SOME ASPECTS ON ECOTOXICOLOGY AND ECOPHYSIOLOGY OF SHRIMP *PENAEUS SEMISULCATUS* (de HAAN, 1844) TO COPPER, CADMIUM AND ZINC

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सारांश

वर्तमान अध्ययन में, हरित पुलि झींगा *पेनिअस सेमीसलकेटस,* जो भारत के समुद्री जलों विशेषतः मंडपम और पाक उपसागर के तटों में पाए जाने वाला एक साधारण चिंगट है, में कोपर, कैड्मियम और ज़िंक के प्रभाव का मूल्यांकन करने का प्रयास किया गया है. कोपर, कैड्मीयम और जिंक में तीव्र विषाक्तता आमापन का परीक्षण किया गया. कोपर (6.98पी पी एम), कैड्मियम (2.8 पी पी एम) और ज़िंक (5.00 पी पी एम) में 96 घंटों में पातक सांद्रता याने LC_{50} 96 h का आकलन किया गया. तीनों घातुओं में विषाक्तता का मापन कैड्मियम > ज़िंक > कोपर (Cd>Zn>Cu) के क्रम में था. जैव संचयन, शारीरिक - जैव रासायनिक अध्ययन और ऊतक - रोगविज्ञान अध्ययन केलिए LC_{50} मूल्यों के आधार पर तीनों धातुओं की उप पातक सांद्रताओं का चयन किया गया जो - कोपर केलिए 0.7 पी पी एम और 1.4 पी पी एम, कैड्मियम केलिए 0.25 पी पी एम और 0.5 पी पी एम तथा जिंक केलिए 0.5 पी पी एम और 1.00 पी पी एम थी. सभी पातकीय एवं उप पातकीय प्रभावनों में क्लोम पटलिका और बहि कंकाल में कालापन देखा गया. धातुओं का संचयन मुख्यतः हेपटा पान्क्रियास, क्लोम, पेशी, पुछ और पृष्ठवर्म में होता है. परीक्षण के परिणाम से यह मालूम पडा किये जीव कोपर, कैड्मियम और जिंक का संचयन नहीं कर सके. उप पातक सांदता में धातुओं की मात्रा के आधार पर शरीर के अंगों में जैव संचयन होता है. शारीरिक - जैव रासायनिक अध्ययनों में, कोपर, कैड्मियम तथा जिंक की आपाती सांद्रता में रखे गए चिंगटों में श्वसन दर की बढ़ती देखी गई. धातुओं के प्रभावन विभिन्न दशाओं में जैव रासायनिक घटकों , कार्बोहाइड्रेट, प्रोटीन और लिपिड की मात्रा में घटती देखी गई. ग्लाइकोजन निम्नीकरण, फोस्फोरिलेस द्वारा षुगर पूल की बढ़ती , लैक्टेट डीहाइड्रोजनेस की बृद्धि से लैक्टिक आसिड का उत्पादन जैसे ऊतक ऊर्जा उत्पादन से होने वाले एन्ज़ाइम के परिवर्तन से सन्निकट घटकों में भी परिवर्तन होता है. डी एन ए और आर एन ए घटकों के परिवर्तन से प्रोटीन संश्लेषण का संदमन होता है. संश्लेषण परिवर्तन से मुक्त वसा अम्ल की बढ़ती होती है और लिपिडों की गतिशीलता बढ़ाने से लिपिड मात्रा में घटती होती है. ऊतक रोग विज्ञान अध्ययनों से, कोपर, कैड्मियम और जिंक को पातक और उप पातक सांद्रताओं से प्रभावित करने पर क्लोम एवं हेपाटोपानक्रियास के कोशों की संरचनात्मक समानता व्यक्त हो गई. परासंरचना अध्ययनों से कोशिकांगों , विशेषतः सूत्रकणिका (माइटोकोन्ड्रिया) , एन्डोप्लास्मिक रेटिकुलम और केंद्रक के परिवर्तनों का आकलन किया जा सका. इन सभी अध्ययनों से यह साबित हुआ कि पानी की गुणता और पर्यावरण प्रबंधन के परीक्षण में उपर्युक्त करनेलायक अनुयोज्य जाति है *पेनिअस सेमीसल्केटस.* कोपर, कैड्मियम और जिंक से प्रभावित चिंगटों के क्लोम पटलिका में काला रंग होने की विशेषता से मालूम पडा कि पर्यावरण में होने वाले भारी धातुओं के प्रदूषण के सूचक के रूप में *पेनिअस सेमीसल्केटस* को उपयुक्त किया जा सकता है.

ABSTRACT

The thesis highlights the effect of copper, cadmium and zinc in green tiger prawn *Penaeus semisulcatus*, one of the penaeids occurring throughout the Indian coastal waters particularly in the Gulf of Mannar and Palk Bay. Acute toxicity bioassays were conducted for copper (Cu), cadmium (Cd) and zinc (Zn). The 96 h LC_{50} for copper, cadmium and zinc were 6.98 ppm, 2.8 ppm and 5.00 ppm respectively. The degree of toxicity of the three metals were in the order Cd > Zn > Cu. Based on the LC_{50} values, two sublethal concentrations of copper (0.7 ppm and 1.4 ppm), cadmium (0.25 ppm and 0.5 ppm) and zinc (0.5 ppm and 1.00 ppm) were selected for bioaccumulation, physio-biochemical and histopathological studies. In all the lethal and sublethal exposures, blackening of the gill lamellae and exoskeleton was noticed. The accumulation of metals was pronounced in the hepatopancreas, gills, muscle, tail and carapace. The results of the study suggest that the shrimps could not regulate the accumulation of Cu, Cd and Zn. The bioaccumulation in the selected organs/tissues was found to be dose dependent. In the physio-biochemical studies, an increase in the respiration rate was found in shrimps exposed to lethal concentrations of copper, cadmium and zinc. The biochemical components, carbohydrate, protein and lipid were found to get reduced during the various phases of metal exposure. The observed change in the proximate components is attributed to the alterations in the enzymes involved in cellular energy generation processes, inhibition of protein synthesis by the alteration in the DNA and RNA content and increases in the free fatty acid content due to changes in the synthesis and mobilization of lipids. Histopathological changes include haemocytic infiltration, swelling of gill lamellae, fusion of gill lamellae, lifting of lamellar epithelium, fusion and necrosis of secondary gill lamellae in gills and tissue debris, necrotic tubules, swelling and abnormal lumen in hepatopancreas. Ultrastructural changes in gills include damaged nuclear membrane, disrupted mitochondria, distorted endoplasmic reticulum, apical cell damage, and damage to mitochondrial and nuclear membrane. In hepatopancreas vacuole formation, breakage of cell membrane, swelling of nuclear membrane, condensed nucleus, aggregations in nucleus, disrupted endoplasmic reticulum and formation of electron dense bodies were the major changes.

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INTRODUCTION

1. INTRODUCTION

In recent years, the role of metals in causing environmental pollution has been well recognized and is gaining significance. Many of these metals occur naturally in the environment and in trace amounts are essential to the normal metabolism of the aquatic organisms. However, industrial and agribusiness wastes, in some cases, have elevated the natural levels of such metals in the aquatic environment.

Toxicity tests, otherwise called "Bioassay tests" are performed widely to evaluate the impact of chemicals both on aquatic and terrestrial organisms. Since 1950, the acute toxicity testing has become the 'work house' for the detection, evaluation and abatement of water pollution. Information generated from toxicity tests can be of use in the management of pollution for the purpose of (i) prediction of environmental effects of a waste, (ii) comparison of toxicants or animals or test conditions and (iii) regulation of discharge (Buikema *et al.*, 1982). Acute toxicity studies have been performed to determine the effects of metals on marine and freshwater invertebrates and fishes (Sprague, 1969, 1970).

Decapod crustaceans form an important component of aquatic biota and are found in fresh, brackish and marine waters. Decapod crustaceans such as prawns, crabs, shrimps and lobsters form an important group of seafood items. The US Environmental Protection Agency has developed guidelines for deriving national water quality criteria for the protection of aquatic organisms and their uses. These guidelines have been in place for a long time and states have used them to establish water quality standards for their water bodies. More recent reports have been directed towards the development of technical regulation based on the concept that bioassessment and biocriteria programs for estuaries and near coastal waters are interrelated and are critical components of comprehensive water resource protection and management. This is a holistic

approach to protection and management, integrating biological assessments into traditional chemical and physical evaluations and augmenting the established water quality criteria (Russo, 2002).

The bioaccumulation or bioconcentration of toxic substances has become a cause for concern in pollution toxicology as it may wreak havoc with the higher trophic members. Despite the relatively low concentrations of trace metals in the surrounding medium, aquatic organisms take up and accumulate them in their soft tissues to concentrations several folds higher than those of ambient levels (Wright, 1978; Bryan, 1979). The ability of many edible aquatic organisms to accumulate metals is potentially hazardous to human health. Shellfishes commonly accumulate much higher concentrations of trace metals than the finfishes. This ability has been found in *Penaeus semisulcatus* and *Mytilus edulis* and so shellfish can be considered as indicators of environmental quality (Eustace, 1974; Philips, 1976).

The pattern of bioaccumulation of heavy metals in animals differs from metal to metal and from organ to organ. Abiotic and biotic factors also influence the accumulation of heavy metal in animal tissues. The biological significance of metal deposition in soft tissues of decapod crustaceans is still a matter of intense debate (Gibson and Barker, 1979; Dall and Moriarty, 1983; Rainbow, 1988; 1995a, b; 1997a, b.). Trace metals are accumulated by marine invertebrates to body concentrations higher than the concentration in the surrounding seawater environment (Esler, 1981; Rainbow, 1990).

Regarding, nonessential and toxic metals like lead, cadmium and mercury, there is a general agreement that intracellular inclusions of these metals reflect detoxification mechanisms. In contrast, the interpretation of respective metal granules is controversial for essential metals like copper, iron and calcium. Some authors consider them as storage compartments for physiological requirements whereas others advocate for their role in removal and detoxification of surplus

metal acquired passively from the feed or water (Al-Mohanna and Nott, 1985, 1986, 1987; Rainbow, 1988).

A sequence of metabolic and biochemical changes are known to precede the histological manifestation of pathological alterations in tissues. Therefore, an understanding of the biochemical changes would appear to be a rational approach to the assessment of the toxic stress caused by the metal poisoning. The biochemical changes that can be studied in organisms include the changes in protein, carbohydrate and lipid. The difference in the changes in these basic components will indicate the changes in the activity of the substrate level metabolites and enzymes involved in the oxidative metabolism, hydrolysis and detoxification. The extent of the deleterious effects of various stress producing substances can be elucidated from the studies on the concentrations or activities of various metabolites and the metabolic processes occurring *in vivo*.

In aquatic organisms the rate of oxygen consumption is considered to be a useful tool to assess the influence of environmental stressors like salinity, pH, temperature etc. The rate of respiration serves as an index of energy expenditure (Vernberg and Vernberg, 1972; Thurberg *et al.*, 1973; Vijayaraman, 1993). The respiratory responses of an organism to any environmental contamination may provide information about the magnitude of the stress posed to the exposed population of organisms. Damage to the physiological processes of the organisms may cause morbidity and, ultimately mortality. The measurement of oxygen consumption is also a sensitive method of establishing the relative importance of various environmental factors. The findings thus obtained may help in the evaluation of the possible resistance or susceptibility of the tissue concerned to the heavy metal. Such studies can also offer clues as to the extent of possible tissue damage.

The biochemical composition of crustaceans changes with development and growth, particularly during moulting and maturation and also due to the

"stressors' that include heavy metals and pesticides. The major biochemical constituents are the energy yielding proteins, carbohydrates and lipids. Various organs differ both in quantity and quality of the protein, carbohydrate and lipid components.

Various indices of metabolism have been formulated to evaluate the intensity of stress in a given organism. Certain metabolites have been known to show pronounced response to the stressors. Biochemical studies afford a rational way of assessing early changes in various tissues caused by exposure to toxic metals. Besides, information on the interplay between certain important metabolites might lead to a better understanding of the aerobic and anaerobic potential of cells and tissues and about which the energy requirements are satisfied.

The exposure of aquatic organisms to pollutants in their environment may result in various biochemical, physiological and histological alterations in the vital tissues. Histological methods have been used to assess the effects of pollutants on aquatic organisms, since such studies bear a direct testimony to the deleterious effects of toxicants. A number of light microscopic studies have been performed on the general structure and function of the crustacean gill and hepatopancreas. Studies on the ultrastructure of the gills and of the hepatopancreas in crustaceans include those of Loizzi (1971) and Talbot *et al.* (1972). However, there have hitherto been few attempts to document histological changes occurring in the tissues of the gills and hepatopancreas of crustaceans following exposure to different concentrations of metal ions.

The present study is carried out to evaluate the effect of copper, cadmium and zinc in the green tiger prawn *Penaeus semisulcatus* (de Haan, 1844), a common shrimp occurring throughout the Indian marine waters with a dominance in the Gulf of Mannar and Palk Bay, with the following objectives.

