# Cadmium induced alterations in certain aspects of protein metabolism of the freshwater mussel, *Lamellidens marginalis* (Lamarck) and freshwater fish, *Labeo rohita* (Hamilton)

M.VENKATA CHANDRUDU AND K. RADHAKRISHNAIAH

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SUMMARY

**The levels of glutamine, ammonia and urea and the activities of alanine and aspertate aminotransferases (AlAT and AAT) and glutamate dehydrogenase (GDH) were estimated in the organs of mussel and fish exposed to acute concentration (7.0mg/l) of cadmium relative to their controls at day 1, 2 and 3. The AlAT and AAT activities decreased with an increase in GDH activity and glutamine levels in all the organs of the mussel and fish, greater in degree in fish than in mussel. The level of ammonia increased with a decrease in urea level in all the organs of mussel and fish. Eeither the increase or decrease in parameters was more in fish than in mussel and they were in the order day 1 < 2 > 3 in mussel and day 1 < 2 < 3 in fish over time of exposure. Among the organs, the degree in all the changes of all the parameters of protein metabolism were more or less insignificant and inconsistent but in general they were in the order ctenidium > mantle > hepatopancreas > foot in mussel and kidney > liver > gill > muscle in fish. In between the two animals, the degree of either the decrease or increase, as the case may be, in the parameters studied was more in the organs of fish and less in mussel. Further, the changes were progressive overtime of exposure in fish, but a slight recovery was observed at day 3 in mussel. The results indicated increased deamination, ammonia accumulation and suppression of urea synthesis in the organs of both the animal groups exposed to acute cadmium stress, with a greater in degree and progressive over time of exposure in fish.**

See end of the article for authors' affiliations

Correspondence to :
**K.RADHAKRISHNAIAH**
Department of Zoology,
Sri Krishnadevaraya University,
ANANTPUR (A.P.)
INDIA

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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 Yamuna has been well reported by Sharma *et al.* (2000). The Ogba river which was once considered safe for drinking water was also contaminated by the heavy metals like Cu, Mn, Zn, Cd, Cr, Ni and Pb and the fishes in it with higher levels of metals bioaccumulated in their tissues reported unsafe for human consumption (Obasohan, 2007). More than permissible levels of heavy metals were reported in water and sediment, and plankton and fish tissues in lake Egirdir, Turkey (Yigit and Altindag, 2006). The physico-chemical properties of heavy metals in aquatic systems are the principal 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 near by ponds and drains without any treatment contain highly toxic heavy metals (Mathur *et al.*, 2005).

Cadmium (Cd) 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$^0$C and boiling point 765$^0$C. 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. Cadmium 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 sulfide is used as co lour pigment in plastics and in various types of paints. Cadmium stearate is used as a stabilizer in plastics. Cadmium thus reaches the water bodies prilimirley 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. Cd has become the focus of intense research globally because of its toxicity to humans and terrestrial and aquatic organisms. The target organs are the liver, placenta, kidneys, lungs, brain and bones. Exposure to cadmium causes anemia, hepatite, renal and cardiovascular diseases in humans. Considerable amounts of cadmium accumulates in the liver and kidney, mostly bound to an inducible low molecular weight protein called metallothionein (Nerdberg and Nerdberg, 2000). The reports available though indicate an imbalance in metabolic homeostasis of aquatic fauna exposed to cadmium (Choi, *et al.*, 2007; Serafim and Bebianno, 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, it was felt 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.

## 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 ± $1^0$C, pH 7 ± 0.1, total hardness 100 ± 5 mg/l CaCO3, 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 the laboratory conditions for ten days prior to the experimentation.

