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THE EFFECT OF CHANGING ENVIROMENTAL PRAMETERS ON RESPIRATION OF BIVLAVE FROM GODVARI RIVER MAJALGOAN, (M.S.), INDIA.

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ABSTRACT

In the present investigation the effect of rise in temperature and atmospheric air was studied on the respiration physiology of *Lamellideans marginalis* from Godavari River. The studies were conducted in three different seasons on 2nd day, 6th day and 12th day of exposure period. The result indicates that the effect of rise in temperature on control group is decreased on 2nd and12th day in every season in rate of respiration was 11.94 on 2nd day 35.29 on 6thday and 13.98 on 12th day. In summer season the rate of respiration followed similar trend in both control and experimental group but in monsoon season the rate reached to steady level. The effect of exposure to atmospheric air in three different seasons on control and experimental groups shows the rate of respiration decreased on the day in all the season in experimental groups in monsoon season there was very little effects of exposure to atmospheric air on the rate of oxygen consumption and more in summer.

KEY WORDS: Atmospheric air, Godavari river, Lamellideans marginalis Temperature,

INTRODUCTION

The effect of environmental parameters is an important aspect for every animal. The physiological studies in relation to ecology of fresh water bivalves are historical based early descriptive work which includes basic taxonomy with notes on habitat, community composition, abundance and distribution. The effect of environmental temperature on animals has been reviewed by many workers (Precht *et.al.*, 1973 and Kuhhemann and precht1979), Since the changes in the Kinetic energy of the environment are usually rapid and quantitatively transferred to the cellular chemistry of the organism, environmental temperature changes cause unique problems. The rate of respiration in aquatic animals is as influenced by activity, body size, stages in life cycle and time of day, as well as previous oxygen experience and genetic background (Prosser1973).

Rate of respiration reflects the metabolic activities of animals and the responses due to change in surrounding environment are an indicator of adjustment capacity of the organism. Lamellidens marginalis is an economically important bivalve. It circulates large quantity of water through respiratory surface to obtain food and oxygen. Thus, it suffers a great risk of pesticide poisoning. Therefore, it is considered as a bio-monitoring tool in toxicological studies (Rittschof and McClellan-Green, 2005). Bivalve molluscs reflect immediate responses to toxic substances present in surrounding water by change in physiological responses (Basha et al., 1988) and histological arrangement (EI-Shenawy et al., 2009; Kumar et al., 2011; Kamble et al., 2012). The abundant found of freshwater mussels, Lamellidens marginals along the bank of Godavari river near Majalgaon, Dist. Beed Maharashtra the present study has planned to find out the effects of some environmental parameters on respiration physiology in three different seasons.

MATERIALS AND METHODS

The freshwater bivalve molluscs *Lamellidens marginalis* were collected from bank of Godavari River, Majalgoan Dist. Beed. After collection of these bivalves, they were immediately brought to the laboratory. The freshly samples as collected during summer (April-may) monsoon (July-August) and winter (December-January), in the year 2019-20, before conducting the experiments studying the environmental conditions like rise in temperature and exposure to atmospheric air.

After 24 hour laboratory adjustment 50 animals were kept in each of the 2 aquaria during summer, monsoon and winter to study the effect of normal laboratory temperature and others were experimental at different temperatures of the water from control group was 29.5°C in summer, 24.5-25°C in monsoon and 20.5-22 °C in winter. The experimental



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temperature of water during summer was 32°C, during monsoon it was 30°C were as during winter the experimental temperatures were 28°C. The temperatures of experimental groups were controlled by using thermostats. In each experimental aquarium care was taken to keep the animal away from the thermostat. The water of appropriate temperatures and of controls was renewed every 12-13 h run for 12 days in each season.

The rate of the respiration of the animals from the control and experimental group described above, while studying the effects of rise 6th day and 12th day (except of atmospheric exposure experiment on 12th day). The technique used for the rate of respiration (measurement of oxygen content from freshwater was similar to that described by Golterman et al. (1978), APHA (1985) All the values obtained for the atmospheric air were statistically analysed for confirmation of significance by using student "t"test (Dowdeswell, 1957).

RESULTS AND DISCUSSION

The effect of rise in temperature and effect of exposure to atmospheric air on oxygen consumption of *Lamellidens* marginalis in different season different seasons in table 1 and 2.

Oxygen concentration is also influenced by temperature, firstly oxygen solubility decreases as temperature rises, secondly, organic pollution (for example, domestic waste water), which produces a biological oxygen demand, will have this demand intensified by temperature change on chemical toxicity in part may be mediated through the arability of dissolved oxygen to aquatic organisms. Many substance are more toxic when dissolved oxygen to the aquatic organisms (Caimes *et al*, 1975). The ability of water to retain dissolved gas is greater and oxygen depleting waste, there can be a significant increase in the effective toxicity due to the reduced oxygen content.

Table 1. Effect of rise in temperature on oxygen consumption of Lamelladens marginalis in different seasons

Day			summer		monsoon			
	control	28°C	control	28°C	control	28°C		
2 nd	02444+_0.018	+_2624+_0.0125 (11.94)	0.1513+_0.05	0.2821+_0.0413 (86.45)	0.2317+_0.0121	+_0.1831+_0.0138(20.98)		
6 Th	0.1564+_0.0121	0.1012+_0.0221(35.29)	0.1917+_0.0190	0.2112+_0.01212(10.17)	0.1619+_0.0218	0.1843+_0.02141913.83)		
12 th	0.1423+_0.01712	0.1622+_0.01712	0.1448+_0.0121	0.1612+_0.082(11.32)	0.1422+_0.013	0.1822+_0.062(28.13)		

All values are expressed in mg/100gm.

Table 2. Effect of exposure to atmospheric air on oxygen consumption Lamelladens marginalis in different seasons.

Day 2 nd	Winter		summer		monsoon			
	Control	Atmospheric exposure	Control	Atmospheric exposure	Control	Atmospheric exposure		
	0.2330+_0.0421	+_0.2413 +_0.01212 (1.125	0.1991+_0.0313	0.2484+_0.0312(29.16)	0.22181+_0.01318	+_21589=-0.204(1.05)		
6 Th	01920+_0.01358	+_02013+_0.01358(484)	0.1741+_0.02858	0.1814+_0.1318(419)	+ 0.2018+ 0.01813	0.1921+ 0.01213(480)		

All values are expressed in mg/100gm, Bracket values represents percentage differential (Compared to control).

