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Glycogen levels in fresh water bivalves (*Lamellidens corrianus* and *Indonaea caerulus*) due to influence of heavy metals

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ABSTRACT
The freshwater bivalves L. corrianus and I. caerulus were exposed to LC_0 and LC_{50} values of 96 hrs. with concentrations of 165.01 ppm for zinc chloride, 130.20 ppm for copper sulphate, 95.05 ppm for cadmium chloride and 0.761 ppm for mercuric chloride. The L. corrianus in monsoon the glycogen content in lower concentration showed more amounts in mercury followed by cadmium zinc and copper. When these contents compared with zinc it was more in mercury than copper and cadmium. In higher concentration mussels the more amounts in zinc followed by copper, mercury and cadmium. In winter season the lower concentration bivalves showed more amount in cadmium followed by copper mercury and zinc. In higher concentration mussels the more amounts in zinc followed by mercury, cadmium and copper. In summer season the lower concentration bivalves showed more amount in cadmium followed by mercury, zinc and copper. In higher concentration mussels the more amounts in mercury followed by cadmium, zinc and copper. In Indonaea caerulus monsoon season the glycogen content in lower concentration bivalves showed more amount in zinc followed by copper, mercury and cadmium. When these contents compared with zinc it was more in cadmium, than mercury and copper. In higher concentration mussels the more amounts in zinc followed by copper, mercury and cadmium. In summer season the lower concentration bivalves showed more amount in zinc followed by copper, mercury and cadmium. In higher concentration mussels the more amounts in zinc followed by copper, cadmium and mercury. Further in present study it was observed when the content compared among seasons the glycogen was more decreased in summer followed by monsoon and winter. Amongst the metals some metals showed more toxicity to both species of bivalves hence more glycogen content were decreased and the more toxicity showed by mercury followed by cadmium, copper and zinc. Significant depletion in glycogen level suggests possibility of its rapid utilization to provide excess energy during abnormal conditions developed in the waterborne heavy metals or cellular biochemical process through glycolysis or hypoxic condition might have been prevailed in the bivalve to provide excess energy by its utilization.
Keywords: *Glycogen, heavy metals, L. corrianus and I. caerulus,, acute toxicity, seasons.*

