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A study on the toxicity of cadmium on certain aspects of protein metabolism of the freshwater mussel *Lamellidens marginalis* (Lamarck) and freshwater fish *Labeo rohita* (Hamilton)

Venkata Chandrudu M[1], Radhakrishnaiah K[2]
1- Department of Zoology, Rayalaseema University, Kurnool - 518 002. A.P., India
2- Department of Zoology Sri Krishnadevaraya University, S.V. Puram, Anantapur - 515 055 A.P., India
chandramidde1979@gmail.com
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ABSTRACT

The activities of alanine and aspertate aminotransferases (AlAT and AAT) and glutamate dehydrogenase (GDH) and the levels of glutamine are estimated in the organs of mussel and fish exposed to subacute concentration of cadmium (0.7 mg/l) and controls. The AlAT, AAT and GDH activities and glutamine level increased relative to controls in ctenidium, mantle, hepatopancreas and foot of mussel in the order: day 10 > 20 > 30. Whereas in gill, kidney, liver and muscle of fish the activities of AlAT and AAT increased at day 10 and 20 (day 10 > 20) with a decrease at day 30. GDH activity and glutamine levels, however, increased at all the days in the order day 10 > 20 < 30. Increased transamination was observed in the organs of mussel on initial days of exposure, but they reached to normal at day 30. In fish even though an initial increase trans-deamination was observed at day 10, it tried to recover at day 20 but failed to attain recovery on further exposure. Among the organs the degree in all the changes of protein metabolism was more or less insignificant and inconsistent, but in general it was in the order hepatopancreas > ctenidium > foot > mantle in mussel and kidney > liver > gill > muscle in fish. The results indicated that in sublethal cadmium stress the mussel gradually attained normal protein metabolic activity on prolonged exposure to 30 days but the fish could sustain upto day 20 later it became susceptible as indicated by more suppressive changes at day 30.

Keywords: L.marginalis, L.rohita, Transdeamination, glutamate dehydrogenase, glutamine.

1. Introduction

Pollution of an environment is mostly due to man's intervention and his rapid progress in colonization, urbanization, industrialization, agriculture, mining, transportation and chemical technology; there by the marine and freshwater habitats have become the repositories of pollutants released from all those anthropogenic activities. Mining and smelting operations and discharge of most of the industrial wastes into the aquatic environment lead to the accumulation of inorganic pollutants like mercury, cadmium, copper, lead, chromium, iron and zinc in dissolved and suspended forms (Chukwu and Ugbeva, 2003). Heavy metals occur naturally in a fairly low concentrations in aquatic environment added principally through erosion, land drainage and volcanic activity. Eventhough they are the marvelous gifts of nature playing a key role in the great outcome of chemical technology, in recent years high concentrations of heavy metals are entering the aquatic system due to the injudicious and unprogrammed discharge of industrial wastes, agricultural effluents and sewage waters, and indirectly from aerial fallout, bioaccumulation of metals in the eutrophicated sections of

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Yamuna has been well reported by Sharma et al., (2000). Recently, the Ogba and Yangtze rivers which were considered safe for drinking water also contaminated by the heavy metals like Cu, Mn, Zn, Cd, Hg, Cr, Ni and Pb and the fishes in it higher levels of metals bioaccumulated in their tissues reported unsafe for human consumption (Obasohan, 2007; Yi and Zhang, 2012). More than permissible levels of heavy metals were reported in water and sediment, and plankton and fish tissues in lakes and seas (Yigit and Altindag, 2006; Luo et al., 2013). The physico-chemical properties of heavy metals in aquatic systems are the principle factors for their accumulation in animals. Chronic pollution of bottom sediments of water bodies leads to a decrease in the biodiversity of fauna and the development of specific metal tolerant communities (Davyd Kova et al., 2005). Jain (2004) stated that heavy metals are causing greatest threat to the health of Indian aquatic ecosystems due to their toxicity and accumulation behaviour. Many effluents discharged into nearby ponds and drains without any treatment contain highly toxic heavy metals (Mathur et al., 2005). Cadmium is discovered by Fredrich Stromesper in 1817, it is the second member of Group II B triad (Zn, Cd, Hg) in the periodic classification of elements. It has the atomic weight 112.4, atomic number 48, density 8.6, melting point 320.9^0C and boiling point 765^0C. It is a hexagonal crystalline, silver-white malleable metal with stable oxidation state +2. It has a medium class B character compared to zinc and mercury. This character imparts moderate covalency in bonds and high affinity for sulfhydryl groups leading to increased lipid solubility, bioaccumulation and toxicity. The chloride, sulphate and nitrates of cadmium are soluble compounds whereas carbonate and hydroxides are not. it is one of the most toxic and widespread heavy metals, and is a recognized carcinogen in mammals (Pruski and Dixon, 2002). There has been rapid and continuous increase in the worldwide production and use of cadmium since 1925. It is used in a number of industrial processes. Because of its ability to protect iron items rusting, it is used for coating such items by electroplating. Cadmium coated parts for automobiles are most resistant to rust than zinc coated (galvanized) objects. Cadmium sulfide is used as color pigment in plastics and in various types of paints. Cadmium stearate is used as a stabilizer in plastics. Cadmium thus reaches the water bodies prilimirly from the industrial sources such as zinc melting and electroplating, combustion of fuels, plastics, phosphate fertilizers, pesticides, domestic wastes, oil refineries etc.