1. To determine the 96 h LC_{50} levels for the above heavy metals.

2. To investigate the bioaccumulation patterns in major soft tissues of *Penaeus semisulcatus* exposed to lethal and sublethal concentrations of the heavy metals.

3. To determine the rate of oxygen consumption in the shrimp exposed to lethal concentrations of heavy metals at various time intervals.

4. To determine the effects of lethal and sublethal concentrations of copper, cadmium and zinc on important biochemical components *viz.* protein, carbohydrate and lipid in various tissues of the shrimp.

5. To elucidate the structural changes in the gill and hepatopancreas of *Penaeus semisulcatus* exposed to lethal and sublethal concentration of copper, cadmium and zinc through light and electron microscopy

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Bioassay Studies

Copper is an essential trace metal required in small doses by organisms for metabolic functions, but it is potentially very toxic if the internal available concentration exceeds the capacity of physiological/ biochemical detoxification processes (Sunda and Hanson, 1987; Rainbow, 1992). Normal transport, storage and metabolism of copper must thus safeguard the cells against toxicity. This is partially true for decapod crustaceans, that require copper for biosynthesis of the respiratory pigment, haemocyanin, dissolved at huge concentrations in haemolymph (Rtal and Truchot, 1996).

Cadmium, a non-essential metal is a micro pollutant with a wide range of ecological and physiological effects. It is considered as a potential hazard to marine organisms under elevated levels and crustaceans are known for their sensitivity to cadmium (White and Rainbow, 1982; Rainbow and White, 1989; Bambang *et al.*, 1994).

Zinc is an essential element and *in vivo* levels are therefore regulated by most organisms. In decapod crustaceans the absorption of zinc and probably other metals from solution is almost certainly a passive process involving absorption on to the surface of the gills and inward diffusion, probably attached to organic molecules (Bryan, 1967; 1971).

Evaluation of the toxic effect of certain heavy metals (Hg, Cd, Pb, As and Se) on the common Indian marine crab *Scylla serrata* showed mercury to be the most toxic among the metals tested (Krishnaja *et al.*, 1987), and that cadmium showed an increase in toxicity with increase in time of exposure.

The acute toxicity of cadmium to different embryo stages of the penaeid shrimp *Penaeus japonicus* and the effect of copper on the survival and osmoregulation of various developmental stages of shrimp *Penaeus japonicus* were reported by Bambang *et al.* (1994; 1995) who found that the tolerance to copper increased with the developmental stage of the shrimp.

In the marine amphipod, *Allorchestes compressa*, copper was found to be 6 times more toxic than cadmium and 4 times more toxic than that of zinc (Ahsanullah *et al.*, 1988). The LC_{50} levels of copper for *Penaeus indicus*, *Penaeus merguensis* and *Crangon crangon* were 0.3, 6.1 and 1.9 ppm Cu/l respectively (Mary Carmel *et al.*, 1983; Denton and Burdon, 1982 and Portman and Wilson, 1971).

Morphological and behavioral studies have emphasized the importance of both the activity and chemical senses of crustaceans (Mc Leese, 1970; 1974; Mc Leese *et al.*, 1977). Studies on the physiology and behavioral responses of marine and estuarine organisms to pollutants often necessitate the establishment of acceptable concentrations of the pollutants (Henderson, 1957; Anderson, 1971; Preston, 1971; Tarzwell, 1971; Sprague, 1971, 1976; Vernberg and Vernberg, 1974; Vernberg, 1975; Vernberg and De Coursey, 1977; Nammalwar, 1982) in the natural habitat.

The acute toxicity levels of *Penaeus indicus* to zinc are reported by Visvanathan and Manisseri (1993). A few data are available on the acute toxicity bioassay of cadmium, copper and zinc to marine crustaceans (Bahner and Nimmo, 1975; Ahsanulla, 1976; Sivadasan *et al.*, 1986) and for freshwater prawns (Nimmo *et al.*, 1977; Vijayaram and Geraldine, 1992 and Vijayaraman, 1993).

2.2. Bioaccumulation

2.2.1. Copper

The marine environment is the final reservoir for heavy metals produced by both anthropogenic and natural activities. The concentrations of metals in coastal and estuarine areas tend to be higher than in the open sea, due to anthropogenic input. The copper level in the unpolluted marine waters is less than 0.05 μg/l, but in polluted coastal zones and estuaries, concentrations of copper exceed by far this value (Saager *et al.*, 1997).

Decapod crustaceans can regulate their body copper concentration, which is required for haemocyanin synthesis (Alikhan, 1972; Alikhan *et al.*, 1990; Alikhan and Storch, 1990; White and Rainbow, 1982). However, above a certain concentration of copper in the external medium, the regulation breaks down and decapod crustaceans accumulate this metal, which becomes toxic at high concentration (Rainbow, 1985; Sunda and Hanson, 1987; Scott-Fordmand and Depledge, 1997; Soegianto *et al.*, 1999).

Elevated concentrations of copper are lethal as demonstrated in *Callianassa australiensis* (Ahsanullah *et al.*, 1981a, b), *Penaeus merguiensis* (Denton and Burdon Jones, 1982) and *Penaeus japonicus* (Bambang *et al.*, 1994). Several adverse effects on physiology of crustaceans have been reported, particularly on respiration and osmoregulation if high concentration of copper was present in the environment. Exposure to high copper levels disrupted respiration in *Carciuns maenas* (Depledge, 1984; Boitel and Truchot, 1989) and *Cancer pagurus* (Spicer and Weber, 1991, 1992), and it affected osmoregulation in *Carcinus maenas* (Thurberg *et al.*, 1977; Boitel and Truchot, 1989; Hansen *et al.*, 1992; Soegianto *et al.*, 1999).

Accumulation of heavy metals from the aquatic milieu has been reported in crabs. In *Callinectes spp*, copper concentrations were higher than other metals in the hepatopancreas. In *Scylla serrata*, the pattern of accumulation of heavy

metals in the tissues in the laboratory experiment was similar to what was observed in the environment: hepatopancreas > gill > muscle. Further, increased uptake of metals by tissues was recorded with increase in the test concentrations (Devi and Yogamoorthy, 1997; Narayanan, 1989; Narayanan *et al.*, 1997; Sastre *et al.*, 1999).

2.2.2. Zinc

The accumulation and regulation of zinc in invertebrates have been described by various authors. The bioaccumulation of zinc in the lobster *Homarus vulgaris*, crab, *Carcinus maenas* and shrimp *Crangon crangon* has been reported (Bryan, 1964, 1976; Rainbow, 1988). Decapod crustaceans regulate the body concentration of zinc over a wide range of ambient zinc bioavailability (Bryan, 1964; 1966; 1967; 1968; 1976; White and Rainbow, 1982; Bryan *et* al., 1986.). White and Rainbow (1982), carefully defined physico-chemical conditions for regulation of zinc in the laboratory using *Palaemon elegans*.

2.2.3. Cadmium

There is no evidence to suggest that any decapod regulates body cadmium concentration to a constant level. The bioaccumulation of cadmium in various tissues has been reported in the crab *Carcinus maenas* (Wright, 1977a, b; Jennings and Rainbow, 1979; Rainbow, 1985, 1988), the prawns *Palaemon elegans* (White and Rainbow, 1982, 1986), *Palaemon serratus* (Devineau and Amiard Triquet, 1985), *Macrobrachium malcolmsonii* (Vijayaraman, 1993) and *Crangon crangon* (Dethlefson, 1978; Amiard *et al.*, 1985), and in the lobster *Homarus americanus* (McLeese *et al.*, 1981). The accumulation of cadmium was found to be more in the hepatopancreas of *Carcinus maenas* (White and Rainbow, 1986), *Cancer pagurus* (Overnell, 1986) and *Homarus americanus* (Engel and Brouwer, 1984).

2.3. Physio-Biochemical studies

The effect of heavy metals on the respiratory efficiency of crustaceans has been studied in crabs (Raymont and Shields, 1963; Vernberg and Vernberg, 1972; Collier *et al.*, 1973; Thurberg *et al.*, 1973; Bubel, 1976; Vernberg and De Coursey, 1977; Reish, 1978; Reddy, 1980; Gokhle and Borgaonkar, 1985; Bharani kumar, 1986; Tulasi *et al.*, 1987; Narayanan, 1989; Sarojini *et al.*, 1989; Sakundala, 1992), cray fish (Anderson, 1978; Costa, 1985; Miaz-Mayans *et al.*, 1986; Torreblanca *et al.*, 1987), fresh water shrimp *Caridina rajadhari* (Chinnayya, 1971) and in prawns *Palaemon serratus* (Papathanassiou,1983) and *Macrobrachium malcolmsonii* (Vijayaraman, 1993).

Reddy and Ramamurthi (1997) reported on the inhibitory effect of pesticide, phosalone in the mitochondrial function and respiratory metabolism of *Penaeus monodon* resulting in lesser production of energy molecules.

Studies pertaining to the effect of heavy metals on biochemical components and function of hepatopancreas, gills and muscle of crustaceans are scanty. The changes in these vital organs due to various stressors following exposure to pesticides, dichlorves, aldrin, endosulphan and phosphamidone have been revealed in *Macrobrachium lamarrei* (Omkar and Shukla, 1985) and in *Macrobrachium malcolmsonii* following exposure to cadmium, copper, chromium and zinc (Vijayaraman, 1993).

In fishes exposed to heavy metals a decrease in glycogen reserves has been observed (Qayyum and Shaffi, 1977; Koundinya and Ramamurthi, 1979; Srinivassalu Reddy *et al.*, 1986). Toxic stress following exposure to heavy metals induce an amplified utilization of carbohydrate and glycogenolysis. The reduction of carbohydrate is possible when concentration of free sugars increases in the

tissue. The elevation in the free sugar level has been observed in *Macrobrachium malcolmsonii* (Vijayaraman, 1993; Ramalingam, 1988, 1989, 1990, 2003; Sakundala, 1992) exposed to heavy metals and also in fishes.

The effect of cadmium leads to a reduction in carbohydrate and lipids in gill, muscle, liver, intestine and kidneys of *Oreochromis mossambicus* (Balasubramanian, *et al.* 1999). The effect of cadmium on carbohydrate reduction in tissues was reported in invertebrates by Vijayaraman (1993); and Indra (1998). The decrease in carbohydrate was also reported by Srinivasalu Reddy *et al.* (1986) in *Penaeus indicus* exposed to phosphamidon. A decrease in glycogen reserves has been reported in *Macrobrachium lamarrei* exposed to dichlorves, aldrin, endosulphan and phosphamidon (Omkar and Shukla, 1985) and in *Macrobrachium malcolmsoni* when exposed to copper, cadmium, chromium and zinc (Vijayaraman, 1993).

Anoxic conditions create an increase in carbohydrate consumption through the activation of phosphorylase enzyme in fishes (Larsson, 1975). *Barytelphusa guerini* (Fingerman *et al.*, 1981). *Therapon jarbua* (Selvakumar, 1981) *Sarotherodon mossambicus* (Ramalingam, 1988). *Spiralotelphusa hydrodroma* (Sakundala, 1992) *Carassius auratus* (Gargiulo *et al.*, 1996) and in the crab *Scylla serrata* (Sreenivasulu Reddy and Bhagyalakshmi, 1994) exposed to cadmium and pesticides.

Depletion of proteins in tissues following exposure to various toxicants has been reported in fishes, *Oncorhynchus kisutch* (Mc Leay and Brown, 1974) *Sarotherodon mossambicus* (Ramalingam and Ramalingam, 1982) and *Mugil cephalus* (Mihelic *et al.*, 1999), crabs *Scylla serrata* (Narayanan, 1989) *Spiralotelphusa hydrodroma* (Sakundala, 1992) *Carcinus aestuari* (Mihelic *et al.*, 1999) shrimp, *Metapenaeus monoceros* (Vijayalakshmi and Ramana Rao, 1985) and the prawn *Macrobrachium malcolmsonii* (Vijayaraman, 1993).

Reduction of protein in the hepatopancreas has been reported in *Macrobrachium kistensis* exposed to pesticides (Nagabhusanam *et al.*, 1987). Jackim *et al.* (1970) observed an inhibition in nucleic acid levels in killi fishes when exposed to heavy metals. Histochemical studies revealed an inhibition of RNA synthesis in *Scylla serrata* when exposed to mercury, cadmium and zinc (Narayanan, 1989).

Kulkarni and Kulkarni (1989) reported elevation in blood glucose, lactic acid, blood serum proteins, sodium, potassium and specific activity of aspartate aminotransferase and alanine aminotransferase in the crab, *Scylla serrata* exposed to malathion. Lee (1988) explained the action of Glutathione S-transferase (GST), an enzyme system which conjugates glutathione to a variety of xenobiotics. Fayi *et al.* (1990) described the activity of cytochrome oxidase and molecular distribution of copper in the hepatopancreas of the prawn *Penaeus orientalis*. Yeragi *et al.* (2000) reported a reduction in protein content in gills, testes, ovaries, larger chelae muscle, smaller chelae muscle and hepatopancreas of the marine crab *Uca marionis* exposed to pesticide malathion. The reduced activity of cytochrome P450 was reported in *Scylla serrata* (Hong *et al.*, 2000) and *Barbus barbus* (Hugla and Thome, 1999), exposed to Cu, Zn, and Cd and polychlorinated biphenyls.