In the present investigation the effect of rise in temperature on oxygen consumption *Lamellidens marginalis* in different season on different day's of exposure period in control groups on 2nd day the ate of respiration in winter was 0.2344+_0.018 mg/l/h, where as in summer season on 6th day ate of respiration in winter was 0.1564+_0.0121 mg/i/h. where as in summer it was 0.1917+_0.0190mg/i/h and in monsoon 0.1619+_0.0218mg/l/h on 12th day the rate of respiration in winter was 0.1423+_0.01712mg/l/h where as in summer it was 0.1448+_0.01211 mg/l/h and in monsoon 0.1422+_0.0131 mg/l/h. The study revealed that the rate of respiration decreased through 2nd and 12th day in every season in summer and monsoon after 6th days it show decrease after 12th days compared to 2nd day in winter season the rate of respiration was 0.2624+_0.125 mg/l/h atwas 28 °C. Thus the study revealed that at 28 °C the percentage decrease in rate of respiration was 11.94 on 2nd day35.29 on 6th day and 13.98on 12th day. In summer the rate of respiration at 32°C was 0.2821+_0.041 mg/l/h and on 12th day it was 0.1621+_0.082 mg/l/h. Thus, the percentage decrease in rate of respiration was 86.45 on 2nd day 10.17 on 6th day and 11.32 on 12th day it significantly increased on 2nd and 12th at 32°C compared to control. In monsoon the rate of respiration 30°C was 0.1831+_0.0138



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mg/l/h on 2nd day $0.1843 + _0.02141$ mg/i/h on 6^{th} day and $0.1822 + _0.0962$ mg/l/h 12th days. This revealed that the rate of respiration increased on non-significantly on 2^{nd} and 6^{th} day significantly increased on 12th day. Comparing the rate of respiration controls in winter and monsoon it is seen that the rate respiration increased on 12th day. Comparing the rate of respiration at 28°C with the respective controls in winter and in winter and it sharply increased from 6th to 12th day. The present investigations the effect of exposure to atmospheric air on oxygen consumption of L. marginalis in different season on different days of exposure period in control groups.

In the recent estimation the effect of exposure to atmospheric air on oxygen consumption of L. marginalis in different season on different days of exposure period in control groups .on 2nd day was 0.23358+_0.0421mg/l/h and in monsoon 0.2181+ 0.01813 mg/l/h in monsoon. Thus the study revealed that the rate of respiration deceased on 6th day in all the seasons. In winter the rate of respiration was 0.2413 + 0.0212 mg/l/h on 2^{nd} day and 0.2013 + 0.01358 mg/l/h on 2^{nd} day and 0.1814 + 0.01318 mg/l/h, on 6^{th} day. In monsoon it was 0.21589 + 0.0204 mg/l/h on 2^{nd} day and 0.1921 + 0.0213 mg/l/h on 6th day. Thus, the study revealed that in all the season the rate of respiration increased nonsignificantly by 1.025% and 4.847% in winter 29.06% increased non-significantly by 1.025% and 4.847 in winter 29.96% and 4.18% in summer and 1.05 and 4.80 in monsoon after 2 and 6 day respectively. It is seen that in monsoon after 2 and 6 days respectively It is seen that in monsoon there was very little effect of exposure to atmospheric air on the rate of oxygen consumption and more in summer similar observation were made by Muley, 1988, Lioydspeek et al (2002) show that after elevation in temperature in Antarctic bivalve at ernulaelliptica and they stated that maximum circulating haemolymph oxygen content fell. Brace (1926) worked on the respiratory exchange of mussel, Mytilus edulis and report that the seasonal pattern of respiration with high values in winter and spring, and low values is summer he concluded that the increasing populations of gonad material in the body through the rate summer and autumn increased oxygen demand of the mussels. Bruce (1926) estimated that in the natural populations temperature would be more important in controlling rate of respiration their innate physiological changes. In the present study the rise in the temperature in summer months as well as at 32°C under experimental conditions resulted in the increase in the rate of respiration which is probably due to the maintain of the body metabolism Bayne (1976).

It is also probable that the increase in the rate of respiration is accounted due to the increases in the day length suggested by Chancel et.al (1979) similar results were observed by Muley 1988. Ansell (1969), Davies (1966 – 1967), Stickle (1973), Akarte (1985), Kulkarni (1987), Rao (1988), worked on freshwater bivalve mollusc and stated that the high temperature and lower nutritional conditions during summer months can significantly bring about alternation in the rate of oxygen consumption in the present study due to exposure also the animals showed increase in the rate of respiration L. manganilis shares rise in temperature using different season was kept in such a way that it was of the maximum during the given period of particular season that means in summer the experimental temperature was raise to 32°C, in monsoon to 30°C and in the view of irregular flow of water on the animal habitat during different season due to dam construction. The rate of respiration was measured during the rise in temperature and changes on 2^{nd} , 6th and 12^{th} day while during exposure to atmospheric air; it was measured on 2^{nd} and 6^{th} day. The rate of respiration from control group showed increased the rate during summer monsoon and winter. But the rate was increased more in summer than monsoon and winter. In all season the rate tended to decrease as the exposure period increased rise in temperature resulted in increase in the respiration in all the seasons, particularly in summer experiment of exposure to atmospheric air caused increased the rate of respiration.

AKNOWLEDGMENT

Authors wish to acknowledge the Principal and Department of Zoology, Sunderrao Solanke Mahavidyalaya, Majalgaon Dist. Beed (M.S), India.

