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Effect of Heavy Metals on Antioxidant Biomarker Enzymes and Biochemical Constituents in Different Tissues of *Lamelleidens marginallis* in Different Reservoirs of Nasik District

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ABSTRACT

Heavy metals are known inducers of oxidative stress by directly producing reactive oxygen species (ROS), which leads to formation of LPO and modulate the activities of antioxidant enzymes and causes disturbances in metabolic functions. The modulation of antioxidant enzyme system like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione *S*-transferase (GST) and concentration of reduced glutathione (GSH) are reported. Accumulated heavy metals also cause conflict in metabolic functions, trigger detoxifying enzymes, and antioxidant system damage leads to oxidative stress and causes damage to protein and other biochemical constituents. In the present investigation, the heavy metals Zn, Cu, Pb and Cd were determined in surface water and the freshwater bivalve *Lamellidens marginalis* collected from Girna, Ozarkhed, Chankapur and Gangapur reservoirs of Nasik district during summer, monsoon and winter seasons to study their effect on the activity of antioxidant enzymes and biochemical constituents like protein and ascorbic acid in soft body tissues of the bivalve.

INTRODUCTION

Occurrence of toxic metals in lakes, ponds, dams, ditches and rivers affect the lives of local people who depend upon these water sources for their daily requirements. Consumption of such aquatic food stuff enriched with toxic metals may cause serious health hazards through food-chain magnification. The supply of quality water remains a major challenge for humanity in the twenty-first century (Schwarzenbach et al. 2010). Research has shown that metals have the ability to bioconcentrate in organisms directly from the water and bioaccumulate and biomagnify within food chains, which causes higher trophic organisms to become contaminated with higher concentrations of chemical contaminants than their prey (Hargrave et al. 2000 and Lee et al. 2000, Boran & Altinok 2010, Shariati et al. 2011). Therefore, heavy metal pollution poses a great potential threat to the environment and human health. Thus, there is a need of regular monitoring of them, not only to prevent diseases and hazards, but also to check the water resources from going further polluted.

MATERIALS AND METHODS

Animals were collected in summer, monsoon and winter seasons from different places of four reservoirs during November 2010 to October 2011 and their digestive glands were removed and used for estimation of oxidative stress indicator biomarkers. Lipid peroxidation (LPO) was assayed by the procedure of Ohkawa et al. (1979). Reduced glutathione (GSH) was determined by the procedure described by Boyne & Ellmen (1972). The activity of superoxide dismutase (SOD) was determined by the procedure of Paoletti et al. (1990). Catalase activity (CAT) was determined by method according to Aebi (1974). Glutathione peroxidase (GPx) was assayed according to the method of Rotruck et al. (1973). Glutathione-S-transferase (GST) was assessed by the procedure of Habig et al. (1974). The total proteins and ascorbic acid contents were estimated from different soft body tissues like mantle, gills, digestive glands and whole soft body tissue of the bivalve. The tissues were removed and dried at 70° to 80°C in the oven till the constant weight of dry tissues was obtained. From each powder, protein contents were estimated by Lowry's method (Lowry et al. 1951) by using Bovine Serum Albumin (BSA) as standard. Ascorbic acid contents were estimated by the procedure of Roe (1967) using the hydrazine reagent. Results are expressed as mean ± standard deviation (S.D.).

RESULTS AND DISCUSSION

The concentrations of heavy metals Zn, Cu, Pb and Cd were

determined during three seasons in surface water sampled from the reservoirs Girna, Ozarkhed, Chankapur and Gangapur of Nasik district and the results are presented in Table 1. Higher concentrations of Zn, Cu, Pb and Cd were found in Girna reservoir than the other three reservoirs. Number of investigators have reported that domestic waste and agricultural waste matter act as a major source of cadmium and lead (Aksoy et al. 2005, Huang et al. 2007). In the present study it was observed that Zn, Cu, Pb and Cd concentrations were significantly higher in summer season and lowest in monsoon season in all the reservoirs. Therefore, these studies indicate that Girna reservoir is more polluted and Gangapur reservoir is less polluted than the other reservoirs. Duman et al. (2007) reported seasonal changes in heavy metal concentrations of Sapanca Lake water, Turkey. Rigollet et al. (2004) pointed out that Mn, Zn, Cu and Ni concentrations increased at the end of summer and in autumn at Thau Lagoon.

