International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 9 Issue 6 ǁ 2021 ǁ PP. 93-97

Effects of heavy metals on protein status of freshwater bivalves (Parreysia corrugata) and (Indonaea caeruleus)

G. D. Suryawanshi
Department of Zoology, Yogeshwari Mahavidyalaya, Ambajogai, Dist. Beed-431517

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 species showed more amount of protein in lower concentration than higher concentration values of metal concentration during all seasons. In monsoon the protein content in bivalves showed more amounts in zinc, followed by cadmium, mercury and copper. In winter the content when compared to monsoon to respective metal groups the protein was increased from mercury followed by cadmium, zinc and copper. In higher concentration it was decreased in all metal groups except the cadmium, followed by mercury than zinc and copper. In summer when the content compared with monsoon and winter of respective metal groups the content showed in lower concentration of bivalves more decreased in cadmium followed by copper, mercury and zinc respectively. In higher concentration the decrease rate was in cadmium followed by copper, mercury and zinc respectively. In I. caeruleus the protein content in monsoon the lower concentration bivalves showed more amount in cadmium and mercury followed by copper and zinc. In higher concentration mussels the more amounts in cadmium and zinc followed by mercury and copper. In winter the content when compared to monsoon to respective metal groups the protein was increased and the more increased rate was in lower concentration bivalves from zinc followed by mercury and copper and cadmium. In higher concentration it was decreased in all metal groups the decrease rate was in mercury than copper, zinc and cadmium. In summer the content compared with monsoon and winter of respective metal groups it showed in lower concentration bivalves more decreased in mercury followed by copper, cadmium and zinc respectively. In higher concentration the decrease rate was in zinc followed by cadmium, mercury and copper respectively.

Keywords: *Protein, heavy metals, P. corrugata and I. caeruleus acute toxicity, seasons.*

Date of Submission: 06-06-2021 Date of acceptance: 19-06-2021

I. INTRODUCTION

Biochemical changes in the animals exposed to different metals are likely to be amongst the first expressions of excess metal accumulation and perhaps the most sensitive indicators of stress and are useful in shaping the mechanism of toxicity and severity of a variety of xenobiotics. Proteins are most abundant intracellular macro-molecules and constitute over half the dry weight of most organisms. They occupy a central position in the architecture and functioning of living matter. They are intimately connected with all phases of chemical and Physical activity that constitutes the life of the cell. Therefore they are, essential to cell structure and cell function. 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). The aim of our study to focus on understanding how bivalves Parreysia corrugata and Indonaea caeruleus from Manjra dam metabolizes and are affected by the wide range of concentration of different heavy metals like zinc, copper, cadmium and mercury in aquatic environment.

II. MATERIALS AND METHODS

The bivalve species habitats are rich in flora and fauna around Manjra dam; it is large sized dam constructed on Manjra River near village Dhanegaon, Dist. Osmanabad 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 Parreysia corrugata and Indonaea caeruleus 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 bivalves' P. corrugata and I. 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 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 0C till constant weights were obtained. The dried product was ground to obtain fine powder. From the replicates of three samples the protein was analyzed by using Lowry's method (Lowry et al., 1951). The amount of protein was calculated by regression equation and expressed in terms mg/100mg dry powder.