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INFLUENCE OF HEAVY METALS UPON EFFICIENCY OF HYDROLYSIS OF GLYCOGEN IN DIFFERENT SPECIES OF FRESHWATER BIVALVES

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ABSTRACT

The freshwater Parreysia corrugata and Indonaea caeruleus were exposed to LC₀ and LC₅₀ 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 P. corrugata in monsoon the glycogen in lower concentration showed more amount in mercury, followed by copper, cadmium and zinc. In higher concentration the more amounts in mercury followed by cadmium, copper and zinc. In winter the lower concentration showed more amount in mercury followed by zinc, copper and cadmium. In higher concentration the more amounts in mercury followed by cadmium, copper and zinc. In summer the lower concentration showed more amount in cadmium followed by mercury, copper and zinc. In higher concentration the more amounts in cadmium followed by mercury, copper and zinc. In I caeruleus in monsoon the content in lower concentration showed more amount in mercury, followed by copper, cadmium and zinc. In higher concentration the more amounts in mercury followed by copper, cadmium and zinc. In winter the lower concentration showed more amount in mercury followed by copper, cadmium and zinc. In higher concentration the more amounts in mercury followed by cadmium, copper and zinc. In summer the lower concentration showed more amount in copper followed by cadmium, zinc and mercury. In higher concentration the more amounts in mercury followed by copper, zinc and cadmium. While, when compared with monsoon and winter of respective metal groups showed in lower concentration more decreased in cadmium, followed by zinc, copper and mercury respectively. In higher concentration the decrease rate was in cadmium followed by zinc, copper and mercury respectively.

KEY WORDS: Glycogen, heavy metals, P. corrugata, I caeruleus acute toxicity.

INTRODUCTION

To investigate the physiological changes after heavy metal treatment, to study the changes in the biochemical constituents i. e. carbohydrate as it is 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. 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 organismal 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 are an ecologically important fauna because they are used as sensitive biomarkers of aquatic ecosystem pollution. are stationary filter-feeding organisms able to bioaccumulate 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).

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*. Chaudhari *et al.*,(2002) observed the effect of heavy metal, on the biochemical component like glycogen of various tissues of freshwater bivalve *Parreysia cylindrica*. Monitoring changes in glycogen levels has been used in various studies dealing with for example to monitor stress (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). 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. The higher concentrations of toxicants bring the



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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 bivalves 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 (Osada and Mori 2000, Satyaparameshwar *et al.*, 2006, Suryawanshi and Shaikh, 2012). Nevertheless, pelecypod molluscs undergo modifications in their functional response as well as biochemical characteristics of tissue in response to ambient pollutant level. Among freshwater mussels, are most commonly available and distributed all over the country, forming important species for pearl culture operation in India (Surajit Das 2005, Shaikh, 2011, Suryawanshi and Sirsat 2018). Hence, the aim of our study to focus on understanding how *Parreysia corrugata* and *Indonaea caeruleus* from Manjara dam metabolizes and are affected by the wide range of concentration of different heavy metals viz. zinc, copper, cadmium and mercury in aquatic environment.

MATERIALS AND METHODS

The bivalves were collected from Manjara dam near Kallamb city, District Osmanabad, Maharashtra, India during the year 2018-19. 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 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 Parreysia corrugata and Indonaea caeruleus were collected for laboratory experiments from study area during monsoon, winter and summer. 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 health 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 P. corrugata and I. caeruleus were exposed to LC0 and LC50 values of 96 hrs with concentrations of 105.01 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. They 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 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 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.

RESULTS

In present study the P. corrugata and I. caeruleus were exposed to different heavy metals for 96 hrs using the values of LC₀ and LC₅₀. 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 in P. corrugata showed more amount of protein in LC₀ bivalves than LC₅₀. In monsoon the glycogen content in LC₀ showed more amount in mercury (8.03) followed by copper (7.55), cadmium (7.39) and zinc (7.01). When these contents compared with zinc it was more in mercury (14.56%), than cadmium (5.43%) and copper (1.71%). In LC₅₀ the more amounts in mercury (7.32) followed by cadmium (7.28), copper (6.56) and zinc (6.09). When compared with the zinc it was more in mercury and copper (5.48%) than cadmium (4.90%). In winter the LC₀ showed more amount in mercury (8.30) followed by zinc (8.14), copper (7.85) and cadmium (7.51). When compared with zinc it was more in cadmium (7.74%; P<0.01) followed copper (3.57%; P<0.01) and mercury (1.97%). In LC₅₀ the more amounts in mercury (7.39) followed by cadmium (6.97), copper (6.86) and zinc (6.21). When compared with the zinc species content it was more in mercury (19.01%) than cadmium (12.24%) and copper (10.47%). Furtherwhen compared to monsoon to respective groups the glycogen was increased in all groups and s. In LC₀ more increased in zinc (16.12%) followed by copper (3.98%), mercury (3.67%) and cadmium (1.63%). In LC₅₀ it was increased in all metal groups and the increase rate was in zinc (10.52%) followed by copper (4.58%), cadmium (4.20%) and mercury (0.96%). In summer the LC₀ showed more amount in cadmium (7.01) followed by mercury (6.94), copper (6.36) and zinc (5.87). When these contents compared with zinc it was more in cadmium (19.43%) than mercury (18.23%) and copper (8.37%). In LC₅₀ the more amounts in cadmium (6.52) followed by mercury (6.25), copper (5.30) and zinc (4.96). When compared with the zinc species content it was more in cadmium (31.46%) than mercury (26.01%) and copper (6.86%). Furtherwhen compared



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with monsoon and winter of respective metal groups showed in LC₀ more decreased in zinc (16.27%; P<0.05), (27.89%; P<0.001) followed by copper (15.77%; P<0.001), (18.98%; P<0.001), mercury (13.58%; P<0.01), (16.39%; P<0.01) and cadmium (5.45%), (6.66%; P<0.05) respectively. In LC₅₀ the decrease rate was in zinc (28.54%; P<0.05), (20.13%; P<0.001) followed by copper (19.21%; P<0.001), (22.75%; P<0.001), mercury (14.62%; P<0.001), (15.43%; P<0.001) and cadmium (10.44%), (6.46%; P<0.05) respectively.