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I. INTRODUCTION

To investigate the physiological changes after heavy metal treatment, points to study the changes in the biochemical constituents like carbohydrate, protein and lipid as they are important metabolites to provide energy for different vital processes. Carbohydrate plays structural role in every living organism and serves as a reservoir of the chemical energy and many decrease or increase according to needs of the organism. The biochemical changes in the organs of animal exposed to heavy metals have no definite pattern and the physiological state of metabolic activity of an organism reflects in the utilization of their biochemical energy to counteract toxic stress. Glycogen is considered to be the major source of energy in animal tissues and maintenance of glycogen reserves is an essential feature of the normal metabolism. The pollutant give the heavy physical irritated stress causing rapid movement and increased respiration rate thus increased the utilization of reserve constituents like lipids and glycogen to meet the high energy demand of body causing decrease in organic constituents content Bhagylakshmi, (1981). The freshwater mussels are an ecologically important fauna because they are used as sensitive biomarkers of aquatic ecosystem pollution. Bivalves are stationary filter-feeding organisms able to bioaccumulation and concentrate most pollutants even if they are present fairly low concentrations (Niyogi *et al.,* 2001). The mode of action of pollutant may be responsible for cellular disorganization offering the storage and metabolism of the organic constituents. Glycogen quickly reacts to changes in the environment (Fisher and Dimock 2006), and it is connected to the nutritional condition, different types of stress, stages of the life cycle, and sexual maturity (de Zwaan and Zandee 1972; Anacleto *et al*. 2013; Cordeiro *et al*. 2017). The evaluation of the condition of macro invertebrates is often determined indirectly from growth and mortality data, but more specific physiological markers can be critical for the early identification of changes in their health status (Fritts *et al*. 2015b). The higher concentrations of toxicants bring the adverse effects on aquatic organisms, at cellular level or molecular level and ultimately lead to disorder in biochemical composition which is useful in determining different toxicants and protective mechanism of the body to resist the toxic effects of the substances. This study addresses freshwater mussels, order Unionida, which are increasingly being propagated in aquaculture facilities for conservation purposes, and there is a need to employ reliable and non invasive methods to assess their energetic status. Many workers evaluated the glycogen content under heavy metal stress conditions (Almeida *et al.*,1999; Osada and Mori 2000, Satyaparameshwar *et al.*,2006). The change in metabolic rate leads towards the change in biochemical composition hence, the change in biochemical composition is an indicator of stress of chemical or physical nature in the surrounding which mainly affects glycogen contents. Chaudhari *et al.*,(2002) observed the effect of heavy metal, on the biochemical component like glycogen of various tissues of freshwater bivalve, Parreysia cylindrical. The significant decreases in total glycogen content of gill, digestive gland were observed due to pollution stress caused by nickel chloride. Ramalingam and Indra, (2002) showed decrease in glycogen level when exposed to copper sulphate toxicity on tissue phosphatases and carbohydrate turned over in Achatina fulcia. Monitoring changes in glycogen levels has been used in various studies dealing with bivalves, for example, to monitor stress (Fritts *et al*. 2015b; Andrade *et al*. 2017) under different conditions such as starvation (Cordeiro *et al*. 2016), transportation (Anacleto *et al*. 2013), in ecotoxicological studies (Hazelton *et al*. 2014). In particular, it would be focus on the link between specific types of tissues and the condition of the individual and its environment during different periods of the year and with respect to environmental conditions. This can help determine the reasons for the variation in the distribution of glycogen in the body so that glycogen evaluations can be put to practical use. Hence the aim of our study to focus on understanding how bivalves *L. corrianus* and *I. caerulus* from Nagapur dam metabolizes and are affected by the wide range of concentration of different heavy metals viz. zinc, copper, cadmium and mercury in aquatic environment.

II. MATERIALS AND METHODS

The animal 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 were selected as per the species abundance and water qualities of dam in different geographic area. The bivalves *L. corrianus* and *I. caerulus* 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 LC_0 and LC_{50} values of 96 hrs with concentrations of 165.01 ppm for zinc chloride, 130.20 ppm for copper sulphate, 95.05 ppm for cadmium chloride and 0.761 ppm for mercuric chloride up to 96 hrs. 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 ^{0}C till constant weights were obtained. The dried product was ground to obtain fine powder. From the replicates of three samples the glycogen was analyzed by anthrone reagent method (Dezwaan and Zandee, 1972). The amount of glycogen was calculated by regression equation and expressed in terms mg/100mg dry powder.

III. RESULTS

The *L. corrianus* and *I. caerulus* were exposed to different heavy metals for 96 hrs using the values of LC_0 and LC_{50}. The control group was runs simultaneously with exposed bivalves and only the mean values expressed in the Table-1. The results were calculated and compared amongst heavy metals and season, in *L. corrianus* showed more amount of protein in LC_0 bivalves than LC_{50} during all seasons and metals also. In monsoon the glycogen content in LC_0 bivalves showed more amount in mercury, (8.07) followed by cadmium (7.96), zinc (7.69) and copper(7.39).When these contents compared with zinc it was more in mercury (4.95%; $P< 0.05$), than copper (3.91%) and cadmium (3.52%). In LC_{50} mussels the more amounts in zinc (10.09) followed by copper (9.94), mercury (9.91) and cadmium (9.72). When compared with the zinc species content it was more in cadmium (3.67%; $P<0.05$) than copper (1.49%; $P<0.01$) and mercury (1.79%). In winter season the LC_0 bivalves showed more amount in cadmium (9.34) followed by copper (8.76), mercury (8.69) and zinc (8.65). When these contents compared with zinc it was more in cadmium (7.98%; $P<0.05$) than copper (1.28%) and mercury (0.47%). In LC_{50} mussels the more amounts in zinc (11.01) followed by mercury (10.48), cadmium (10.40) and copper (10.39). When compared with the zinc species content it was more in copper (5.64%;