The study of environmental effects of cadmium is now very timely. Cadmium has become the focus of intense research globally because of its toxicity to humans and terrestrial and aquatic organisms. Cadmium inhalation accounts for 15-50% through the respiratory system and 2-7% is absorbed through gastro-intestinal system. The most target organs are the liver, kidneys, lungs, brain, bones and placenta and exposure to cadmium causes anemia, hepatite, renal and cardiovascular diseases and also some epidemiological and clinical features of itai-itai disease (Nawrot et al., 2010; Aoshima, 2012). After adsorption into the gastro-intestinal tract cadmium is transferred to the liver and kidneys and finally excreted via urine, however, considerable amounts of cadmium accumulates in the gill, liver and kidneys mostly bound to an inducible low molecular weight protein called metallothionein (Bouraoui et al., 2008; Isani et al., 2009; Vergauwen et al., 2013). Cadmium is concentrating more and more in recent years in freshwaters through the disposal of effluent from the industries like zinc smelting, mining and electroplating (Zantopoulos, 1999). The reports available though indicate an imbalance in metabolic homeostasis of aquatic fauna exposed to cadmium (Choi et al., 2007), A comparative study on the toxicity of it at physiological and biochemical levels in freshwater fauna are scanty. As the prime recipients of cadmium contaminated effluents are freshwater bodies, the shellfish and finfish inhabiting in them are first prone to the effects of it, and on consumption of them later biotransformated in human beings. Hence, the author feels necessary to make a comparative study on the effects of cadmium on some aspects of

protein metabolism in a freshwater shellfish, the bivalve *Lamellidens marginalis*, and in a finfish, the teleost *Labeo rohita*, in order to fill the lacuna to a possible extent.

2. Materials and methods

The freshwater mussel *L. marginalis*, weighing 25 g ± 2 g, and the freshwater fish *L. rohita*, weighing 10 g ± 2 g, were collected from the local freshwater canals and lakes, and they were maintained in laboratory in 5' x 3' x 3' cement tanks, thirty in each. Water from the local wells was used for their maintenance. It has temperature 28 ± 1^0C, pH 7 ± 0.1, total hardness 100 ± 5 mg/l $CaCO_3$, Chlorinity 0.08 ± 0.003% and dissolved oxygen 5.8 ± 0.4 mg /l (Sivaramakrishna and Radhakrishnaiah, 2000). The mussels were fed *ad libitum* with freshwater plankton, whereas the fish were fed daily with groundnut cake milled with rice bran (having around 40% protein content). Both the animal groups were adapted to laboratory conditions for ten days prior to the experimentation.

A stock solution of cadmium was prepared by dissolving of 2.74g of cadmium nitrate ($Cd(NO_3)$ $4H_2O$) in one litre of distilled water, that consists of 1g of cadmium. Appropriate amounts of stock solution were taken to obtain the desired concentrations of cadmium. 96h LC_{50}s were determined by exposing the mussel and fish to different concentrations of cadmium (Finney, 1971). Based on the percent and probit mortality curves as well as through Dragstedt and Behren's method, the 96h LC_{50}s obtained to the mussel and fish were 11.04 mg/l and 6.98 mg/l respectively. Of the two, the lowest concentration (7.0 mg/l) and one tenth of the LC_{50} of the fish (0.7 mg/l) was considered suitable for study to both the animal groups as subacute concentration. Further, as the period of exposure is an important factor in assessing the effects of a metal on an organism, 10, 20 and 30 days were selected controls were maintained alongside for comparison. After the period of exposure the mussles and fish, along with the controls, were sacrificed and ctenidium, mantle, hepatopancreas and foot of mussels and gill, kidney, liver and muscle of fish were dissected out and put in the ice-packed petridishes for biochemical analysis

The activities of alanine (AlAT), aspertate aminotransferases (AAT) and glutamate dehydrogenase (GDH) and the levels of glutamine were estimated in the organs of mussels and fish of both the controls and experimentals by standard experimental procedures. For the activities of AlAT (µM pyruvate/mg protein/h) and AAT (µM oxaloacetate/mg protein/h) Reitman and Frankel (1957), GDH (µM formozan/mg protein/h) Lee and Lardy (1965) and levels of glutamine (µM/g wet wt) Colowick and Kaplan (1957) are adopted. Each experiment included a minimum of 10 animals and the mean was taken into consideration. The statistical analysis is made through DMR test and the significance is calculated at 5% level.

