after exposure to heavy metal stress. Bivalves are benthic, widespread in distribution and ecologically important because of their biological filtration activity. They can accumulate heavy metals in their tissues at concentrations in excess of the ambient water (Potter *et al.* 2013) through ingestion of sediment particles, food and directly from overlaying water. Heavy metals are responsible for biochemical and physiological changes in the organisms. Biochemical composition serve as the initial sensitive indicators of toxic effect on tissues (Thaker and Haritos, 1989). Ascorbic acid is an “enediol-lactone” of an acid with a configuration similar to that of the sugar L-glucose. The vitamin C is L-ascorbic acid is essential for the normal development while D-ascorbic acid is antiscorbutic. Glucose and hexoses are utilized for the synthesis of ascorbic acid. Similarly, these toxic water pollutants enter into the body of human through food chain. The toxicants produce cumulative deleterious effects not only on particular group of animals but also inhabiting the ecosystem. Biochemical responses in aquatic organisms have been used in several monitoring programs to study the anthropogenic pollution (Cajaraville *et al.*, 2000). Recently, more attention has been given to propose biomarkers of exposure and effect, in toxicity testing aiming an application in pollution monitoring. The physiological and biochemical responses of the molluscs to environmental toxicants have been expanded significantly. Lackner (1998) studied that ascorbic acid level was decreased during chronic exposure to different stressors. Waykar *et al.*, (2001) studied the effect of cypermethrin on ascorbic acid content in the mantle, foot, gill, digestive gland and whole body tissues of freshwater bivalve, study of change in ascorbic acid content in molluscs exposed to heavy metals can be useful as an indicator of toxicant stress condition. Rahane and Bhalla (2018) studied effect of heavy metals on antioxidant biomarker enzymes and biochemical constituents in different tissues of *Lamellidens marginallis*. The ascorbic acid has antioxidant property which helps to prevent free radical formation from toxic water soluble molecules that may cause cellular injuries. It acts as a hydrogen carrier and plays an important role in carbohydrate, protein or both metabolisms (Kaya, 2003). Biochemical analysis of benthic organisms as monitors is the best option for conventional metal pollution monitoring system which may be insufficient, that leads to inaccurate water quality assessment. Nandi *et al.*, (2005) reported that, ascorbic acid prevents oxidative damage to the membranes against peroxydation by increasing the activity of

tochoperol. Ascorbic acid is well known to play protective and therapeutic role against pollutant or metal toxicity (Rao *et al.*, 2001). A maximum depletion in ascorbic acid contents were observed in whole soft tissue of *Lymnaea acuminata* in response to accumulated levels of Zn, Cu and Pb while *Mellanoides tuberculata* for Cd as compare to studied snail species. Changes in biochemical components such as proteins, ascorbic acid, DNA and RNA of organisms are useful to know effect of different toxicants and defensive mechanism against toxic effects of heavy metals of the bcomponents are indices of pollution as they are useful to determine nutritional status, health and vigor of an organism. Among all treated heavy metals, Cd caused more decrease in ascorbic acid as compared to Zn, It is important antioxidant that prevents oxidative damage in tissues of organisms. The ascorbic acid has antioxidant property which helps to prevent free radical formation from toxic water soluble molecules that may cause cellular injuries. It has a hydrogen carrier and plays an important role in carbohydrate, protein or both metabolisms. When animals are exposed to polluted aquatic environment containing metals, these metals accumulate in various tissues significantly (Fernandes *et al.*, 2008). Many researchers reported that heavy metals stress leads to alterations in protein, ascorbic acid, DNA and RNA in molluscs (Deshmukh, 2013, Nandurkar 2013; Jie *et al.*, 2017). This study helps to develop database about bioaccumulation response under laboratory condition, which might be used for future biomonitoring of heavy metal pollution. This study also helps to determine the most sentinel snail species for monitoring of heavy metal pollution in the freshwater ecosystem. Ascorbic acid readily forms salts of several metals and reduces their activity. Hence, the present study investigates the effect of heavy metal salts induced variation in ascorbic acid contents in the body of the fresh water bivalve, *Lamellidens marginalis* and *Lamellidens corrianus* after acute toxicity at 96 hrs.

