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EFFECT OF SUBACUTE CONCENTRATION OF CADMIUM ON THE ENERGETICS OF FRESHWATER MUSSEL *LAMELLIDENS MARGINALIS* (LAM.) AND FISH *LABEO ROHITA* (HAM.)

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ABSTRACT

The levels of pyruvate and lactate, and the activities of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) were estimated in the organs of freshwater mussel *Lamellidens marginalis* and fish *Labeo rohita* at days 10, 20 and 30 after exposure to the subacute (0.7 mg/L) concentration of cadmium. A decrease was observed in succinate dehydrogenase (SDH) activity along with a corresponding decrease in pyruvate level with an increase in lactate dehydrogenase (LDH) activity and lactate level in ctenidium, mantle, hepatopancreas and foot of the mussel and in the kidney, liver and muscle of the fish at all the days of exposure. Interestingly, an increase was observed in SDH activity and pyruvate level with a decrease in LDH activity and lactate levels in the gills of the fish. Among the exposure periods, either the percent suppression or elevation progressively recovered and reached to near normal over time of exposure in the order: day 10>20>30 in the organs of mussel. Whereas, in the organs of fish it was in the order: day 10>20<30. Thus, between the two groups of animals, either the percent suppression or elevation was progressively recovered over time of exposure in the organs of mussel, but the fish exhibited a little recovery at day 20 with a significant fall at day 30. These results indicated an adaptive ability to subacute concentration of cadmium in the organs of mussel by normalizing the oxidative and glycolytic pathways on prolonged exposure; whereas the fish exhibited susceptibility due to the suppression of oxidative metabolism and increased dependency on anaerobic glycolysis.

INTRODUCTION

In fact, pollution of the environment is mostly due to man's intervention and his rapid progress in colonization, urbanization, industrialization, agriculture, mining, transportation and chemical technology. The marine and freshwater habitats have become the repositories of pollutants released from all the anthropogenic activities. Mining and smelting operations and discharge of most 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 & Ugbeva 2003). In recent years high concentrations of heavy metals are entering the aquatic systems 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). Many effluents discharged into ponds and drains without any treatment contain highly toxic dyes, bleaching agents and heavy metals (Mathur et al. 2005). The physico-chemical properties of heavy metals in aquatic systems are the principal factors for their accumulation in animals. Jain (2004) stated that heavy metals are causing greatest threat to the health of Indian aquatic ecosystems due to their toxicity and accumulation behaviour.

Cadmium is one of the most toxic and widespread heavy metals, and is a recognized carcinogen in mammals (Pruski & Dixon 2002). Cadmium reaches the water bodies from combustion of fuels, and plastics, phosphate fertilizers, pesticides, domestic wastes, oil refineries and electroplating industries. Even though some reports are available on the effects of cadmium toxicity in different groups of animals, comparative studies at physiological and biochemical levels on freshwater fauna are scanty. As the prime recipient of cadmium contaminated effluents are freshwater bodies, the shellfish and finfish inhabiting them are first prone to the effects of it. The cadmium after bioaccumaulation in the bodies of fishes is transferred to human beings through food. Hence, it is felt necessary to make a comparative study on the effects of cadmium 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 25g ± 2g, and the freshwater fish *L. rohita*, weighing 10g ± 2g, were collected from the local freshwater canals and lakes, and were maintained in laboratory in 5 × 3 × 3 feet cement tanks, thirty in each. Water from the local wells was used for their maintenance. It has temperature of 28 ± °C, pH 7 ± 0.1, total hardness 100 ± 5 mg/L, chlorinity 0.08 ± 0.003% and dissolved oxygen 5.8 ± 0.4 mg/L (Sivaramakrishna & 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 2.74g of cadmium nitrate in one litre of distilled water, which consists of 1 g of cadmium. 96h LC_{50} was 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 & Behren's method, the 96h LC_{50} values obtained for the mussel and fish, were 11.04 mg/L and 6.98 mg/L respectively. Of the two, the lowest was the LC_{50} of the fish; therefore one tenth of it i.e., 0.7mg/L, was considered suitable for study for 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 considering them as short-term and long-term periods. Controls were maintained alongside for comparison. After the period of exposure the mussels and fish, along with the respective control, 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 petri dishes for biochemical analysis.

