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Protein, Ascorbic Acid and Antioxidative Enzymes Alterations In The Digestive Gland of Lamellidenscorrianus Due to Heavy Metals from Different Reservoirs of Nashik District. (M.S.)

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ABSTRACT

The heavy metals Zn, Cu, Pb and Cd concentrations were determined in surface water and the freshwater bivalve lamellidens corrianus were collected from Girna, Ozarkhed, Chankapur and Gangapur reservoirs of Nasik district during summer, monsoon and winter seasons.The biochemical components proteins, ascorbic acid and oxidative stress indicator parameters like activity of antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), the levels of antioxidant scavenger molecules, reduced glutathione(GSH) and lipid peroxidation (LPO) were estimated from digestive glands of the freshwater bivalve lamellidenscorrianus.The results demonstrate that the level of proteins, ascorbic acid, LPO and activity of GST were lowest and activity of antioxidant enzyme CAT, GPx and SOD were highest at Gangapur reservoir and lowest at Girna reservoir. The results also indicates that the level of LPO and activity of GST were lowest and CAT, GPxand SOD activity were highest in monsoon, while level of LPO and activity of GST were highest and CAT, GPx and SOD activity were lowest in summer season at four reservoirs in digestive glands oflamellidenscorrianus. 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.

Keywords: Lamellidenscorrianus,heavy metals, proteins, ascorbic acid, antioxidant enzymes.

I. INTRODUCTION

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 (Otitoloju and Don-Pedro, 2004; Lodhi et. al., 2006; Yigit and Altindag, 2006; Sarabject and Dinesh, 2007; Medeiros et al., 2012).The toxicant bioaccumulation became a topic of public and scientific concern early in the 1950s (Barron, 2003). Heavy metal pollution poses a great potential threat to the environment and human health.A wide range of metal pollution or stresses are also responsible for the secretion or suppression of the proteins (Iwata et al., 1998 and Kohler et al., 2001) in the body of organism. Ascorbic acid is well known to inhibit oxidative damage against metal toxicity (Houston and Johnson, 2000; Rao et al., 2001; Nandi et al., 2005). Ascorbic acid helps to maintain the oxidation-reduction potential of the cell at the stabilized level. Antioxidant property of ascorbic acid helps to prevent free radical formation from toxic water-soluble molecules which may cause cellular injuries and diseases.The study of antioxidant enzymes in conjunction with trace metal body burden contribute to a more comprehensive picture of environmental pollution and biological responses in bivalves representing useful reference value for future heavy metal pollution assessment. Several studies reported that accumulated heavy metal stress causes biochemical alterations in organism (Verlecar et al., 2008; Zhanget al., 2010; Rajkumar and Milton, 2011).

II. METHODS AND MATERIAL

Four reservoirs of Nasik district were selected for the study. The digestive glands of five animals of lamellidens corrianus, species was collected seasonally during November 2010 to October 2011 from four water reservoirs of Nashik district.Protein content of the tissues was estimated by method of Lowry's (Lowry et al., 1951). Estimation of ascorbic acid was carried out by the method of Roe (1967). Lipid peroxidation (LPO) was determined by the method of Oshkawa et al., (1979). Glutathione-S-transferees (GST) activity was assessed by the method of Habig et al., (1974). The amount of reduced glutathione (GSH) in the samples was estimated by the method of Boyne and Ellman (1972). Superoxide dismutase (SOD) activity was estimated by the method of Beauchamp and Fridovich (1973). Catalase (CAT) is measured using hydrogen peroxide as a substrate by (Aebi, 1984). Glutathione peroxidase (GPx) was assayed according to the procedure of Rotruck et al., (1973) with some modifications. 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.