II. MATERIALS AND METHODS

The bivalves habitat was rich in flora and fauna around and in as there was no any industry on both sides as well as in catchment area. The availability of bivalves for present study depend upon the topography of the dam weather condition and human activities like pollution, heavy water force, interfering the cattle, washing the cloths along the dam etc. The localities in dam was selected as per the species abundance and water qualities of dam in different geographic area. The bivalves *L. marginalis* and *L. corrianus* were collected for laboratory experiments from study area during monsoon, winter and summer seasons. They were brought to the laboratory and kept in plastic troughs containing five liters of dechlorinated tap water for three days to acclimatize to laboratory conditions. Water from the plastic trough was changed after every 12 hours. The healthy bivalves of approximately same size and weight were selected for the experiments. Since the animals are micro feeders no special food was supplied during the experiment. The acclimatized bivalves were exposed to LC₀ and LC₅₀ values of 96 hrs with concentrations of 182.02 ppm for zinc chloride, 1.62 ppm for copper sulphate, 1.04 ppm for cadmium chloride and 0.687 ppm for mercuric chloride up to 96 hours. The bivalves were divided into two groups and the first group was maintained as control and each of the remaining group was exposed to different metal concentrations. After 96 hrs exposure the control and experimental bivalves were dissected and whole body were weighed and they were then kept in hot air oven at 92 °C till constant weights were obtained. The dried product was ground to obtain fine powder. From the replicates of three samples the ascorbic acid was estimated using methods by Roe (1967). The amount of ascorbic acid were calculated by regression equation and expressed in terms mg/100mg dry powder.

III. RESULTS

In *L. marginalis* monsoon the ascorbic acid content in LC₀ showed more amount in copper, (1.93) followed by cadmium (1.91), zinc (1.87) and mercury (1.77). When these contents compared with zinc it was more in mercury (5.35%), than copper (3.21%) and cadmium (2.14%). In LC₅₀ the more amounts in copper (1.95) followed by zinc (1.91), cadmium (1.85) and mercury (1.81). When compared with the zinc species content it was more in mercury (5.24%) than cadmium (3.15%) and copper (2.10%). In winter the LC₀ showed more amount in copper (2.02) followed by zinc (2.01), cadmium (2.0) and mercury (1.94). When these contents compared with Zinc it was more in mercury (3.49%; P<0.05) than copper (0.50%) and cadmium (0.50%). In LC₅₀ the more amounts in copper (2.13) followed by zinc (2.07), cadmium (2.03) and mercury (1.99). When compared with the zinc species content it was more in mercury (3.87%) than copper (2.80%) and cadmium (1.94%). On the other hand the content when compared to monsoon to respective groups the ascorbic acid was increased in all groups and sizes also. In LC₀ the content more increased in mercury (9.61%; P<0.05) followed by zinc (7.49%), cadmium (4.72%) and copper (4.67%). In LC₅₀ it was increased in all groups and the increase rate was in mercury (9.95%) followed by cadmium (9.73%), copper (9.24%) and zinc (8.38%) all were non significant. In summer the LC₀ showed more amount in copper (1.28) followed by mercury (1.25), cadmium (1.20) and zinc (1.06). When these contents compared with zinc it was more in copper (20.76%; P<0.05) than mercury (17.93%) and cadmium (13.21%). In LC₅₀ the more amounts in zinc (1.32) followed by copper (1.30), mercury (1.20) and cadmium (1.06). When compared with the zinc species content it was more in cadmium (19.17%) than mercury (9.10%) and copper (1.52%) all were non significant level. On the other hand when the