The levels of pyruvate and lactate, and activities of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) were estimated in the organs of mussel and fish of both, the controls and experimentals, by standard experimental procedures: Succinate dehydrogenase (µM formozan/mg protein/h as per Nachlas et al. 1960), lactate dehydrogenase (µM formozan/mg protein/h as per Srikantan & Krishnamoorthi 1955), the levels of pyruvate (mg/g wet wt. as per Friedman & Hangen 1942) and lactate (mg/g wet wt. as per Barker & Summerson 1941).

Each experiment was conducted with a minimum of 10 animals and the mean was taken into consideration. The results were analysed statistically subjecting them to Duncan multiple range test.

RESULTS AND DISCUSSION

Relative to controls a significant ($P < 0.05$) decrease was observed in SDH activity along with a corresponding decrease in pyruvate level and an increase in LDH activity and lactate level in ctenidium, mantle, hepatopancreas and foot of mussels, and in kidney, liver and muscle of fish on all the days of exposure to subacute cadmium stress. In the gills of fish an increase was observed in SDH activity and pyruvate level with a decrease in LDH activity and lactate level. The degree of decrease or increase in SDH and LDH activities and pyruvate and lactate levels, however, differed among the three periods of exposure. In the organs of mussel the degree was more at day 10 than at day 20 and 30; it was progressively recovered over time of exposure in the order: 10 > 20 > 30 days. Whereas, in the organs of the fish, a little recovery was seen at day 20 from day 10, but again the degree of decrease or increase was significantly more at day 30 in the order: 10 > 20 < 30 days. Among the

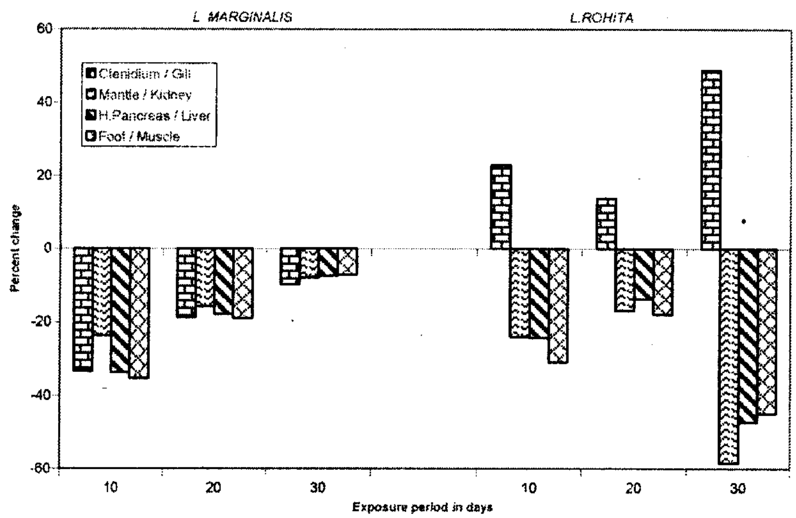

Fig. 1: Percentage change over control in the SDH activity in the organs of freshwater mussel *L. marginalis* and freshwater fish *L rohita* at different periods of exposure to the subacute concentration of cadmium.

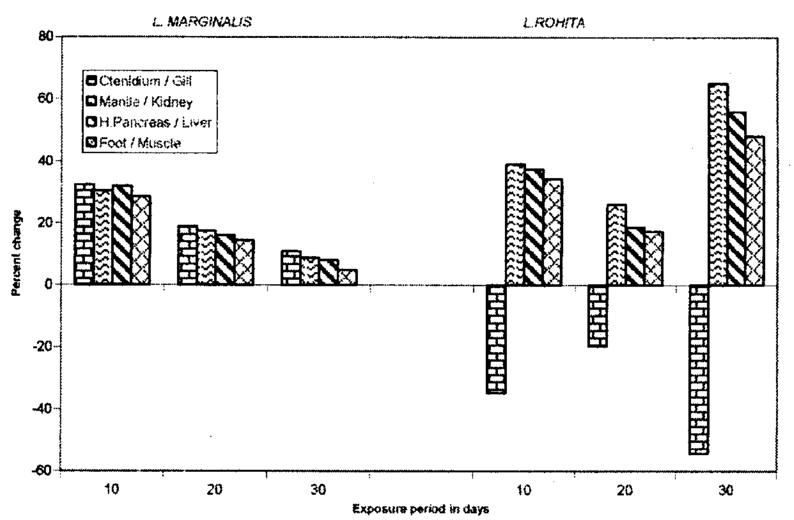

Fig. 2: Percentage change over control in the LDH activity in the organs of freshwater mussel *L. marginalis* and freshwater fish *L rohita* at different periods of exposure to the subacute concentration of cadmium.