Reduction in the fat content of the liver was reported in vertebrates exposed to mercury, arsenic, phosphorous and chloroform exposure (Bell *et al.*, 1972). The fat content of *Metapenaeus monoceros* exposed to phosphamidon and methyl parathion was found reduced (Srinivasalu Reddy *et al.*, 1986). Due to the exposure to copper, cadmium and zinc in *Macrobrachium malcolmsonii*, an increase in lipase activity has been explained by Vijayaraman (1993).

Xenobiotic detoxification mechanisms have been explained in vertebrates and invertebrates by various studies. The role of metallothioneins in detoxification of metals is reported in *Palaemon elegans* when exposed to

copper, zinc and cadmium (White and Rainbow, 1984) and in *Callinectes sapidus* exposed to cadmium (Syring *et al.*, 1992; Brouwer *et al.*, 1994; 2000).

2.4. Light and Transmission Electron microscopy (TEM)

The exposure of aquatic organisms to pollutants in their environment may result in biochemical, physiological and histological alterations in the vital tissues (Hinton *et al.*, 1973; Rao *et al.*, 1982; Anbu and Ramaswamy, 1991; Geraldine *et al.*, 1999; Bhavan and Geraldine, 2000).

Histological methods have been used to assess the effects of pollutants on aquatic organisms, since such studies bear a direct testimony to the deleterious effects of toxicants (Hinton, *et al.*, 1973). A number of light microscopic studies have been performed on the general structure and function of the crustacean gill and hepatopancreas (Loizzi, 1971; Barker and Gibson, 1978; Gibson and Barker, 1979). Similarly histological alterations have been characterized in crustaceans such as *Palaemonetes pugio* and *Macrobrachium spp* exposed to various chemicals such as copper, mercury, cadmium, zinc, pentachlorophenol and dithiocarbomates (Ghate and Mulherkar, 1979; Rao *et al.*, 1982; Doughtie and Rao, 1983; Rao and Doughtie, 1984; Victor *et al.*, 1990; Vijayaraman, 1993; Maniseeri and Menon, 1995; Bhavan and Geraldine, 2000).

However, there have been a few attempts to examine the histopathological changes effected by pollutants in crustaceans, including cadmium in *Penaeus duorarum* (Couch, 1977), *Palaemonetes vulgaris* and *P. pugio* (Nimmo *et al.*, 1977), *Palaemon serratus* and *Crangon crangon* (Papathanassiou, 1983, 1985) and *Marobrachium malcolmsonii* (Vijayaraman, 1993), copper in *Macrobrachium spp* and *caridina spp* (Ghate and Mulherkar, 1979), *Marobrachium malcolmsonii* (Vijayaraman, 1993), *Metapenaeus dobsoni* (Manisseri and Menon, 1995) and in *Penaeus japonicus* (Soegianto *et* al., 1999), chromium in *Palaemonetes pugio* (Doughtie and Rao, 1983; Rao and Doughtie, 1984) and mercury in *Scylla serrata* (Narayanan, 1989).

Studies on the ultrastructure of the gills and of the hepatopancreas in crustaceans include those of Loizzi (1971) and Talbot *et al.* (1972). However, there have been few studies to document ultrastructural changes occurring in the tissues of the gills and hepatopancreas of crustaceans following exposure to of metal ions (Bubel, 1976; Nimmo *et al.*, 1977; Papathanassiou, 1983, Papathanassiou and King, 1986; Doughtie and Rao, 1983; Vijayaraman, 1993; Manisseri and Menon, 1995; Soegianto *et* al., 1999).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Collection and transportation of test shrimps

The shrimp, *Penaeus semisulcatus* were collected from both Palk Bay and Gulf of Mannar (Lat $8^0 55^I$ - 9^0 15^I N and Long 78^0 0^I – 79^0 16^I E) using a traditional type of net, which can be operated by two persons along the inshore shallow areas, called 'Thalluvala'. Shrimp collections were done mainly at night (21.00-23.00 h), since the shrimp is a nocturnal feeder, comes out from the substrate during night for feeding. The collected shrimps were sorted out according to the size and the required size group 6-8 cm (4.5 - 7.1 g) for the experiment was selected. The shrimps were transported in plastic can of 40 L capacity with 20 L seawater from the place of collection and aeration was provided through portable battery operated aerators at a stocking density of approximately 5-6 no/l. The shrimps were immediately transferred to Shrimp Hatchery of Mandapam Regional Centre of CMFRI, where they were acclimatized in the seawater (30-32 ppt) collected from Gulf of Mannar. The shrimps were maintained in two-ton capacity tanks filled with clear, filtered seawater. Hydrological characteristics, such as temperature, salinity, dissolved oxygen and pH were recorded concurrently. Shrimps were fed with boiled clams *ad libitum* once in a day at night (21.00 h) during maintenance. The fecal matter and other waste materials were siphoned off daily and the water quality was maintained.

3.2. Bioassay tests under laboratory conditions.

Static bioassay for 96 h was conducted under laboratory conditions with the methods recommended for toxicity test with aquatic organisms (APHA, 1976). Healthy *P. semisulcatus* in intermoult stage of size 6-8 cm total length (4.5-7.1 g) were separated using the method of Darch (1939) for bioassay

experiments. These shrimps were starved for 24 h in order to remove the stomach contents during acclimatization prior to experiments.

Stock solutions were prepared from analar grade copper sulphate ($CuSO_4$ $5H_2O$), cadmium chloride ($CdCl_2$) and zinc sulphate ($ZnSO_4$ $7H_2O$) with deionised water following the dilution technique adopted by committee on methods for toxicity test on aquatic organisms (Sprague, 1970; 1973).

3.3. Evaluation of LC_{50} Levels.

Ten numbers of *P. semisulcatus* of the size group of 6-8 cm weighing (4.5 to 7.1 g) were starved for 24 hrs prior to experimentation, and released into tanks containing 50 L of seawater (Sprague, 1970; 1973). A series of ten static bioassay tests in triplicates were conducted with shrimps using known concentrations of copper, zinc and cadmium and the occurrence of mean percentage mortality of 20%, 50% and 80% for 24, 48, 72 and 96 hrs after exposure was recorded. Concurrently, three control experiments without the toxicant were maintained for each set of experiments (Buikema *et al.*, 1982; Ward and Parrish, 1982). Hydrological characteristics, such as temperature, salinity, dissolved oxygen and pH were recorded concurrently.

The data so obtained were plotted on a log probit chart no. 32.376 (supplied by Codex Co. Norwood, Massachusetts, USA) which gives a simple solution of the dose effect curve with 95% confidence limits for LC_{50} values. (Sprague, 1970; 1973; Mohapatra, and Rengarajan, 1995; 2000). Further lower and upper limits and slope function were also computed. The slope functions (S) were calculated as per the method of Reish and Oshida (1987)

3.4. Morphological and Behavioural observations

The morphological and behavioral changes in the shrimp *P. semisulcatus* were studied during the acute bioassay test. External body parts were observed

particularly; the exoskeleton, gills, appendages, telson and uropod and any colour change in these parts were recorded for morphological changes. Behavioral changes observed during the bioassay study included the pattern of movement/ swimming behavior of the shrimp.

3.5. Sublethal Studies

Sublethal studies of metal effects were conducted for 14 days in two different concentrations (10% of LC_{50} and 20% of LC_{50}). For 10% of LC_{50} sublethal levels the concentration of copper (0.7 ppm/l), zinc (0.5 ppm/l) and cadmium (0.25 ppm/l) and for 20% of LC_{50} copper (1.4 ppm/l), zinc (1.0 ppm/l) and cadmium (0.5 ppm/l) were selected. Triplicates were conducted in all the experimental and control exposures. The physicochemical parameters of water during the study were salinity (30.1-30.3 ppt), Temperature (25.1-26.4 ^{0} C), pH (8.01-8.03) and dissolved oxygen (5.03-5.82 mg/l).

Each of the experiments was conducted in 50 litre plastic tubs with 10 numbers of shrimps (5 such tubs forms an experimental group). Water exchange (50 %) was done daily and refilled with the same concentration of the element. Complete water exchange was done on the 3rd, 6th, 9th and 12th day of the experiment. The shrimps were fed with boiled clam meat *ad libitum*, daily at night (21.00 h). After 14 days, shrimps were collected from the tanks.

For analysis, whole shrimp (5 numbers) from the control and experiment group was taken. The remaining shrimps were dissected and tissues such as hepatopancreas, gills, tail and carapace were taken and dried. The temperature was maintained at 60°C for 2 days to get a constant weight. The dried samples were taken for analysis of bioaccumulation of zinc, copper and cadmium and for biochemical changes in protein, carbohydrate and lipid content due to the effect of metals. For bioaccumulation and biochemical analysis, the dried tissues from each experimental and control groups were combined to get appropriate weight of the tissues.

3.5.1. Bioaccumulation Studies

For bioaccumulation analysis, dried samples of approximately 100 mg were taken and then digested in perchloric acid: nitric acid mixture (1:4 ratio). Dried powdered sample (1 g) was taken and soaked in the above mixture for 4 h in a glass beaker. The soaked samples were digested on a hotplate for 5 h at 80°C. When the sample colour turned from yellow to white the samples were taken out, cooled and filtered using Whatmann No. 2 filter paper. They were made into 5 ml aliquots and used for determining the level of the selected metals in an atomic absorption spectrophotometer (ICP) (Agemian and Chau, 1976).

3.5.2. Physio-Biochemical Studies

3.5.2.1. Respiratory Physiology

A static method (Mohapatra and Rengarajan 1995) was used to determine the rate of oxygen consumption in the shrimps. The shrimps were exposed to LC_{50} levels of copper, cadmium and zinc. The shrimps were left undisturbed for a period of 2 h prior to the commencement of the experiment so as to allow them to get acclimatized to the test concentration, *i.e.* copper @ 6.98 ppm, cadmium @ 2.8 ppm and zinc @ 5.00 ppm. Three shrimps from each concentration were introduced into a chamber with 5 L of seawater with the same concentration of the test metal. Liquid paraffin was poured carefully to make a thin layer on the surface of the medium to avoid diffusion of atmospheric oxygen. The amount of oxygen consumed by the experimental and control shrimps were measured at different time intervals (1, 2, 3, and 4 h) by Winkler's method (Strickland and Parsons, 1972) and the respiratory rate was expressed in ml/g (wet wt)/hr.

3.5.2.2. Biochemical studies

From the dried samples, 50 mg of sample was taken for protein and carbohydrate analysis and approximately 3 g was used for lipid analysis. Protein

content in the tissues was determined by Biuret method, using bovine serum albumin as standard (Gornall *et al.*, 1949). Total lipid was estimated by gravimetric method (Folch *et al.*, 1957) and modified by Linford (1965). Carbohydrate was estimated by phenol sulphuric acid method of Dubois *et al.* (1956).

3.5.3. Light Microscopy

The hepatopancreas and gills of the shrimp were fixed in Davidson's fixative for 48 h. The preserved tissues were processed by following routine histological methods with slight modification (Bell and Lightner, 1988); dehydrated in alcohol series and embedded in paraffin wax. They were cut into sections of 6 μm thickness by a rotary microtome. The thin sections of the hepatopancreas and gills were stained using haematoxylin and eosin for observation in light microscope.

3.5.4. Transmission Electron Microscopy (TEM)

1mm^3 tissues were incised from the gills and hepatopancreas and fixed in 3 % sodium cacodylate buffered gluteraldehyde for 4 hrs under refrigeration. After decanting the solution, the tissues were subjected to sodium cacodylate buffer wash for 3 times and postfixation was done with 1% osmium tetroxide (OsO_4) for 1h under refrigeration. After decanting the OsO_4, the tissues were washed with buffer for three times of 15 mts duration. The tissues were then dehydrated in different grades of acetone (30, 50, 70, 90 and 100) each of 15 mts duration in SPURR embedding resin. The tissues were made into blocks after infiltration by keeping the mould in the incubator setting the temperature initially at 60°C for two hours and raising the temperature to 70°C for another 10 hrs. The blocks were trimmed and ultrathin sections were made by LKB Ultramicrotome (Ultranova) and stained in uranyl acetate and lead citrate for enhanced contrast and sections were observed in the Hitachi (H-600) Electron microscope choosing different magnifications.

3.6. STATISTICAL ANALYSIS

3.6.1. Bioaccumulation

Data on bioaccumulation in whole shrimp and in different tissues at lethal and sublethal levels of heavy metals were analysed by using ANOVA (SYSTAT Version 7.0).