III. 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 protein content in LC0 bivalves showed more amount in zinc, (57.38) followed by cadmium (56.99), mercury (56.38) and copper (55.88). When these compared with zinc it was more in copper (2.62%), followed by mercury (1.75) and cadmium (0.68). In LC50 mussels the more amounts in mercury (55.50) followed by zinc (55.13), copper (54.75) and cadmium (53.63). When compared with the zinc species content it was more in cadmium (2.73%) than copper and mercury showed equal amount of content i e.(0.69%) all were non significant levels. In winter the LC0 bivalves showed more amount in mercury (62.63) followed by cadmium (60.38), zinc (58.38) and copper (56.39). When these contents compared with zinc it was more in mercury (7.28%) than cadmium (3.43%) and copper (3.41%) (all non significant level). In LC50 mussels the more amounts in mercury (59.25) followed by cadmium (58.13), zinc (56.99) and cadmium (5.95). When compared with the zinc species content it was more in mercury (9.90%) than cadmium (5.95%) and copper (1.29%). On the other hand the content when compared to monsoon to respective metal groups in LC0bivalves the protein was increased rate was from mercury (11.08%) followed by cadmium (5.95%), zinc (1.75%) and copper (0.92). In large sizes it was decreased in all metal groups except the cadmium (8.40%), followed by mercury (6.76%) than zinc (3.38%) and copper (2.74%). In summer the LC0 bivalves showed more amount in zinc (61.13) followed by mercury (43.14), cadmium (45.75) and copper (44.99).When these contents compared with zinc it was more in copper (26.41%; $P<0.01$) than cadmium (25.16; $P<0.001$%) and mercury (24.54; $P<0.001$%). In LC50 mussels the more amounts in zinc (51.38) followed by mercury (44.63), copper (43.50) and cadmium (42.38). When compared with the zinc species content it was more in cadmium (17.52%; $P<0.01$) than copper (15.34%; $P<0.001$) and mercury (13.14%; $P<0.01$). On the other hand when the content compared with monsoon and winter of respective metal groups the content showed in LC0bivalves more decreased in cadmium (19.73%; $P<0.001$), (24.23%; $P<0.001$) followed by copper (19.49%; $P<0.01$), (20.22%; $P<0.01$), mercury (18.19%; $P<0.01$), (26.35%; $P<0.001$) and zinc (6.54%), (4.72%) respectively. In LC50 the decrease rate was in cadmium (20.98%; $P<0.05$), (27.09; $P<0.001$%) followed by copper (20.55%; $P<0.01$), (22.67%; $P<0.01$), mercury (19.59%; $P<0.01$), (24.38%; $P<0.001$) and zinc (6.81%; $P<0.05$), (9.85%) respectively.

In I. caeruleus the protein content in monsoon the LC0 bivalves showed more amount in cadmium and mercury, (63.38) followed by copper (60.75) and zinc (50.63). When these contents compared with zinc it was more in mercury (25.19%), the copper (19.99%) and cadmium (1.42%). In LC50 mussels the more amounts in cadmium and zinc (61.88) followed by mercury (60.38) and copper (58.50). When compared with the zinc species content it was more in copper (5.47%) than mercury (2.43%) (all non-significant levels). In winter the LC0 bivalves showed more amount in mercury (66.38) followed by cadmium (65.63), copper (62.99) and zinc (62.25). When these contents compared with zinc it was more in mercury (6.64%) than cadmium (5.43%) and copper (1.19%). In LC50 mussels the more amounts in mercury (64.88) followed by cadmium (63.75), copper (61.88) and zinc (59.25). When compared with the zinc species content it was more in mercury (9.51%) than cadmium (7.60%) and copper (4.44%). On the other hand the content when compared to monsoon to respective metal groups the protein was increased and the more increased rate was in LC0 bivalves from zinc (22.96%) followed by mercury (4.73%) and copper (3.69%) and cadmium (3.56%). In LC50 it was decreased in all metal groups the decrease rate was in mercury (7.46%) than copper (5.78%), zinc (4.26%) and cadmium (3.03%). In summer the LC0 bivalves showed more amount in mercury (58.50) followed by cadmium (58.13), copper (55.13) and zinc (53.25). When these contents compared with zinc it was more in mercury (9.86%) than cadmium (9.17%) and copper (3.54%) (all non significant level). In LC50 mussels the more amounts in cadmium (56.25) followed by mercury (55.88), copper (54.75) and zinc (50.25). When compared with the zinc species content it was more in cadmium (11.95%) than mercury (11.21%) and copper (8.96%). On the other hand when the content compared with monsoon and winter of respective metal groups the content showed in LC0 bivalves more decreased in mercury (20.33%; $P<0.05$), (23.93%; $P<0.01$) followed by copper (9.26%; $P<0.01$), (12.48%; $P<0.05$), cadmium (8.29%; $P<0.05$), (11.43%; $P<0.01$) and zinc (5.18%), (14.46%; $P<0.05$) respectively. In LC50 the decrease rate was in zinc (18.80%; $P<0.01$), (15.19%; $P<0.05$) followed by cadmium (9.09%; $P<0.05$), (11.77%; $P<0.05$), mercury (7.46%; $P<0.01$), (13.88%; $P<0.01$) and copper (6.42%), (11.52%) respectively.

