The Cauvery and Coleroon rivers in South India are examples of aquatic ecosystems which, in recent years, are facing the threat of eco-destruction due to the intentional as well as unintentional release of various pollutants. The river system promotes the growth and breeding of *Macrobrachium malcolmsonii*, a major export variety of prawn. Studies on the effects of heavy metal toxicity on *M. malcolmsonii* would provide a wealth of information on biophysiological aspects of this prawn; in addition, these studies would aid the identification of various factors that lead to the contamination of the body of water. This knowledge would, hopefully help in ensuring the survival of the species and in marshalling resources for the management of their freshwater ecological status.

In the above context, an integrated study has been performed on *M. malcolmsonii*, dealing with various important aspects such as bioaccumulation, depuration, respiration, ionic regulation, bioenergetics, biochemistry and histopathology.

The observation recorded in the studies on bioaccumulation or bioconcentration suggested that all four test heavy metals accumulated in the tissues of the hepatopancreas, gill and muscle. The pattern of bioaccumulation differed from metal to metal and from organ to organ (Tables 4-7), an observation consistent with that made by other investigators as well (Bryan, 1964, 1966).

In the present study, the hepatopancreas exhibited a greater degree of residue accumulation, than did the other two tissues, at the end of the experimental period. The concentration of metals in the gill tissue was possibly influenced by the absorption and the flow of water over the gill surface. The accumulation of the heavy metals in muscle was possibly due to the transport of these metals in the haemolymph, a possibility suggested by Bryan (1967). The higher quantum of residue in the hepatopancreas could
easily be explained by the well-known preeminent role of this organ in detoxification. The accumulation of all four heavy metals was most marked in the gills during the initial phase of the experiment, probably due to the permeability of the gills. The subsequent decline in concentrations was possibly due to the translocation of the accumulated metals from the gill to internal peripheral tissues; the formation of a mucous coating might also have impeded the rapid entry of metals, therein leading to a decrease in concentration.

The accumulation of heavy metals in the gills is likely to impair respiratory and osmoregulatory functions. An analysis of the results obtained in the present studies on tissue oxygen concentrations, whole animal respiratory rates and electron microscopic structures suggested that such an impairment occurred in test prawns exposed to the various heavy metals (Fig. 9; Table 10).

The uptake of cadmium was apparently greater in prawns exposed to the mixture of metals than in those exposed to Cd alone (Table 4). In the case of Cu, Cr and Zn, the degree of accumulation of metal was less in prawns exposed to the combination of metals than that in prawns exposed to the individual metals (Tables 5-7). This would suggest that when the metals were present in combination, the concentration of one particular metal might have been increased or decreased by the presence of the other metals. In the present study, the prawns were apparently able to regulate entry of zinc, when exposed to the lower sublethal concentration of this metal (Section 6.4.1.3.).

Depuration studies revealed that the concentrations of accumulated metals in the tissues began to decline during the depuration period; an exception was in the case of prawns exposed to cadmium, where the concentration of the metal in the hepatopancreas remained high in spite of depuration (Table 8).

The observed decreases in concentrations of metals in tissues during depuration were possibly due to several factors; the sequestration and accumulation of metals in non-toxic forms; detoxification by the formation of sulfhydryl-rich, low molecular weight proteins or metallothioneins; the utilization of infiltrated metals, such as zinc, for the functioning of metabolic enzymes such as carbonic anhydrase. The higher concentration of metals noted in the hepatopancreas during depuration were also perhaps indicative of the redistribution of accumulated metals in tissues.

In studies on the functioning of the respiratory processes in prawns exposed to heavy metals, two phases were noted: (1) an initial phase of increase, in tissue respiration as well as in whole animal respiration (Fig. 9; Table 10) which could be construed as 'overshoot responses' to the stress of heavy metal toxicity; (2) subsequent consistent
decreases in the respiratory rates which would suggest that a state of hypoxia ensued in tissues during the latter phase of the studies.

Dose and time dependent increases were observed in the levels of Na$^+$ and Mg$^{++}$ and dose and time dependent decreases in the levels of K$^+$, Ca$^{++}$ and Cl$^-$ were also observed (Tables 11-15); these changes were suggestive of disturbances in the ionic balance, occurring as a result of exposure to heavy metals. These disturbances possibly originated in ultrastructural lesions in the tissues, in renal dysfunction or in modifications in the metabolic fortifications of tissues, wrought by exposure to heavy metals.

The studies on bioenergetics revealed generalised decreases in the feeding rate, gross conversion efficiency, net conversion efficiency and metabolic rate in prawns exposed to heavy metals (Table 16). In prawns exposed to the higher sublethal concentration of cadmium and to the mixture of metals, the disturbances were so severe that the prawns failed to grow.

The biochemical studies on various enzymes and substrates, when viewed together, revealed the following features in prawns exposed to heavy metals:

i. The levels of glycogen were found to be decreased in all three tissues (Table 17).

ii. The concentrations of free sugars were found to be increased in all three tissues studied (Table 18).

iii. The activity levels of phosphorylase 'a' were found to be increased while the activity of phosphorylase 'b' was decreased (Tables 27,28); these changes suggest that the conversion of the 'b' form to the 'a' form occurred to facilitate the breakdown of tissue glycogen.

iv. The level of aldolase was found to be increased (Table 29); this change was suggestive of the conversion of hexoses to trioses, possibly to accelerate glycolysis.

v. A decrease in the concentrations of pyruvate was observed, coinciding with an increase in the quantum of lactate in the tissues (Tables 19,20); an increase in the activity levels of LDH was also noted (Table 30). These changes implied that the stress induced by exposure to heavy metals resulted in the conversion of pyruvate to lactate and in a consequent shift from aerobiosis to anaerobiosis.