3.6.2. Biochemical analysis

The test of significance for variations in the biochemical contents in whole animal and among tissues with the heavy metals at lethal and sublethal levels were analysed by using ANOVA (SYSTAT Version 7.0).

The test for significance among the tissues of each exposure and control were done by using "t" test (SYSTAT Version 7.0).

RESULTS

4. RESULTS

4.1. Lethal Concentration Studies

4.1.1. Lethal Concentration of copper

The results of the experiment to determine lethal concentration levels of *P. semisulcatus* exposed to copper are given in Table 1.

The 24, 48, 72 and 96 h LC_{50} levels *P. semisulcatus* to copper were found to be 16.74 ± 0.19, 12.64 ± 0.14, 8.86 ± 0.11 and 6.98 ± 0.09 ppm Cu/l respectively. The experiments showed no significant difference between replicates when tested with 1.96 SE_{diff} explained by Litchfield and Wilcoxon, 1949.

4.1.2. Lethal concentration of cadmium

The results of the experiment to determine lethal concentration levels of shrimp exposed to cadmium are given in Table 2.

The 24 h, 48, 72 and 96 h LC_{50} values for *P. semisulcatus* were 8.18 ± 1.3, 5.16 ± 0.48, 4.00 ± 0.122 and 2.68 ± 0.15 ppm Cd/l respectively. The 1.96 SE_{diff} Litchfield and Wilcoxon (1949) formula showed no significant difference between replicates of the experiments.

4.1.3. Lethal concentration of zinc

The results of the experiment to determine lethal concentration levels of shrimp exposed to zinc are given in Table 3.

The 24, 48, 72 and 96 h LC_{50} levels for *P. semisulcatus* were 11.5 ppm ± 0.25, 7.73 ± 0.18, 6.4±0.11 and 4.9 ppm Zn/l. There was no significant difference between replicates of the experiments.

Table 1: LC_{50} values and slope values of copper to *P. semisulcatus*

Time in hrs	LC_{50} value (ppm)	Slope (S)	Confidence limits (CL)	Filucidal Values (95%)	
				Upper ((ppm)	Lower (ppm)
24	16.74	2.06	1.80	29.40	9.00
48	12.64	1.72	1.50	18.96	8.42
72	8.86	1.49	1.30	11.52	6.81
96	6.98	1.39	1.22	8.51	5.72

Table 2: LC_{50} values and slope values of cadmium to *P. semisulcatus*

Time in hrs	LC_{50} value (ppm)	Slope (S)	Confidence limits (CL)	Filucidal Values (95%)	
				Upper ((ppm)	Lower (ppm)
24	8.18	2.34	2.05	16.76	4.00
48	5.16	2.13	1.86	9.6	2.8
72	4.00	1.97	1.72	6.9	2.32
96	2.68	1.58	1.34	3.6	2.00

Table 3: LC_{50} values and slope values of zinc to *P. semisulcatus*

Time in hrs	LC_{50} value (ppm)	Slope (S)	Confidence limits (CL)	Filucidal Values (95%)	
				Upper ((ppm)	Lower (ppm)
24	11.5	1.84	1.61	18.515	7.15
48	7.73	1.64	1.43	11.05	5.4
72	6.4	1.64	1.43	9.19	4.48
96	4.90	1.5	1.31	6.4	3.74

4.2. Morphological and Behavioral Characters

Various body parts of the control shrimps during the study are shown in Plate 1.

4.2.1. Cadmium toxicity changes

Shrimps exposed to 2.8 ppm Cd/l had the following morphological and behavioral changes.

4.2.1.1. Morphological

The various morphological changes after exposure are shown in Plate 2.

Among the three metals, cadmium showed more toxicity effects at a lower concentration. It caused blackening of the rostrum tip, antennae and the antennules. Melanisation was observed in the scaphognathite of the antennae, the flagella of the antennules and the pereiopods. The Black deposits were visible clearly on the exoskeleton of the cephalothorax and on the pleura. The tip of the uropod had blackened areas.

Gills showed major changes since it is directly in contact with the exposed medium. It turned black on the third day of the exposure.

4.2.1.2. Behavioral

When shrimps were exposed to cadmium, on the first day itself, they showed some erratic movements. On the second day it came to normal. They mostly found attached near to the air stone. When disturbed, they showed less reaction. On the third day, the shrimps settled down at the bottom, the movement became slow. Continuous movement of the pleopods showed that the shrimps were under stress due to the pollutant and lead to mortality.

4.2.2. Copper toxicity changes

4.2.2.1. Morphological

The various morphological changes observed in *P. semisulcatus* when exposed to copper are shown in Plate 3 A-E.

The flagella of the antennules, which are regarded as the chemosensory functional organ, got blackened due to the exposure. The exopodites of the pereiopods showed blackening and is associated very close to the gills. It is this part which gets blackened first when exposed to copper.

The melanisation of the pereiopods are prominent at the joints of ischium, merus, carpus, propodus and dactylus. Copper toxicity resulted in breakage of these parts/joints. The uropod showed blackening. The gills turned black at the third day of exposure.

4.2.2.2. Behavioral Changes

Shrimp showed erratic movements after two days of exposure in copper. They were found attached to the air tubes and stones most of the time.

4.2.3. Zinc toxicity changes

4.2.3.1. Morphological

The telson became dark and in some case even breakage occurred in the shrimps exposed to zinc (Plate 3 F, G)

4.2.3.2. Behavioral Changes

Behavioral changes include erratic movements, continuous movement of the pleopods, settling at the bottom without much movement and when disturbed with a probe, it showed slight movement of the pereiopods.

Plate 1. Various parts of control *Penaeus semisulcatus*

Dorsal view of cephalothorax

Lateral view of cephalothorax

Lateral view of abdomen

Uropods and telson

Control (A) and shrimp exposed to cadmium (B)

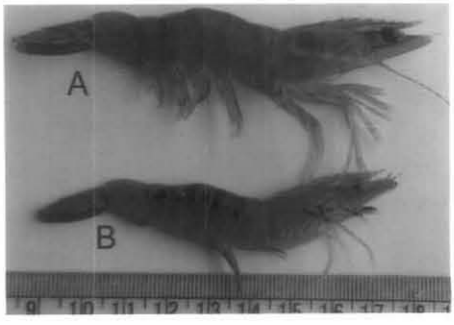
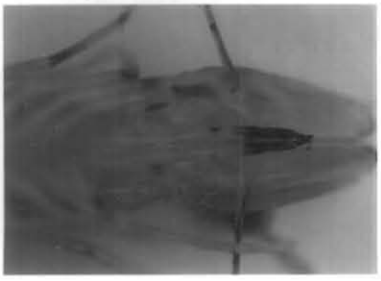
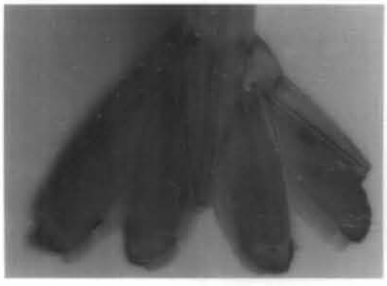


Plate 2. *Penaeus semisulcatus* exposed to cadmium



A



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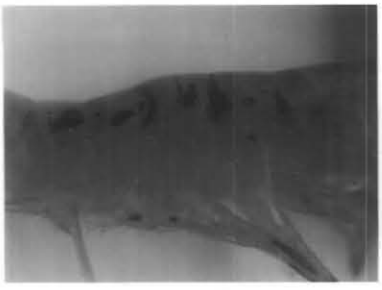
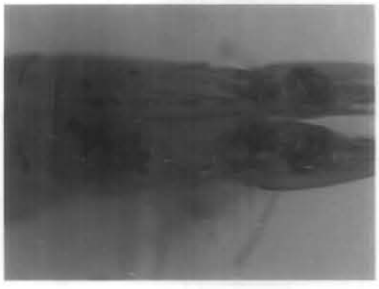
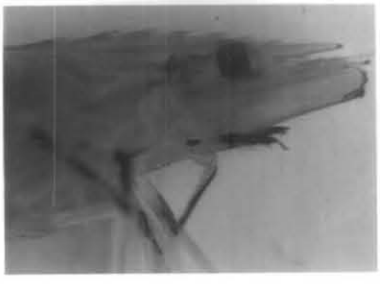


C



D



E

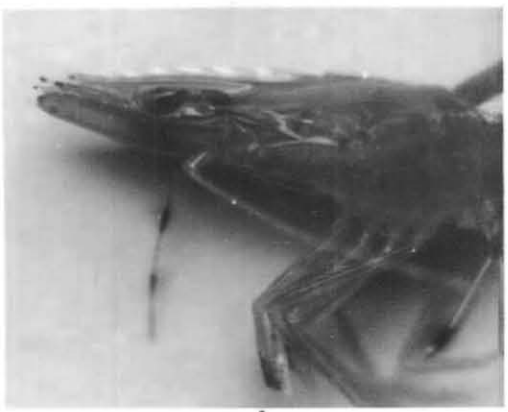
A. Ventral view showing blackening of antennules and antennae
B. Uropods showing blackening and breakage
C. Pleura with blackened areas
D. Cephalothorax with blackened areas
E. Blackening of rostrum, antennules and pereiopods

Plate 3

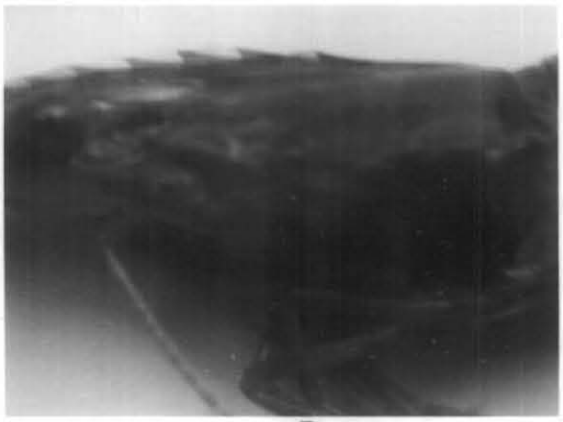
A. Melanisation in appendage and flagella
B. Blackened Gills
C. Blackening in appendages
D. Blackening of uropod
E. Blackened exopodites

F. Blackened uropod
G. Blackening of telson

Plate 3. Effects of exposure to copper



A



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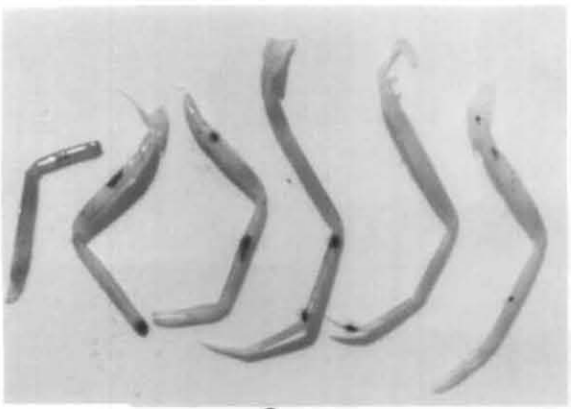
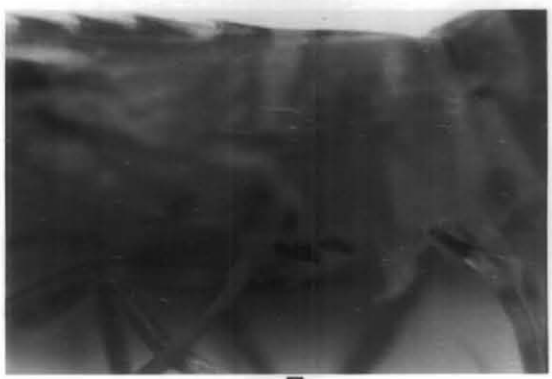


C

D



E

Effects of exposure to zinc

F

G

4.3. Bioaccumulation Studies

Bioaccumulation of copper in various organs

The bioaccumulation of copper in various tissues at lethal and two sublethal doses are given in the Table 4.

Bioaccumulation of cadmium in various organs

The bioaccumulation of cadmium in various tissues at lethal and two sublethal doses are given in the Table 5

Bioaccumulation of zinc in various organs

The bioaccumulation of zinc in various tissues at lethal and two sublethal doses are given in the Table 6.