III. RESULTS AND DISCUSSION

To monitor the heavy metal pollution of Girna, Ozarkhed, Chankapur and Ganapur reservoirs of Nasik district, biochemical components, Proteins, Ascorbic acid and the activity of antioxidant enzymes (SOD, CAT, GPx and GST) and level of GSH and LPOwere measured in digestive glands of the bivalve species, Lamellidens corrianus, collected during three seasons. The obtained results are presented in table nos. 1 to 4.The knowledge of these biomarkers will provide information on metal pollution in the reservoirs. In the present investigation the digestive glands were selected for the study because in bivalve the digestive glands are the main site of metal accumulation as it contains higher level of metallothenin (Pipe et al., 1999; Canesi et al., 2008; Waykar and Shinde, 2011; Deshmukh, 2013). In the present investigation the biochemical constituents like protein, ascorbic acid contents were determined from soft body tissues like mantle, gills, digestive glands and whole soft body tissues of bivalve species, Lamellidenscorrianusinhabiting the four reservoirs of Nasik district during three seasons.

In the presence of reactive oxygen species (ROS), proteins can be damaged by oxidative attack, results in site-specific amino acid modifications, fragmentation of the peptide chain, and aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis (Grune, 2000). 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 (Vincent et al., 1995; Waykar and Lomte, 2001a). Pottinger et al., (2002) reported that at high pollution stress, protein synthesis can be suppressed representing disturbance of regular metabolic processes.

The results showed low level of ascorbic acid contents in different soft body tissues of bivalves collected from Girna reservoir than other three studied reservoirs, might be due to bivalve species inhabiting at Girna reservoir were exposed to higher level of pollutant, the contaminants may exert stress on bivalves.

Number of researchers reported that due to toxicant stress ascorbic acid content was decreased. Nawale (2008) reported a decrease in ascorbic acid content in freshwater bivalve, Lamellidenscorrianus after chronic exposure to lead nitrate and sodium arsenate.

In the present seasonal study, the lowest protein, ascorbic acid contents were observed in different soft body tissues of bivalve sampled during summer season, might be due to bivalves were exposed to higher level of pollutant in summer than winter and monsoon seasons.Digestive glands often show higher level of antioxidant enzymes (Irato et.al., 2003). In the present investigation obtained results showed the highest level of lipid peroxidation and glutathione-S-transferase activity and lowest activity of superoxide dismutase, catalase and glutathione peroxidase and low level of reduced glutathione (GSH) in the digestive glands of freshwater bivalve Lamellidens corrianuscollected from Girna reservoir than other three studied reservoirs. On the other hand results showed the lowest level of lipid peroxidation and glutathione-S-transferase activity and highest activity of superoxide dismutase, catalase and glutathione peroxidase and level of reduced glutathione (GSH) in the digestive glands of bivalve species collected from Gangapur reservoir than other three studied reservoirs. Rajkumar and Milton (2011) reported increase of lipid peroxidation in P. viridis along with increase in concentration of cadmium, copper, lead and zinc in short-term chronic toxicity test.

In the present investigation the highest activity of glutathione-S-transferase (GST) was observed in digestive glands of three bivalve species collected from Girna reservoir than other three reservoirs might be due to bivalve species were exposed to higher level of pollutants than other three reservoirs. Higher GST activity at Girna reservoir in the digestive glands of the freshwater bivalve might be related to the capacity of the digestive glands to metabolize xenobiotics, eliminate waste products (Gamble et al., 1995) and it also suggests the protective action against reactive oxygen radicals. Increase of GST enzyme activity indicating activation of detoxification mechanism in the digestive glands could be a good indicator of pollutant exposure. Increase of GST activity can therefore be due to increased detoxification of hydroperoxides. Bouraoui et al., (2009) reported a parallel increase in GST activities as well as in LPO levels in H. diversicolor exposed to a mixture of BaP and Cu (1 μM) for a short-period.