Table 1:- Changes in glycogen in different species of freshwater bivalves in different seasons during acute toxicity

				MON	SOON			WIN	TER		SUMMER				
Species Course Code Manual The Course Code Manual The Course Code in															
	Contr ol group	metalsval ues	Zinc chlori de	Copper chloride	Cadmiu m chloride	Mercuri c chloride	Zinc chloride	Copper chloride	Cadmium chloride	Mercuri c chloride	Zinc chloride	Copper chloride	Cadmium chloride	Mercuric chloride	
Parreys ia corruga ta	8.20 ±0.1 6	LC ₄	7.01 ±0.34	7.55 ±0.19 (1.71%	7.39 ±0.18 (5.43 %)	8.03 ±0.24 (14.56 %)	8.14 ±0.14 (16.12%)	7.85 ±0.14 (3.57%)	7.51 ±0.07 (7.74%) ** (1.63%)	8.30 ±0.24 (1.97%) (3.67%	5.87 ±0.24 (16.27%)a (27.89%)\(\Delta\)	6.36 ±0.14 (8.37%) (15.77%)□ (18.98%)△	7.01 ±0.37 (19.43%) (5.45%) (6.66%)Δ	6.94 ±0.27 (18.23%) (13.58%)□ (16.39%)∆	
		LC ₅₀	6.09 ±0.66	6.56 ±0.14 (5.48%	7.28 ±0.07 (4.90 %)	7.32 ±0.12 (5.48%	6.21 ±0.07 (10.52%)	6.86 ±0.20 (10.47%) (4.58%)	6.97 ±0.12 (12.24%) (4.26%)	7.39 ±0.07 (19.01 %) (0.96%	4.96 ±0.07 (28.54%)α (20.13%)Δ ΔΔ	5.30 ±0.18 (6.86%) (19.21%)□ □□ (22.75%)Δ	6.52 ±0.70 (31.46%) (10.44%) (6.46%)∆	6.25 ±0.18 (26.01%) (14.62%)□ (15.43%)Δ	
Indona ea caerule us	8.12 ±0.1 3	LC,	7.05 ±0.02	7.62 ±0.14 (08.09 %)	7.39 ±0.07 (4.83 %)	7.80 ±0.35 (10.64 %)	7.24 ±0.14 (2.69%)	8.08 ±0.14 (11.61%) (5.52%)	7.73 ±0.07 (6.77%) (4.61%)	8.27 ±0.35 (14.23 %) (6.03%	6.25 ±0.18 (11.35%)□ □ (13.68%)∆	7.16 ±0.24 (14.56%) (5.04%)□ (11.39%)△ △	6.32 ±0.18 (1.12%) (14.48%)□ □□ (18.25%)△	5.85 ±0.25 (17.76%) (5.65%)© (11.01%)∆ ΔΔ	
		LC56	6.21 ±0.07	6.97 ±0.18 (12.21 %)	6.75 ±0.13 (0.65 %)	7.16 ±0.07 (15.30 %)	6.44 ±0.07 (3.71%)	7.04 ±0.18 (9.32%) (1.01%)	7.19 ±0.12 (11.65%) (6.52%)	7.62 ±0.07 (18.33 %) (6.43%	5.68 ±0.14 (8.54%)□□ (11.81%)Δ ΔΔ	6.40 ±0.19 (12.68%) (8.18%)□ (9.09%)△	4.98 ±0.27 (12.33%)* (26.23%)□ (30.74%)△	6.59 ±0.07 (16.03%) (7.97%)00 (13.52%)4	

(Bracket values represent percentage differences) (*, \Box , \triangle -P < 0.05, **, \Box D, \triangle -P < 0.01, ***, \Box D, \triangle A-P < 0.001, **- compared to zinc, \Box - compared to monsoon, \triangle - compared to winter of respective metal groups)

Further the I caeruleus in monsoon the glycogen content in LC₀ showed more amount in mercury, (7.80) followed by copper (7.62), cadmium (7.39) and zinc (7.05). When these contents compared with zinc it was more in mercury (10.64%), than copper (8.09%) and cadmium (4.83%). In LC₅₀ the more amounts in mercury (7.16) followed by copper (6.97), cadmium (6.75) and zinc (6.21). When compared with the zinc species content it was more in mercury (15.30%) than copper (12.21%) and cadmium (0.65%). In winter the LC₀ showed more amount in mercury (8.27) followed by copper (8.08), cadmium (7.73) and zinc (7.24). When these contents compared with zinc it was more in mercury (14.23%) than copper (11.61%) and cadmium (6.77%). In LC₅₀ the more amounts in mercury (7.72) followed by cadmium (7.19), copper (7.04) and zinc (6.44). When compared with the zinc species content it was more in mercury (18.33%) than cadmium (11.65%) and copper (9.32%). Furtherwhen compared to monsoon to respective metal groups the glycogen was increased in all metal groups and s. In LCo more increased in mercury (6.03%) followed by copper (5.52%), cadmium (4.61%; P<0.05) and zinc (2.69%). In LC₅₀ it was increased in all metal groups and the increase rate was in cadmium (6.52%) followed by mercury (6.43%), zinc (3.71%) and copper (1.01%). In summer the LC₀ showed more amount in copper (7.16) followed by cadmium (6.32), zinc (6.25) and mercury (5.85). When these contents compared with zinc it was more in mercury (17.76%) than copper (14.56%) and cadmium (1.12%). In LC₅₀ the more amounts in mercury (6.59) followed by copper (6.40), zinc (5.68) and cadmium (4.98). When compared with the zinc species content it was more in mercury (16.03%) than copper (12.68%) and cadmium



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(12.33%). Furtherwhen compared with monsoon and winter of respective metal groups showed in LC_0 more decreased in cadmium (14.48%, P<0.001), (18.25%, P<0.001) followed by zinc (11.35%, P<0.01), (13.68%; P<0.01), copper (6.04%; P<0.05), (11.39%; P<0.01) and mercury (5.65%; P<0.01), (11.01%; P<0.001) respectively. In LC_{50} the decrease rate was in cadmium (26.23%; P<0.01), (30.74%; P<0.001) followed by zinc (8.54%; P<0.01), (11.81%; P<0.001), copper (8.18%; P<0.05), (9.09%; P<0.05) and mercury (7.97%; P<0.001), (13.52%; P<0.001) respectively.