Table 4. Bioaccumulation of copper in various tissues (µg/g dry wt of the tissue)

Tissues	Concentration	SD (±)
Lethal Concentration (6.98 ppm)		
Hepatopancreas	1587.29	59.81
Gills	284.96	24.37
Muscle	18.79	3.72
Tail	38.73	1.54
Exoskeleton	19.94	2.56
Whole shrimp	223.20	13.62
Sub Lethal (0.7 ppm)		
Hepatopancreas	2431.29	39.98
Gills	114.28	6.70
Muscle	11.17	0.79
Tail	22.25	1.79
Exoskeleton	11.26	1.43
Whole shrimp	81.43	6.24
Sub Lethal (1.4 ppm)		
Hepatopancreas	5236.32	48.53
Gills	95.37	8.95
Muscle	19.36	0.74
Tail	50.99	3.79
Exoskeleton	31.63	3.93
Whole shrimp	181.23	8.42
Control		
Hepatopancreas	334.50	10.60
Gills	78.30	3.40
Muscle	7.94	1.45
Tail	16.86	1.90
Exoskeleton	8.92	3.32
Whole shrimp	36.68	4.16

Table 5. Bioaccumulation of cadmium in various tissues (µg/g dry wt of the tissue)

Tissues	Concentration	SD (±)
Lethal Concentration (2.8 ppm)		
Hepatopancreas	1057.85	87.89
Gills	144.84	11.39
Muscle	1.07	0.18
Tail	7.39	1.51
Exoskeleton	6.38	1.42
Whole shrimp	38.73	5.78
Sub Lethal (0.25 ppm)		
Hepatopancreas	100.06	5.33
Gills	38.34	3.48
Muscle	1.16	0.12
Tail	2.71	0.70
Exoskeleton	1.55	0.68
Whole shrimp	6.42	0.82
Sub Lethal (0.5 ppm)		
Hepatopancreas	376.37	8.56
Gills	95.37	8.95
Muscle	5.07	0.80
Tail	5.89	0.64
Exoskeleton	0.82	0.26
Whole shrimp	20.93	1.84
Control		
Hepatopancreas	nd	
Gills	nd	
Muscle	nd	
Tail	nd	
Exoskeleton	nd	
Whole shrimp	nd	

nd. Not detected

Table 6. Bioaccumulation of zinc in various tissues (µg/g dry wt of the tissue)

Tissues	Concentration	SD (±)
Lethal Concentration (5.00 ppm)		
Hepatopancreas	1145.72	93.75
Gills	271.99	34.42
Muscle	54.52	2.66
Tail	94.19	1.24
Exoskeleton	39.67	3.19
Whole shrimp	77.05	4.90
Sub Lethal (0.5 ppm)		
Hepatopancreas	181.33	14.59
Gills	138.75	7.38
Muscle	59.16	3.98
Tail	84.22	12.15
Exoskeleton	18.39	5.08
Whole shrimp	62.69	3.98
Sub Lethal (1.00 ppm)		
Hepatopancreas	447.02	11.92
Gills	223.64	3.47
Muscle	84.72	6.31
Tail	122.29	8.01
Exoskeleton	37.57	2.05
Whole shrimp	70.92	3.63
Control		
Hepatopancreas	11.66	3.06
Gills	124.23	6.56
Muscle	43.50	3.85
Tail	64.76	3.24
Exoskeleton	20.74	0.59
Whole shrimp	57.48	3.11

4.3.1. Comparison of copper, cadmium and zinc accumulation in various organs

4.3.1.1. Lethal concentration

Hepatopancreas

A marked difference among the metals in bioaccumulation was observed in hepatopancreas ((f = 36.06; $p < 0.05$) (Appendix 1a). The mean concentrations of copper, cadmium and zinc in hepatopancreas were 1587.29 ± 59.81, 1057.85 ± 87.9 and 1145.72 ± 93.74 µg/g respectively. Copper concentration was five fold greater than that of control while zinc showed a 98.00 fold increase than that of control shrimp.

Gills

In the gills of *P. semisulcatus* also a significant difference among the metals were observed in bioaccumulation pattern (f = 28.27; $p < 0.05$) (Appendix 1b). The mean concentrations of copper, cadmium and zinc in gills were 284.96 ± 24.36, 144.83 ± 11.39 and 271.77 ± 34.70 µg/g respectively. Copper and zinc contents were 3.5 and 2.00 fold greater than that of the control.

Muscle

A significant difference in accumulation of copper, cadmium and zinc were noticed in the muscle of *P. semisulcatus*, exposed to lethal concentrations of the metals (f = 318.62; $p < 0.05$) (Appendix 1c). The mean concentration of copper, cadmium and zinc in muscle were 18.79 ± 3.72, 1.07 ± 0.183 and be 54.52 ± 2.65 µg/g respectively. Copper was 2.36 fold greater, while zinc was 1.25 fold greater than that of control.

Tail

In the tail, 38.73 ± 1.54 µg/g copper, 7.39 ± 1.5 µg/g cadmium and 94.76 ± 1.24 µg/g of zinc were recorded with a significant variation in bioaccumulation among metals ($f = 2808.76$; $p < 0.05$) (Appendix 1d). Copper was 2.3 fold greater while zinc showed 1.50 fold increase than that of the control.

Exoskeleton

The concentration of copper, cadmium and zinc were 19.94 ± 2.55, 6.37 ± 1.41 and 39.67 ± 3.18 µg/g respectively in exoskeleton of *P. semisulcatus* exposed to metals. A marked variation in the bioaccumulation pattern among the metals ($f = 134.88$; $p < 0.05$) (Appendix 1e) was observed in the exposure. Copper was 2.24 fold greater while zinc showed 1.81 fold increase than that of the control.

Whole shrimp

A significant difference was found in bioaccumulation among metals ($f = 351.04$; $p < 0.05$) (Appendix 1f). The concentration of copper, cadmium and zinc were 223.19 ± 13.62, 38.74 ± 5.75 and 77.04 ± 4.86 µg/g respectively. Copper concentration was six fold and zinc 1.35 fold greater than that of the control shrimp.

4.3.1.2. Sublethal concentration (10 % of LC_{50})

Hepatopancreas

P. semisulcatus showed significant difference in bioaccumulation among the metals ($f = 8564.77$; $p < 0.05$) (Appendix 2a). The concentration of copper, cadmium and zinc were 2431.29 ± 40, 100.06 ± 5.3 and 181.4 ± 14.6 µg/g in the

hepatopancreas. There was a seven fold increase in copper and 15.00 fold increase in zinc more in the tissue than that of the control level.

Gills

Copper, cadmium and zinc levels in gills of *P. semisulcatus* were 114.28 ± 6.7, 38.34 ± 3.48 and 138.75 ± 7.37 µg/g respectively and the observed differences were significant ($f = 221.49$; $p < 0.05$) (Appendix 2b). Copper and zinc levels were 1.2 fold greater than that of the control tissue.

Muscle

Metals showed a significant difference among themselves in the accumulation pattern in the muscle of *P. semisulcatus* ($f = 525.81$; $p < 0.05$) (Appendix 2c). The concentrations of copper, cadmium and zinc were 11.17 ± 0.78, 1.16 ± 0.115 and 84.21 ± 12.31 µg/g respectively in the muscle of shrimp after the exposure. The copper level was 1.40 fold while was two fold greater than the control tissue.

Tail

Copper, cadmium and zinc in the tail of shrimp were 22.24 ± 1.79, 2.71 ± 0.69 and 84.21 ± 12.14 µg/g of metals respectively, with a significant difference among the metals ($f = 107.74$; $p < 0.05$) (Appendix 2d). Both copper and zinc showed 1.30 fold increase in bioaccumulation than that of the control.

Exoskeleton

In the exoskeleton of *P. semisulcatus*, there was a significant difference in the accumulation among metals ($f = 22.67$; $p < 0.05$) (Appendix 2e). The

concentrations of copper, cadmium and zinc were 11.25 ± 1.4, 1.55 ± 0.67 and 23.39 ± 0.25 µg/g.

Whole shrimp

Copper, cadmium and zinc in shrimps were 81.43 ± 6.43, 6.41 ± 0.82 and 62.68 ± 3.97 µg/g and the observed differences were ($f = 247.61$; $p < 0.05$) (Appendix 2f).

4.3.1.3. Sublethal Concentration (20 % of LC_{50})

Hepatopancreas

The hepatopancreas of *P. semisulcatus* showed significant difference in bioaccumulation among the metals ($f = 27168$; $p < 0.05$) (Appendix 3a). The concentration of copper, cadmium and zinc were 5236 ± 48.53, 376.36 ± 8.56 and 447.02 ± 11.97 µg/g in the hepatopancreas. Copper accumulates fifteen fold while zinc had 38 fold increase in the concentration in the tissue than that of the control shrimp.

Gills

In the gills of *P. semisulcatus*, significant differences among the metals were observed in bioaccumulation pattern ($f = 286.78$; $p < 0.05$) (Appendix 3b). The mean concentration of copper, cadmium and zinc in gills were 95.37 ± 8.94, 90.99 ± 5.92 and 223.54 ± 3.47 µg/g respectively. Copper concentration was 1.2 fold greater than that of the control while zinc showed an increase of 2.00 fold with its control.

Muscle

A significant difference in accumulation of copper, cadmium and zinc was noticed in muscle of *P. semisulcatus*. ($f = 395.66$; $p < 0.05$) (Appendix 3c). The mean concentration of copper, cadmium and zinc in muscle were 95.37 ± 8.94, 5.07 ± 0.8 and 84.71 ± 6.31 µg/g respectively.

Tail

In the tail, a concentration of 50.99 ± 3.9 µg/g copper, 5.89 ± 0.64 µg/g cadmium and 122.28 ± 8.00 µg/g of zinc was observed. A marked variation in bioaccumulation among metals were observed ($f = 392.75$; $p < 0.05$) (Appendix 3d). A 3.02 fold increase in copper and 1.88 fold increase in zinc were observed in shrimps exposed to metals.

Exoskeleton

The concentration of copper, cadmium and zinc were 31.62 ± 3.93, 0.82 ± 0.26 and 37.57 ± 2.04 µg/g respectively in exoskeleton of *P. semisulcatus* exposed to metals and the bioaccumulation pattern ($f = 177.76$; $p < 0.05$) (Appendix 3e) was statistically significant. A 5.71 fold increase in copper was observed during the exposure.

Whole shrimp

The concentration of copper, cadmium and zinc were 181.20 ± 8.39, 20.93 ± 1.84 and 70.91 ± 3.63 µg/g respectively. A significant difference in bioaccumulation among metals ($f = 691.97$; $p < 0.05$) (Appendix 3f) was observed with a five fold increase in copper accumulation.

4.4. Physio-Biochemical studies

4.4.1. Respiration

The rates of oxygen consumption by *Penaeus semisulcatus* exposed to the heavy metals are shown in Table 7. The rate of oxygen consumption in shrimps exposed to copper, cadmium and zinc increased with increase in time when compared to that of the control.

Table 7. Rate of oxygen consumption in *Penaeus semisulcatus* exposed to lethal levels of copper, cadmium and zinc at various time intervals (ml/g (wet wt)/h) (± SD)

	1h	2h	3h	4h
Control	0.51 (±0.02)	0.78 (± 0.02)	1.002 (± 0.05)	1.1428 (± 0.05)
Copper	0.971 (± 0.025)	1.084 (±0.03)	1.195 (± 0.193)	2.134 (± 0.102)
Cadmium	1.093 (± 0.05)	1.830 (± 0.025)	1.965 (± 0.048)	**
Zinc	0.95 (±0.02)	1.063 (±0.05)	1.562 (± 0.05)	1.635 (± 0.05)

** Shrimps died after 3 hrs

4.4.2. Biochemical composition in *Penaeus semisulcatus*

The biochemical composition in various tissues of control shrimp is given in Table 8.

Table 8. Biochemical composition of various organs in *Penaeus semisulcatus* (% dry weight of the tissue)

	Concentration	SD (±)
Hepatopancreas		
Protein	66.46	0.42
Carbohydrate	5.33	0.11
Lipid	32.42	0.14
Gills		
Protein	40.85	0.10
Carbohydrate	1.11	0.05
Lipid	1.10	0.09
Muscle		
Protein	79.99	0.13
Carbohydrate	1.67	0.31
Lipid	9.59	0.08

4.4.3. Lethal concentration exposure

4.4.3.1: Copper

The biochemical composition of various organs of *Penaeus semisulcatus* exposed to copper at the lethal concentration of 6.98 ppm is given in table 9. In the hepatopancreas and the muscle, the carbohydrate, lipid and protein levels were significantly ($p<0.05$) lower than that of control, whereas in the gills, protein and lipid levels were significantly lower at 1% level. However, the carbohydrate concentration did not show any significant variation in the gills of the test shrimp.

4.4.3.2. Cadmium

The biochemical composition of various organs of *P. semisulcatus* exposed to cadmium at the lethal concentration of 2.8 ppm is given in table 10. At this level of cadmium, significant low levels of carbohydrate ($p<0.01$), lipid ($p<0.01$) and protein ($p<0.01$) were observed in the hepatopancreas and muscle when compared to that of control. However, in the gills only the protein showed a significant variation than that of control and the other two variables were not showing any level of significance.

4.4.3.3. Zinc

The data for assessing the effect of zinc on the biochemical composition of various organs of *P. semisulcatus* is given in table 11. The results indicated that all the variables such as carbohydrate ($p<0.01$), lipid ($p<0.01$) and protein ($p<0.01$) levels were significantly lower in the hepatopancreas, muscle and gills of the test shrimp than that of control.