organs, in general, the percent suppression and/or elevation in the said parameters was in the order: ctenidium > mantle > hepatopancreas > foot in mussel, and kidney > liver > muscle in fish, with an entirely opposite trend in the gills (Figs. 1-4).

The initial suppression of SDH activity along with an elevation in LDH activity in the organs of mussel at day 10 of exposure to subacute concentration of cadmium indicate the interference of cadmium with the enzymes of oxidative metabolism. Hence, the animal started deriving the necessary energy to meet the stress, partly through energetically more efficient oxidative metabolism and partly by stepping up of energetically less efficient glycolysis. This situation, however, gradually decreased with the increase in exposure period by the recovery of SDH activity, followed by the decrease in the elevation of LDH activity. This indicates the ability of adaptation of the animal and

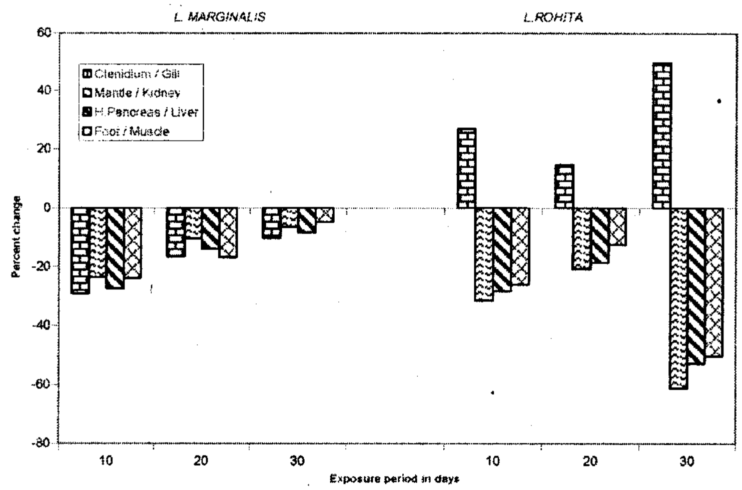

Fig. 3: Percentage change over control in the pyruvate level in the organs of freshwater mussel *L. marginalis* and freshwater fish *L. rohita* at different periods of exposure to the subacute concentration of cadmium.

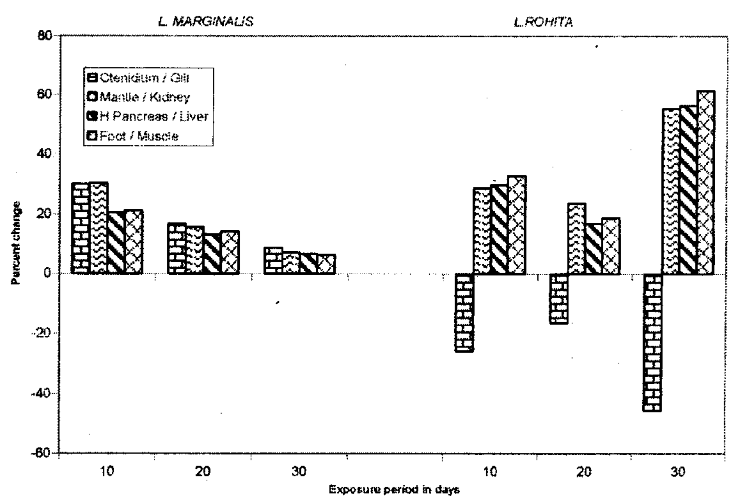

Fig. 4: Percentage change over control in the lactate level in the organs of freshwater mussel *L. marginalis* and freshwater fish *L. rohita* at different periods of exposure to the subacute concentration of cadmium.

