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# ALTERATIONS IN BIOCHEMICAL CONSTITUENTS DUE TO HEAVY METALS IN BODY TISSUES OF *LAMELLIDENS MARGINALLIS* FROM GANGAPUR RESERVOIRS AT NASIK, MAHARASHTRA, INDIA

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## ABSTRACT

The present study investigates the effect of heavy metals Zn, Cu, Pb and Cd on the total proteins, ascorbic acid, DNA and RNA content from different soft body tissues of bivalve species, *Lamellidens marginalis* collected seasonally (summer, monsoon and winter seasons) from Gangapur reservoir of Nasik district during November 2010 to October 2011. In the present investigation results revealed highest concentrations of heavy metals Zn, Cu, Pb and Cd during summer season in surface water and bivalve species sampled from Gangapur reservoir. The results also revealed the lowest protein, ascorbic acid, DNA and RNA concentrations in different soft body tissues in bivalves sampled during summer season, from Gangapur reservoir might be due to bivalves were exposed to higher concentration of heavy metals in summer than winter and monsoon seasons.

**KEY WORDS:** Heavy metals, total proteins, Ascorbic acid, DNA, RNA, Gangapur reservoir, *Lamellidens marginallis* 

#### INTRODUCTION

In an aquatic ecosystem, heavy metals are considered as the most important pollutants due to their highly bio accumulative nature, persistent behaviour and potential of higher toxicity (Niyogi and Wood, 2004; Censi et al., 2006; Rahane et al; 2016). Some of these metals (e.g. Cu, Ni, Cr and Zn) are essential trace metals to living organisms, but become toxic at higher concentrations (Albergoni and Piccinni, 1983). Many aquatic organisms have the ability to accumulate and biomagnifies metals in food chain (Labonne et al., 2001; Goodwin et al., 2003; Casas et al., 2008). Consumption of such aquatic food stuff highly contaminated with toxic metals may cause serious health hazards through food-chain magnification (Khan et al., 2000). The biochemical composition varies according to the situation like seasonal changes, environmental factors (temperature, salinity), starvation and toxicants in the water (Verlecar et al., 2007; Nandurkar and Zambare, 2010; Salaskar and Nayak 2011). Seasonal changes in biochemical composition in two unionid mussels, Actinonaias ligamentina and Amblema plicata was observed by Baker and Hornbach (2001). Rahane and Bhalla (2018) studied toxic effects of heavy metals on DNA, RNA and ascorbic acid content in soft tissues of freshwater bivalve, Lamellidens corrianus. The seasonal variation in RNA content in Austrovenus stutchburyi at different sites was studied by Norkko and Thrush (2006). Hence, the study of biochemical components would be much meaningful to estimate the nutritive value of the organism and its further analysis with the metal effect would provide an intricate relation between the metal pollutants and the metabolism of the basic biochemical constituents. Therefore, it is an attempt to investigate the effect of metal stress on

the biochemical components.

# MATERIALS AND METHODS

The samples were collected in summer, monsoon and winter seasons during November 2010 to October 2011 from Gangapur reservoir of Nashik district. The heavy metals Zn, Cu, Pb and Cd concentrations were determined in surface water samples and obtained results were summarized in Table 1. The heavy metals Zn, Cu, Pb and Cd concentrations were determined from whole soft body tissues of freshwater bivalve, Lamellidens marginalis and obtained results were presented in Table 2. The total protein, ascorbic acid, DNA and RNA contents were determined from soft body tissues like mantle, gills, digestive glands and whole soft body tissues of Lamellidens marginalis. Mantle, gills, digestive glands and whole soft body tissues were removed and dried at 70° to 80°C in the oven till the constant weight of dry tissues were obtained. From each powder total protein, ascorbic acid, DNA and RNA content were estimated. The total protein content of the tissues was estimated by method of (Lowry et al., 1951A). Estimation of ascorbic acid was carried out by the method of Roe (1967). DNA content of the tissue was estimated by using Diphenylamine method of Burton (1956). RNA content of the tissue was estimated by following Orcinol method of Volkin and Cohn (1954). The results are presented in the Table 3.