Oxidative stress as biomarkers: To monitor the heavy metal pollution of reservoirs of Nasik district, the activity of antioxidant enzymes (SOD, CAT, GPx and GST) and level of GSH and LPO were measured in digestive glands of the bivalve species, *Lamellidens marginalis* collected during the three seasons. The results are presented in Table 2 and Figs. 1-6 respectively. Increased lipid peroxide (LPO) is one of the most important contributors to the loss of cell function in oxidative stress conditions (Hermes-Lima et al. 1995). The higher level of LPO revealed that the bivalve species sampled from Girna reservoir was under oxidative stress and indicate the prevalence of free radical reaction in digestive glands, which could be attributed to the low levels of GSH and highest activity of GST, and low activity of antioxidant enzyme SOD, CAT and GPx in digestive glands indicating effect of bioaccumulated metals. Usually the increased LPO level can be linked to decreased antioxidant levels and/or increased ROS production (Faria et al. 2010). In the present investigation, the highest activity of glutathione-S-transferase (GST) was observed in digestive glands of the bivalve species collected from Girna reservoir than the other three reservoirs, which might be due to bivalve species were exposed to higher level of pollutants. The obtained data also revealed higher concentrations of Zn, Cu, Pb and Cd in Girna reservoir and the bivalve species inhabiting here than the other reservoirs. In the bivalve, GST activity is predominantly located in digestive glands (Petushok et al. 2002). 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. Reduced glutathione (GSH) is the major non-protein thiol and is an important endogenous antioxidant which plays a central role in the defence against oxidative damage (Sies 1999). It has been proved that reduced glutathione is one of the most efficient scavengers of ROS arising as by-products of cellular metabolism or during oxidative stress (O'Brien et al. 2001, Tsukamoto et al. 2002, Han et al. 2008). It was observed that the bivalve species collected from Girna reservoir show low level of GSH in digestive glands than the other three reservoirs, which might be related to the bioaccumulated level of heavy metals in bivalve species. The antioxidant defence enzyme system comprises of several enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). In the present investigation, it was observed that the bivalve species collected from Girna reservoir showed the lower activity of SOD, CAT and GPx, than the bivalves collected from the

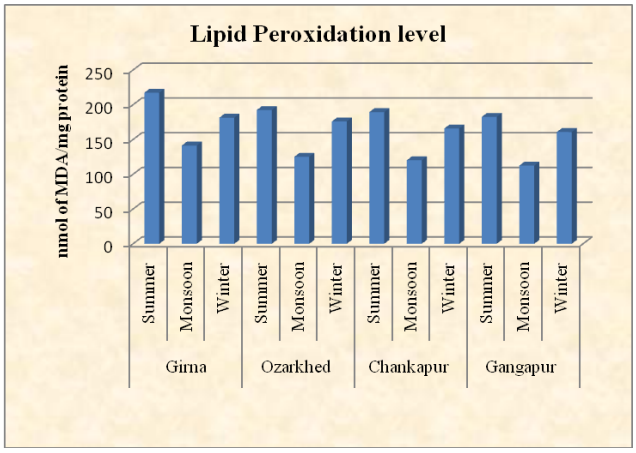

Fig. 1: Profile of lipid peroxidation (LPO) level, in the digestive glands of freshwater bivalve, *Lamellidens marginalis* from different reservoirs of Nasik district.