DISCUSSION

A tissue-specific glycogen evaluation can provide more detailed data for the monitoring the health and condition of and can provide new valuable information for future sampling, where more than one type of tissue for the glycogen analysis can be quantified. In the present study results clearly indicate that biochemical constituent. In present study glycogen in the body of experimental bivalves P. corrugata and I caeruleus decreases significantly as the period of acute concentration of increases. In P. corrugata in monsoon the glycogen content in lower concentration showed more amount in mercury, followed by copper, cadmium and zinc. In higher concentration the more amounts in mercury followed by cadmium, copper and zinc. In winter the lower concentration showed more amount in mercury followed by zinc, copper and cadmium. In higher concentration the more amounts in mercury followed by cadmium, copper and zinc. In summer the lower concentration showed more amount in cadmium followed by mercury, copper and zinc. In higher concentration the more amounts in cadmium followed by mercury, copper and zinc. While, when compared with monsoon and winter of respective metal groups showed in lower concentration more decreased in zinc followed by copper, mercury and cadmium respectively. In higher concentration the decrease rate was in zinc followed by copper, mercury and cadmium respectively. Significant depletion in glycogen level suggests possibility of its rapid utilization to provide excess energy for cellular biochemical process through glycolysis. 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 Sujatha et al.,(1996). Gabbott, and Bayne, (1973), have shownthat all 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.

The greater breakdown of glycogen may suggest the need of high energy to animal in stress conditions caused due to pollutants. 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. Further, in present study the I caeruleus in monsoon the glycogen content in lower concentration showed more amount in mercury, followed by copper, cadmium and zinc. In higher concentration the more amounts in mercury followed by copper, cadmium and zinc. In winter the lower concentration showed more amount in mercury followed by copper, cadmium and zinc. In higher concentration the more amounts in mercury followed by cadmium, copper and zinc. In summer the lower concentration showed more amount in copper followed by cadmium, zinc and mercury. In higher concentration the more amounts in mercury followed by copper, zinc and cadmium. While, when compared with monsoon and winter of respective metal groups showed in lower concentration more decreased in cadmium, followed by zinc, copper and mercury respectively. In higher concentration the decrease rate was in cadmium followed by zinc), copper and mercury respectively. The present results are corobates with Mandal and Ghose (1970) observed glycogen depletion in the body of the snail, Achatina fulica (Bawdich) when exposed to calcium arsenate. 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).

The depletion of glycogen content was greater in the digestive gland as compared to the foot and mantle of the bivalve, when exposed to pollutants. This indicates that the digestive gland is the principal metabolic center for various metabolic functions. 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) and Baby et al., (2010) observed that the glycogen content in Lamellidens margianalis was increased during winter. The amount of glycogen present in different tissues is closely



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linked with food availability and gonadal development. Glycogen is found maximum in gonad during winter season, which shows utilized as reserve material during unavailability of food, to increase inflow and turbidity of water and to cope up with new environmental change. Similar results are observed by Pandit (2005) in L. marginalis of Godavari River. Significant reductions in bivalves glycogen greatly reduce their ability to cope with natural stressors present in the new environment. Glycogen storage fluctuates ally during ebb periods of gametogenisis and decrease rapidly in response to reduced food availability and environmental stress Patterson et al., (1997, 1999). Further, in present study the results showed during winter caused some how different trend was observed, revealing different type of substrate utilization to meet the energy demand. The study showed maximal reduction of glycogen in body of the both bivalves mollusc may be the result of heavy metal stress induced hypoxia reflecting the elevation of glutamate dehydrogenase activity in body parts for mobilization of glucose from tissue glycogen to meet the high-energy demand. Similar observations were made by Das and Jana (2003) on cadmium induced stress in Lamellidens marginalis. Kulkarni et al. (2005) reported significant decrease in total sugar and glycogen content in Lamellidens corianus under sublethal impact of hildan and concluded it to be due to energy demand by the animal in stress conditions.

ACKNOWLWDGEMENT

The authors are grateful to Principal, Yogeshwari Mahavidyalaya, Ambajogai for providing laboratory facilities

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HEAVY METAL INDUCED PROTEIN ALTERATIONS IN THE FRESHWATER BIVALVES FROM NAGAPUR DAM DIST BEED, (M. S.) INDIA

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ABSTRACT

The freshwater Lamellidens marginalis and lamellidens corrianus were exposed to LCo and LCo 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 L. marginalis in monsoon the protein content in LC₀ bivalves showed more amount in copper, followed by cadmium, zinc and mercury. In LC₅₀ mussels the more amounts in copper followed by cadmium, zinc and mercury. In winter the LC₀ bivalves showed more amount in copper followed by cadmium, zinc and mercury. In LC₅₀ mussels the more amount in copper followed by zinc, cadmium and mercury. In summer the LC₀ bivalves showed more amount in copper followed by cadmium, zinc and mercury. When these contents compared with zinc it was more in mercury than copper and cadmium. In LC₅₀ mussels the more amounts in copper followed by zinc, cadmium and mercury. When compared with the zinc species content it was more in mercury than cadmium and copper. In L. corrianus in monsoon the protein content in LC₀ bivalves showed more amount in zinc followed by copper, cadmium and mercury. In LC₅₀ mussels the more amounts in copper followed by cadmium, zinc and mercury. In winter the LCo bivalves showed more amount in copper followed by zinc, mercury and cadmium. In LC50 mussels the more amounts in copper followed by zinc, cadmium and mercury. In summer the LC₀ bivalves showed more amount in zinc followed by mercury, copper and cadmium. When these contents compared with zinc it was more in cadmium than copper and mercury. In LC₅₀ mussels the more amounts in copper followed by zinc, cadmium and mercury. When compared with the zinc species content it was more in mercury than copper and cadmium.

KEY WORDS: acute toxicity, heavy metals, L. marginalis, L. corrianus, Protein.