3. Results and discussion

Tables 1 and 2 show that relative to controls both AlAT and AAT activities significantly ($P < 0.05$) increased in ctenidium, mantle, hepatopancreas and foot of mussel, whereas in gill, kidney, liver and muscle of fish they increased more at day 10 and less at day 20 with a steep decrease at day 30. The elevation observed in both AlAT and AAT activities progressively restored near to normalcy over time of exposure in the organs of mussel in the order: 10 > 20 > 30 days, whereas in the organs of fish the elevation was in the order: 10 > 20 days with a significant suppression at day 30. Either the elevation or suppression, as the case may be, was more in AAT than the AlAT in the organs of mussel and fish. Among the organs, in general, either the degree of elevation or suppression of AlAT activity was in the order:

hepatopancreas > foot > ctenidium > mantle of the mussel; whereas in fish it was in the order: kidney > gill > liver > muscle (Figure 1), and the percent elevation or suppression of AAT activity was more in hepatopancreas and less in ctenidium in mussel and it was more in kidney and less in muscle in fish (Figure 2). In general, AlAT activity was less in control and experimental organs of mussel than in those of fish; but in AAT activity no significant differences were observed between the organs of mussels and fish. Though an initial significant increase in the enzyme activities was observed at day 10 but it gradually came down and reached to almost normal at day 30 in the organs of mussel, but the increase observed at day 10, came down at day 20 with a significant decrease of at day 30 in the organs of fish (Figures 1-2).

The initial increase at day 10 in AlAT and AAT activities in the organs of mussels and fish exposed to subacute cadmium stress suggests either the increase in their activation or increased synthesis. It is reported that AlAT and AAT activities increase on stepwise induction of these enzymes during exposure of animals to subacute toxicity (Kulkarni and Kulkarni, 1987; Oner et al., 2008). Elevation in the activities of these enzymes could be helpful to the mussels and fish for the structural reorganisation of proteins and for the incorporation of keto acids into the TCA cycle to favour gluconeogenesis (Jurss and Bastrop, 1995). The increase in transaminases can also be linked with urea (Ramana Rao and Ramamurthi, 1983). A steady decrease in the elevation of these enzyme activities in the organs of mussels at day 20 and day 30 indicates the metabolic compensation for their adaptation to the imposed toxic stress. Probably, in mussels both these enzymes might have equally participated in cellular metabolic homeostasis. In fishes though the elevation in these enzyme activities exhibited a little recovery at day 20, the significant suppression of them at day 30 indicates the failure of homeostasis. The decrease could be due to the interaction of metal ions with the active sites of these enzymes and/or decreased de novo synthesis. Both elevation and suppression in AlAT and AAT activities were reported in the organs of fishes and mussels exposed to heavy metals (Ramana Rao and Ramamurthi, 1983; Kulkarni and Kulkarni, 1987; Das et al., 2004; Oner et al., 2009).

From the data presented in tables 3 and 4 relative to controls the GDH activity and glutamine level significantly ($P < 0.05$) increased in ctenidium, mantle, hepatopancreas and foot of mussel and in gill, kidney, liver and muscle of fish exposed to subacute concentration of cadmium. Among the exposure periods the degree of their in the organs of mussel was in the order: 10 > 20 > 30 days; whereas in the organs of fish it was in the order: 10 > 20 < 30 days. Among the organs in general, the degree of increase in GDH activity was in the order: hepatopancreas > foot > ctenidium > mantle in mussel and liver > kidney > gill > muscle in fish (Figure 3), and the degree of increase in glutamine level was more in ctenidium and less in foot in mussel and it was more in muscle and less in liver in fish (Figure 4). No significant differences were observed in GDH activity and glutamine levels between the organs of control mussels and fish, however, the elevation was less in the organs of mussel than in those of fish at subacute cadmium concentration. The elevation gradually came down over time of exposure in the organs of mussel and reached to normal at day 30 whereas the fish though exhibited a little recovery at day 20 but again a significant increase was noticed at day 30 (Figures 3-4). The initial increase in GDH activity in the organs of mussels and fish exposed to subacute concentration of cadmium could lead to increased production of glutamate, which is necessary to prevent ammonia toxicity. Further, the equilibrium constant of GDH reaction could favour glutamate formation. Increased amino acid levels could also partly be responsible for the hike in GDH activity (Ramanadikshitulu et al., 1976). The increased glutamate levels in the organs of mussels and fish exposed to subacute cadmium