#### **RESULTS AND DISCUSSION**

The study clearly demonstrates that the mean

values of Pb and Cd in surface water of Gangapur reservoirs were higher than the WHO recommended limits for drinking water standard; where as those of zinc and copper were within the limits. Therefore, this study indicates that the surface water of Gangapur reservoirs were polluted by heavy metals Pb and Cd.

The results indicate that the Zn, Cu, Pb and Cd concentration were highest in surface water in summer season and lowest in monsoon season at Gangapur reservoir. The highest concentrations of heavy metals Zn, Cu, Pb and Cd were recorded in surface water sampled from Gangapur reservoirs, might be due to heavy input of various pollutants. It was also observed that the mean concentrations of Zn, Cu, Pb and Cd were highest in summer season and lowest in monsoon season in whole body tissue of bivalve species, *Lamellidens marginalis* sampled from the Gangapur reservoir.

In the present seasonal study, the lowest total protein, ascorbic acid, DNA and RNA contents were observed in different soft body tissues like mantle, gills, digestive glands and whole soft body tissue of bivalves sampled during summer season, might be due to bivalves were exposed to higher level of pollutant in summer than winter and monsoon seasons.

The observed low level of protein contents in different tissues indicate that, environmental stress reduces the rate of protein synthesis or increase the proteolysis to cope with the high energy demands under toxicants stress (Waykar and Lomte, 2001). Decrease in protein content in *Lamellidens marginallis* on exposure to methomyl and lambdacyalothrin

 Table 1. Seasonal variations of heavy metal concentrations (mg/L) in surface water of Gangapur reservoir of Nasik district.

Name of reservoir	Seasons	Zn	Cu	Pb	Cd
Gangapur reservoir	Summer	0.1008±0.0005	0.0193±0.0005	0.0250±0.0002	0.0070±0.0003
Monsoon	$0.0573 \pm 0.0004$	$0.0128 \pm 0.0003$	$0.0207 \pm 0.0003$	$0.0054 \pm 0.0001$	
Winter	$0.0812 \pm 0.0006$	$0.0145 \pm 0.0004$	$0.0228 \pm 0.0005$	$0.0068 \pm 0.0002$	
WHO standard,1993 mg/L	03	02	0.01	0.003	

± indicate standard deviation

**Table 2.** Seasonal variations in heavy metal concentrations ( $\mu g/g \, dry$  tissue weight) in whole soft body tissues offreshwater bivalve Lamellidens marginalis at Gangapur reservoir of Nasik district.

Seasons	Zn	Cu	Pb	Cd
Summer	359.15±5.72	98.26±2.14	95.37±2.42	12.51±0.82
Monsoon	225.09±5.27	$74.42 \pm 1.45$	62.53±1.86	06.37±0.65
Winter	254.70±4.75	85.11±1.63	79.23±1.92	$08.48 \pm 0.74$

± indicate standard deviation.

exposure and recovery due to ascorbic acid was reported by Bhalla, (2014, 2019).

At stressful condition on exposure to toxicants ascorbic acid indicates positive role in detoxification (Mahajan and Zambare, 2001) and also perform therapeutic role against pollutant toxicity in mollusc (Waykar, 2006; Waykar and Pulate, 2012).Decrease in ascorbic acid content indicated its involvement in counteracting oxidative damage. Stress caused alterations in the normal physiology of animal leading to enhanced utilization and mobilization of ascorbic acid (Chinoy and Kamalakumari, 1976) as ascorbic acid is recognized as anti-stress factor (Kutsky, 1973; Bhalla, 2014, 2019). Rahane and Bhalla, (2018 a, b) observed decrease in ascorbic acid content in different freshwater bivalve species on exposure to heavy metals.