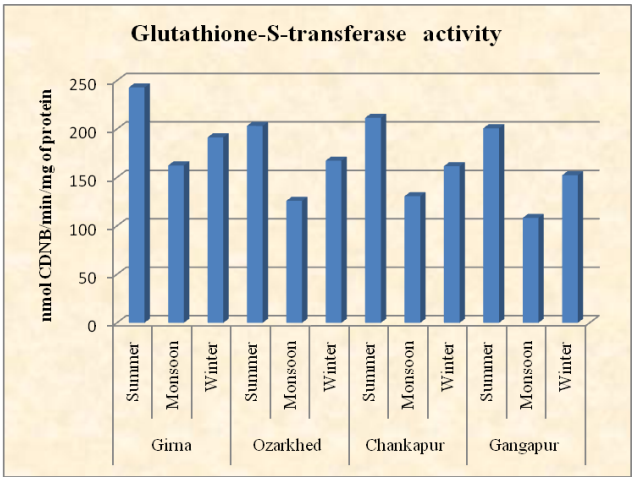

Fig. 2: Profile of glutathione-S-transferase (GST) activity in the digestive glands of freshwater bivalve, *Lamellidens marginalis* from different reservoirs of Nasik district

Table 1: Seasonal variations of heavy metal concentrations (mg/L) in surface water of different reservoirs of Nasik district.

Name of 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, mg/L		03	02	0.01	0.003

± indicate standard deviation

Table 2: Profile of lipid peroxidation level, reduced glutathione level and activity of antioxidant enzymes in the digestive glands of freshwater bivalve, *Lamellidens marginalis* from different reservoirs of Nasik district.

Name of reservoir	Sampling seasons	Lipid peroxidation (LPO)(nmol of MDA formed/ mg protein)	Glutathione-S-transferase (GST) (nmol of 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	217.26±2.81	242.82±4.38	8.05±0.65	118.05±2.14	89.34±1.72	32.63±1.33
	Monsoon	141.29±3.07	162.38±3.29	9.24±0.81	163.02±2.94	132.08±2.44	45.97±1.92
	Winter	181.37±2.82	191.35±3.26	8.93±0.72	128.72±3.05	117.82±2.16	36.12±1.48
Ozarkhed	Summer	192.42±2.01	203.14±4.03	8.42±0.67	125.24±2.63	98.01±1.82	37.24±1.23
	Monsoon	125.08±1.93	125.81±2.72	9.53±0.86	175.02±2.42	140.15±2.37	49.17±1.68
	Winter	175.91±1.68	167.33±3.27	8.97±0.74	146.83±2.18	124.25±2.15	41.64±1.57
Chankapur	Summer	189.37±2.05	211.50±3.81	14.26±1.05	135.28±3.00	98.38±1.92	39.16±1.43
	Monsoon	120.09±2.16	130.62±2.11	15.08±1.16	192.68±2.32	151.26±2.08	48.28±2.15
	Winter	165.82±2.33	161.58±2.43	14.79±1.08	145.95±2.28	119.13±1.89	43.77±2.03
Gangapur	Summer	182.57±1.72	200.71±3.62	14.91±1.17	147.39±2.47	107.21±2.17	44.15±1.82
	Monsoon	112.43±1.89	108.18±2.18	16.18±1.25	192.34±2.38	157.42±2.48	53.49±2.25
	Winter	160.73±1.65	152.19±3.25	15.22±1.12	154.82±2.64	130.91±2.26	47.28±2.07

± indicates the standard deviation

other three reservoirs, which might be due to bioaccumulated levels of metals in bivalves. A deficiency in these cellular defence enzymes might decline the capacity of aquatic organism to neutralize the production of ROS. Numerous researchers have shown that the toxicants induces the LPO formation, increase the activity of GST, decrease the GSH level and alter the antioxidant enzyme (SOD, CAT and GPx) activities in molluscs (Vasseur & Leguille 2003, Box et al. 2007, Osman et al. 2007, Deshmukh 2013). In the present study, results demonstrate the higher level of LPO and activity of GST and lower activity of antioxidant enzymes SOD, CAT and GPx and levels of GSH in summer season than monsoon and winter seasons.