stress partly aids in meeting the energy demands under toxic stress by its entry into TCA cycle, thus links nitrogen metabolism with energetics. Similar result was observed in freshwater crab *Sinopotamon yangtsekiense* exposed to cadmium stress (Xuan et al., 2011). However, more accumulation of glutamine in the tissues of fishes on prolonged exposure indicates their inability to utilize this metabolite in various metabolic processes. The ornithine produced can mobilize the formation of glutamine by accepting ammonia. This is also evident by the increased glutamine levels in the organs of fish under subacute toxic stress.

Changes in nitrogen metabolism in ctenidium and mantle of the mussel exposed to subacute concentration of cadmium indicate their functional reoganisation over time of exposure to meet the imposed subacute toxic stress. An increase in GDH activity was reported in clam *Polymesoda expansa* exposed to sublethal concentrations of toxic metals (Hiong et al., 2004). Since the hepatopancreas is the major site for all metabolic reactions, increased activities of transdeaminases can be linked with urea (Ramana Rao and Ramamurthi, 1983) as a strategic step of adaptation to the ambient medium. Glutamine synthetase, one of the enzymes involved in ammonia detoxification, was mostly limited to the hepatopancreas and this might be responsible for the increased levels of glutamine in this organ. In the foot of mussels the compensatory cycle is well maintained and accumulation of nitrogenous waste product in it may be for balancing the osmoregulatory activity.

As the gills of fish are in direct content with the surrounding water medium, the initial elevation of AAT activity provides the oxaloacetate required for the gluconeogenic pathway to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism. But at day 30 exposure a significant suppression observed in AAT and AlAT activities indicates the inhibition of transdeamination reaction on prolonged exposure. In the kidney of fishes an increase in GDH activity and glutamine levels also indicate impaired osmo-regulatory function of this organ to long-term exposure. In the liver of fish the increase in AlAT and AAT could be for the turnover of aminoacids (Todorovic and Vujanovic, 2002). Similar observation was reported in the liver of fish exposed to cadmium toxicity (De Smet and Blust, 2001; Velmurugan et al., 2008). At day 30, a significant decreased in AlAT and AAT activities indicates the failure of incorporation of amino acids into the TCA cycle. A decrease in trasaminases was reported in the liver of fish *Oreochrmis niloticus* exposed to metals (Oner et al., 2009). So, there is an effective metabolic reorgnization in the liver of the fish under subacute toxic stress. The changes observed in protein metabolism in the muscle of the fish, suggests gearing up of protein synthetic potentials, but at day of 30 the significant decrease in AlAT and AAT activities indicates the inability of the animal to overcome to stress. However, high glutamine production in the muscle be one of the favourable compensatory mechanisms to meet ammonia stress.

In between the mussel and fish the mussels could easily overcome the stress by activating the protein synthetic potentials necessary for metal detoxification and disposal. The fish could not succeed in activating the protein synthesis, hence on prolonged exposure it went under the influence of toxic impact. The ammonia formed due to transdeamination reactions, is mostly converted into glutamine in the organs of mussels. The fish though tried to resist upto day 20 but on prolonged exposure all the mechanisms failed to operate may be due to the domination of metal accumulation over its detoxification and disposal. Thus, the mussels could bring the shifts in protein metabolisms to normal level at day 30 with more adaptive ability but not the fish. Probably the subacute concentration becomes acute to fish on long-term exposure.

Table 1: AlAT activity (µM pyruvate/mg protein/h) in the organs of the freshwater mussel *Lamellidens marginalis* and freshwater fish *Labeo rohita* at different days of exposure to the subacute concentration of Cadmium. Each value is a mean of six estimations. Percent (%) change over control is given in parenthesis