Several reports are available on the reduction in DNA and RNA levels on exposure to different toxicants. Zahran et al., (2005) reported decrease in DNA and RNA contents in rat after exposure to pollutant. Nwani et al., (2010) demonstrated DNA damage after treatment with carbosulfan in freshwater fish, Channa punctatus. Singh et al., (2010) reported a significant decline in RNA levels in various tissues of Labeo rohita after cypermethrin intoxication. Bhosale et al., (2011) reported that DNA and RNA content in soft body tissues of Corbicula striatella was decreased due to toxicants stress. Pandey et al., (2011) reported toxicants induced DNA damage in freshwater fish, Channa punctatus. Thenmozi et al., (2011) showed significant decrease in DNA and RNA content in the liver, muscle and gill of freshwater fish, Labeo rohita after treatment of malathion.

This work provides information regarding the heavy metal level in surface water, in bivalve species and overall result showed the low protein, ascorbic acid, DNA and RNA contents in different soft body tissues of bivalve species collected from Gangapur reservoir. This indicated that bivalve species inhabiting reservoir are more under environmental stress.

# CONCLUSION

The study clearly demonstrates that the mean values of Pb and Cd in surface water of Gangapur reservoir were higher than WHO recommended limits for drinking water standard; whereas those of zinc and copper were within the limits. The results showed, lowest protein, ascorbic acid, DNA and

mg/10	0 mg dry tissu	e weight).										
Parameter		Mantle			Gills		Di	gestive glanc	ls	Whol	e soft body ti	ssue
	Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win
Total												
Protein	$44.05 \pm 1.28$	$54.73\pm1.38$	$52.48\pm 1.18$	$53.18 \pm 1.83$	$66.16\pm 1.84$	$61.14\pm 2.14$	$51.54\pm 2.62$	$64.28\pm 1.93$	$59.32 \pm 1.82$	$49.17\pm 2.08$	63.47±2.08	$57.06 \pm 1.44$
Ascorbic Acid	$0.701 \pm 0.016$	$0.981 \pm 0.017$	$0.908 \pm 0.018$	$0.855\pm0.012$	$1.203\pm0.019$	$1.097\pm0.026$	$0.970 \pm 0.014$	$1.403\pm0.029$	$1.198 \pm 0.018$	$0.814 \pm 0.012$	$1.172 \pm 0.011$	$1.024 \pm 0.018$
DNA	$1.03 \pm 0.070$	$1.45\pm0.052$	$1.21 \pm 0.041$	$1.29\pm0.049$	$1.89 \pm 0.052$	$1.70\pm0.041$	$1.37\pm0.040$	$2.01\pm0.048$	$1.74\pm0.041$	$1.50\pm0.056$	$2.30\pm0.048$	$2.12\pm0.072$
RNA	$3.18 \pm 0.11$	$4.41 \pm 0.22$	$3.52 \pm 0.18$	$5.38 \pm 0.25$	6.26±0.26	$5.98 \pm 0.22$	$6.69 \pm 0.20$	$8.72 \pm 0.27$	$8.05 \pm 0.30$	$4.85 \pm 0.25$	$6.81 \pm 0.16$	$6.13 \pm 0.31$
± indicate stand	ard deviation											

Profile of Total protein, Ascorbic acid, DNA and RNA in different body tissues of Lanellidens marginalis from Gangapur reservoir of Nashik district (Values are in

Table 3.

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RNA contents in soft body tissues of bivalve species, indicated that bivalve species inhabiting Gangapur reservoir are under environmental stress.