4.4.4. Sub lethal exposure (10% LC_{50})

4.4.4.1. Copper

The mean concentration along with their SD for the biochemical composition of the various organs of *P. semisulcatus* exposed to copper at the sub lethal level of 0.7 ppm is given in table 12. The results showed that the carbohydrate ($p<0.05$), lipid ($p<0.05$) and protein ($p<0.05$) levels were significantly lower in hepatopancreas, whereas lipid ($p<0.05$) was alone significantly reduced in the gills and a low level of protein ($p<0.05$) was observed in the muscle of the test shrimp as compared to the control.

4.4.4.2. Cadmium

The mean concentration along with their SD for the biochemical composition of the various organs of *P. semisulcatus* exposed to cadmium at the sub lethal concentration of 0.25 ppm is given in table 13. Significant reductions in protein ($p<0.05$) and lipid ($p<0.05$) levels were noted in the hepatopancreas and gills as compared to the control. On the other hand, the protein content was alone significantly ($p<0.05$) lower in the muscle. However, the carbohydrate level in all these organs did not show any level of significance during the period of experiment.

4.4.4.3. Zinc

The biochemical composition of various organs of *P. semisulcatus* exposed to zinc at the sub lethal level of 0.5 ppm is given in table 14. At this level of zinc, the concentrations of carbohydrate ($p<0.05$), lipid ($p<0.05$) and protein ($p<0.05$) were significantly reduced in the gills than that of control. The levels of carbohydrate ($p<0.05$) and lipid ($p<0.05$) in the hepatopancreas exhibited a significant variation between the test and control. In the muscle, the lipid ($p<0.05$)

recorded its least value than that of control. It was also observed that, both carbohydrate and protein levels did not show any significance in the muscle when compared to the same at the other tissues (hepatopancreas and gills).

4.4.5. Sub lethal exposure (20% LC_{50})

4.4.5.1. Copper

The biochemical composition in various organs of *Penaeus semisulcatus* exposed to copper at 1.4 ppm is given in table 15. At this level of copper, both the protein ($p<0.05$) and lipid ($p<0.05$) levels significantly varied in the hepatopancreas and muscle of the test shrimp as compared to the control. In the gills, the lipid was alone significant ($p<0.05$).

4.4.5.2. Cadmium

The biochemical composition of the various organs of *Penaeus semisulcatus* exposed to cadmium at 0.5 ppm is given in the table 16. Protein and lipid levels in the hepatopancreas and muscle were found to be significant ($p<0.05$) whereas, in gills, lipid was alone significant ($p<0.05$).

4.4.5.3. Zinc

The biochemical composition in various tissues of *Penaeus semisulcatus* exposed to zinc at 1.00 ppm is given in table 17. The results showed that all the variables such as carbohydrate, lipid and protein were significantly lower ($p<0.05$) in hepatopancreas. Similarly in the gills, both protein and lipid varied significantly ($p<0.05$) between the experiment and the control. In the muscle, significantly ($p<0.05$) lower levels of lipid were observed as compared to the control.

Table 9. Biochemical composition of various organs of *Penaeus semisulcatus* exposed to copper at 6.98 ppm (% dry weight of the tissue)

	Concentration	SD (±)	t	p
Hepatopancreas				
Protein	62.24	0.55	9.22	0.05
Carbohydrate	4.96	0.05	6.87	0.05
Lipid	26.43	0.90	9.48	0.05
Gills				
Protein	39.06	0.48	4.78	0.01
Carbohydrate	1.05	0.04	1.26	NS
Lipid	1.01	0.03	1.77	0.05
Muscle				
Protein	74.64	1.08	6.56	0.01
Carbohydrate	1.58	0.11	5.7	0.01
Lipid	6.79	0.29	11.5	0.01

If $p < 0.05$ (significant); if $p < 0.01$ (highly significant);
NS: Not Significant

Table 10. Biochemical composition of various organs of *Penaeus semisulcatus* exposed to cadmium at 2.8 ppm (% dry weight of the tissue)

	Concentration	SD (±)	t	p
Hepatopancreas				
Protein	60.64	0.54	13.46	0.01
Carbohydrate	4.32	0.24	5.9	0.01
Lipid	25.91	1.57	5.9	0.01
Gills				
Protein	38.89	0.29	7.23	0.01
Carbohydrate	0.90	0.06	2.94	NS
Lipid	0.82	0.09	3.74	0.01
Muscle				
Protein	73.95	0.50	13.43	0.01
Carbohydrate	1.47	0.15	5.13	0.01
Lipid	6.48	0.37	10.61	0.01

If $p < 0.01$ (highly significant)
NS: not significant

11. Biochemical composition of various organs of *Penaeus semisulcatus* exposed to zinc at 5.00 ppm (% dry weight of the tissue)

	Concentration	SD (±)	t	p
Hepatopancreas				
Protein	62.58	0.40	11.21	0.01
Carbohydrate	5.01	0.03	7.14	0.01
Lipid	28.20	0.30	19.95	0.01
Gills				
Protein	38.59	1.56	2.08	NS
Carbohydrate	0.99	0.04	2.05	NS
Lipid	0.89	0.10	2.54	NS
Muscle				
Protein	71.66	1.17	9.49	0.01
Carbohydrate	1.26	0.19	5.64	0.01
Lipid	6.44	0.31	12.34	0.01

If $p < 0.01$ (highly significant)
NS: Not Significant

Table 12. Biochemical composition of various organs of *Penaeus semisulcatus* exposed to copper at 0.7 ppm (% dry weight of the tissue)

	Concentration	SD (±)	t	p
Hepatopancreas				
Protein	64.49	0.70	4.17	0.05
Carbohydrate	5.27	0.06	2.01	NS
Lipid	30.61	0.71	4.36	0.05
Gills				
Protein	40.67	0.56	0.53	NS
Carbohydrate	1.06	0.04	0.95	NS
Lipid	0.82	0.10	3.36	0.05
Muscle				
Protein	78.75	0.35	5.8	0.05
Carbohydrate	1.97	0.04	1.85	NS
Lipid	9.35	0.30	1.12	NS

If $p < 0.05$ (significant)
NS: Not Significant

Table 13. Biochemical composition of various organs of *Penaeus semisulcatus* exposed to cadmium at 0.25 ppm (% dry weight of the tissue)

	Concentration	SD (±)	t	p
Hepatopancreas				
Protein	63.53	0.91	5.06	0.05
Carbohydrate	5.10	0.20	1.29	NS
Lipid	29.44	0.73	6.97	0.05
Gills				
Protein	39.46	0.73	3.27	0.05
Carbohydrate	0.84	0.08	5.18	0.05
Lipid	0.67	0.10	5.48	0.05
Muscle				
Protein	76.57	1.10	5.35	0.05
Carbohydrate	2.07	0.13	1.61	NS
Lipid	7.81	1.39	2.33	NS

If $p < 0.05$ (significant)
NS: Not Significant

Table 14. Biochemical composition of various organs of *Penaeus semisulcatus* exposed to zinc at 0.5 ppm (% dry weight of the tissue)

	Concentration	SD (±)	t	p
Hepatopancreas				
Protein	65.36	0.92	1.42	NS
Carbohydrate	4.50	0.29	4.36	0.05
Lipid	30.83	0.61	4.39	0.05
Gills				
Protein	37.01	0.60	11.00	0.05
Carbohydrate	0.99	0.03	3.43	0.05
Lipid	0.83	0.05	4.52	0.05
Muscle				
Protein	79.39	0.65	1.32	NS
Carbohydrate	1.99	0.02	1.69	NS
Lipid	8.70	0.30	5.03	0.05

If $p < 0.05$ (significant)
NS: Not Significant

Table 15. Biochemical composition of various organs of *Penaeus semisulcatus* exposed to copper at 1.4 ppm (% dry weight of the tissue)

	Concentration	SD (±)	t	p
Hepatopancreas				
Protein	62.48	0.78	9.47	0.05
Carbohydrate	5.42	0.21	0.52	NS
Lipid	28.84	0.76	7.05	0.05
Gills				
Protein	40.84	0.42	0.93	NS
Carbohydrate	0.98	0.03	2.03	NS
Lipid	0.74	0.08	4.35	0.05
Muscle				
Protein	77.04	0.61	7.66	0.05
Carbohydrate	1.84	0.10	0.83	NS
Lipid	7.84	0.25	8.89	0.05

If $p < 0.05$ (significant); NS: not significant

Table 16. Biochemical composition of various organs in *Penaeus semisulcatus* exposed to cadmium at 0.5 ppm (% dry weight of the tissue)

	Concentration	SD (±)	t	p
Hepatopancreas				
Protein	63.37	0.90	6.53	0.05
Carbohydrate	5.37	0.27	0.39	NS
Lipid	29.69	0.86	4.7	0.05
Gills				
Protein	40.00	0.38	1.97	NS
Carbohydrate	1.11	0.08	0.1	NS
Lipid	0.82	0.04	3.65	0.05
Muscle				
Protein	76.40	1.04	5.85	0.05
Carbohydrate	2.01	0.02	1.88	NS
Lipid	7.97	0.21	9.45	0.05

If $p < 0.05$ (significant); NS: Not Significant

Table 17. Biochemical composition of various organs in *Penaeus semisulcatus* exposed to zinc at 1.00 ppm (% dry weight of the tissue)

	Concentration	SD (±)	t	p
Hepatopancreas				
Protein	65.69	0.65	2.90	0.05
Carbohydrate	4.34	0.07	9.39	0.05
Lipid	30.70	0.60	3.8	0.05
Gills				
Protein	37.00	0.58	5.46	0.05
Carbohydrate	1.31	0.36	0.89	NS
Lipid	0.79	0.03	4.26	0.05
Muscle				
Protein	79.92	0.67	0.15	NS
Carbohydrate	1.99	0.02	1.81	NS
Lipid	8.40	0.40	3.54	0.05

If $p < 0.05$ (significant); NS: Not Significant

4.4.6. Effect of heavy metals on the constituents

4.4.6.1. Lethal exposure

In the hepatopancreas significant differences among the metals in protein ($f = 8.67$; $p < 0.05$) (Appendix 4a) and carbohydrate ($f = 14.24$; $p < 0.05$) (Appendix 4b) contents were observed but no difference in lipid ($f = 3.70$; $p > 0.05$) (Appendix 4 c) to lethal exposure.

In the gills, there was no significant difference among the metals in the biochemical contents (Appendix 4d-f).

In the muscle, protein ($f = 5.19$; $p < 0.05$) (Appendix 4g) showed a significant difference among exposed metals in lethal concentration.

4.4.6.2. Sublethal (10 % of LC_{50})

In hepatopancreas, the carbohydrate ($f = 11.78$; $p < 0.05$) (Appendix 5b) content differed significantly among metal treatments while protein and lipid did not show any difference among the metals (Appendix 5a and 5c).

Protein ($f = 26.15$; $p < 0.05$) (Appendix 5d) and carbohydrate levels ($f = 13.27$; $p < 0.05$) (Appendix 5e) in the gills showed a significant change while lipid had no change among the metals.

In the muscle, protein ($f = 11.28$; $p < 0.05$) (Appendix 5g) showed a significant change while carbohydrate and lipid showed no difference (Appendix 5h-i) among the metals.

4.4.6.3. Sublethal (20 % of LC_{50})

The protein ($f = 13.4$; $p < 0.05$) (Appendix 6a) and carbohydrate ($f = 27.95$; $p < 0.05$) (Appendix 6b) contents changed significantly among the exposed metals while the lipid had no significant change among copper, cadmium and zinc exposures (Appendix 6c) in hepatopancreas.

Protein ($f = 56.02$; $p < 0.05$) (Appendix 6d) in the gills showed a significant change while carbohydrate and lipid did not differ significantly among the metals (Appendix 6e-f).

In the muscle, protein ($f = 16.13$; $p < 0.05$) (Appendix 6g) and carbohydrate ($f = 7.9$; $p < 0.05$) (Appendix 6h) levels showed a significant change among the metals while lipid levels were not significantly different (Appendix 6i).

4.5. Histopathology

4.5.1. Light microscopy

Histoarchitecture of the control shrimp

The hepatopancreas of control shrimp exhibited the well organized glandular, tubular structure seen in shrimps (Bell and Lightner, 1988). The tubules were closed distally but opened out proximally into ducts which in turn united to form longer ducts that were ultimately connected to the digestive tract. The tubule lumen has "star" like appearance (Plate 4: A, B). A single layer of epithelial cells was found lining the tubules. The cells showed normal differentiation into E (Embryonic) cells at the normal distal end of the tubule, young R (Restzellen) cells and F (Fibrllenzellen) cells a short distance away from the distal region, and B (Blasenzellen) cells in the middle and proximal region of the tubule (Plate 4: A, B). The interstitial sinuses between tubules are normal.

The gills of control shrimps showed uniform arrangement of lamellae with uniform interlamellar space. The secondary gill filaments were normal and both branched and non-branched gill filaments were seen (Plate. 5: A, B). The septum (sep) dividing the afferent vessel (Afs) and efferent vessel (efs) were visible (Plate 5: A).