P<0.01) than cadmium (5.55%; P<0.05) and mercury (4.82%; P<0.05). On the other hand the content when compared to monsoon season to respective groups the glycogen was increased in all groups and sizes also. In LC_0 mussels the content more increased in copper (18.54%; P<0.01) followed by cadmium (17.34%; P<0.001), zinc (12.49%; P<0.01) and mercury (8.22%; P<0.05). In LC_{50} it was increased in all groups and the increase rate was in zinc (9.12%; P<0.01) followed by cadmium (6.99%; P<0.05), mercury (5.76%; P<0.05) and copper (4.53%; P<0.01). In summer season the LC_0 bivalves showed more amount in cadmium (7.20) followed by mercury (7.0), zinc (6.55) and copper (6.40). When these contents compared with zinc it was more in cadmium (9.93%; P<0.05) than mercury (6.88%) and copper (2.30%). In LC_{50} mussels the more amounts in mercury (6.13) followed by cadmium (6.11), Zinc (5.58) and Copper (4.33). when compared with the zinc species content it was more in copper (23.76%; P<0.001) than mercury (8.81%; P<0.05) and cadmium (7.58%; P<0.05). On the other hand when the content compared with monsoon and winter of respective groups the content showed in LC_0 bivalves more decreased in zinc (14.83%; P<0.01), (24.28%; P<0.001) followed by copper (13.40%; P<0.01), (26.95%; P<0.001), cadmium (9.55%; P<0.01), (22.92%; P<0.001) and mercury (13.26%; P<0.001), (19.45%; P<0.01) respectively. In LC_{50} the decrease rate was in zinc (43.71%; P<0.001), (48.42%; P<0.001) followed by copper (56.44%; P<0.001), (58.33%; P<0.001), cadmium (37.14%; P<0.001), (41.25%; P<0.001) and mercury (37.64%; P<0.001), (41.04%; P<0.001) respectively.

In *I. caerulus* monsoon season the glycogen content in LC_0 bivalves showed more amount in zinc, (7.77) followed by copper (7.75), mercury (7.51) and cadmium (7.31). When these contents compared with zinc it was more in cadmium (5.93%), than mercury (3.35%) and copper (0.26%). In LC_{50} mussels the more amounts in zinc (9.53) followed by copper (9.26), mercury (8.85) and cadmium (8.69). When compared with the zinc species content it was more in cadmium (8.82%; P<0.01) than mercury (7.14%; P<0.01) and copper (2.84%). In winter season the LC_0 bivalves showed more amount in zinc (9.34) followed by cadmium (9.11), copper (9.07) and mercury (8.80). When these contents compared with zinc it was more in mercury (5.79%) than copper (2.90%) and cadmium (2.47%). In LC_{50} mussels the more amounts in zinc (10.71) followed by cadmium (10.52), copper (10.40) and mercury (10.06). When compared with the zinc species content it was more in mercury (6.07%; P<0.05) than copper (2.90%) and cadmium (1.78%). On the other hand the content when compared to monsoon season to respective groups the glycogen was increased in all groups and sizes also. In LC_0 mussels the content more increased in cadmium (24.63%; P<0.01) followed by zinc (20.21%; P<0.01), mercury (17.18%; P<0.01) and copper (17.04%; P<0.01). In LC_{50} it was increased in all groups and the increase rate was in cadmium (21.06%; P<0.001) followed by mercury (13.68%; P<0.001), zinc (12.38%; P<0.01) and copper (12.32%; P<0.001). In summer season the LC_0 bivalves showed more amount in zinc (6.15) followed by copper (5.99), mercury (5.85) and cadmium (5.75). When these contents compared with zinc it was more in cadmium (6.51%) than mercury (4.88%) and copper (2.61%). In LC_{50} mussels the more amounts in zinc (5.91) followed by copper (5.81), cadmium (5.70) and mercury (5.40). When compared with the zinc species content it was more in mercury (8.63%) than cadmium (3.56%) and copper (1.70%). On the other hand when the content compared with monsoon and winter of respective groups the content showed in LC_0 bivalves more decreased in zinc (20.85%; P<0.01), (34.16%; P<0.001) followed by copper (22.71%; P<0.01), (33.96%; P<0.001), cadmium (21.35%; P<0.001), (36.89%; P<0.001) and mercury (22.11%; P<0.01), (33.53%; P<0.001) respectively. In LC_{50} the decrease rate was in zinc (37.99%; P<0.001), (44.82%; P<0.001) followed by copper (37.26%; P<0.001), (44.14%; P<0.001), cadmium (34.41%; P<0.01), (45.82%; P<0.001) and mercury (38.99%; P<0.001), (46.33%; P<0.001) respectively.