INTRODUCTION

Protein is the most efficient organic reserves of most of the bivalve and other animals and it is responsible for variety of function in organisms. The proteins represent an energetic reserve because of their high caloric value and are mainly used in chronic stress conditions. The trace metals are known to be non-biodegradable and highly toxic to most organisms (Kaoud and Dahshan, 2010). Some metals are ubiquitous and important biochemical constituent of the earth's crust and trace amounts can be released into aquatic environments through the processes of weathering and erosion (Batty et al., 2010). Small doses of metals are essential for almost all living organisms as it has a major role in numerous biochemical and physiological processes acting as a co-factor of proteins; metabolism of proteins, nucleic acids, carbohydrates and lipids (Rosabal et al., 2012). The studies on biochemical response of a bivalve to stressors have led to the better understanding as to how bivalve cope with the stressor at the biochemical level (Suryawanshi and Deshpande, 2016). The inorganic constituents of water have effect on the diversity of the bivalves, the texture of the sediment and the quantity of organic matter seemed to have played a role in their distribution and bivalves are able to survive even in the presence of sandy soil and lesser organic matter Shafakatullah Nannu et al., (2014). The effects of chronic exposure of color pigments which is using in paintings on changes in the biochemical constituents like protein, glycogen and lactic acid, in different body parts of fresh water mussels L. marginalis (Phadnis et al., 2013). Exposure to environmental stressors can induce oxidative stress in cells and result in a decrease in reducing potential and metabolic transformation to reactive intermediates (Simmons et al., 2011). Benthic biota can acquire metals through ingestion of sediment particles, food and directly from pore water and overlaying water (Griscom and Fisher, 2004).

The biochemical assay provides both qualitative and quantitative changes of tissue level in the bivalve. The aspect of energy metabolism and reproduction has been reported for a number of species of bivalves due to their commercial importance and edibility values In this regards many researchers devoted to study on the biochemical composition of bivalve molluscs (Kapil Manoj and Ragothaman, 1999, Shaikh, 2011, Kamble *et al.*, 2019, Suryawanshi and Kamble 2020). Hence, the aim of study to focus on understanding how bivalves *Parreysia corrugata* and *Lamellidens*



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corrianus from Nagapur dam metabolizes and are affected by the wide range of concentration of different heavy metals like zinc, copper, cadmium and mercury in aquatic environment.

MATERIALS AND METHODS

The bivalve species habitats are rich in flora and fauna around Nagapur dam; it is large sized dam constructed on Nagapur River near village Nagapur, Dist. Beed hence selected for study and in as there is no any industry on both sides as well as in catchment area. The locality in dam is 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 of year 2018-19. 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 LCo and LCo 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 °C till constant weights were obtained. The dried product was ground to obtain fine powder. From the replicates of three samples the protein was estimated using Lowry method (Lowry et al., 1952). The amount of protein were calculated by regression equation and expressed in terms mg/100mg dry powder.

RESULTS

In L. marginalis in monsoon the protein content (Table-1) in LC₀ bivalves showed more amount in copper, (4.98) followed by cadmium (4.89), zinc (4.83) and mercury (4.73).

Table 1:- Changes in protein levels in different species of freshwater bivalves in different seasons during acute toxicity to heavy metals from Nagapur dam Dist Beed (M. S.) India.

Species				MONSOON			WINTER				SUMMER			
	Control group	metalsvalues	Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride	Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride	Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride
amellidens narginalis	3.93 ±0.22	LC ₀	4.83 ±0.11	4.98 ±0.10 (3.11%)	4.89 ±0.18 (1.25%)	4.73 ±0.04 (16.57%)	5.63 ±0.16 (0.42%)	5.11 ±0.13 (1.60%) (2.62%)	5.09 ±0.09 (1.19%) (4.09%)	4.97 ±9.14 (1.19%) (5.08%)	4.52 ±0.07 (6.42%)Ω (10.14%)ΔΔ	4.60 ±0.14 (1.77%) (7.64%)∩ (9.99%)ΔΔ	4.58 ±916 (1.33%) (6.34%) (10.02%)ΔΔ	4.06 ±0.13 (10.18%)** (14.17%)∩- (18.31%)∆∆
		LC ₅₀	4.73 ±0.07	4.93 ±0.07 (4.23%)*	4.78 ±0.11 (1.06%)	4.53 ±0.07 (4.23%)*	4.85 ±0.35 (2.54%)	4.99 ±0.09 (2.89%)* (1.22%)	4.82 ±0.17 (0.62%) (0.09%)	4.75 ±0.04 (2.07%) (4.86%)⊓	4.33 ±0.23 (8.46%):: (10.73%)	4.35 ±0.07 (0.47%) (11.77%)nnn (12.83%)ΔΔΔ	4.14 ±0.13 (4.39%) (13.39%)nn (14.11%)ΔΔ	3.99 ±0.10 (7.86%) (11.93%)
amellidens orrianus	4.09 ±0.05	LCu	4.98 ±0.13	4.95 ±0.16 (0.61%)	4.91 ±0.10 (1.61%)	4.89 ±0.03 (1.81%)*	5.20 ±0.10 (4.42%)	5.22 ±0.13 (0.39%) (5.46%)	4.45 ±0.11 (1.93%)** (7.95%)GG	5.09 ±0.14 (2.12%) (4.09%)	4.67 ±0.07 (6.23%)□ (10.20%)△△	4.65 ±0.16 (0.43%) (6.07%) (10.92%)ΔΔΔ	2.80 ±0.07 (2.15%)*** (6.93%)000 (11.89%)\\D\D\D\D\D\D\D\D\D\D\D\D\D\D\D\D\D\D\	4.65 ±0.16 (0.43%) (4.91%)□ (8.65%)ΔΔ
		LC 50	473 ±0.04	4.93 ±0.10 (4.23%)	4.77 ±0.07 (0.85%)	4.57 ±0.10 (3.39%)	4.97 ±0.04 (5.08%)	5.11 ±0.09 (2.82%) (3.66%)	4.91 ±0.09 (1.21%) (2.94%)	4.87 ±0.04 (2.02%) (6.57%)	4.53 ±0.07 (4.23%) (8.26%).\(\Delta\)	4.65 ±0.11 (2.65%) (5.68%)□ (9.01%)ΔΔ	4.49 ±0.04 (0.89%) (5.88%)©© (8.56%)∆∆	4.41 ±0.11 (2.65%) (3.51%) (9.45%)ΔΔ

(Bracket values represent percentage differences) (*, \Box , Δ -P < 0.05, **, \Box \, $\Delta\Delta$ -P < 0.01, ***, \Box \, $\Delta\Delta\Delta$ -P < 0.001, *- compared to zinc, \Box - compared to monsoon, Δ - compared to winter of respective metal groups)



