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RESEARCH ARTICLE

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.)

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ABSTRACT

The present study investigates the heavy metals concentrations in surface water and soft body tissues of the freshwater bivalve *lamellidens corrianus* collected from Girna, Ozarkhed, Chankapur and Gangapur reservoirs of Nasik district during summer, monsoon and winter seasons. The mean values of heavy metals Zn, Cu, Pb and Cd concentrations in surface water were highest at Girna reservoir and lowest at Gangapur reservoir. Therefore, it was concluded that Girna reservoir was more polluted than other studied reservoirs. The biochemical components DNA, RNA and ascorbic acid, were estimated from soft tissues of the freshwater bivalve *lamellidens corrianus.* The results showed lowest DNA, RNA and ascorbic acid contents in soft body tissues of bivalve species sampled from Girna reservoir while highest DNA, RNA and ascorbic acid contents were observed in soft body tissues of bivalve species sampled from Gangapur reservoir. This indicated that bivalve species inhabiting at Girna reservoir are under more environmental stress than bivalve species inhabiting at Gangapur reservoir.

Key words: Lamellidens corrianus, Heavy Metals, DNA, RNA, Ascorbic Acid, Reservoirs.

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INTRODUCTION

The most important metals from the point of view of water pollution are As, Zn, Cu, Pb Cd, Hg, Ni and Cr (Li *et al*., 2002). Consumption of aquatic food highly contaminated with heavy metals may form a significant pathway to metal contamination in the human being and creating public health problems wherever man is involved in the food chain (Yigit and Altindag, 2006; Sarabjeet and Dinesh, 2007; Medeiros *et al.,* 2012). Deoxyribose nucleic acid contents can be the index of capacity of an organism for protein synthesis in different stress conditions affected by heavy metals or any toxic metals or pesticides. DNA damage results from exposure to many contaminants and is widely used as an indicator or biomarker of biological effects (van der Oost *et al.,* 2003). RNA is an important molecule with long chains of nucleotides. Alterations in RNA content due to heavy metal exposure was reported by several researchers (Gulbhile, 2006; Nawale, 2008; Srivastava and Verma, 2009; Andhale and Zambare, 2011).Ascorbic acid is important micronutrient, which functions as a factor in several metabolic reactions (Kaya, 2003).

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Antioxidant property of ascorbic acid helps to prevent free radical formation from toxic water-soluble molecules which may cause cellular injuries and diseases.

MATERIALS AND METHODS

Four reservoirs of Nasik district were selected for the study. The analysis of metal concentrations in surface water and different body tissue of bivalve was carried out by using Atomic Absorption Spectrophotometer (AAS) (Thermo Scientific, U. K. make, Solaar A series model). The DNA, RNA and ascorbic acid contents were determined from soft body tissues like mantle, gills, digestive glands and whole soft body tissues of *Lamellidens corrianus*, collectd seasonally during November 2010 to October 2011 from four water reservoirs of Nashik district. 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 Volk in and Cohn (1954).Estimation of ascorbic acid was carried out by the method of Roe (1967). Results are expressed as mean ± standard deviation (S.D.). The ANOVA test was used in order to access whether biochemical constituents are varied significantly between the reservoirs, seasons and bivalve species.

The probabilities less than 0.05 ($p<0.05$) were considered statistically significant. All statistical calculations were performed with SPSS 21.0 version.

RESULTS

Heavy metal concentrations in surface water of four reservoirs of Nasik district

The heavy metals Zn, Cu, Pb and Cd concentrations were determined seasonally in surface water samples collected from Girna, Ozarkhed, Chankapur and Gangapur reservoirs of Nasik district and obtained results were summarized in Table.1.

Heavy metal accumulationS in bivalve sampled from four reservoirs of Nasik district.

The heavy metals Zn, Cu, Pb and Cd concentrations were determined from whole soft body tissues of freshwater bivalve, *Lamellidnes corrianus*, sampled from Girna, Ozarkhed, Chankapur and Gangapur reservoirs of Nasik district during summer, monsoon and winter seasons and obtained results were presented in Table 2. Overall results indicate that the mean concentrations of Zn, Cu, Pb and Cd were highest in thewater samples collected from Girna reservoir than Ozarkhed, Chankapur and Gangapur reservoirs and lowest at Gangapur reservoir than other studied reservoirs.