content compared with monsoon and winter of respective groups the content showed in LC₀ the decrease rate was in zinc (43.32%; P<0.01), (47.27%; P<0.001) followed by copper (33.68%; P<0.01), (36.64%; P<0.001), cadmium (37.18%; P<0.01), (40.03%; P<0.01) and mercury (29.38%; P<0.01), (35.57%; P<0.01). In LC₅₀ more decreased in zinc (44.51%; P<0.05), (36.24%; P<0.05) followed by copper (33.34%), (38.97%; P<0.01), cadmium (42.71%; P<0.05), (47.79%; P<0.05) and mercury (33.72%), (39.70%; P<0.05) respectively.

In *L. corrianus* monsoon the ascorbic acid content in LC₀ showed more amount in zinc (1.65) followed by copper (1.61), cadmium (1.51) and mercury (1.29). When these contents compared with zinc it was more in mercury (21.82%), than cadmium (8.49%) and copper (2.43%). In LC₅₀ the more amounts in zinc (1.85) followed by copper (1.77), cadmium (1.63) and mercury (1.60). When compared with the zinc species content it was more in mercury (13.52%) than cadmium (11.90%) and copper (4.33%). In winter the LC₀ showed more amount in mercury (1.76) followed by copper (1.75), zinc (1.73) and cadmium (1.61). When these contents compared with zinc it was more in cadmium (6.94%) than mercury (1.74%) and copper (1.16%). In LC₅₀ the more amounts in mercury (1.87) followed by copper (1.83), cadmium (1.78) and zinc (1.69). When compared with the zinc species content it was more in mercury (10.66%) than copper (8.29%) and cadmium (5.33%). On the other hand the content when compared to monsoon to respective groups the ascorbic acid was increased in all groups and sizes also. In LC₀ the content more increased in mercury (36.44%; P<0.05) followed by copper (8.70%), cadmium (6.63%) and zinc (4.85%). In LC₅₀ it was increased in all groups and the increase rate was in mercury (16.88%) followed by cadmium (9.21%), zinc (8.65%) and copper (3.39%) all were non significant. In summer the LC₀ showed more amount in zinc (1.02) followed by copper (0.90), cadmium (0.85) and mercury (0.67). When these contents compared with zinc it was more in mercury (34.32%) than cadmium (16.67%) and copper (11.77%). In LC₅₀ the more amounts in zinc (0.88) followed by copper (0.68), cadmium (0.60) and mercury (0.56). When compared with the zinc species content it was more in mercury (65.0%; P<0.01) than cadmium (63.20%; P<0.01) and copper (61.59%; P<0.01). On the other hand when the content compared with monsoon and winter of respective groups the content showed in LC₀ the decrease rate was in zinc (38.19%; P<0.05), (41.05%; P<0.05) followed by copper (44.10%; P<0.01), (48.58%; P<0.01), cadmium (43.71%; P<0.05), (47.21%; P<0.05) and mercury (48.07%; P<0.01), (61.94%; P<0.01). In LC₅₀ more decreased in zinc (52.44%; P<0.01), (47.93%; P<0.01) followed by Copper (61.59%; P<0.01), (62.85%; P<0.01), cadmium (63.20%; P<0.01), (66.30%; P<0.001) and mercury (65.0%; P<0.01), (70.06%; P<0.001) respectively.