A stock solution of cadmium was prepared by dissolving 2.74g of cadmium nitrate ($Cd(NO_3)$ $4H_2O$) in one liter of distilled water, consisting of 1 g of cadmium. Appropriate amount of stock solution was taken to obtain the desired concentrations of cadmium. 96h LC50s were determined by exposing the mussel and fish to different concentrations of cadmium (Finney, 1971). Based on the per cent and probit mortality curves as well as through Dragstedt and Behren's method, the 96h LC50s obtained to the mussel and fish were 11.0 mg/l and 7.0 mg/l, respectively. Of the two, the lowest concentration (7.0 mg/l) was considered suitable for study to both the animal groups as acute concentration. Further, as the period of exposure is an important factor in assessing the effects of a metal on an organism, 1, 2 and 3 days 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), the levels of glutamine, ammonia and urea 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) by Rietman and Frankel (1957), GDH (µM formozan/mg protein/h) by Lee and Lardy (1965) and levels of glutamine (µ M/gwet wt) by Colowick and Kaplan (1957), ammonia (µ M/g wet wt) by Bergmeyer (1965) and urea (µ M/g wet wt) by Natelson (1971) were adopted. Each experiment included a minimum of 10 animals and the mean was taken into consideration. The statistical analysis was made through DMR test and the significance was calculated at 5% level.

## RESULTS AND DISCUSSION

Fig. 1 and 2 show that relative to controls Both AlAT and AAT activities decreased significantly ($P < 0.05$) in ctenidium, mantle, hepatopancreas and foot of mussel and gill, kidney, liver and muscle of fish exposed to acute concentration of cadmium (Fig. 1 and 2). Based on per cent values obtained, it was observed that the suppression of AAT was more than AlAT in all the organs of mussel and fish exposed to acute cadmium concentration. The suppression in AlAT and AAT activities were differed in degree between the three exposure periods to acute cadmium concentration. In the organs of mussel, the degree was in the in the order: 1 < 2 > 3 days, whereas in fish it increased over time in the order: 1 < 2 < 3 days.

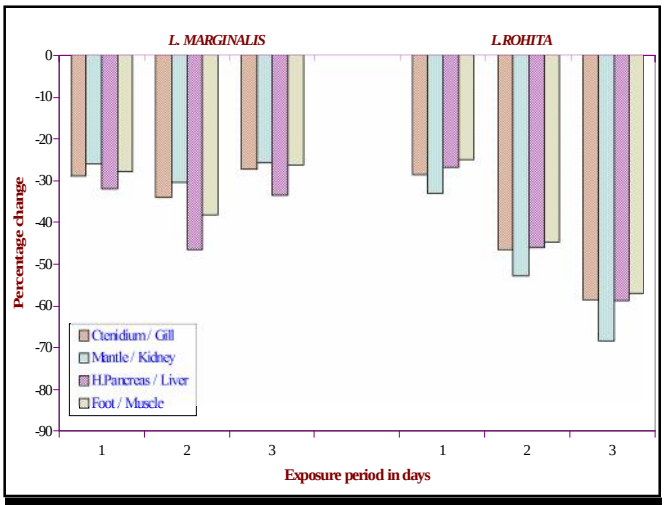


**Fig. 1 : Percentage change over control in the AIAT activity in the organs of freshwater mussel *L. marginalis* and freshwater fish *L. rohita* at different periods of exposure to the acute concentration of cadimum**

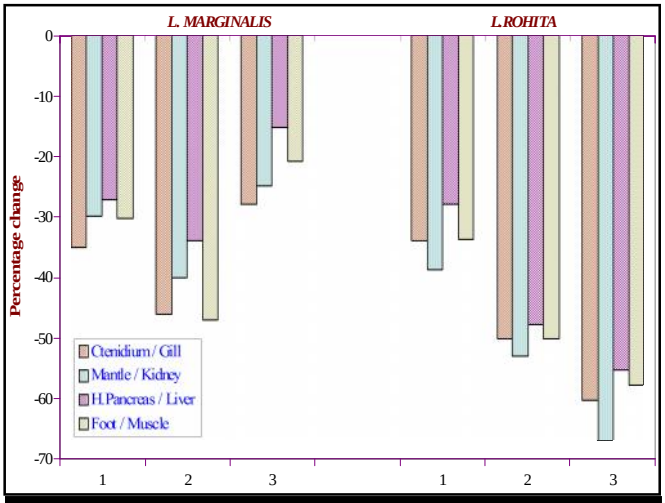


**Fig. 2 : Percentage change over control in the AAT activity in the organs of freshwater mussel *L. marginalis* and freshwater fish *L. rohita* at different periods of exposure to the acute concentration of cadimum**