SD: Standard Deviation					H.P: Hepatopancreas				P: Level of significance					
Exposure period in days														
Organ	Control		10		20		30		Organ	Control		10		20		30	
L. marginalis									*L. rohita*								
Ctenidium	1.200	a	1.500	d	1.370	c	1.280	b	Gill	1.380	b	1.760	d	1.630	c	0.936	a
SD ±	0.076		0.054		0.062		0.067		SD ±	0.082		0.130		0.010		0.150	
%			(+25.4)		(+14.2)		(+6.7)		%			(+27.5)		(+18.1)		(-32.2)	
Mantle	0.645	a	0.789	d	0.726	c	0.685	b,c	Kidney	2.290	b	2.980	d	2.690	c	1.490	a
SD ±	0.038		0.030		0.034		0.036		SD ±	0.150		0.170		0.160		0.200	
%			(+22.3)		(+12.5)		(+6.2)		%			(+30.1)		(+17.5)		(-35.0)	
H.P.	1.930	a	2.540	d	2.280	c	2.110	b	Liver	2.710	b	3.380	d	3.100	c	2.030	a
SD ±	0.130		0.079		0.094		0.110		SD ±	0.160		0.190		0.180		0.220	
%			(+31.6)		(+18.1)		(+9.3)		%			(+24.7)		(+14.4)		(-24.9)	
Foot	0.835	a	1.038	d	0.966	c	0.892	b	Muscle	1.260	b	1.530	d	1.430	c	0.952	a
SD ±	0.070		0.034		0.042		0.050		SD ±	0.082		0.100		0.086		0.110	
%			(+24.3)		(+15.7)		(+6.8)		%			(+21.4)		(+13.5)		(-24.4)	

a,b,c,d alphabets of each organ denote that the values differ significantly differently with each other ($P<0.05$).

Same alphabets of each organ denote that the values do not differ significantly with each other (P>0.05).

Table 2: AAT activity (µM oxaloacetate/mg protein/h) in the organs of fresh water mussel *Lamellidens marginalis* and freshwater fish *Labeo rohita* at different days of exposure to the subacute concentration of Cadmium. Each value is a mean of six estimations. Percent (%) change over control is given the parenthesis

SD: Standard Deviation			H.P: Hepatopancreas					P: Level of significance							
Exposure period in days															
Organ	Control		10		20		30		Organ	Control		10		20		30	
L. marginalis									*L. rohita*								
Ctenidium	0.417	a	0.532	d	0.486	c	0.448	b	Gill	0.138	b	0.185	d	0.162	c	0.105	a
SD ±	0.020		0.028		0.024		0.022		SD ±	0.007		0.009		0.008		0.010	
%			(+27.6)		(+16.5)		(+7.4)		%			(+34.0)		(+17.4)		(-23.6)	
Mantle	0.324	a	0.424	d	0.384	c	0.353	b	Kidney	0.381	b	0.513	d	0.461	c	0.270	a
SD ±	0.016		0.020		0.019		0.018		SD ±	0.019		0.025		0.023		0.028	
%			(+30.9)		(+18.5)		(+8.9)		%			(+34.6)		(+21.0)		(-29.1)	
H.P	0.340	a	0.453	d	0.406	c	0.381	b,c	Liver	0.232	b	0.302	d	0.260	c	0.173	a
SD ±	0.017		0.022		0.020		0.019		SD ±	0.011		0.015		0.013		0.019	
%			(+33.2)		(+19.4)		(+12.0)		%			(+30.2)		(+12.1)		(-25.6)	
Foot	0.115	a	0.149	c	0.134	b	0.123	a,b	Muscle	0.373	b	0.463	d	0.429	c	0.258	a
SD ±	0.008		0.009		0.006		0.006		SD ±	0.018		0.023		0.021		0.026	
%			(+29.6)		(+16.5)		(+6.9)		%			(+24.1)		(+15.0)		(-30.8)	

a,b,c,d alphabets of each organ denote that the values differ significantly with each other (P<0.05).

Same alphabets of each organ denote that the values do not differ significantly with each other (P>0.05).

Table 3: GDH activity (µM formozan/mg protein/h) in the organs of the freshwater mussel *Lamellidens marginalis* and freshwater fish *Labeo rohita* at different days of exposure to the subacute concentration of Cadmium. Each value is a mean of six estimations. Percent (%) increase over control is given in parenthesis.