Table 1. Seasonal variations of heavy metal concentrations (mg/l) in surface waterof different reservoirsof Nasik district

Reservoir	Seasons	Zn	Cu	Pb	Cd
Girna **reservoir**	Summer	0.1442±0.0007	0.0261±0.0005	0.0338±0.0005	0.0094±0.0003
	Monsoon	0.0854±0.0005	0.0172±0.0003	0.0282±0.0003	0.0078±0.0001
	Winter	0.1137±0.0006	0.0184±0.0004	0.0293±0.0004	0.0083±0.0002
Ozarkhed **reservoir**	Summer	0.1298±0.0007	0.0237±0.0002	0.0296±0.0002	0.0082±0.0003
	Monsoon	0.0723±0.0004	0.0153±0.0003	0.0253±0.0003	0.0068±0.0001
	Winter	0.1023±0.0005	0.0162±0.0004	0.0267±0.0004	0.0074±0.0002
Chankapur **reservoir**	Summer	0.1167±0.0007	0.0208±0.0005	0.0279±0.0005	0.0077±0.0003
	Monsoon	0.0658±0.0004	0.0144±0.0003	0.0227±0.0003	0.0062±0.0001
	Winter	0.0925±0.0006	0.0153±0.0004	0.0241±0.0004	0.0072±0.0002
Gangapur **reservoir**	Summer	0.1008±0.0005	0.0193±0.0005	0.0250±0.0002	0.0070±0.0003
	Monsoon	0.0573±0.0004	0.0128±0.0003	0.0207±0.0003	0.0054±0.0001
	Winter	0.0812±0.0006	0.0145±0.0004	0.0228±0.0005	0.0068±0.0002
WHO standard,1993 mg/L		03	02	0.01	0.003

± indicate standard deviation

Table 2. Seasonal variations in heavy metal concentrations (µg/g drytissue weight), in the fresh water bivalve Lamellidens corrianus from different reservoirs of Nasik district

Parameters	Seasons	Zn	Cu	Pb	Cd
Girna reservoir	Summer	432.08± 5.37	112.26±1.63	129.72±2.24	20.16±0.78
	Monsoon	293.68± 4.76	91.37 ±1.07	93.68±1.09	13.07±0.68
	Winter	322.76±4.94	103.71±1.16	107.64±1.63	15.73±0.78
Ozarkhed reservoir	Summer	405.63±5.42	103.03±2.47	105.03±1.87	14.68±1.37
	Monsoon	255.07±3.62	82.91±1.29	82.91±1.15	08.32±1.08
	Winter	291.44±4.56	92.11±1.58	94.11±1.39	10.93±1.25
Chankapur reservoir	Summer	374.64±5.08	97.52±1.83	99.52±2.07	13.86±1.15
	Monsoon	230.07±3.28	76.82±1.24	76.82±1.12	07.73±0.54
	Winter	272.42±4.13	88.17±1.65	88.17±1.45	9.38±0.67
Gangapur reservoir	Summer	353.34±4.93	91.19±1.83	97.63±2.33	11.78±1.24
	Monsoon	213.24±3.64	64.56±1.65	64.56±2.01	05.92±0.87
	Winter	248.61±3.92	78.71±1.24	82.35±1.92	07.88±0.95