Among the organs, in general, the suppression of AlAT and AAT activities were more in ctenidium and kidney and less in mantle and muscle of the mussel and fish, respectively. In general, AlAT activity was less in control and experimental organs of mussel than in those of fish; but no significant differences were observed in AAT activity between the organs of mussels and fish. The degree of suppression in the activities, however, was less in the organs of mussel than in those of fish exposed to acute concentration. The suppression slightly recovered at day of 3 in the organs of mussel whereas its progressed over time of exposure in the organs of fish (Fig. 1 and 2).

The transaminases are an important wing of amino acid catabolism that is mainly involved in transferring of an amino group from one amino acid to another keto acid, thus forming another amino acid. They operate at crossover points between carbohydrates and proteins by interconnecting the metabolites like α-keto glutarate, pyruvate and oxaloacetate on one hand and alanine, aspartate and glutamate on the other hand (Knox and Greengard, 1965; Martin *et al*., 1981). The aspartate aminotransferase (AAT) catalyses the interconversion of asparatic acid and a-keto glutric acid to oxaloacetic acid and glutamic acid, while the alanine aminotransferase catalyses the interconversion of alanine and a-keto glutric acid to pyruvic acid and glutamic acid (Tilak *et al*., 2005). Localization of AlAT and AAT in both mitochondrial and cytosolic fractions of cell has been confirmed and a close correlation appears to exist between the mitochondrial integrity and transamination levels (Bonitenko, 1974). Any alteration in mitochondria is bound to alter the levels of enzymes associated with them. AlAT and AAT function as a strategic link between carbohydrate and protein metabolism. Hence, these activities are considered to be sensitive indicators of stress (Ramana Rao *et al*., 1990; Bag *et al*., 1999). In the present study, the steady decrease in AlAT and AAT activities in the organs of mussels and fish from 1 to 3 days on exposure to acute concentration of cadmium is not clearly understood. But, it indicates the dysfunctioning of cell organelles and probable degradation of them in high metal concentrations (David *et al*., 2003). The decrease in transaminases could even point to decrease syntheses of amino acids. It may be even due to mitochondrial swelling as a consequence of lactate accumulation. Suppression of AlAT activity can cause the accumulation of alanine thereby decrease the pyruvate level and prevent excessive build up of lactate. The decrease in AAT could be due to the decreased availability of aspartate, since the amino group of aspartate is supposed to reaminate by inosine monophosphate (IMP) in purine nucleotide cycle. In the organs of mussels, however, the suppression in these enzyme activities observed at day 2 was prevented to a little degree on day 3, that indicates their ability of recovery. Contrary to it, severe suppression of those enzyme activities from day 1 to day 3 in the organs of fish reveals their susceptibility. There are reports that the activities of phosphatases and transaminases decrease in the muscle of *Oreochromis mossambicus* subjected to cartap hydrochloride (Palanivelu *et al*., 2005).