SD: Standard Deviation			H.P: Hepatopancreas						P: Level of significance							
Exposure period in days																
Organ	Control		10		20		30		Organ	Control		10		20		30	
L. marginalis									*L. rohita*								
Ctenidium	0.083	a	0.099	c	0.095	b,c	0.089	a,b	Gill	0.032	a	0.041	c	0.037	b,c	0.048	d
SD ±	0.005		0.006		0.004		0.005		SD ±	0.002		0.002		0.001		0.003	
%			(+19.3)		(+14.4)		(+7.2)		%			(+28.1)		(+15.6)		(+50.0)	
Mantle	0.047	a	0.054	b	0.052	a,b	0.049	a,b	Kidney	0.059	a	0.076	c	0.069	b	0.090	d
SD ±	0.003		0.003		0.002		0.002		SD ±	0.003		0.004		0.003		0.005	
%			(+14.9)		(+10.6)		(+4.2)		%			(+28.8)		(+16.9)		(+52.5)	
H.P.	0.121	a	0.153	d	0.143	c	0.133	b,c	Liver	0.074	a	0.097	c	0.088	b	0.118	d
SD ±	0.008		0.009		0.008		0.007		SD ±	0.004		0.006		0.003		0.007	
%			(+26.4)		(+18.2)		(+9.9)		%			(+31.1)		(+18.9)		(+59.4)	
Foot	0.020	a	0.024	b	0.023	a,b	0.021	a,b	Muscle	0.038	a	0.047	b	0.043	a,b	0.056	d
SD ±	0.001		0.002		0.002		0.001		SD ±	0.003		0.003		0.004		0.004	
%			(+20.0)		(+15.0)		(+5.0)		%			(+23.7)		(+13.1)		(+47.4)	

a,b,c,d alphabets of each organ denote that the values differ significantly with each other ($P<0.05$).

Same alphabets of each organ denote that the values do not differ significantly with each other ($P>0.05$).

Table 4: Glutamine levels (µM glutamine/g wet wt) in the organs of the freshwater mussel *Lamellidens marginalis* and freshwater fish *Labeo rohita* at different days of exposure to the subacute concentration of Cadmium. Each value is a mean of six estimations. Percent (%) increase over control is given in parenthesis.

SD: Standard Deviation			H.P: Hepatopancreas						P: Level of significance							
Exposure period in days																
Organ	Control		10		20		30		Organ	Control		10		20		30	
L. marginalis									*L. rohita*								
Ctenidium	1.800	a	2.300	c	2.060	b	1.960	a,b	Gill	5.930	a	7.370	c	6.970	b,c	9.070	d
SD ±	0.090		0.110		0.100		0.098		SD ±	0.290		0.360		0.340		0.450	
%			(+27.8)		(+14.4)		(+8.9)		%			(+24.3)		(+17.5)		(+52.9)	
Mantle	10.150	a	12.550	c	11.720	b,c	10.940	a,b	Kidney	4.640	a	5.700	c	5.350	b	6.820	d
SD ±	0.510		0.720		0.590		0.550		SD ±	0.230		0.200		0.220		0.300	
%			(+23.6)		(+15.5)		(+7.8)		%			(+22.8)		(+15.3)		(+46.9)	
H.P.	24.410	a	29.320	d	27.540	c	25.630	b	Liver	8.420	a	10.410	c	9.670	b	12.240	d
SD ±	1.220		1.400		1.380		1.290		SD ±	0.400		0.610		0.480		0.700	
%			(+20.1)		(+12.8)		(+5.0)		%			(+23.6)		(+14.8)		(+45.4)	
Foot	1.240	a	1.440	a	1.410	a	1.320	a	Muscle	4.340	a	5.520	c	5.130	b	6.840	d
SD ±	0.084		0.089		0.085		0.066		SD ±	0.220		0.270		0.250		0.340	
%			(+16.1)		(+13.7)		(+6.4)		%			(+27.2)		(+18.2)		(+57.6)	

a,b,c,d alphabets of each organ denote that the values differ significantly with each other ($P<0.05$).

Same alphabets of each organ denote that the values do not differ significantly with each other ($P>0.05$).