IV. DISCUSSION

Qualitative study of changes in most important biochemical components of organisms is valuable to identify different toxicants and protective mechanism of the body against toxic property of heavy metal pollution. 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 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 *P. corrugata* 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. In winter the content when compared to monsoon to respective metal groups in LC_0 bivalves the protein was increased rate was from mercury followed by cadmium, zinc and copper. In LC_{50} it was decreased in all metal groups except the cadmium, followed by mercury than zinc and copper. In summer when the content compared with monsoon and winter of respective metal groups the content showed in LC_0 bivalves more decreased in cadmium followed by copper, mercury and zinc respectively. In LC_{50} the decrease rate was in cadmium followed by copper, mercury and zinc respectively. Further, the study showed upon 96 hrs exposure of metals caused some how different trend was observed, revealing different type of substrate utilization to meet the energy demand. The mussel *P. corrugata* during exposure with different heavy metals and time period showed that the protein levels in their body parts decreased continuously when increases the time period. Overall in study the zinc metal group bivalves showed less amount of protein decreases. It is evident that decrease in the protein from in mussels in all the metal concentration probably caused metabolism restricted to lipogenesis and maintenance by utilizing protein substrate. When exposed mussels at metal concentration showed that more decrease was in mercury followed by cadmium and copper. The decrease of protein content, suggests possible utilization for metabolic purposes enhancement of proteiolysis to meet the high-energy demand under metal pollution stress condition. 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).

Further, in *I. caeruleus* the protein content in monsoon the LC_0 bivalves showed more amount in cadmium and mercury followed by copper and zinc. In LC_{50} mussels the more amounts in cadmium and zinc followed by mercury and copper. In summer the content compared with monsoon and winter of respective metal groups it showed in LC_0 bivalves more decreased in mercury followed by copper, cadmium and zinc respectively. In LC_{50} the decrease rate was in zinc followed by cadmium, mercury and copper respectively. 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 (Patil, 2011). On the other hand in present study decrease in protein might be due to increased proteolysis activity or might be due to changes in the metabolic substrate during anaerobic condition produced in the bivalves by metal. 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. corrianus* 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 author is grateful to Principal, Yogeshwari Mahavidyalaya, Ambajogai for providing laboratory facilities.

REFERENCES

[1]. Batty L. C., Auladell M., Sadler J. and Hallberg, K. (2010) The impacts of metalliferous drainage on aquatic communities in streams and rivers. Ecol. Indust. Pollut. (2), 70-100.

[2]. Gagnon C., Gagne F., Turcotte P., Saulnier I., Blaise C., Salazar M.H. and Salazar S. (2006) Exposure of caged mussels to metals in a primary—treated municipal wastewater plume. Chemosphere. (62) 998–1010.

[3]. Genton, Laurence; Melzer, Katarina; Pichard, Claude (2010) "Energy and macronutrient requirements for physical fitness in exercising sub-jects".Clinical Nutrition. 29 (4), 413–423

[4]. Griscom S.B., Fisher N.S. (2004) Bioavailability of sediment-bound metals to marine bivalve molluscs: an overview. Estuaries. (27), 826–838.