From the data presented in Fig. 3 and 4 relative to controls the GDH activity and glutamine levels significantly ($P < 0.05$) increased in ctenidium, mantle,

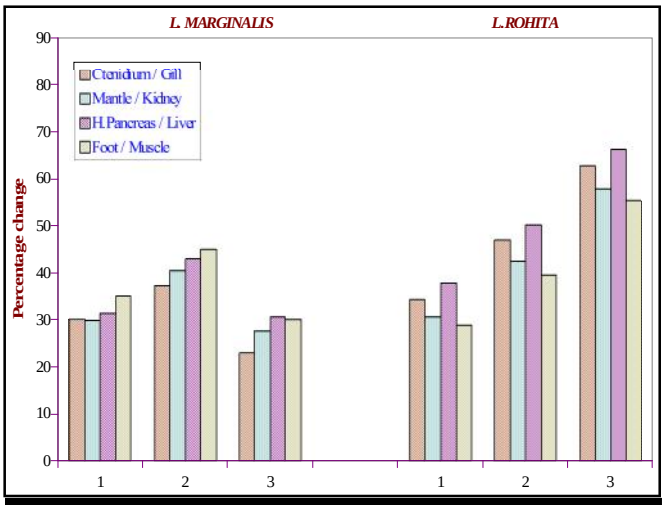


**Fig. 3 : Percentage change over control in the GDH activity in the organs of freshwater mussel *L. marginalis* and freshwater fish *L. rohita* at different periods of exposure to the acute concentration of cadimum**

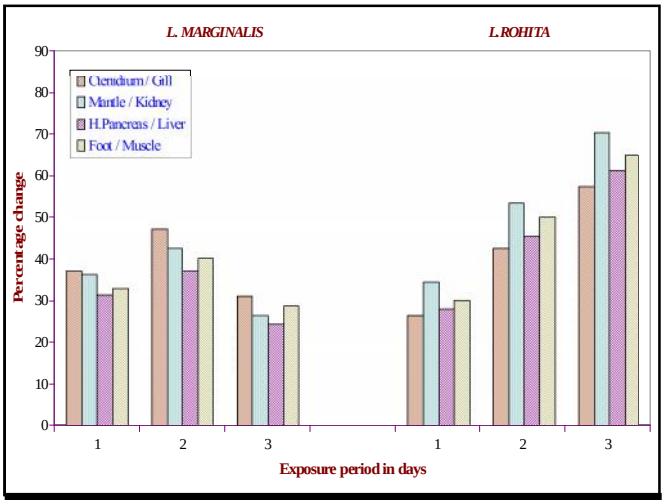


**Fig. 4 : Percentage change over control in the glutamine levels in the organs of freshwater mussel *L. marginalis* and freshwater fish *L. rohita* at different periods of exposure to the acute concentration of cadimum**

hepatopancreas and foot of mussel and in gill, kidney, liver and muscle of fish exposed to acute concentration of cadmium. Among the exposure period the degree of increase in GDH activity and glutamine levels differed at day1, 2 and 3, in the organs of mussel the degree of increase was in the order: 1 < 2 > 3 days; whereas in fish the increase was in the order: 1 < 2 < 3 days. Either in mussel or fish the degree of difference in the elevation of GDH activity among the organs was less and inconsistant. However, in general, it was in the order: foot > hepatopancreas > ctenidium > mantle in mussel and liver > gill > kidney> muscle in fish. No significant differences were observed in GDH activity and glutamine levels between the organs of control mussels and fish. However, the increase in them was less in the organs of mussel than in those of fish and it was more at day 2 than at day 3 in the organs of mussel whereas it progressed over time of exposure in the organs of fish (Fig. 3 and 4).

Glutamate dehydrogenase (GDH), a mitochondrial enzyme, has also been reported to be localized in the nuclear fraction (Kato and Lowry, 1972). It catalyses the reversible oxidative deamination of glutamate to a-ketoglutarate and ammonia (Tilak *et al*., 2005); depending on the availability of intracellular NAD and NADH (Prameelamma and Swami, 1975). It has several metabolic functions with great physiological significance, and is known to be closely associated with the detoxification mechanisms of tissues. GDH in extra hepatic tissues could be utilized for channeling of ammonia released during proteolysis for its ultimate detoxification to urea in liver. In the present study, significant increase observed in GDH activity in the organs of the mussel and fish in acute cadmium concentration could be due to the rapid utilization of glutamate for the formation of glutamine. This is also evident by the increase in glutamine levels in the organs of mussel and fish (Hiong *et al*., 2004). Even, the dysfunctioning of the organ systems of the mussel and fish on exposure to acute concentration could elevate the GDH activity, along with suppression of AlAT and AAT activities (Pal and Chatterjee, 2004). Further, increased production of ammonia and lactic acid in the organs of these animals may also elevate the activity of GDH. The increased GDH level and increased ammonia level in extra hepatic tissues reflect the inability of the animals to maintain the homeostasis. However, there appears to be an attempt on the part of these animals to detoxify the excess ammonia by converting a part of it to glutamine.