IV. DISCUSSION

Most of the toxicants interact with enzymes, metabolites or other cellular components of the organisms and affect the integrated functions like survival, growth, reproduction and behavior of the organism (Abou *et al.*, 2005). The results showed significant depletion in ascorbic acid is summarized in table (1). Biochemical study of animals in laboratory condition is an important diagnostic tool in the assessment of risk and hazards of potential for human (Krishna and Ramchandran, 2009). In *L. marginalis* monsoon the ascorbic acid content in LC₀ showed more amount in copper followed by cadmium, zinc and mercury. In LC₅₀ the more amounts in copper followed by zinc, cadmium and mercury. In winter the LC₀ showed more amount in copper followed by zinc, cadmium and mercury. In LC₅₀ the more amounts in copper followed by zinc, cadmium and mercury. On the other hand the content when compared to monsoon to respective groups the ascorbic acid was increased in all groups and seasons also. In summer the LC₀ showed more amount in copper followed by mercury, cadmium and zinc. In LC₅₀ the more amounts in zinc followed by copper, mercury and cadmium. On the other hand when the content compared with monsoon and winter of respective groups. The high levels of ascorbic acid found in the ovaries have been considered a reflection of the endocrine function of the organ (Levine and Morita, 1985). Muley and Mane (1987) have reported in gills and hepatopancreas the ascorbic acid content decreased in fresh water bivalves when it was exposed to different concentrations of pesticides. Nagpure (2004) studied the decrease in ascorbic acid in different tissues of *Lamellidens corrianus* and *Parreysia cylindrica* after the exposure of antibiotics. Among the tested heavy metals, Cd caused more depletion in ascorbic acid content in three experimental freshwater snail species as compared to Zn, Cu and Pb. Highest depletion of ascorbic acid content was observed in whole soft tissues of *Lymnaea acuminata* in response to accumulated levels of Zn, Cu and Pb and in *Mellanoides tuberculata* for Cd. Mahajan (2013) observed depletion in the ascorbic acid contents in *Bellamyia bengalensis* on chronic exposure to lead and copper as compare to control. Ascorbic acid has a coenzyme or cofactor necessary for normal functioning of cellular and sub-cellular structures necessary for the synthesis of collagen. Nawale, (2008) reported that depletion in the ascorbic acid contents in bivalve, *Lamellidens corrianus* after chronic exposure to lead nitrate and sodium arsenate. Deshmukh (2013) observed decrease in ascorbic acid level in different tissues of experimental bivalves after chronic exposure to heavy metals for 10 and 20 days as compare to control bivalves. The decreased level of ascorbic acid might be due to its contribution in detoxification or impairments in its synthesis, repairing of injuries in tissues and to face the toxic stress caused by heavy metals (Waykar *et al.*, 2001, Mahajan and Zambare, 2006)

In *L. corrianus* monsoon the ascorbic acid content in LC₀ showed more amount in zinc, followed by copper, cadmium and mercury. In LC₅₀ the more amounts in zinc followed by copper, cadmium and mercury. In winter the LC₀ showed more amount in mercury followed by copper, zinc and cadmium. In LC₅₀ the more amounts in mercury followed by copper, cadmium and zinc. On the other hand the content when compared to monsoon to respective groups the ascorbic acid was increased in all groups and seasons also. In summer the LC₀ showed more amount in zinc followed by copper, cadmium and mercury. In LC₅₀ the more amounts in zinc followed by copper, cadmium and mercury. On the other hand when the content compared with monsoon and winter of respective groups the content showed in LC₀ the decrease rate was in zinc followed by copper cadmium and mercury. In LC₅₀ more decreased in zinc followed by copper, cadmium and mercury respectively. In biochemical components like proteins, ascorbic acid, DNA and RNA, are useful to study different toxicant defense mechanisms of the body in response to toxic effects of heavy metals. Most biochemical changes in the laboratory studies are evaluated after exposure to toxicants like metals, pesticides, etc. These changes provide sensitive and specific response to specific toxicant. Seasonal variation of heavy metals in surface water, soil sediment and, bioaccumulation in whole body tissue and their effect on biochemical constituents are focused in this study. The biochemical components are indicators of pollution, which are useful to determine health and nutritional status of an organism. The results obtained during the present study were clearly revealed progressive depletion in ascorbic acid content along with the increase in exposure period. The obtained results are in harmony with the results reported by various researchers Hargrave *et al.* 2000 and Lee *et al.* 2000, Boran & Altinok 2010, Shariati *et al.* 2011). From the above results, in present study it can be stated that ascorbic acid act as detoxifying agent which results in the excretion of metals might have occurred. Because of this, the level of ascorbic acid in different seasons and metal concentrations might have decreased from all the metals and during all seasons. We can further stated from our finding that, due to metal toxicity in bivalves needs more energy, hence most of the tissue glycogen might have utilized for the energy purpose (glycogen content decreased from all the seasons in all heavy metals) Therefore, there may be reduced synthesis of ascorbic acid since, the production needs glucose molecule. Similar results were observed by Buttner and Jurkiewicz (1996) further they stated that ascorbic acid is an excellent reducing agent, act as a detoxifier and reduce the toxicity of heavy metal, serves as donor antioxidant in free radical mediated oxidation and reduces the metal toxicity