IV. DISCUSSION

In present study the *L. corrianus* in monsoon the glycogen content in LC_0 bivalves showed more amount in mercury followed by cadmium zinc and copper. When these contents compared with zinc it was more in mercury than copper and cadmium. In LC_{50} mussels the more amounts in zinc followed by copper, mercury and cadmium. When compared with the zinc species content it was more in cadmium than copper and mercury. In winter season the LC_0 bivalves showed more amount in cadmium followed by copper mercury and zinc. In LC_{50} mussels the more amounts in zinc followed by mercury, cadmium and copper. On the other hand the content when compared to monsoon season to respective groups the glycogen was increased in all metal groups. In LC_0 mussels the content more increased in copper followed by cadmium, zinc and mercury. In LC_{50} it was increased in all groups and the increase rate was in zinc followed by cadmium, mercury and copper. In summer season the LC_0 bivalves showed more amount in cadmium followed by mercury, zinc and copper. In LC_{50} mussels the more amounts in mercury followed by cadmium, zinc and copper. On the other hand when the content compared with monsoon and winter of respective groups the content showed in LC_0 bivalves more decreased in zinc followed by copper, cadmium and mercury respectively. In LC_{50} the decrease rate was in zinc followed by copper, cadmium and mercury respectively. At the later stages, changes may occur at higher biological levels and they affect the ability of organisms to grow, to reproduce or to survive. Significant depletion in glycogen level suggests possibility of its rapid utilization to provide excess energy for cellular biochemical process through glycolysis. This study addresses freshwater mussels, order Unionida, which are increasingly being propagated in aquaculture facilities for conservation purposes, and there is a need to employ reliable and non invasive methods to assess their energetic status. Many workers evaluated the glycogen content under heavy metal stress conditions (Almeida *et al.*,1999; Osada and Mori 2000, Satyaparameshwar *et al.*,2006. The greater breakdown of glycogen may suggest the need of high energy to animal in stress conditions caused due to pollutants. Depletion in glycogen level might be because of the anoxia and hypoxia caused due to stress conditions which are known to increase carbohydrate consumption (Dezwan and Zandee, 1972). According to Koundinya and Ramamurthi (1979), the decrease in glycogen may be due to enhanced breakdown of glycogen to glucose through glycolysis. The greater decrease in the glycogen level, in the digestive gland might be due to high potential of digestive gland for glycolysis, similar to that of the vertebrate liver as suggested by Kabeer *et al.*, (1977).The mode of action of pollutants may be responsible for cellular dis-organization offering the storage and metabolism of the glycogen. Glycogen, the primary energy reserve in bivalves, drives many important physiological processes and could be used to ensure short-term exposure to anoxia, emersion and reduced food supplies. The gradual increased content of glycogen from summer onwards could be due to the development of the gonads Shettigar and Seetharamaiah, (2013).). Hypoxic condition might have been prevailed in the bivalve to provide excess energy by its utilization. Depletion in glycogen levels in the present study might be attributed to hypoxic conditions under heavy metals, our results are in agreement with Kharat, (2007). The decrease in glycogen content form the whole body of the bivalve, *L. corrianus* suggests the possibility of the glycogenolysis which in turn produce energy to cope up the adverse stress conditions. Kabeer (1979) stated that decrease in glycogen content in Malathion exposed tissues can also be due to decrease in glycogen synthesis. Gabbott, and Bayne, (1973), have shown that seasonal variations in biochemical composition of molluscs depend on environmental parameters such as temperature and available phytoplankton and factors such as timing of the reproductive cycle and the rate of turnover of stored energy.).In present study the results showed during summer season caused some how different trend was observed, revealing different type of substrate utilization to meet the energy demand. Glycogen, the primary energy reserve in bivalves, drives many important physiological processes and could be used to ensure short-term exposure to anoxia, emersion and reduced food supplies. The gradual increased content of glycogen from March onwards could be due to the development of the gonads Shettigar *et al.,* (2013), an intimate association of glycogen with the period of sexual activity was also observed in *Corbicula sp*. Greseth *et al*., (2003) & Baby *et al.*, (2010) observed that the glycogen content in *Lamellidens margianalis* was 4.94%. Nagabhushan and Lomte (1971) in *P. corrugata* observed that the glycogen content varied from 4.57 to 5.73% of dry weight in various size groups.