Biochemical study: In the present study, the results given in Table 3 and Figs. 7 and 8 showed the lower level of proteins and ascorbic acid contents in soft body tissues of the bivalve species collected from Girna reservoir than the other three studied reservoirs. This might be due to bivalves inhabiting the Girna reservoir were exposed to higher load of pollutants than the other three reservoirs. The results revealed higher concentration of Zn, Cu, Pb and Cd in surface water and the bivalve species from Girna reservoir than the other three reservoirs. The accumulated heavy metals were able to disturb the natural oxidation/reduction balance in the cells by inducing generation of reactive oxygen species that leads to oxidative stress (Abdullah et al. 2004). Satyaparameshwar et al. (2006) observed decrease in total protein content on exposure to chromium in three different tissue viz. adductor muscles, gills and mantle of freshwater mussel, *Lamellidens marginalis*. Siddiqui et al. (2010) re-

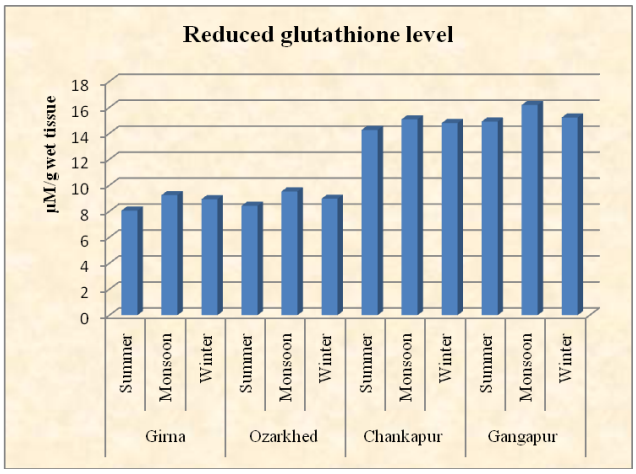

Fig 3: Profile of reduced glutathione (GSH) level, in the digestive glands of freshwater bivalve, *Lamellidens marginalis* from different reservoirs of Nasik district.

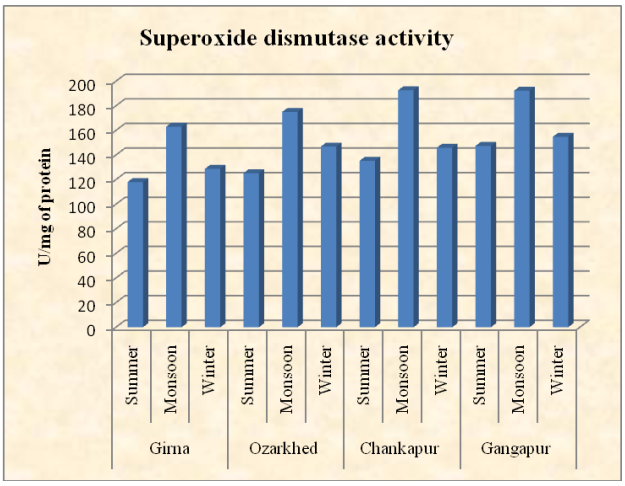

Fig. 4: Profile of superoxide dismutase (SOD) actvity in the digestive glands of freshwater bivalve, *Lamellidens marginalis* from different reservoirs of Nasik district.