minimizing the energy utilization during adaptation to subacute toxic stress. Even in the organs of fish severe inhibition of oxidative metabolism and the reliance on glycolysis at day 10 could suggest the shift in its energetic levels on cadmium stress. The recovery changes observed at day 20 indicate the animal's efforts in resisting the stress and the regain of normal metabolic rate. But, may be due to the domination of accumulation of metal ions over detoxification and disposal, the animal could not tolerate the stress on further exposure, therefore at day 30 once again a significant suppression is observed in SDH activity with a considerable elevation in LDH followed by a decrease of pyruvate and increase of lactate levels. Thus, the mussels could succeed in adapting the new environment by maximum recovery in their energy metabolism over time of exposure. Whereas in the organs of fish, though a little recovery in oxidative metabolism was observed at day 20, again the suppression at day 30 could suggest the metabolic imbalance and fluctuations in their energetics.

Among the organs a high resistance was observed in ctenidium of mussel to the metal. It is a positive signal for adaptation. Such adaptive ability was also reported in the gills of snail *Pila globosa* exposed to low concentrations of mercury (Sivaramakrishna 1992). As the mantle is one of the important structures in maintaining the integrity of soft parts of the body, maximum recovery of in energetics has been observed in it so as to bring the animal to metabolic homoeostasis. In the hepatopancreas the progressive elevation in SDH from its suppression along with the lowering of elevated LDH activity, indicates its role in metal detoxification and disposal. Kadar et al. (2005) reported that the mussels have evolved detoxification mechanisms to many of the toxicants. Gherardi et al. (2002) also reported that the decapods possess all the physiological mechanisms for the uptake, accumulation, detoxification and excretion of trace metals. The muscular foot is the chief locomotory organ of mussel, a significant recovery in oxidative metabolism could be a measure of lactate oxidation and effective function of this organ on prolonged exposure to cadmium.

In fish, the gills are the important organ of respiration. Interestingly, an increase is observed in SDH activity and pyruvate level along with a decrease in LDH activity and lactate level in it as against the other organs. Probably, as this organ is in direct contact with the external medium, oxygen from water might have been slowly diffused into the cells of it thereby the oxidative mechanism has been geared up in order to resist the direct exposure to the toxic ions. Further, as the gills require more energy to perform its respiratory, osmoregulatory and detoxification functions, the reliance of this organ on energetically more efficient oxidative metabolism indicates a strategic step for survival of the animal for a longer period under the imposed toxic stress. Kidney of fish is an important organ for osmoregulation and metal elimination. Decrease in SDH activity and increase in LDH along with lactate level in subacute concentration of cadmium indicate less supply of energy to perform its energy expensive function. In general, the osmoregulatory organs are more oxidative in nature and maintain a high energy demand. As there is a significant decrease in its energy levels, may be due to the lowered transport of oxygen to this organ and more accumulation of cadmium, it results in osmoregulatory failure on prolonged exposure to toxic stress. Failure of osmoregulation is one of the important causes for death of the fish on exposure to heavy metals (Schmidt-Nielsen 1974). In the liver of fish less suppression in oxidative metabolism and less elevation in glycolysis at day 20 may indicate the efforts taken by the fish in detoxification and disposal of metal ions in order to normalize the metabolic activities. But, may be due to domination of accumulation over detoxification the animal could not totally resist hence once again high suppression of oxidative metabolism is observed at day 30. In the muscle of fish, increase in the lactate level could be one of the reasons for its inactive state on prolonged exposure to subacute concentration. The recovery at day 20 indicates enhancement of its resting energy demands with a corresponding increase in its performance efficiency. Such a recovery in the enzyme activities was observed in the muscle of *H. fossils* exposed to sublethal level of mercury (James et al.1996).

On the whole an imbalance is evident in the energetics by the decrease in SDH activity and pyruvate levels and increase in LDH activity and lactate levels in the organs of the mussel and fish on exposure to subacute cadmium stress. But, the mussels could slowly regain the normal metabolic status through gradual adaptation; whereas fish, though tried to resist by elevating the energy levels at day 20, the total energy derived might have become insufficient to detoxify the influx of toxic ions thereby the animal gradually became inactive. This situation could lead to the damage of structural integrity of organs, as energy is the source for maintenance of structural organization. Probably, the fish may die on prolonged exposure to subacute cadmium stress. The results thus indicate that the mussel can adapt to the subacute concentration of metal with less energy fluctuations (Kadar et al. 2001), whereas the fish requires more energy to adapt to such stress. Thus, chronic pollution of water bodies with cadmium may lead to a decrease in the biodiversity of active fauna and to the development of specific metal tolerant communities (Davyd Kova et al. 2005).