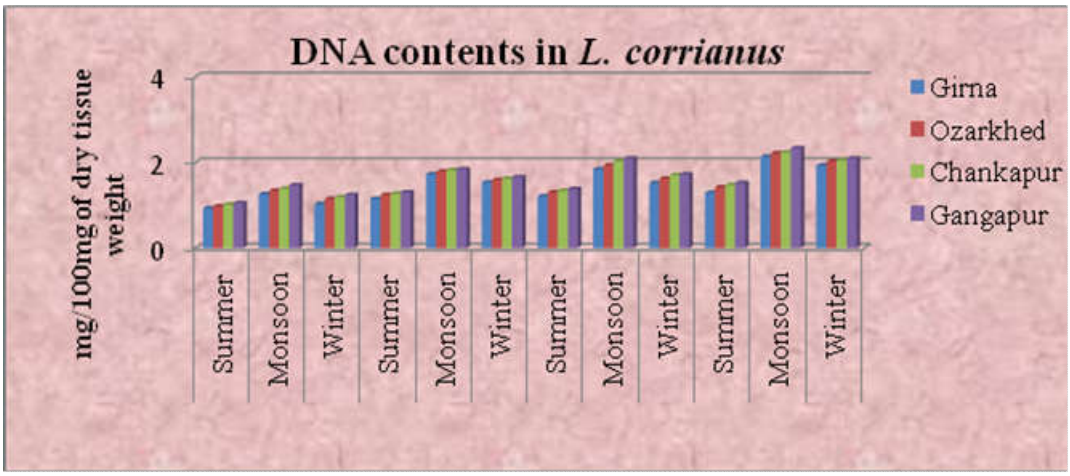

Fig-1. .Profile of DNA contents in different tissues of fresh water bivalve *Lamellidenscorrianus* from different reservoirs of Nasik district (Values are in mg/100mg of dry tissue weight)

Table 4: Profile of RNA contents in different soft body tissues of freshwater bivalve *Lamellidens corrianus* from different reservoirs of Nasik district (Values are in mg/100mg dry tissue weight).

Name of reservoir	Mantle			Gills			Digestive glands			Whole soft body tissue		
	Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter
Girna reservoir	3.01±0.007	4.21±0.19	3.32±018	5.12±0.23	6.03±0.32	5.72±0.25	6.42±0.18	8.51±0.32	7.94±0.30	4.68±0.15	6.59±0.25	5.89±0.29
Ozarkhed reservoir	3.14±0.15	4.32±0.21	3.43±0.26	5.29±0.21	6.15±0.23	5.83±0.28	6.54±0.22	8.64±0.35	8.09±0.35	4.85±0.23	6.74±0.34	5.96±0.18
Chankapur reservoir	3.19±0.08	4.42±0.31	3.49±0.12	5.34±0.18	6.19±0.28	5.91±0.25	6.62±0.19	8.71±0.41	8.13±0.24	4.91±0.31	6.81±0.41	6.02±0.16
Gangapur reservoir	3.28±0.12	4.51±0.33	3.54±0.15	5.41±0.23	6.26±0.24	6.07±0.29	6.69±0.15	8.75±0.26	8.16±0.35	4.97±0.28	6.88±0.37	6.04±0.13

± indicate standard deviation

Table 5. Profile of Ascorbic acid contents in different soft body tissues of freshwater bivalve *Lamellidens corrianus* from different reservoirs of Nasik district (Values are in mg/100mg dry tissue weight)

Name of reservoir	Mantle			Gills			Digestive glands			Whole soft body tissue		
	Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win
Girna reservoir	0.627±0.009	0.934±0.018	0.835±0.018	0.767±0.0.18	1.108±0.026	1.002±0.021	0.876±0.018	1.307±0.0.29	1.093±.0.022	0.714±0.010	1.103±0.025	0.926±0.018
Ozarkhed reservoir	0.662±0.016	0.957±0.013	0.869±0.012	0.795±0.023	1.143±0.018	1.048±0.017	0.897±0.019	1.364±0.026	1.154±0.014	0.741±0.009	1.135±0.021	0.987±0.014
Chankapur reservoir	0.681±0.014	0.974±0.019	0.893±0.016	0.819±0.017	1.154±0.016	1.103±0.019	0.943±0.014	1.394±0.022	1.165±0.018	0.785±0.014	1.146±0.018	1.007±0.010
Gangapur reservoir	0.694±0.012	0.985±0.012	0.903±0.010	0.848±0.015	1.187±0.012	1.109±0.028	0.968±0.016	1.397±0.019	1.193±0.023	0.797±0.016	1.164±0.015	1.034±0.016