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# REFERENCES

- Albergoni, V. and Puccinne, E. 1983. Biological response to trace metals and their biochemical effects. In: *Trace Element Speciation in Surface Water and its Ecological Implications.* Leppard G. G. (Ed.): 159-174.
- Baker, S.M. and Hornbach, D.J. 2001. Seasonal metabolism and biochemical composition of two unionid mussels, *Actinonaias ligamentina* and *Amblema plicata. Journal of Molluscan Studies* 67(4): 407-416.
- Bhalla, R. 2014. Role of L-ascorbic acid in the detoxification due to methomyl induced alterations in the protein content in different tissues of fresh water bivalve Lamellidens marginallis (Lamarck). ESSENCE. International Journal for Environmental Rehabilitation and Conservation. V (2): 41-49.
- Bhalla, R. 2019. Methomyl and lambdacyalothrininduced alterations in the protein content and recoverydue to L-ascorbic acid in different tissues of the freshwaterbivalve, *Lamellidensmarginalis* (lamarck). *International Journal of Global Science Research*. 6 (1): 653-662.
- Bhosale, P.A., Andhale, A.V. and Zambare, S.P. 2011. Biochemical alterations in DNA content of gill and gonad tissues of *Corbicula striatella* due to 5-Fluorouracil toxicity. *Rec Res Sci Tech.* 3 : 73-74.
- Burton, K. 1956. Study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *J. Biochem.* 62: 315-323.
- Casas, S., Gonzalez, J. L., Andral, B. and Cossa, D. 2008. Relation between metal concentration in water and metal content in marine mussels (*Mytilus galloprovincialis*): impact of physiology. *Environ Toxicol Chem.* 27 : 1543-1552.
- Censi, P., S.E. Spoto, F. Saiano, M. Sprovieri and S. Mazzola 2006. Heavy metals in coastal water system. A case study from the North Western Gulf of Thailand. *Chemosphere*. 64 : 1167-1176.
- Chinoy, N.J. and Kamalakumari, D. 1976. M.Sc. Dissertation, Zoology Department, Gujarat University, Ahmadabad. Clams and Commercial Clam Meats from the State of Miranda (Venezuela)

employing ICP-OES, GF-AASand WD-XRF. At. *Spectrosc.* 25 : 112-124.

- Goodwin, T. H., Young, A., Holmes, M., Old, G., Hewit, N., Leks, G., Packman, J. andSmith, B. 2003. The Temporal and Spatial Variability of Sediment Transport and Yield within the Bradford Beck Catchment, West Yorkshire. *Science of the Total Environment.* 314 : 475-494.
- Khan A.G., Kuek T.M., Chaudhury, T.M., Khoo, C.S, Hayes, W.J. 2000. Role of plants, mycorrhizae and Phyto chelators in heavy metal contaminated land remediation. *Chemosphere*. 41: 197-203.
- Kutsky, R.J. 1973. *Hand Book of Vitamins and Hormones.* Van Nostrand Rain hold, New York.
- Labonne, M., D. B. Othman and J. M. Luck, 2001. Lead isotopes in muscles as tracers of metal sources and water movements in a Lagoon (Thau Basin, S. France). *Chem. Geology.* 181 : 181-191.
- Lowry, O.M., Rosenbrough, N.J., Farr, A.C. and Randall, R.F. 1951. Protein estimation with Folin Phenol reagent. J. Biol. Chem. 193 : 265-275.
- Mahajan, A. Y. and Zhambre, S. P. 2001. Ascorbate effect on CuSO<sub>4</sub> and HgCl<sub>2</sub> induced alteration of protein levels in fresh water bivalve *Corbiculastriatella*. *Asian. J. Micro. Biotech and Environ. Sci.* 3(1-2) : 95-100.
- Nandurkar, H. P. and Zambare, S. P. 2010. Effect of broad-spectrum antibiotics on oxygen consumption rate of freshwater bivalve, *Parreysia cylindrica* (Annandale and Prashad). *J. Env. Bio. Sci.* 24(2): 183-187.
- Niyogi, S. and Wood, C.M. 2004. Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. *Environmental Science and Technology*. 38 : 6177-6192.
- Norkko, J. and Thrush, S.F. 2006. Ecophysiology in environmental impact assessment: implications of spatial differences in seasonal variability of bivalve condition. *Marine Ecology Progress Series*. 326 : 175-186.
- Nwani, C.D., Nwachi, D.A., Ogokwu, O.I., Ude, E.F. and Odoh, G.E. 2010. Heavy metalsin fish species from lotic freshwater ecosystem at Afikpo, Nigeria. *J. Environ. Biol.* 31(5) : 595-601.
- Pandey, N., Meena, R.M., Rai, S.K. and Rai, S.P. 2011. Medicinal plants derived nutraceuticals: a reemerging health aid. *Int J Pharm Biosci.* 2 : 419-441.
- Roe, J.H. 1967. *Methods of Biochemical Analysis.* 5, (Eds. By Glick Inter Science, NewYork), 5: 44-45.
- Rahane, B., Bhalla, R.and Waykar B. 2016. Bio accumulation of heavy metals in three freshwater bivalve species from Girna reservoir, Nasik (M.S.) *The Bioscan.* 11(1): 27-31.
- Rahane, B. and Bhalla, R. 2018 a. Toxic effect of heavy metals on DNA, RNA and ascorbic acid content in soft tissues of the fresh water bivalve *Lamellidens*