vi. The activity levels of certain enzymes of the TCA cycle, namely, MDH and SDH, were found to have decreased in prawns exposed to the metals (Tables 30-32). An inhibition of NAD$^+$ dependent ICDH activity, as well as an increase in the activity of NADP$^+$ dependent ICDH, was also noted (Tables 33,34). The NADH$_2$ formed from the activity of NADP$^+$ dependent ICDH is known to play an
important role in the detoxification of heavy metals. The reduced activity levels of the enzymes of the TCA cycle might have arisen due to mitochondrial damage or due to a lower availability of substrates. The observed reduction in activity of the enzymes of the TCA cycle implied that the aerobic pathway of metabolism was not being favoured in prawns exposed to heavy metals; rather, there was a shift from aerobiosis to anaerobiosis.

vii. The activity level of G6-PDH (Table 36) in the present study was suggestive of the operation of the alternative pathway, namely, the HMP shunt, to satisfy energy requirements.

viii. A reduction in the activity of ATPases (Table 37) and elevations in the activity levels of ACP and ALP (Tables 38,39), were also noted.

ix. Increased concentrations of free fatty acids (Table 26) were noted in test prawns exposed to heavy metals; this would imply that the lipids served as an important energy source during the periods of stress in test prawns.

x. The levels of DNA, RNA and protein were found to be reduced (Tables 22-25) in the tissues; this finding is consistent with the belief that protein molecules serve as a source of energy at times of demand.

The various metabolic interactions consequent to heavy metal toxicity is schematically illustrated in Chart 1.

Distinct pathological changes were observed in the gills and hepatopancreas of prawns exposed to heavy metals. The prominent changes observed in the gill were thickening of the gill lamellae, fusion of lamellae, increase in the number of haemocytes, cuticular lifting and malformations at the tips of the gill lamellae (Figs. 21-24, 26-38). Blackening of the gill lamellae was observed in prawns that had been exposed to sublethal concentrations of Cd. The ultrastructural changes observed in test prawns included a reduction in the number of organelles in epithelial cells, degeneration of mitochondrial cristae and swelling and trabeculation of the endoplasmic reticulum (Figs. 55-67). The 'R' and 'F' cells of the hepatopancreas exhibited alterations in their structural integrity. Vesicles containing metal were observed in the 'R' cells of prawns exposed to Cd (Figs. 88-93).

Based on the results obtained in the present investigation it would appear that the changes observed in various parameters in test prawns were an attempt to counter the deleterious effect of exposure to sublethal concentrations of the four heavy metals and the mixture of metals. Thus, the heavy metals were accumulated in the vital tissues of the animals, and could also be eliminated by them to a considerable extent. The
accumulation of metals led to respiratory distress in the animals and an attempt was made to overcome this distress by deriving energy from biochemical reserves, hence the alterations in the biochemical parameters noted in the present investigation. The activation of the alternate energy generating pathways and the modulation of certain enzymes could be conceived as compensatory mechanisms aimed to counter the stress of exposure to heavy metal toxicity. Unfortunately, these various compensatory processes apparently did not guarantee the success of or the survival of the species in its ecological niche. There was a progressive death of cells, as revealed by the histopathological studies; these changes indicate that the functional organelles were ultimately unable to perform their functions effectively, leading to progressive augmental stress in the animals. Apparently, the compensatory process could not be persisted with for long since the changes involved led to reductions in the metabolic cost, stunting the growth rate and, above all, depleting essential metabolites and sources of energy which were necessary for breeding and reproduction of the species. These changes constitute a cause for concern in aquaculture practices and in environmental management.

It is evident that the problem of heavy metal pollution is likely to worse unless serious measures are resorted to inorder to study and manage the environmental consequences. In this context, the following may be considered:

1. Sensitive techniques need to be devised for the biological monitoring of heavy metal pollution in the environment. The exposure of prawns to heavy metal pollution in the field can be routinely monitored by the measurement of metals in tissues and by assessing the presence of metallothionein in tissues, using techniques such as Radio Immuno Assay (RIA) and Enzyme Linked Immuno Sorbent Assay (ELISA).

2. Research should be conducted on the molecular basis of metal induction and metal transfer and on the hormonal control of metallothionein metal interactions. This may elucidate the physiological role of metallothionein in cellular functions and in the detoxification of heavy metals.

*Macrobrachium malcolmsonii*, a most important decapod prawn, is a readily marketable commodity, the worldover. The present study has vividly demonstrated the extent to which heavy metals can damage this important animal species, and emphasise the need to protect this important economic resource from the perils of pollution due to heavy metals. In addition, *M. malcolmsonii* may play an important role as a biological indicator of heavy metal pollution; in the present study, black pigmentation was noted, especially in the gill region, of prawns exposed to the toxic effects of cadmium. This
phenomenon could possibly be exploited as a measure in quality control. Thus, *M. malcolmsonii* could be regarded as a suitable species for the evaluation of the quality of water and for use in environmental management.
Chart 1. Schematic illustration of various metabolic interactions consequent to heavy metal toxicity

**HMP SHUNT**

- **G6-PDH**
- NADPH<sub>2</sub> (Detoxification)
- ATP
- FFA

**FREE SUGAR POOL**

- **Phosphorylase 'a'**
- **AMP**
- **ATP**
- **Aldolase**

**PROTEIN**

- DNA
- RNA

**PYRUVIC ACID**

- **LDH**
- **Lactic Acid**

**KREBS CYCLE**

- **ICDH**
- **NAD<sup>+</sup> dependent**
- **NADP<sup>+</sup> dependent** (Detoxification)
- **MDH**
- **SDH**

**ADP + Pi**

**Total ATPases**

**ATP**

- Increase in the level of concerned parameter
- Decrease in the level of concerned parameter