[5]. Kamble, S. G., R. D. Sonwane and G. D. Suryawanshi (2019) Biochemical variation in freshwater bivalve Lamellidens marginalis.Think India Journal (22), Special Issue-31

[6]. Kaoud H. A. and El-Dahshan A. R. (2010) Bioaccumulation and histopathological alterations of the heavy metals in Oreochromis niloticus fish Nature Sci. (8) 4-8

[7]. Kapil Manoj and Ragothaman, G (1999) Mercury, copper and cadmium induced changes in the proteins levels in muscle tissue of an edible esturine fish, Bolephthalmus dussumeri (Cuv). J. Environ. Biol. 20 (3), 231-234

[8]. Lehtonen K.K. and Leiniö S. (2003) Effects of Exposure to Copper and Malathion on Metallothionein Levels and Acetylcholinesterase Activity of the Mussel Mytilus edulisand the Clam Macoma balthicafrom the Northern Baltic Sea. Bull. Environ. Contam. Toxicol. (71), 489–496.

[9]. Lodeiros, C. J., Rengel, J. J., Guderley, H. E., Nuseni, O. and Himmelman, J. H. (2001) Biochemical composition and energy allocation in the tropical scallop Lyropecten (Nodipecten) nodosus during the months leading up to and following the development gonads. Aquaculture. (199), 63-72.

[10]. Lowry O.H.,Rosenbrough N.J., Farr A.L., and Randall R.J. (1951) Protein measurement with Folin phenolreagent. J. Biol. Chem. (193), 265-27.

[11]. Mahajan A. Y. and Zambare S. P. (2001) Ascorbate effect on copper sulphate and mercuric chloride induced alterations of protein levels in freshwater bivalve, Corbicula striatella. Asian. J. Microbiol. Biotech. and Env. Sci. (3), 95-100.

[12]. Pardeshi Anilkumar and Gapat Meenakshi (2012) Ascorbate effect on protein content during nickel intoxication the freshwater bivalve, Lamellidens corrianus, Bioscience Discovery, 3, (2), 270 -274.

[13]. Patil A. G. (2011) Protein changes in different tissues of freshwater bivalve, Parreysia cylindrical after exposure to indoxa carb. Recent Res. Sci. Tech. (3), 140-142.

[14]. Phadnis, S. D.; Chandagade, C. A.; Jadhav, V. V. (2013) Impact of colour pigments on biochemical parameters of bivalve, Lamellidens corrianus, J. of Env. Biol. 34, (2), 267-271.

[15]. Rosabal M., Hare L., Campbell P. G. (2012) Subcellular metal partitioning in larvae of the insect Chaoborus collected along an environmental metal exposure gradient (Cd, Cu, Ni and Zn). Aquatic Toxicol. (120) 67–78.

[16]. Satyaparameshwar K., T. Ravinder Reddy and N. Vijaya Kumar, (2006) Effect of chromium on protein metabolism of fresh water mussel, Lamellidens marginalis J. Env. Biol. (2), 401-403.

[17]. Shafakatullah Nannu and M. Krishnamoorthy (2014) Nutritional Quality in Freshwater Mussels, Parreysiaspp. of PeriyarRiver, Kerala, India, Res. J. Recent Sci. (3), 267-270.

[18]. Shaikh, M.J. (2011) Seasonal variation in biochemical constituents in different body tissues of freshwater bivalve mollusk, Lamellidens corrianus (Lamark) from Pravara river in Maharashtra. The Bioscan 6, (2), 287-288.

[19]. Simmons, S. O., Fan, C. Y., Yeoman, K., Wakefield, J., and Ramabhadran, R. (2011) NRF2 oxidative stress induced by heavy metals is cell type dependent, Curr. Chem. Genomics. (5), 1–12.

[20]. Smaal, A. C., Vonck, A. P. M. A. and Bakker, M. (1997) Seasonal variation in physiological energetics of Mytilus edulis and Cerastoderma edule of different size classes. J. Mar. Biol. Assoc. U. K. (77), 817-838.