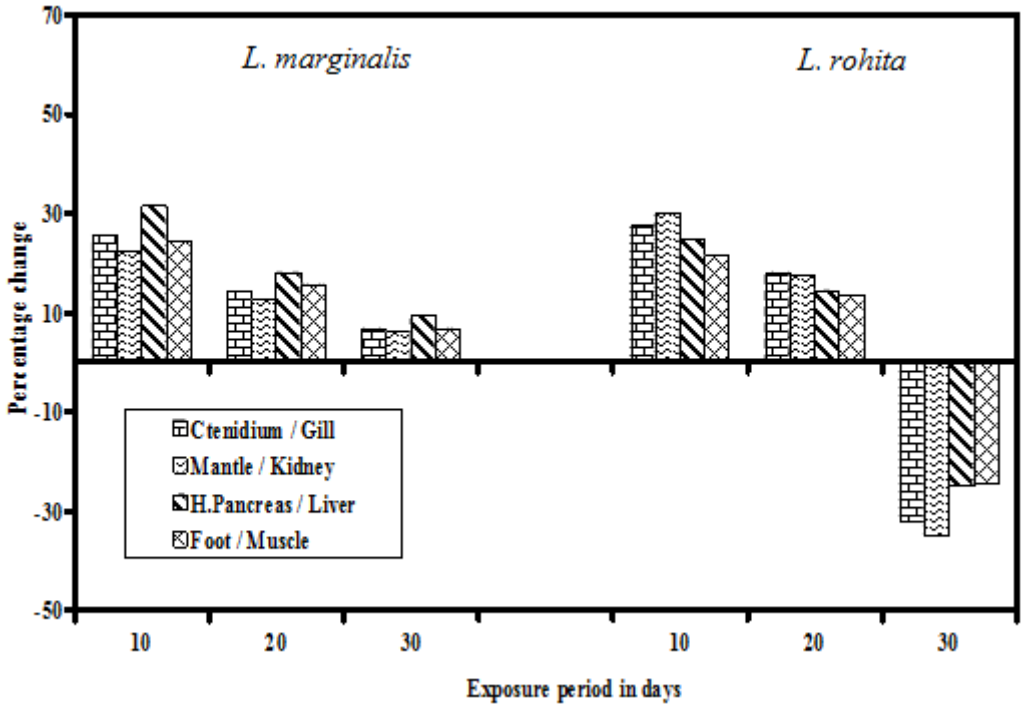

Figure 1: Percentage change over control in the AlAT activity in the organs of freshwater mussel *L. marginalis* and freshwater fish *L. rohita* at different periods of exposure to the subacute concentration of cadmium.

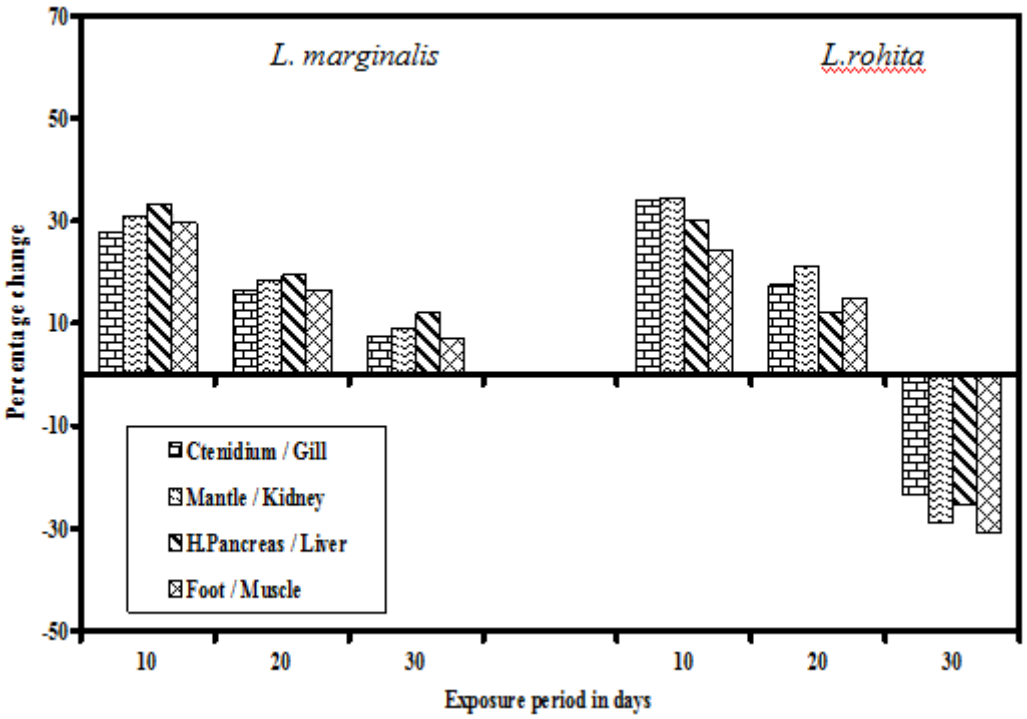

Figure 2: Percentage change over control in the AAT activity in the organs of freshwater mussel *L. marginalis* and freshwater fish *L. rohita* at different periods of exposure to the subacute concentration of cadmium.