Histoarchitecture of gills and hepatopancreas exposed to copper, cadmium and zinc at various concentrations showed the following:

Exposure of the shrimp to copper caused slight change in the histoarchitecture of the hepatopancreatic cells. Formation of tissue debris (TD), swelling of the intercellular membrane that separates the adjacent cells (SW), necrotic tubules near the areas of swelling (NT) were the main observed effects at 0.7 ppm of copper (Plate 6. A). When exposed to 1.4 ppm the changes include necrotic tubules (NT), tissue debris (TD) and abnormal lumen (ALU) (plate 6. B).

Exposure to lethal concentration led to massive necrosis of the cells in the hepatopancreas (NCH) (Plate 6. C).

The exposure to copper (0.7 ppm) showed fusion of the gill lamellae (FL), haemocytic infiltration (HI) and swelling of the secondary gill lamellae (SW) (Plate 7. A). In 1.4 ppm lifting of lamellar epithelium (LLE) necrosis (N) and infiltration of haemocytes (HC) became evident (Plate 7. B). The exposure to lethal concentration led to necrosis of the basal membrane of the secondary gill lamellae (N), swelling of the intercellular membrane that separates the adjacent cells (SW), fusion and necrosis of the gill filament (FN) and haemocytic infiltration (HI) (plate 7. C).

The histoarchitectural changes in the hepatopancreas of shrimps exposed to 0.25 ppm cadmium include abnormal lumen (ALU) and increase in the number of R cells (Plate 8. A). When exposed to 0.5 ppm cadmium necrosis of the hepatopancreatic cells (NCH) and abnormal lumen (ALU) (Plate 8. B) were observed. The lethal concentration exposure resulted in severe damages such as swelling of the intercellular membrane that separates the adjacent cells (SW), tissue debris (TD) and necrosis of the cells (N) (Plate 8. C).

Exposure to 0.25 ppm led to deformity in the secondary gill lamellae (DL) leading to its necrosis (N) (Plate 9. A). The exposure to 0.5 ppm caused malformation of the gill tip (ML), necrosis (N) and haemocytic infiltration (plate 9. B). A complete deformity in the gills occurred when exposed to 2.8 ppm, the common features include necrosis (N) and haemocytic infiltration (HI) (Plate 9. C).

The hepatopancreas of shrimp exposed to zinc showed varied alterations in the histoarchitecture of the cells. When exposed to 0.5 ppm abnormal lumen (ALU) and slight swelling in the basal membrane (SW) (Plate 10. A) were observed. When exposed to 1.00 ppm, deformity in the architecture of the

cellular organization, swelling of the intercellular membrane that separates the adjacent cells (SW) and necrosis (N) (Plate 10. B) occured. When exposed to 5.00 ppm zinc there was complete damage to the cell architecture which included destruction of the cells, swelling of the intercellular membrane and abnormal lumen (Plate 10. C).

Not much changes were noticed in the gill filaments of the shrimp exposed to zinc @ 0.5 ppm. The alterations in the secondary branching gill filaments showed hemocytic infiltration (HI), fused basal membrane of the secondary branching gill filaments (FN) and thickening of basal membrane (T) (Plate 11. A). When exposed to 1.00 ppm, the alterations in the histoarchitecture included, the necrosis of the basal membrane of the secondary gill lamellae (N) and deformity at the tip of the secondary gill filaments (DF) (Plate 11. B). When exposed to 5.00 ppm zinc the cells showed damage to the whole architecture of the gills including the fusion of the gill filament by the thickening of the outer layer of the membrane in the secondary lamellae (FL) (Plate 11. C).

Plate 4. Control Hepatopancreas

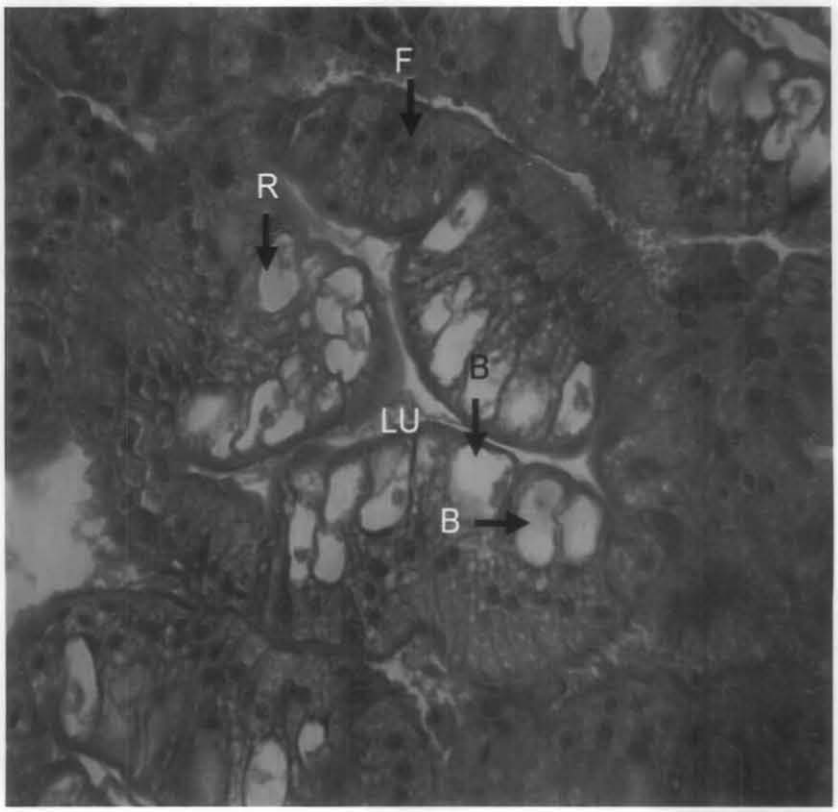
A. Histoarchitecture of the hepatopancreas in *Penaeus semisulcatus* X 40x

E - E-cell
B - B- cell
R - R-cell
F - F cell
IS – Interstitial space
LU – Lumen

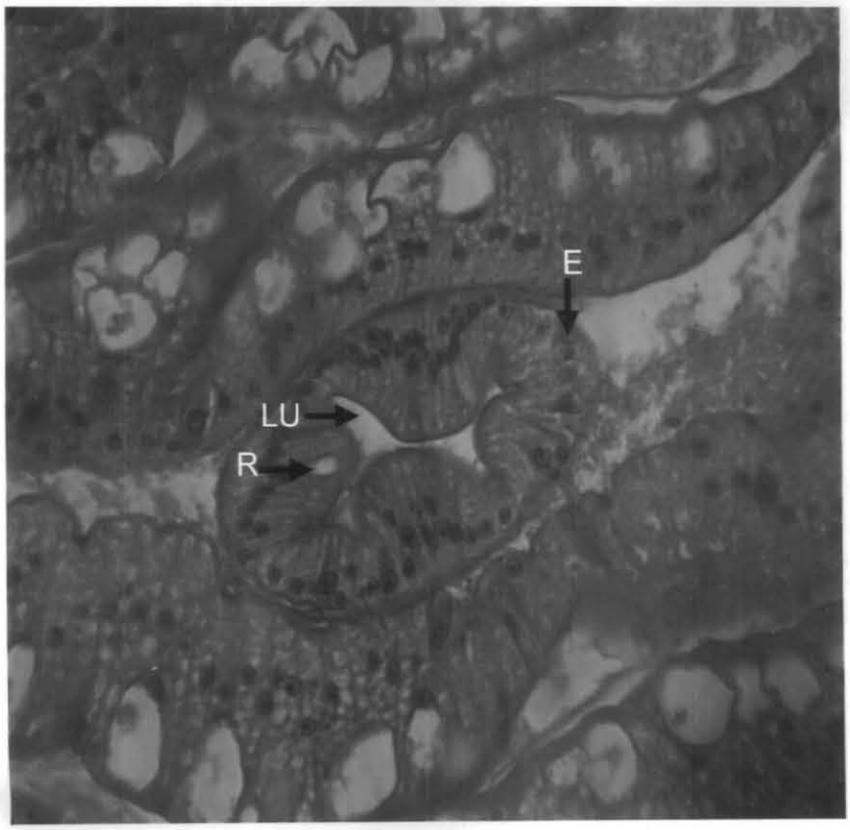
B. Histoarchitecture of the hepatopancreas in *Penaeus semisulcatus* X 40x

E - E-cell
B - B- cell
LU – Lumen

Plate. 4. Hepatopancreas of control shrimp



A



B

Plate 5. Histoarchitecture of control gills

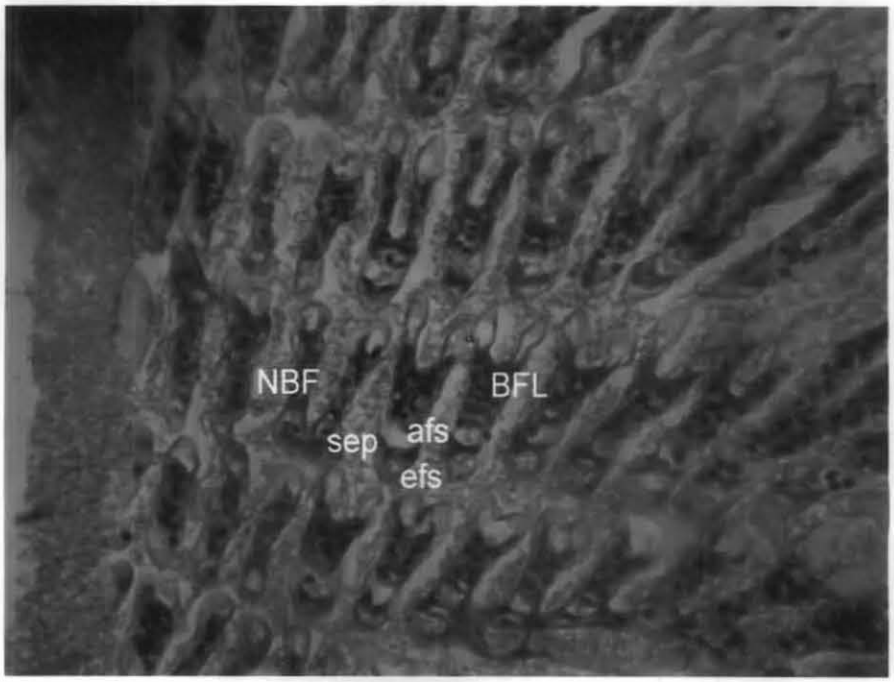
A. Histoarchitecture of control gills of *Penaeus semisulcatus* X 40x

NBF – Non branching secondary gill filament
afs – Afferent vessel
efs – efferent vessel
sep – septum
BFL – Branching secondary gill filament

B. Histoarchitecture of control gills of *Penaeus semisulcatus* X 40x

CEN - Primary gill lamellae with a portion of the central axis.
efp – efferent vessel of primary gill lamellae.
afp – afferent vessel of primary gill lamellae.

Plate. 5. Gills of control shrimp



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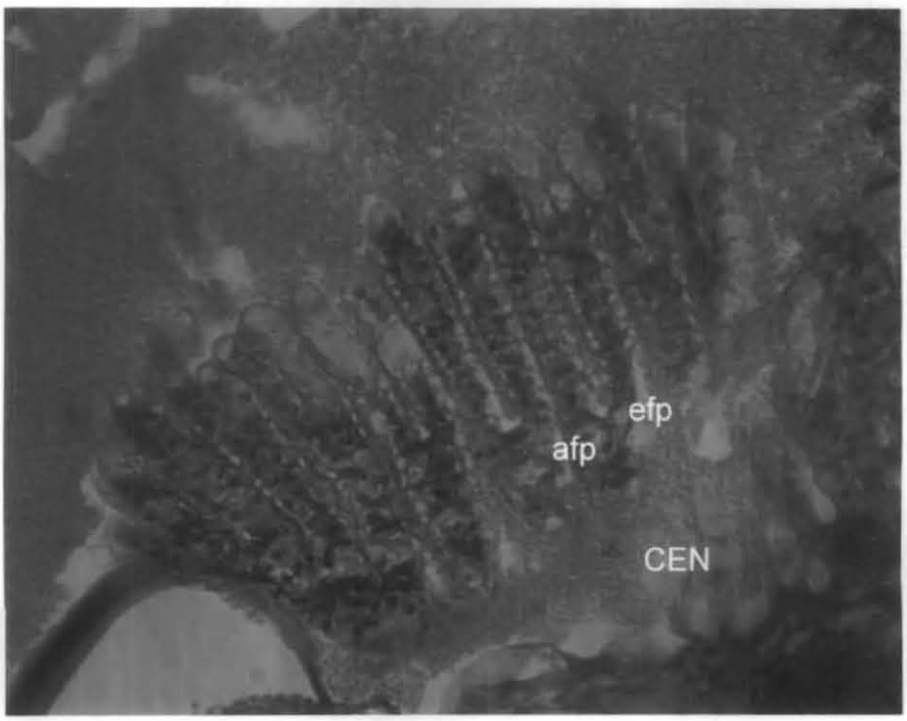


B

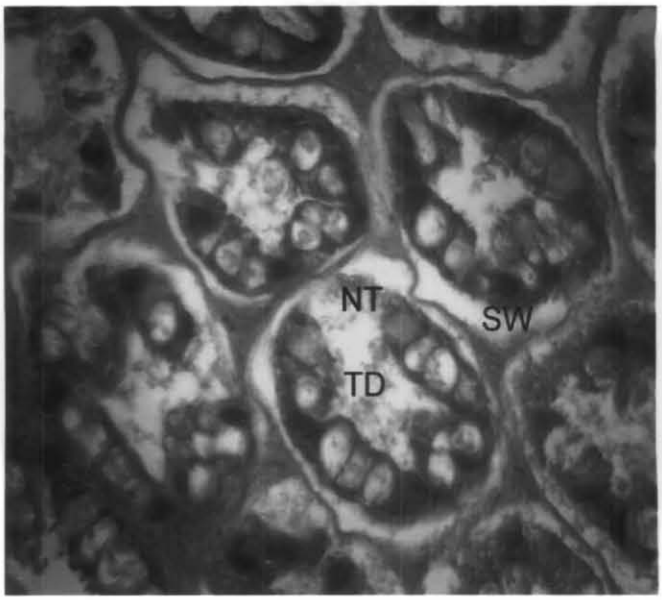
Plate 6 Changes in the hepatopancreas of *Penaeus semisulcatus* exposed to copper

A. Copper induced alteration in the histoarchitecture of hepatopancreas in shrimp. Formation of necrotic tubules (NT), tissue debris (TD) and swelling (SW) leading to the deformity in basal lamina of the test shrimp exposed to 0.7 ppm of copper. X 40x

B. Copper induced alteration in the histoarchitecture of hepatopancreas in shrimp. Formation of abnormal lumen (ALU), necrotic tubule (NT) and tissue debris (TD) while exposed to 1.4 ppm of copper. X 40x.