REFERENCES

Barker, S.B. and Summerson, W.H. 1941. J. Biol. Chem., 138**:** 535.

Chukwu, L.O. and Ugbeva, B.O. 2003. Acute toxicity of textile mill effluents to estuarine macro-invertebrate *Clibinarius africanus* (Auri Villus) and *Tilapia zilli* (Gerr) fingerlings. J. Nig. Environ. Soc., 1(2): 223-228.

Davyd Kova, I.H., Fadeeva, N.P., Kovekovdova, L.T. and Fadeev, V.I. 2005. Heavy metal contents in tissues of dominant species the benthos and in bottom sediments of Zolotoi Rog Bay, sea of Japan. Russian J. Marine Biology, 31(3): 176-180.

Finney, D.J. 1971. Probit analysis, 3rd edition, London and New York, Cambridge University Press, pp. 333.

Friedman, T.E. and Hangen, G.E. 1942. Collection of blood for the determination of pyruvic and lactic acids. J. Biol. Chem., 144: 67-77.

Gherardi, F., Barbaresi, S., Vaselli, O. and Bencini, A. 2002. A comparison of trace metal accumulation in indigenous and Alien freshwater Macro-Decapods. Marine and Freshwater Behaviour and Physiology, 35(3): 179-188.

Jain, C.K. 2004. Metal fractionation study on bed sediments of River Yamuna, India. Water Res., 38(3): 569-578.

James, R., Sampath, K., Deakiamma and Pattuv, J. 1996. Recovery of dehydrogenase enzymes activities in *Heteropneustes fossilis* (Bloch) exposed to sublethal levels of mercury. Indian J. Fish, 43: 293-297.

Kadar, E., Salanki, J., Jugdaohsingh, R., Powell, J.J., McCrohan, C.R. and White, K.N. 2001. Avoidance responses to aluminium in the freshwater bivalve *Anodonta cygnea.* Aquat. Toxicol., 55(3-4): 137-148.

Kadar, E., Costa, V., Santos, R.S. and Lopes, H. 2005. Behavioural response to the bioavailability of inorganic mercury in the hydrothermal mussel *Bathymodiolus azoricus.* J. Exp. Biol., 208 (pt 3): 50.

Mathur, N., Bhatnagar, P., Nagar, P. and Bijarnia, M.K. 2005. Mutagenicity assessment of effluents from textile/dye industries of Sanganer, Jaipur (India): A case study. Ecotoxicol. Environ. Saf., 61(1): 105-113.

Nachlas, M.M., Margulies, S.I. and Seligman, A.M. 1960. A colorimetric method for the estimation of succinic dehydrogenase activity. J. Biol. Chem., 235: 499.

Pruski, A.M. and Dixon, D.R. 2002. Effects of cadmium on nuclear integrity and DNA repair efficiency in the gill cells of *mytilus edulis* L. Aquat. Toxicol., 57(3): 127-137.

Schmidt-Nielsen, B. 1974. Osmoregulation: Effect of salinity and heavy metals. Fed. Proc., 33: 2137-2146.

Sharma, H.R., Trivedi, R.C. and Gupta, A. 2000. Bioaccumulation and biomagnification of heavy metals and organochloride pesticides in benthic macroinvertebrates from River Yamuna. Indian. J. Environ. Prot., 20(9): 674-680.

Sivaramakrishna, B. 1992. Comparative studies of mercury toxicity in the freshwater snail *Pila globosa* (Swainson) and mussel *Lamellidens marginalis* (Lamarck) at lethal and sublethal concentration. Ph.D. Thesis, S.K. University, Anantapur, India.

Sivaramakrishna, B. and Radhakrishnaiah, K. 2000. Mercury induced alterations in the energetics of hepatopancreas of two freshwater molluscs, *Pila globosa* and *Lamellidens marginalis.* In: Trace Elements Their Distribution and Effects in the Environment. (Eds.) Markert. B & Friese, K., Elsevier, Amsterdam, pp. 389-409.

Srikantan, T.N. and Krishnamoorthi, C.R. 1955. Tetrazolium test for dehydrogenase. J. Sci. Industrial Res., 14: 206-207.