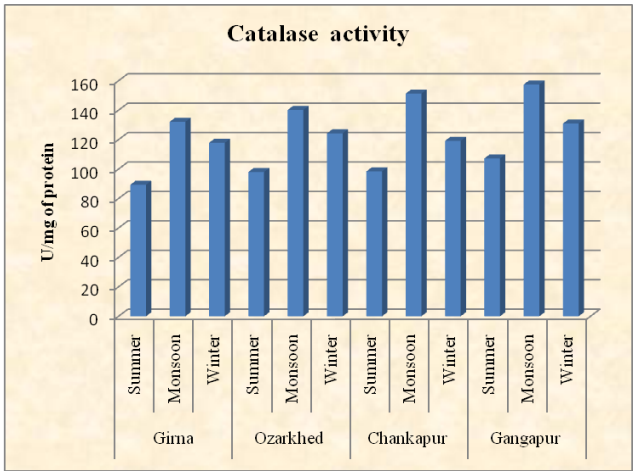

Fig. 5: Profile of catalase (CAT) actvity in the digestive glands of freshwater bivalve, *Lamellidens marginalis* from different reservoirs of Nasik district.

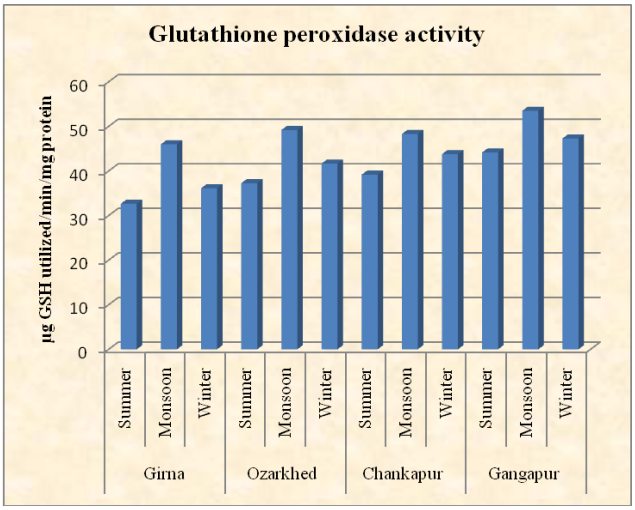

Fig. 6: Profile of glutathione peroxidase(GPx) actvity in the digestive glands of freshwater bivalve, *Lamellidens marginalis* from different reservoirs of Nasik district.

ported depletion of protein in the gills of freshwater crab, *Barytelphusa gureini* when exposed to sub lethal concentration of copper sulphate solution. Decrease in ascorbic acid content indicated its involvement in counteracting oxidative damage. Nawale (2008) reported a decrease in ascorbic acid content in freshwater bivalve, *Lamellidens corrianus* after chronic exposure to lead nitrate and sodium arsenate. Deshmukh (2013) reported that bivalve species inhabiting the higher level polluted site showed low level of ascorbic acid content, while bivalve species inhabiting the low level polluted site showed higher level of ascorbic acid content.

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Table 3: Profile of protein and ascorbic acid contents in different soft body tissues of freshwater bivalve *L. marginallis* from different reservoirs of Nashik district (values are in mg/100mg dry tissue weight).