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Table 1:- Changes in ascorbic acid levels in different species of freshwater bivalves in different seasons during acute toxicity to heavy metals from Manjara dam

Species	Control group	Metals values	MONSOON				WINTER				SUMMER			
			Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride	Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride	Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride
<i>Lamellidens marginallis</i>	2.05 ±0.22	LC ₀	1.87 ±0.13	1.93 ±0.16 (3.21%)	1.91 ±0.10 (2.14%)	1.77 ±0.08 (5.35%)	2.01 ±0.15 (7.49%)	2.02 ±0.10 (0.50%) (4.67%)	2.00 ±0.10 (0.50%) (4.72%)	1.94 ±0.08 (3.49%)* (9.61%)	1.06 ±0.10 (43.32%)	1.28 ±0.09 (20.76%)* (33.68%)	1.20 ±0.18 (13.21%) (37.18%)	1.25 ±0.13 (17.93%) (29.38%)
		LC ₅₀	1.91 ±0.12	1.95 ±0.35 (210%)	1.85 ±0.35 (3.15%)	1.81 ±0.17 (5.24%)	2.07 ±0.13 (8.38%)	2.13 ±0.35 (2.80%) (9.24%)	2.03 ±0.4 (1.94%) (9.73%)	1.99 ±0.17 (3.87%) (9.95%)	1.32 ±0.35 (44.51%)	1.30 ±0.27 (1.52%) (33.34%)	1.06 ±0.17 (19.70%) (42.71%)	1.20 ±0.38 (9.10%) (33.72%)
<i>Lamellidens corrianus</i>	1.85 ±0.11	LC ₀	1.65 ±0.41	1.61 ±0.16 (2.43%)	1.51 ±0.17 (8.49%)	1.29 ±0.16 (21.82%)	1.73 ±0.18 (4.85%)	1.75 ±0.09 (1.16%) (8.70%)	1.61 ±0.15 (6.94%) (6.63%)	1.76 ±0.18 (1.74%) (36.44%)	1.02 ±0.22 (38.19%)	0.90 ±0.15 (11.77%) (44.10%)	0.85 ±0.28 (16.67%) (43.71%)	0.67 ±0.21 (34.32%) (48.07%)
		LC ₅₀	1.85 ±0.19	1.77 ±0.13 (4.33%)	1.63 ±0.09 (11.90%)	1.60 ±0.19 (13.52%)	1.69 ±0.18 (8.65%)	1.83 ±0.17 (8.29%) (3.39%)	1.78 ±0.19 (5.33%) (9.21%)	1.87 ±0.17 (10.66%) (16.88%)	0.88 ±0.19 (52.44%)	0.68 ±0.20 (22.73%) (61.59%)	0.60 ±0.12 (31.82%) (63.20%)	0.56 ±0.11 (36.37%) (65.09%)

(Bracket values represent percentage differences) (*, □, Δ- P < 0.05, **, □□, ΔΔ-P < 0.01, ***, □□□, ΔΔΔ- P < 0.001, *- compared to zinc, □- compared to monsoon, Δ- compared to winter of respective metal groups)