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When these contents compared with zinc it was more in mercury (16.57%) than copper (3.11%) and cadmium (1.25%). In LC₅₀ mussels the more amounts in copper (4.93) followed by cadmium (4.78), zinc (4.73) and mercury (4.53). When compared with the zinc species content it was more in mercury and cadmium showed equal amount of content i e. (4.23%; P<0.05) and cadmium (1.06%). In winter the LC₀ bivalves showed more amount in copper (5.11) followed by cadmium (5.09), zinc (5.03) and mercury (4.97). When these contents compared with zinc it was more in copper (1.60%) than cadmium (1.19%) and mercury (1.19%). In LC₅₀ mussels the more amount in copper(4.99) followed by zinc (4.85), cadmium (4.82) and mercury (4.75). When compared with the zinc species content it was more in copper (2.89%; P<0.05) than mercury (2.07%) and cadmium (0.62%). On the other hand the content when compared to monsoon to respective groups the protein was increased in all groups. In LC₀ mussels the content more increased in mercury (5.08%) followed by cadmium (4.09%), copper (2.62%) and zinc (0.42%). In LC₅₀ it was increased in all groups and the increase rate was in mercury (4.86%; P<0.05) followed by zinc (2.54%), copper (1.22%) and cadmium (0.09%). In summer the LC₀ bivalves showed more amount in copper (4.60) followed by cadmium (4.58), zinc (4.52) and mercury (4.06). When these contents compared with zinc it was more in mercury (10.78%; P<0.05) than copper (1.77%) and cadmium (1.33%). In LC₅₀ mussels the more amounts in copper (4.35) followed by zinc (4.33), cadmium (4.14) and mercury (3.99). When compared with the zinc species content it was more in mercury (7.86%) than cadmium (4.39%) and copper (0.47%). On the other hand when the content compared with monsoon and winter of respective groups the content showed in LC₀ bivalves more decreased in mercury (14.17%, P<0.01), (18.31%, P<0.01) followed by copper (7.64%, P<0.05), (9.99%, P<0.01), zinc (6.42%, P<0.05), (10.14%, P<0.01) and cadmium (6.34%), (10.02%; P<0.01) respectively. In LC₅₀ the decrease rate was in cadmium (13.39%; P<0.01), (14.11%; P<0.01) followed by mercury (11.93%; P<0.01), (16.0%; P<0.001), copper(11.77%; P<0.001), (12.83%; P<0.001) and zinc (8.46%; P<0.05), (10.73%) respectively.

On the other hand the *L. corrianus* in monsoon the protein content in LC₀ bivalves showed more amount in zinc (4.98) followed by copper (4.95), cadmium (4.91) and mercury (4.89). When these contents compared with zinc it was more in mercury (1.81%; P<0.05) than cadmium (1.61%) and copper (0.61%). In LC₅₀ mussels the more amounts in copper (4.93) followed by cadmium (4.77), zinc (4.93) and mercury (4.57). When compared with the zinc species content it was more in copper (4.23%) than mercury (3.39%) and cadmium (0.85%). In winter the LC₀ bivalves showed more amount in copper (5.22) followed by zinc(5.20), mercury (5.09) and cadmium (4.45). When these contents compared with zinc it was more in mercury (2.12%) than cadmium (1.93%; P<0.001) and copper (0.39%). In LC₅₀ mussels the more amounts in copper (5.11) followed by zinc (4.97), cadmium (4.91) and mercury (4.87). When compared with the zinc species content it was more in copper (2.32%) than mercury (2.02%) and cadmium (1.21%). On the other hand the content when compared to monsoon to respective groups the protein was increased in all groups.

In LC₀ mussels the content more increased in cadmium (7.95%; P<0.01) followed by copper (5.46%), zinc (4.42%) and mercury (4.09%). In LC₅₀ it was increased in all groups and the increase rate was in mercury (6.57%) followed by zinc (5.08%), copper (3.66%) and cadmium (2.94%). In summer the LC₀ bivalves showed more amount in zinc (4.67) followed by mercury (4.65), copper (4.65) and cadmium (2.80). When these contents compared with zinc it was more in cadmium (2.15%; P<0.001) than copper (0.43%) and mercury (0.43%). In LC₅₀ mussels the more amounts in copper (4.65) followed by zinc (4.53), cadmium (4.49) and mercury (4.41). When compared with the zinc species content it was more in mercury (2.65%) than copper (2.65%) and cadmium (0.89%). On the other hand when the content compared with monsoon and winter of respective groups the content showed in LC₀ bivalves more decreased in cadmium (6.93%; P<0.001), (11.89%; P<0.001) followed by zinc (6.23%; P<0.05), (10.20%; P<0.01), copper (6.07%), (10.92%; P<0.001) and mercury (4.91%; P<0.05), (8.65%; P<0.01) respectively. In LC₅₀ the decrease rate was in cadmium (5.88%; P<0.01), (8.56%; P<0.01) followed by copper (5.68%; P<0.05), (9.01%; P<0.01), zinc (4.23%; P<0.05), (8.26%; P<0.01) and mercury (3.51%), (9.45%; P<0.01) respectively.

DISCUSSION

Freshwater bivalves are performing efficient role in transformation and reserved of energy in food chains coupled with their sessile made of life. Proteins play a nutritionally and physiologically important role in bivalves by providing an efficient source of high energy content and essential fatty acids. Seasonal changes in protein content may be of great importance in relation to energy metabolism necessary for growth and reproduction (Lodeiros et al., 2001). Thus, food availability may be the important source of nutrients required for the gonadal repining process. Seasonal variation in temperature and availability of food appear to be closely related to energy available for growth and reproduction in



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other bivalve species (Smaal et.al., 1997). The protein seems to be its only alternative resource of energy under conditions of food scarcity. However, it cannot be certain without further studies and proper investigation about the possible advantage of using protein as an energy reserve and the mechanisms of regulation. In present study the L. marginalis showed more amount of protein in monsoon the protein content in bivalves showed more amounts in zinc, followed by cadmium, mercury and copper. When compared with the zinc species content it was more in cadmium than copper and mercury showed equal amount of content.