It was observed that bivalve species collected from Girna reservoir showed low level of GSH in digestive glands than other three studied reservoirs, this might be related to the bioaccumulated level of heavy metals in bivalve species. The results indicate that bivalve species inhabiting in environments with higher level of metals. Dafre et al., (2004) observed decreased GSH level in the mussel Perna perna, after exposure to lead. Nedjoud et al., (2009) also reported a decrease in GSH in H. aspera after exposure to high concentrations of metalicdust.The antioxidant defense enzyme system comprises several enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx). Many of these antioxidants interact in a concerted manner to eliminate reactive oxygen species and prevent damage to cellular components. These enzymes activities can be altered by reactive oxygen species (ROS) and therefore they may represent indicators of oxidative stress (Pavlovic et al., 2004; Valavanidis et al., 2006). Altered antioxidant enzyme activities are frequently used as indicators of oxidative stress (Cargnelutti et al., 2006; Banni et al., 2008; Bocchetti et al., 2008 Zhou et al., 2008). In the present investigation it was observed that, bivalve specis collected from Girna reservoir showed the lowest activity of SOD, CAT, and GPx than bivalves collected from other three studied reservoirs, might be in response to bioaccumulated levels of metal in bivalves. Numerous researchers showed that the toxicants induces the LPO formation, increases the activity of GST, decrease the GSH level and alter the antioxidant enzyme (SOD, CAT and GPx) activities in mollusk (Vasseur and Leguille, 2003; Box, et.al.2007; Osman et.al.2007; Deshmukh, 2013)

IV. CONCLUSION

In the present study obtained results showed the low level of proteins, ascorbic acid, highest level of lipid peroxidation and glutathione-S-transferase activity and lowest activity of antioxidants enzymes superoxide dismutase, catalase and glutathione peroxidase, low level of reduced glutathionein digestive glands of freshwater bivalve species, Lamellidens corrianus collected from Girna reservoir than other three studied reservoirs. Thus results clearly indicated that Girna reservoir was more polluted by heavy metals than other three studied reservoirs. The results demonstrate that bivalves living at Girna reservoir were more under environmental stress than bivalves living at other three studied reservoirs. In the present study results also showed the lowest levels of proteins, ascorbic acid, GSH and lowest activity of SOD, CAT and GPxand highest level of LPO and highest activity of GSTin the digestive glands of bivalve species in summer season than monsoon and winter seasons. This indicates that in summer bivalve species wereunder more environmental stress than in winter and monsoon seasons.

Table 1. Seasonal variations in heavy metal concentrations from surface water samples from different reservoirs of Nasik district.

Parameters	Seasons	Zn	Cu	Pb	Cd
Girna	Summer	437.21±5.81	134.27±1.56	110.72±1.95	23.92±0.95
	Monsoon	293.50±3.69	97.98±1.09	92.61±1.07	12.42±0.63
	Winter	329.07±4.73	113.42±1.42	105.73±1.64	16.72±0.79
Ozarkhed	Summer	408.39±5.46	112.51±2.17	104.42±2.42	15.57±1.24
	Monsoon	258.39±4.12	85.32±1.53	81.20±1.82	08.62±0.83
	Winter	293.65±4.59	98.62±1.90	93.62±2.13	11.40±0.92
Chankapur	Summer	381.32±5.81	108.83±1.94	98.81±1.94	15.12±1.14
	Monsoon	235.16±4.26	80.93±1.08	74.38±1.62	08.16±0.72
	Winter	276.64±4.56	95.84±1.17	85.42±1.87	09.83±0.87
Gangapur	Summer	359.15±5.72	98.26±2.14	95.37±2.42	12.51±0.82
	Monsoon	225.09±5.27	74.42±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±0.74

± indicate standard deviation

Table 2. Profile of Protein contents in different soft body tissues of freshwater bivalve Lamellidenscorrianusfrom different reservoirs of Nashik district (Values are in mg/100mg dry tissue weight).