On the other hand the content when compared to monsoon season to respective groups the glycogen was increased in all groups and sizes also. In LC_0 mussels the content more increased in cadmium followed by zinc, mercury and copper. In LC_{50} it was increased in all groups and the increase rate was in cadmium followed by mercury, zinc and copper. In summer season the LC_0 bivalves showed more amount in zinc followed by copper, mercury and cadmium. When these contents compared with zinc it was more in cadmium than mercury and copper. In LC_{50} mussels the more amounts in zinc followed by copper, cadmium and mercury. When compared with the zinc species content it was more in mercury than cadmium and copper. On the other hand when the content compared with monsoon and winter of respective groups the content showed in LC_0 bivalves more decreased in zinc followed by copper, cadmium, and mercury respectively. In LC_{50} the decrease rate was in zinc followed by copper, cadmium and mercury respectively. In present study the *I. caerulus* monsoon season the glycogen content in LC_0 bivalves showed more amount in zinc followed by copper, mercury and cadmium. When these contents compared with zinc it was more in cadmium, than mercury and copper. In LC_{50} mussels the more amounts in zinc followed by copper, mercury and cadmium. When compared with the zinc species content it was more in cadmium than mercury and copper. In winter season the LC_0 bivalves showed more amount in zinc followed by cadmium, copper and mercury. When these contents compared with zinc it was more in mercury than copper and cadmium. In LC_{50} mussels the more amounts in zinc followed by cadmium, copper and mercury. When compared with the zinc species content it was more in mercury than copper and cadmium. They further observed that the variation in the glycogen content was not size dependent. Histochemical preparation showed that maximum glycogen concentration was noticed in the mantle, muscles, gills, gonads and digestive diverticula of mussels. Another possibility of decrease in organic constituents showed in gill, might be due to unfavorable conditions generally occurred during summer, such as increase in water and atmospheric temperature, low dissolved oxygen, unavailability of food, and no bloom of phytoplankton. Glycogen storage fluctuates seasonally during ebb periods of gametogenisis and decrease rapidly in response to reduced food availability and environmental stress Patterson *et al.,* (1999).