According to our study it was observed that the protein concentration altered due to metals and it was increased during exposure. In present study L. marginalis in monsoon the protein content in LC0 bivalves showed more amount in copper, followed by cadmium, zinc and mercury. When these contents compared with zinc it was more in mercury than copper and cadmium. In LC₅₀ mussels the more amounts in copper followed by cadmium, zinc and mercury. When compared with the zinc species content it was more in mercury and cadmium showed equal amount of content and cadmium. In winter the LC₀ bivalves showed more amount in copper followed by cadmium, zinc and mercury. When these contents compared with zinc it was more in copper than cadmium and mercury. In LC₅₀ mussels the more amount in copper followed by zinc, cadmium and mercury. When compared with the zinc species content it was more in copper than mercury and cadmium. On the other hand the content when compared to monsoon to respective groups the protein was increased in all groups. In LC₀ mussels the content more increased in mercury followed by cadmium, copper and zinc. In LC50 it was increased in all groups and the increase rate was in mercury followed by zinc, copper and cadmium. In summer the LC₀ bivalves showed more amount in copper followed by cadmium, zinc and mercury. When these contents compared with zinc it was more in mercury than copper and cadmium. In LC50 mussels the more amounts in copper followed by zinc, cadmium and mercury. When compared with the zinc species content it was more in mercury than cadmium and copper. When comparison between heavy metals the mercury was more affected the body parts and zinc was less affected.

The fall in the protein content during pollutant exposure may be due to increase protein catabolism and decrease anabolism of protein. Mahajan and Zambare (2001) showed that after acute and chronic exposure to mercuty, protein contents in different tissues of freshwater bivalve Corbicula striatella were found that highly depleted and maximum protein depletion was found in foot. The results obtained in the present study are supported by several investigators who reported decrease in protein of various organisms under influence of different metals. However, total protein content decreased on exposure to chromium in all the three tissues like gill followed by adductor muscle and mantle of freshwater bivalve L. marginalis (Satyaparameshwar et al., 2006). Our present data is compatible with many studies such as (Suryawanshi et al., 2014) the fall in the protein content during pollutant exposure may be due to increase protein catabolism and decrease anabolism of bivalves L. marginalis. Whereas, the protein decreases in organism due to largest need of energy for the metabolic process which leads to increases utilization of protein to meet energy and increase the proteolysis to reach the high energy demands under heavy metal stress in fresh water bivalves (Suryawanshi, 2017). On the other hand, upon 96 hrs exposure to metals the protein decreased in all heavy metals but the more decrease was in mercury, cadmium and copper and zinc of mussels this showed greater demand of energy over the utilization of body reserves in this organs, where in protein metabolites decreased. The increase in MT levels and concomitant decrease in the accumulation of various heavy metals (Gagnon et al., 2006) and labile zinc in gonad and gill tissues might be explained by the inflammation hypothesis.

When organism expose to stress tends to shift all the metabolic processes to face the toxic effects of stress and this lead to changes in biochemical and physiological mechanism in the body of organism, both duration of exposure and heavy metal concentrations important in determination of the level of biomarker response (Lehtonen *et al.*, 2003). It is therefore essential to study the effect of environmental variables on the the protein contents. However, it appears that the some group of bivalves showed protein decreases and protein synthesizes might be due to high concentration of metal and irrespective time of exposure period such as 96 hrs. While, the detected as shown in the results, enable us to make conclusions about the idea of variation in protein and protein was observed and we think that differences might be due to type of heavy metals, tolerance capacity, physiological status and metabolic rate of animals. However, increase in the protein content in some mussels groups was also seen which suggest the inhibition of lipase activity and protein synthesis likely due to impairment in metabolism and to the inhibition of enzyme activity in protein metabolism. Similar finding also do support to above results. (Rashmi *et al.*,2005). Further in present study the protein in lower concentration *L. corrianus* in monsoon the protein in LC₀ bivalves showed more amount in zinc followed by



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copper, cadmium and mercury. When these contents compared with zinc it was more in mercury than cadmium and copper.

In LC₅₀ mussels the more amounts in copper followed by cadmium, zinc and mercury. When compared with the zinc species content it was more in copper than mercury and cadmium. In winter the LC₀ bivalves showed more amount in copper followed by zinc, mercury and cadmium. When these contents compared with zinc it was more in mercury than cadmium and copper. In LC₅₀ mussels the more amounts in copper followed by zinc, cadmium and mercury. When compared with the zinc species content it was more in copper than mercury and cadmium. On the other hand the content when compared to monsoon to respective groups the protein was increased in all groups. In LC₀ mussels the content more increased in cadmium followed by copper, zinc and mercury. In LC₅₀ it was increased in all groups and the increase rate was in mercury followed by zinc, copper and cadmium. In summer the LC₀ bivalves showed more amount in zinc followed by mercury, copper and cadmium. When these contents compared with zinc it was more in cadmium than copper and mercury. In LC₅₀ mussels the more amounts in copper followed by zinc, cadmium and mercury. The results obtained in the present study indicate severe disturbance in the protein metabolism of the fresh water bivalves exposed to different heavy metals.

The results obtained in present study are in agreement of most of the above observations and showed decrease in the protein in the body parts of bivalves shows its prime utilization in gearing of the metabolism. Another possible explanation for the decrease in the protein might be due to diapedesis and mucoprotein which is eliminated in the form of mucous. During the experiments in laboratory it was noticed that the excessive secretion of mucous and diapedesis on the water surface might be scrubbing the body by bivalves due to metals and avoiding the water into the body hence supports this possibility. Pardeshi and Gapat, (2012) were noticed that protein contents were significantly reduced after nickel exposure in all tissues of the bivalve *L. corriamus* as compared to control group of animals. However, total protein content decreased on exposure to various metals in different body parts of freshwater bivalve *L. marginalis* were observed (Suryawanshi *et al.*, 2016). The results obtained in present study are in agreement of most of the above observations and showed decrease in the protein in the body parts of bivalves shows its prime utilization in gearing of the metabolism. Our present study showed when comparison between the metals the zinc was not affected much hence the protein was not depleted more in the body of bivalves but mercury metal concentration showed more pronounced to the bivalves hence more protein was depleted and this indicates that the Zn is essential and Hg is not essential metal to the body of mussels so the variation in protein concentration was observed.

ACKNOWLWDGEMENT

The authors are grateful to Principal, Yogeshwari Mahavidyalaya, Ambajogai for providing laboratory facilities to smooth conduct the experiments.

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