Reservoir	Mantle			Gills			Digestive glands			Whole soft body tissue		
	Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win
Girna	39.28 ±0.78	50.90 ±1.38	47.51± 1.28	50.43± 1.63	60.11± 1.43	58.05± 1.63	48.52± 1.32	59.57± 2.43	56.10 ±2.34	46.19± 1.93	57.13 ±2.32	54.13± 1.85
Ozarkhed	41.73 ±1.98	52.64± 1.85	49.34± 1.59	51.42± 1.97	62.78± 2.14	60.46± 1.92	49.29± 1.82	61.72± 1.87	58.24± 1.93	48.00± 1.63	59.63 ±2.03	55.82± 1.74
Chankapur	42.08 ±2.04	53.72± 1.87	49.92± 1.68	52.60± 1.24	64.18± 2.05	60.90± 2.42	50.46± 1.93	63.04± 1.73	58.92± 2.04	48.83± 1.58	60.07 ±2.28	56.19± 1.83
Gangapur	43.51 ±0.71	53.98± 1.68	51.38± 1.76	53.81± 1.39	64.89± 1.92	61.00± 2.08	51.72± 1.84	63.91± 1.86	60.13± 2.28	49.47± 1.47	62.01 ±1.96	58.05± 1.77

± indicate standard deviation

Table 3. Profile of Ascorbic acid contents in different soft body tissues of freshwater bivalve Lamellidenscorrianus from different reservoirs of Nasik district (Values are in mg/100mg dry tissue weight).

Reservoir	Mantle			Gills			Digestive glands			Whole soft body tissue		
	Sum	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**
Girna	0.627 ±0.009	0.934 ±0.018	0.835 ±0.018	0.767 ±0.018	1.108 ±0.026	1.002 ±0.021	0.876 ±0.018	1.307 ±0.029	1.093 ±.0.022	0.714 ±0.010	1.103± 0.025	0.926 ±0.018
Ozarkhed	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	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	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

Table 4. Profile of lipid peroxidation level, reduced glutathione level and activity of antioxidant enzymes in the digestive glands of freshwater bivalve, Lamellidenscorrianus from different reservoirs of Nasik district.

Reservoir	**Sampling seasons**	**Lipid Peroxidation (LPO)**(nmol MDAformed / mg protein)	**Glutathione-S-transferase (GST)**(nmol CDNB conjugate formed / min / mg protein	**Reduced glutathione (GSH)** (µM / gm wet tissue)	**Superoxide dismutase (SOD)** (U / mg of protein)	**Catalase (CAT)** (U/mg of protein)	**Glutathione peroxidase (GPx)** (µg of GSH utilized / min/ mg of protein)
Girna	Summer	211.08±3.24	231.57±4.08	6.05±0.53	114.82±2.12	91.32±1.72	35.54±1.88
	Monsoon	142.84±3.05	150.18±2.92	8.79±0.61	156.42±2.82	129.46±2.46	49.75±1.63
	Winter	185.20±2.92	193.48±3.34	7.81±0.59	123.06±2.63	115.39±2.09	45.83±1.55
Ozarkhed	Summer	197.32±2.04	217.73±4.18	7.93±0.62	126.91±2.46	96.38±1.72	36.39±1.21
	Monsoon	128.25±1.93	132.92±2.38	8.64±0.58	172.04±2.58	137.81±2.08	51.04±1.55
	Winter	172.14±1.90	172.38±2.13	8.07±0.71	149.28±2.61	121.08±1.94	48.57±1.36
Chankapur reservoir	Summer	191.48±1.72	208.35±4.35	11.23±0.87	137.47±2.76	101.35±2.34	38.76±1.29
	Monsoon	123.16±2.07	127.11±3.81	12.56±1.14	189.28±3.04	148.17±1.93	54.69±1.57
	Winter	163.40±2.18	165.54±3.72	11.98±1.12	148.61±3.17	123.51±2.05	49.63±1.42
Gangapurreservoir	Summer	179.33±3.05	203.93±4.03	13.27±1.21	145.39±2.90	105.47±2.00	42.32±1.35
	Monsoon	109.52±2.85	112.42±3.35	14.78±1.29	195.72±2.47	154.82±1.83	59.17±1.69
	Winter	158.32±3.04	159.02±3.72	14.09±1.08	157.67±2.18	128.62±1.62	51.08±1.47

± indicates the standard deviation

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