Parameter	Reservoir	Mantle			Gills			Digestive glands			Whole soft body tissue		
		Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win
Protein	Girna	38.22 ±0.58	49.62 ±1.74	48.09 ±1.55	49.06 ±1.51	61.24 ±1.85	55.28 ±2.01	47.33 ±1.92	60.28 ±1.84	54.05 ±2.18	45.52 ±1.63	58.92 ±1.83	52.19 ±2.12
	Ozar-khed	43.19 ±1.44	52.47 ±0.98	50.07 ±1.72	51.82 ±1.52	63.18 ±1.87	58.38 ±1.46	50.74 ±2.17	62.17 ±1.85	56.98 ±1.49	47.62 ±2.67	60.82 ±2.18	54.92 ±1.64
	Chan-kapur	43.92 ±1.19	53.62 ±127	51.39 ±1.88	52.86 ±2.43	64.99 ±2.11	60.02 ±2.26	50.91 ±1.82	63.14 ±1.74	58.21 ±1.63	47.95 ±2.23	61.29 ±1.93	55.68 ±1.89
	Gang-apur	44.05 ±1.28	54.73 ±1.38	52.48 ±1.18	53.18 ±1.83	66.16 ±1.84	61.14 ±2.14	51.54 ±2.62	64.28 ±1.93	59.32 ±1.82	49.17 ±2.08	63.47 ±2.08	57.06 ±1.44
Ascor-bic acid	Girna	0.615 ±0.017	0.917 ±0.015	0.841 ±0.019	0.774 ±0.009	1.102 ±0.028	0.993 ±0.026	0.884 ±0.013	1.305 ±0.014	1.118 ±0.026	0.725 ±0.012	1.108 ±0.023	0.941 ±0.019
	Ozar-khed	0.642 ±0.009	0.956 ±0.18	0.887 ±0.013	0.803 ±0.017	1.139 ±0.026	1.019 ±0.019	0.938 ±0.015	1.379 ±0.030	1.143 ±0.024	0.759 ±0.017	1.158 ±0.028	0.994 ±0.012
	Chan-kapur	0.667 ±0.012	0.972 ±0.012	0.892 ±0.011	0.829 ±0.016	1.158 ±0.024	1.045 ±0.025	0.957 ±0.017	1.387 ±0.028	1.167 ±0.020	0.792 ±0.016	1.165 ±0.015	1.045 ±0.012
	Gang-apur	0.701 ±0.016	0.981 ±0.017	0.908 ±0.018	0.855 ±0.012	1.203 ±0.019	1.097 ±0.026	0.970 ±0.014	1.403 ±0.029	1.198 ±0.018	0.814 ±0.012	1.172 ±0.011	1.024 ±0.018

± indicate standard deviation

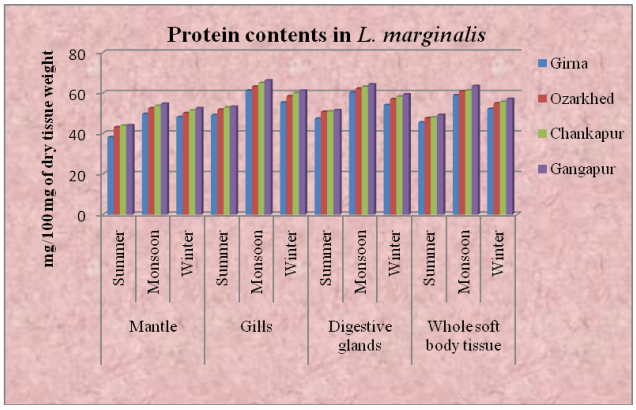

Fig. 7: Profile of protein contents in different tissues of freshwater bivalve *Lamellidens marginalis* from different reservoirs of Nasik district (values are in mg/100mg of dry tissue weight).

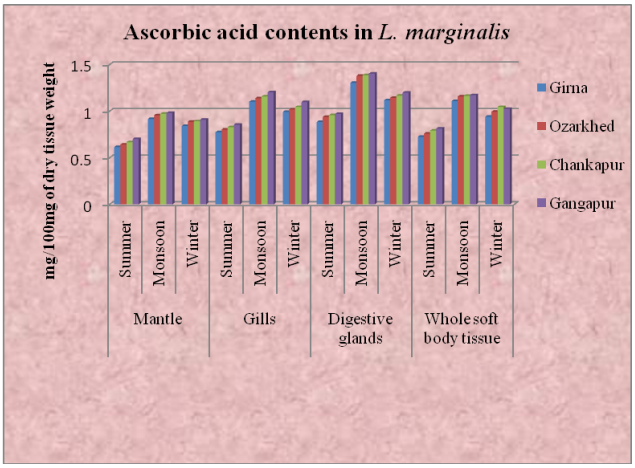

Fig. 8: Profile of ascorbic acid contents in different tissues of freshwater bivalve *Lamellidens marginalis* from different reservoirs of Nasik district (values are in mg/100mg of dry tissue weight).

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