± indicate standard deviation

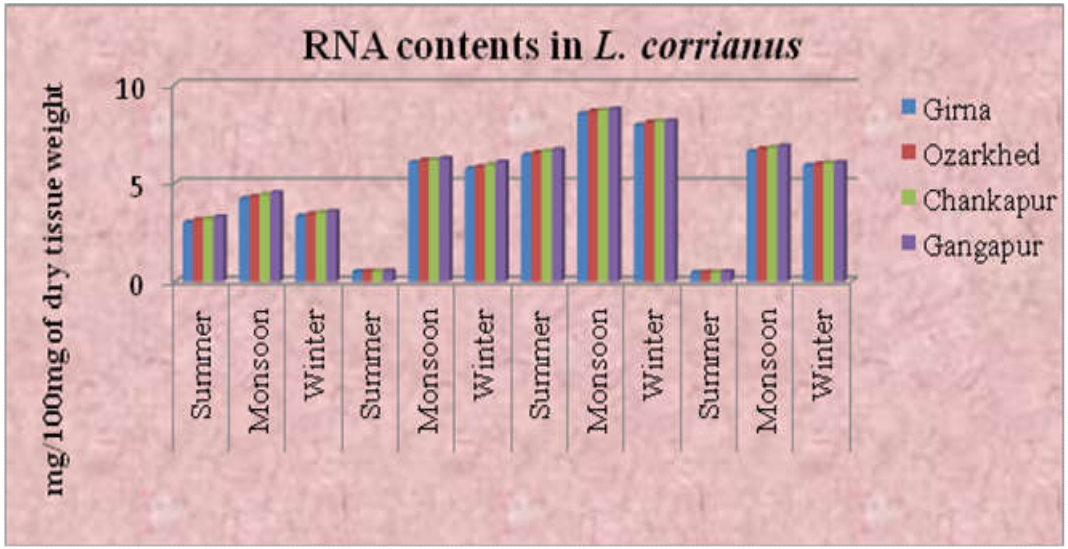

Fig-2. .Profile of RNA contents in different tissues of fresh water bivalve *Lamellidenscorrianus* from different reservoirs of Nasik district (Values are in mg/100mg of dry tissue weight)

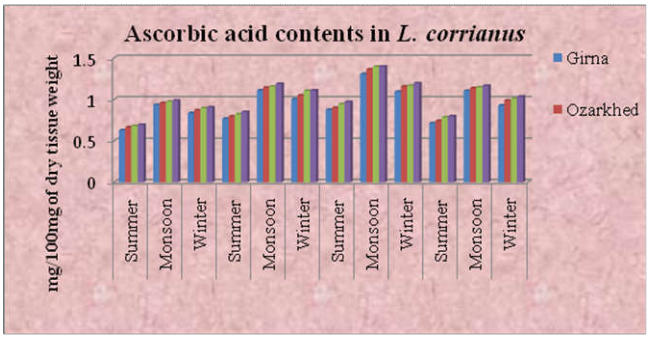

Fig-3.Profile of Ascorbic acid contents in different tissues of fresh water bivalve *Lamellidenscorrianus* from different reservoirs of Nasik district (Values are in mg/100mg of dry tissue weight).

It was observed that the mean concentrations of Zn, Cu, Pb and Cd were highest in summer season and lowest in monsoon season in bivalve species, *Lamellidens corrianus*, sampled from four studied reservoirs. The results of ANOVA test indicate that the difference between the mean values of Zn, Cu, Pb and Cd varied significantly between reservoirs, seasons and bivalve species ($P<0.05$).

Biochemical Study

The freshwater bivalve, *Lamellidens corrianus* were collected during summer, monsoon and winter seasons from Girna, Ozarkhed, Chankapur and Gangapur reservoirs of Nashik district. The biochemical constituent like DNA, RNA and ascorbic acid were determined from soft body tissues like mantle, gill, digestive gland and whole soft body tissues and results are presented in the Table3 to 5 and Fig.1 to 3.

DISCUSSION

The obtained results demonstrated that the mean concentrations of heavy metals Zn, Cu, Pb and Cd were highest in surface water sampled from Girna reservoir. The Ozarkhed reservoir ranked second, Chankapur reservoir ranked third in accumulation of metal in water, while the Gangapur reservoir was the less polluted reservoir. Abaychi and DouAbul (1985) reported that treated and untreated municipal, industrial wastes, agricultural run-off contribute to heavy metal Cu, Pb, Cd and Zn pollution source.