*corrianus* from different reservoirs of Nashik district (M.S.) *International Journal of Current Research in Life Sciences.* 07, (05): 2101-2105.

- Rahane, B. and Bhalla, R. 2018 b. Toxicant stress on protein and ascorbic acid contents in different tissues of fresh water bivalve *Parreysia cylindrica* from different reservoirs of Nashik District, India. *Ecology, Environment and Conservation.* 24 (2): 856-860.
- Salaskar, G.M. and Nayak, V. N. 2011. Nutritional quality of bivalve, *Crassostrea madrasensis* and *Perna viridis* in the Kali estuary, Karnataka, India. *Recent Research in Science and Technology* 3(4) : 6-11.
- Singh, V., Bhatt, I., Aggarwal, A., Tripathi, B., Munjal, A. and Sharma, V. 2010. Proline improves copper tolerance in chickpea (*Cicer arientinum*). *Protoplasma.* 245 : 173-181.
- Thenmozhi, C., V. Vignesh, R. Thirumurugan, S. Arun. 2011. Impacts of malathion on mortality and biochemical changes of freshwater fish *Labeorohita*. *Iran. J. Environ. Health. Sci. Eng.* 8 (4) : 387-394.
- Verlecar, X.N., Jena, K.B., Chainy, G.B.N. 2007. Biochemical markers of oxidative stress in *Perna*

*viridis* exposed to mercury and temperature, *Chemico-Biological Interactions.* 167: 219- 226.

- Volkin, E. and Cohn, W. E. 1954. *Methods of Biochemical Analysis* (Ed. Click, D) Vol. 1: 287, Wiley (Inter science) New York.
- Waykar, B. and Lomte, V. S. 2001. Total protein alteration in different tissues of fresh water bivalve, *Parreysia cylindrica* after cypermethrin exposure. *Ecol. Env. and Cons.* 7(4): 465-469.
- Waykar, B. 2006. Role of L-ascorbic acid in the protection of thehepatopancreas of the fresh water bivalves, *Parreysia cylindrica. Nat. J. Life Sciences.* 3(supp): 501-508.
- Waykar, B. and Pulate, P. 2012. Ameliorating effect of Lascorbic acid on profenofos induced alterations in the protein contents of the freshwater bivalve, *Lamellidens marginalis* (Lamarck). *The Bioscan*. 7(1): 35-38.
- Zahran, M.M., Abdel-Aziz, K.B., Abdel-Raof, A.andNahas, E.M. 2005. The effect of subacute doses of organo phosphorous insecticide, nuvacron, on the biochemical and cytogenetic parameters of mice and their embryos. *Research Journal of Agriculture and Biological Sciences*.1 (3) : 277 - 283.