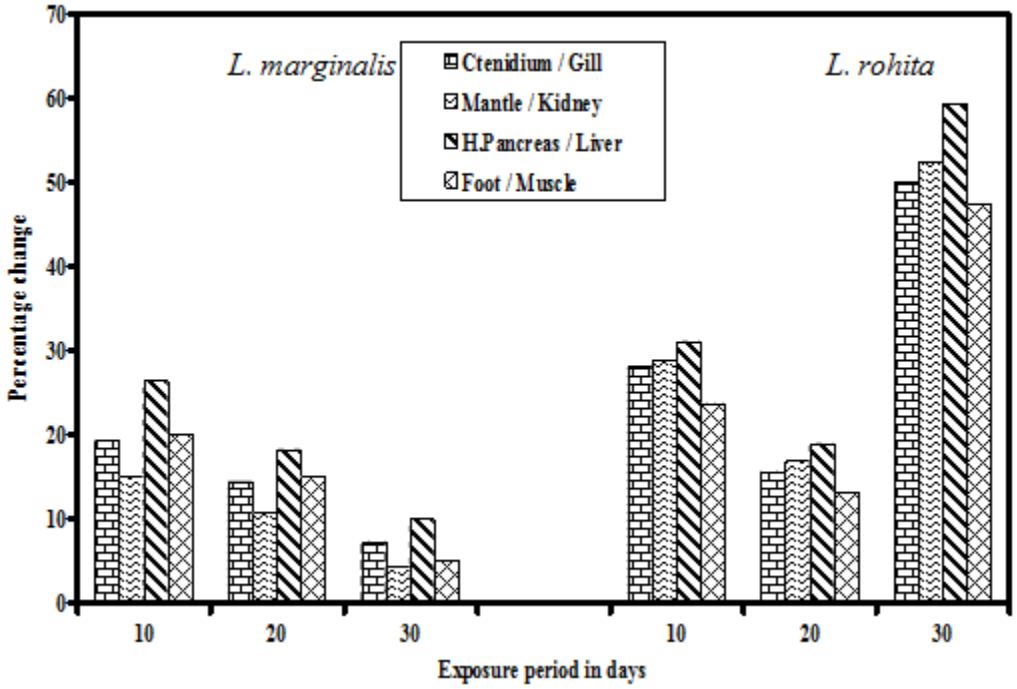

Figure 3: Percentage change over control in the GDH activity in the organs of freshwater mussel L. marginalis and freshwater fish L. rohita at different periods of exposure to the subacute concentration of cadmium**.**

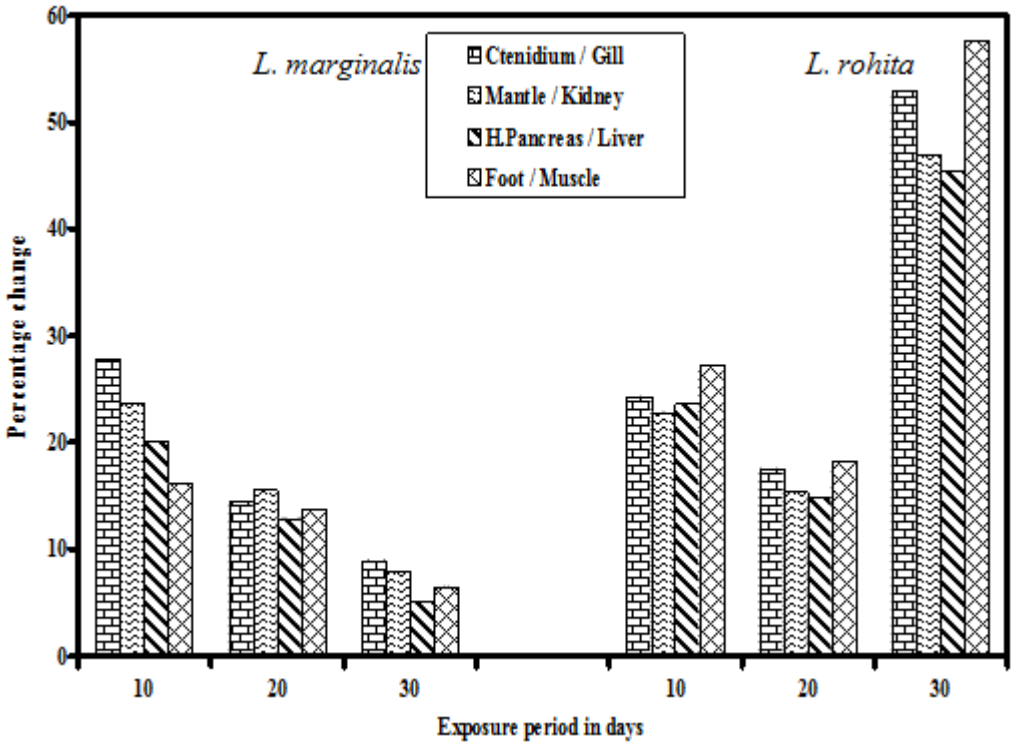

Figure 4: Percentage change over control in the glutamine levels in the organs of freshwater mussel *L. marginalis* and freshwater fish *L. rohita* at different periods of exposure to the subacute concentration of cadmium.

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