Deepali and Gangwar (2010) reported that the textile industries effluent contains higher concentrations of Cu, Pb and Cd. Lokhande et.al (2011) reported that dyes, paints, textile industries are the major industries contributing to the Cd, Pb, and Cu pollution in the aquatic environment. Jaishree and Khan (2014) reported that waste water effluents from textile dyeing and printing industries contains dyes, bleaching agents, salts, acids and heavy metals like Cr, Cu, Pb, and Zn. Udayakumar (2011) reported seasonal variations in metal concentration copper (Cu), lead (Pb), chromium (Cr), nickel (Ni), zinc (Zn), cadmium (Cd) and mercury (Hg) in surface waters of southwest coast of India. Virha *et al.,* (2011) reported seasonal variation in heavy metals concentration in water of upper Lake of Bhopal. Further results also showed the highest concentrations of heavy metals Zn, Cu, Pb and Cd in surface water sampled from Girna reservoir than Ozarkhed, Chankapur and Gangapur reservoirs and lowest concentration of heavy metals Zn, Cu, Pb and Cd in surface water collected from Gangapur reservoir than other three studied reservoirs.

Therefore, this study indicate that Girna reservoir is more polluted than Ozarkhed, Chankapur and Gangapur reservoirs and Gangapur reservoir is less polluted than Girna, Ozarkhed and Chankapur reservoirs. The low level of ascorbic acid might be due to its involvement in detoxification and repairing of injuries in tissues and to cope up against the toxic stress caused by pollutants. This also suggests the increased demand of energy being provided by utilization of ascorbic acid in responses to toxic stress. Gulbhile (2006) reported a decrease in the ascorbic acid content after acute exposure to mercuric chloride and sodium arsenate in freshwater bivalve, *Lamellidens corrianus*. Nawale (2008) reported a decrease in ascorbic acid content in freshwater bivalve, *Lamellidens corrianus* after chronic exposure to lead nitrate and sodium arsenate.

DNA content is significantly influenced due to the stress condition caused by exposure of pollutants. The metals generate reactive oxygen species (ROS) and other reactive intermediates or react directly with DNA (O'Brien *et al.,* 2003) and cause damage to DNA through inhibition of DNA repair enzymes (Hartwig *et al.,* 2002), or by binding to histone protein (Bal *et al.,* 2000). Bioaccumulated metal induces oxidative stress that leads damage to DNA (Leonard *et al.,* 2004). Nwani *et al.,* (2010) demonstrated DNA damage after treatment with carbosulfan in freshwater fish, *Channa punctatus.* The low level of RNA in soft body tissues of bivalves might be due to damage in DNA, poor rate of synthesis of enzymes necessary for transcription or increased catabolism of RNA due to their abnormalities on binding to abnormal. The cellular degradation, rapid histolysis and decreased rate of protein synthesis are the possible reasons (Andhale and Zambare 2011). Several reports are available on the reduction in DNA and RNA levels on exposure to different toxicants. 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.,* (2001) reported toxicants induced DNA damage in freshwater fish, *Channa punctatus*.

Conclusion

Therefore, results indicate that surface water of Girna, Ozarkhed Chankapur and Gangapur reservoirs were polluted by heavy metals. The results showed lowest DNA, RNA and ascorbic acid contents in soft body tissues of bivalve species sampled from Girna reservoir while highest DNA, RNA and ascorbic acid contents were observed in soft body tissues of bivalve species sampled from Gangapur reservoir.This indicated that bivalve species inhabiting at Girna reservoir are under more environmental stress than bivalve species inhabiting at Gangapur reservoir.

These results also clearly indicate that Girna reservoir was more polluted while Gangapur reservoir was less polluted. Therefore, it is suggested that there is need of treating water by central water treatment plant and removing heavy metals before utilizing this water for crop and drinking purpose. Heavy consumption of other aquatic animals from these four reservoirs by humans, as in the current situation, is therefore, at a risk of health implications. Hence it is recommended that regular monitoring is needed to maintain water quality.

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