[21]. Suryawanshi G. D., Kurhe A. R. and Miguel A. Rodriguez (2014) Mercury Exposure Produce Changes in Protein Content in Different Body Parts of Oyster Crassostrea Cattuckensis (Newton and Smith). J. Environ. Sci. Comp. Sci. Eng. Tech. (1), 0065-0071.

[22]. Suryawanshi, G. D. and Deshpande P. A. (2016) Changes in protein in different body parts of bivalve L. marginalis due to heavy metals. Int. J. of Trends in Fisheries Research. 5, (3), 25-29.

[23]. G. D. Suryawanshi and S. G. Kamble (2020) Changes in Protein Metabolism of Freshwater Bivalves (L. Marginalis and L. Corrianus) Due To Short Term Exposed To Heavy Metals." Journal of Environmental Science, Toxicology and Food Technology, 14, (9), 28-32.

Table 1:- Changes in proteins in different species of freshwater bivalves in different seasons during acute toxicity to heavy metals from Manjara 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
Parreysia corrugata	59.35 1.95	LC_0	57.38 ±2.25	55.88 ±2.35 (2.62%)	56.99 ±1.30 (0.68%)	56.38 ±1.95 (1.75%)	58.38 ±3.90 (1.75%)	56.39 ±2.98 (3.41%) (0.92%)	60.38 ±0.65 (3.43%) (5.95%)	62.63 ±2.35 (7.28%) (11.08%)	61.13 ±1.72 (6.54%) (4.72%)	44.99 ±1.95 (26.41%)*** (19.49%)□□ (20.22%)ΔΔ	45.75 ±1.30 (25.16%)*** (19.73%)□□□ (24.23%)ΔΔΔ	46.14 ±1.95 (24.54%)*** (18.19%)□□ (26.35%)ΔΔΔ
		LC_{50}	55.13 ±1.13	54.75 ±1.30 (0.69%)	53.63 ±2.84 (2.73%)	55.50 ±1.72 (0.68%)	56.99 ±3.25 (3.38%)	56.25 ±3.90 (1.29%) (2.74%)	58.13 ±1.30 (5.95%) (8.40%)	59.25 ±1.72 (9.90%) (6.76%)	51.38 ±0.65 (6.81%)□ (9.85%)	43.50 ±1.30 (15.34%)*** (20.55%)□□□ (22.67%)ΔΔ	42.38 ±2.84 (17.52%)** (20.98%)□ (27.09%)ΔΔΔ	44.63 ±1.45 (13.14%)** (19.59%)□□ (24.68%)ΔΔΔ
Indonaea caeruleus	55.13 ±1.45	LC_0	50.63 ±2.98	60.75 ±1.13 (19.99%)	63.38 ±2.84 (1.42%)	63.38 ±2.35 (25.19%)	62.25 ±2.60 (22.96%)	62..99 ±2.98 (1.19%) (3.69%)	65.63 ±1.30 (5.43%) (3.56%)	66.38 ±2.25 (6.64%) (4.73%)	53.25 ±2.60 (5.18%) (14.46%)Δ	55.13 ±1.13 (3.54%) (9.26%)□□ (12.48%)Δ	58.13 ±1.13 (9.17%) (8.29%)□ (11.43%)ΔΔ	58.50 ±1.13 (9.86%) (20.33%)□ (23.93%)ΔΔ
		LC_{50}	61.88 ±2.98	58.50 ±3.90 (5.47%)	61.88 ±1.95 (equal%)	60.38 ±1.72 (2.43%)	59.25 ±2.35 (4.26%)	61.88 ±3.89 (4.44%) (5.78%)	63.75 ±1.90 (7.60%) (3.03%)	64.88 ±1.72 (9.51%) (7.46%)	50.25 ±2.60 (18.80%)□□ (15.19%)Δ	54.75 ±3.62 (8.96%) (6.42%) (11.52%)	56.25 ±1.95 (11.95%) (9.09%)□ (11.77%)Δ	55.88 ±1.72 (11.21%) (7.46%)□□ (13.88%)ΔΔ

(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)**