Hence, the present investigation was undertaken. It is need of further investigation to evaluate the extent of toxic effect of heavy metals to focus the degree of bioaccumulation and bio assessment of glycogen in tissues of freshwater bivalve, *L. corrianus*. Decrease in glycogen content indicates disrupted carbohydrate metabolism. The pollutants give the heavy physical irritate stress causing rapid movement and increased respiration rate thus increased utilization of reserved glycogen to meet higher energy demand of body causing decrease in glycogen content (Bhagyalaxmi, 1981) Glycogen is the stored food material in animal tissue which is used as an immediate source of energy when required and is an essential feature of the normal organism metabolism (Thunberg and Manchaster, 1972).

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

Species			MONSOON				WINTER				SUMMER			
	Control group	metals values	Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride	Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride	Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride
Lamellidens corrianus	8.90 ±0.34	LC_0	7.69 ±0.13	7.39 ±0.18 (3.91%)	7.96 ±0.18 (3.52%)	8.07 ±0.07 (4.95%) *	8.65 ±0.24 (12.49%)□ □	8.76 ±0.27 (1.28%) (18.54%)□□	9.34 ±0.18 (7.98%)* (17.34%)□□ □	8.69 ±0.31 (0.47%) (8.22%)□	6.55 ±0.33 (14.83%)□□ (24.28%)ΔΔ Δ	6.40 ±0.18 (2.30%) (13.40%)□□ (26.95%)ΔΔΔ	7.20 ±0.07 (9.93%)* (9.55%)□□ (22.92%)ΔΔΔ	7.00 ±0.13 (6.88%) (13.26%)□□□ (19.45%)ΔΔΔ
		LC_{50}	10.09 ±0.18	9.94 ±0.12 (1.49%) **	9.72 ±0.12 (3.67%)*	9.91 ±0.14 (1.79%)	11.01 ±0.18 (9.12%)□□	10.39 ±0.12 (5.64%)** (4.53%)□□	10.40 ±0.23 (5.55%)* (6.99%)□	10.48 ±0.18 (4.82%)* (5.76%)□	5.68 ±0.14 (43.71%)□□ □ (48.42%)ΔΔ Δ	4.33 ±0.12 (23.76%)*** (56.44%)□□□ (58.33%)ΔΔΔ	6.11 ±0.27 (7.58%)* (37.14%)□□□ (41.25%)ΔΔΔ	6.13 ±0.24 (8.81%)* (37.64%)□□□ (41.04%)ΔΔΔ
Indonaea caerulus	9.65 ±0.29	LC_0	7.77 ±0.35	7.75 ±0.18 (0.26%)	7.31 ±0.07 (5.93%)	7.51 ±0.24 (3.35%)	9.34 ±0.24 (20.21%)□ □	9.07 ±0.22 (2.90%) (17.04%)□□	9.11 ±0.43 (2.47%) (24.63%)□□	8.80 ±0.31 (5.79%) (17.18%)□ □	6.15 ±0.39 (20.85%)□□ (34.16%)ΔΔ Δ	5.99 ±0.16 (2.61%) (22.71%)□□□ (33.96%)ΔΔΔ	5.75 ±0.09 (6.51%) (21.35%)□□□ (36.89%)ΔΔΔ	5.85 ±0.25 (4.88%) (22.11%)□□ (33.53%)ΔΔΔ
		LC_{50}	9.53 ±0.18	9.26 ±0.12 (2.84%)	8.69 ±0.23 (8.82%)* *	8.85 ±0.19 (7.14%) **	10.71 ±0.28 (12.38%)□ □	10.40 ±0.12 (2.90%) (12.32%)□□ □	10.52 ±0.07 (1.78%) (21.06%)□□ □	10.06 ±0.12 (6.07%)* (13.68%)□ □□	5.91 ±0.51 (37.99%)□□ □ (44.82%)ΔΔ Δ	5.81 ±0.67 (1.70%) (37.26%)□□□ (44.14%)ΔΔΔ	5.70 ±0.81 (3.56%) (34.41%)□□ (45.82%)ΔΔΔ	5.40 ±0.55 (8.63%) (38.99%)□□□ (46.33%)ΔΔΔ

(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)**