From the data presented in Fig. 5 and 6 it is seen that relative to controls the level of ammonia increased with a decrease in urea level in ctenidium, mantle, hepatopancreas and foot of mussel and in gill, kidney, liver and muscle of fish exposed to acute concentration of cadmium. Among the exposure period, the degree in the increase of ammonia level and decrease in urea level differed, and it was more at day 2 than at day 1 and less at day 3 in the organs of mussel, in the order: 1 < 2 > 3 days. Whereas, in fish the increase progressed over time of exposure in the order: 1 < 2 < 3 days. Among the organs, in general, the elevation and/or suppression of ammonia and urea levels was in the order: mantle > hepatopancreas > ctenidium > foot in mussel and gill > kidney > liver > muscle in fish. Insignificant differences were observed in ammonia and urea levels between the

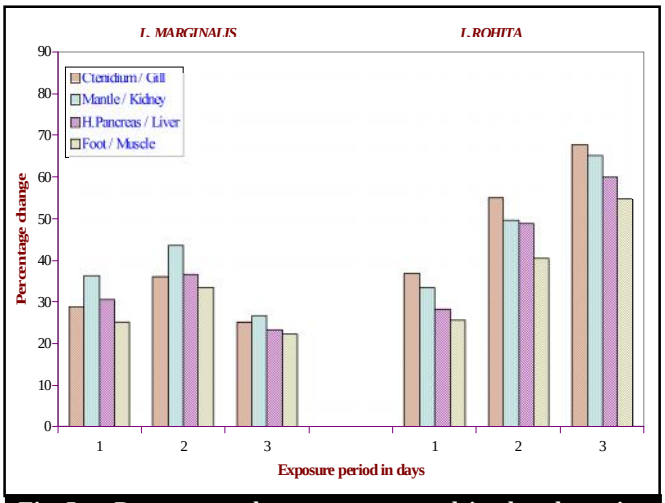


**Fig. 5 : Percentage change over control in the glutamine levels in the organs of freshwater mussel *L. marginalis* and freshwater fish *L. rohita* at different periods of exposure to the acute concentration of cadimum**

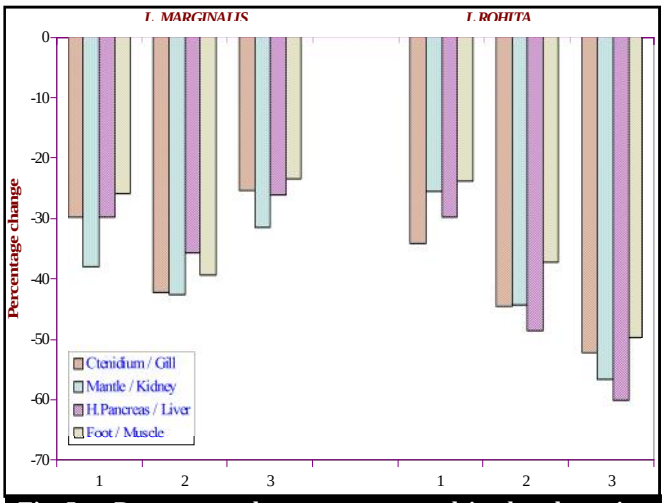


**Fig. 5 : Percentage change over control in the glutamine levels in the organs of freshwater mussel *L. marginalis* and freshwater fish *L. rohita* at different periods of exposure to the acute concentration of cadimum**

control organs of mussel and fish. But, either the degree of increase and/or decrease was more in the organs of fish than in those of mussel exposed to acute stress; and the elevation partially restored back at day of 3 in the organs of mussel, whereas it was progressive over time of exposure in the organs of fish (Fig. 5 and 6).

Ammonia is a toxic nitrogenous end product and is released exogenously in the digestive tract and endogenously in tissues through the catabolism of the amino acids, pyrimidines, purines, amino sugars and gangliosides (Lowenstein and Goodman, 1978). A number of mussel and fish organs are the sites for endogenous production of ammonia (Wilbur and Yonge, 1966). Many amino acids and their derivatives yield sufficient quantities of ammonia by deamination reactions (Braunstein, 1947). Further, hydrolysis of urea by urease results in ammonia formation (Wilbur and Yonge, 1966). There are three basic mechanisms to detoxify ammonia in biological system. Conversion of ammonia to glutamate by GDH activity (Martin *et al*., 1981), glutamine synthesis and urea formation (Schoffeniels and Gilles, 1970). In the present study, the elevation in ammonia levels in the organs of mussel and fish in acute cadmium concentration was not clear, as there was a decrease in the activity of transaminases (Fig. 3 and 4). Since GDH activity increased there could be other ammonogenic systems that are responsible for enhanced production of ammonia. It is known that profused ammonia production takes places through the operation of purine nucleotide cycle that indicates decreased stimulation of urea cycle in the organs of mussel and fish. Increased glutamine levels in the organs of mussel and fish indicates an attempt on the part of animals to detoxify ammonia. Still the excess accumulation of ammonia could lead to the development of an added ammonia toxicity with metal toxicity. Higher concentration of ammonia accumulation is noticed in the organs of fish with the increase in duration of exposure. Under these conditions the animals either succumb to death or excrete the excess ammonia formed. During excretion, more water should be eliminated from the organs of the body and the loss of water may cause dehydration leading to death. Increase in ammonia itself may be toxic to the animal for its survival.

One of the functional pathways to dispose ammonia is the synthesis of glutamine from glutamate by glutamine synthetase (Norenberg, 1979). The glutamine and the enzyme involved in its synthesis have a major role in nitrogen metabolism since the amide group of glutamine can be channeled to the synthesis of several amino acids and nucleotides. Hyperammonia generally results in elevation of urea synthesis, while under stress the glutamine synthetic mechanism is adapted by the organs in overcoming ammonia toxicity. The production of glutamine will be switched on when tissues detect the accumulation of ammonia (David *et al*., 2004). The increase in GDH activity along with increase in ammonia in the organs of mussel and fish exposed to acute concentration indicate higher proteolysis and deamination of amino acids, even though part ammonia is utilized for glutamine synthesis (Fig. 3). The progressive decrease and/or increase in AlAT, AAT GDH, urea, ammonia and glutamine levels in the organs of mussels and fish from 1 to 3 days of exposure to acute concentration indicates

stepwise structural and functional derangement and formation of severe pathological conditions in their organs. But the partial recovery of them observed at day 3 exposure in the tissues of mussels indicate the efforts taken by them in reducing the impact of stress.

Between the mussel and fish, more increase in ammonia, glutamine levels and GDH activity in the organs with a decrease in urea level indicate a greater degree of proteolysis and increase in the accumulation of ammonia in the organs of fish than in the organs of mussels, eventhough there is more glutamate production On the whole, it appears that the acute concentration of cadmium is exerting a greater influence on fish protein metabolism compared to the mussel and the mussel could exhibit some resistance on prolonged exposure to acute cadmium concentrations.

**Authors' affiliations**
**M.VENKATA CHANDRUDU,** Department of Zoology, Sri Krishnadevaraya University, ANANTPUR (A.P.) INDIA

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