C Copper induced alteration in the histoarchitecture of hepatopancreas in shrimp. Necrotic cells of the hepatopancreas (NCH) after exposed to 6.98 ppm of copper. X 40x

Plate. 6. Changes in the hepatopancreas of *Penaeus semisulcatus* exposed to copper



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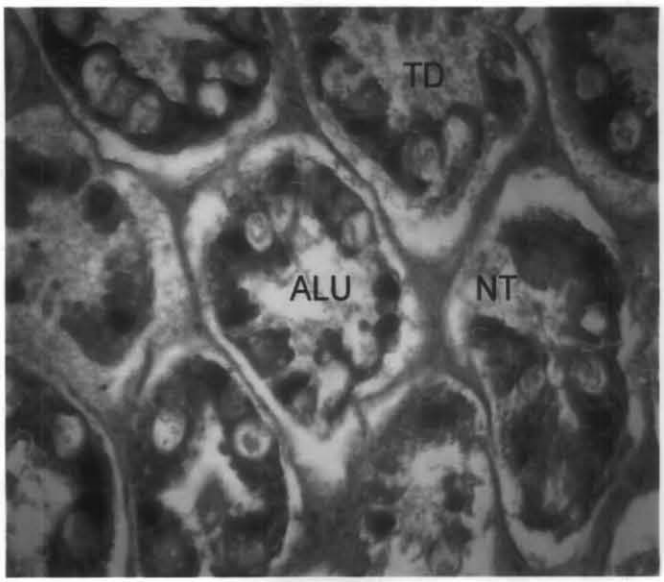

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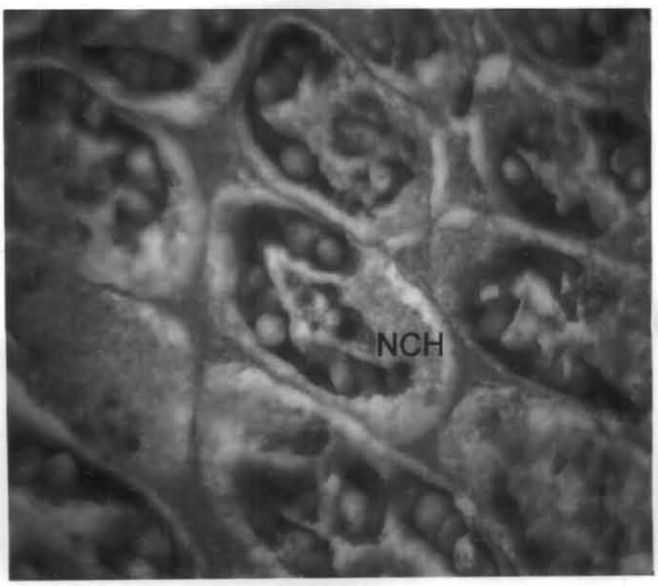


C

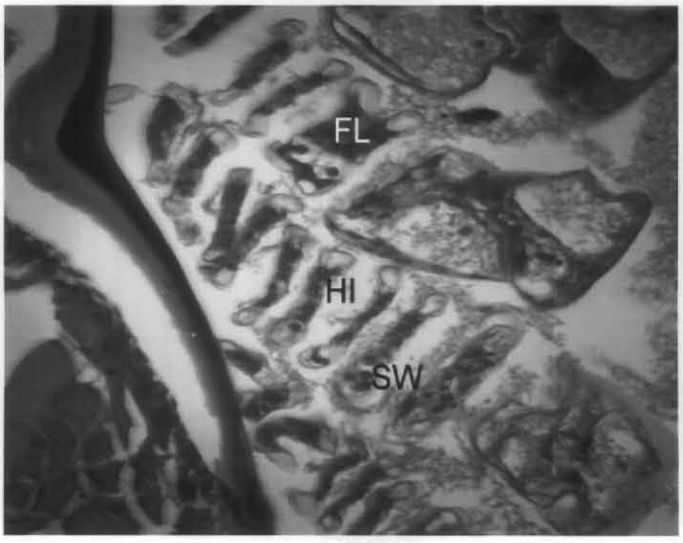
Plate 7. Changes in the gills of *Penaeus semisulcatus* exposed to copper

A. Copper induced alterations in the histoarchitecture in the gills. Haemocytic infiltration (HI), swelling of gill lamellae (SW) and fusion of the gill lamellae (FL) in the test shrimp exposed to 0.7 ppm copper. X 40x

B. Copper induced alteration in the histoarchitecture of gills in shrimp. Lifting of lamellar epithelium (LLE), necrosis (N) and accumulation of haemocyte (HC) in the test shrimps exposed to 1.4 ppm copper. X 40x

C. Copper induced alteration in the histoarchitecture of gills in shrimp. Fusion and necrosis of the basal part of the gills (FN) necrosis (N) and swelling of gill lamellae (SW) in the test shrimp when exposed to 6.98 ppm copper. X 40x

Plate. 7. Changes in the gills of *Penaeus semisulcatus* exposed to copper



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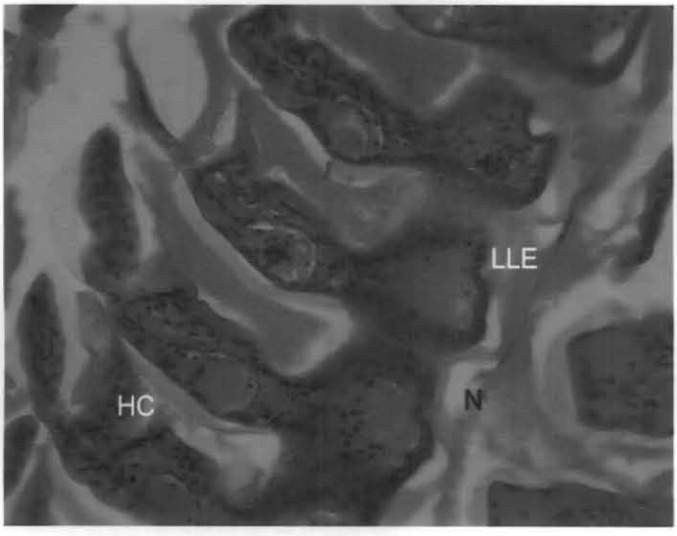

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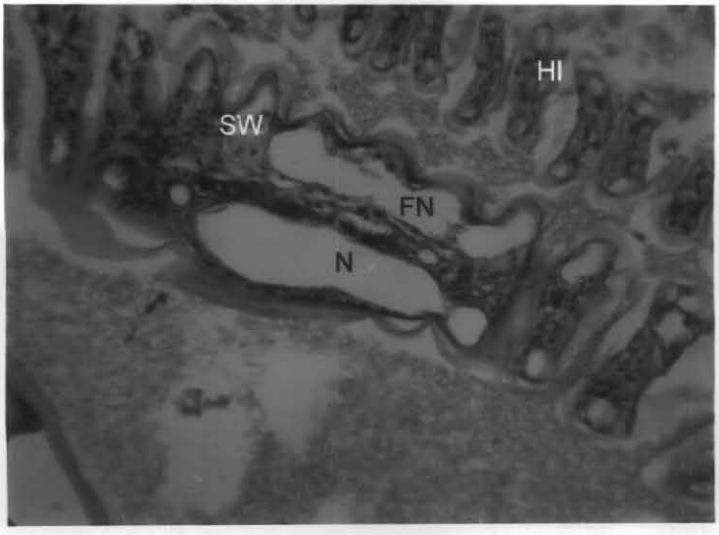


C

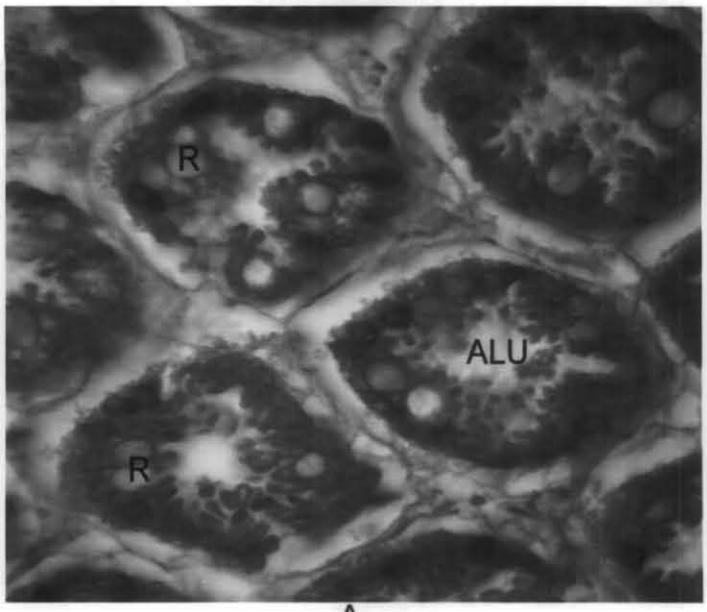
Plate 8. Changes in the hepatopancreas of *Penaeus semisulcatus* exposed to cadmium

A. Cadmium induced alteration in the histoarchitecture of hepatopancreas in shrimp. Abnormal lumen (ALU) in the test shrimp while exposed to 0.25 ppm cadmium. X 40x

B. Cadmium induced variations in the histoarchitecture of hepatopancreas in shrimp. Necrosis of the hepatopancreatic cells (HCN) and abnormal lumen (ALU) in the test shrimp when exposed to 0.50 ppm cadmium. X 40x

C. Cadmium induced changes in the histoarchitecture of hepatopancreas in shrimp. Tissue debris (TD), necrosis of the cells (N) in the test shrimp when exposed to 2.80 ppm cadmium. X 40x

Plate. 8. Changes in the hepatopancreas of *Penaeus semisulcatus* exposed to cadmium



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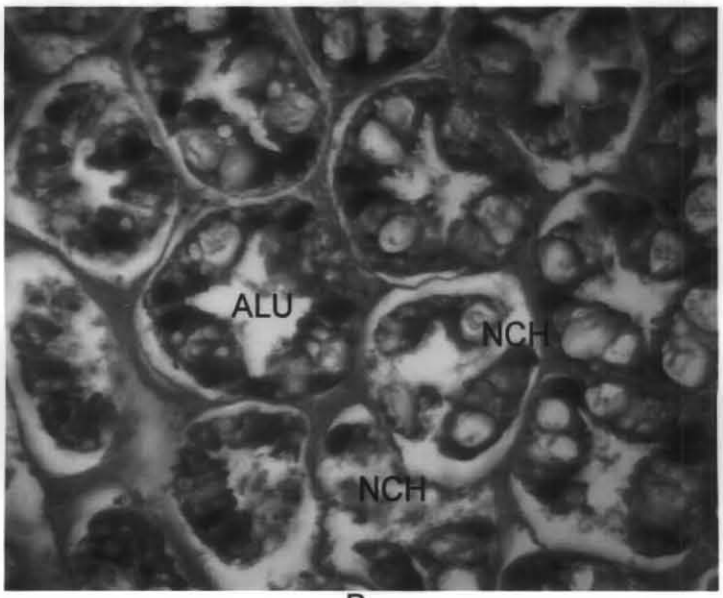

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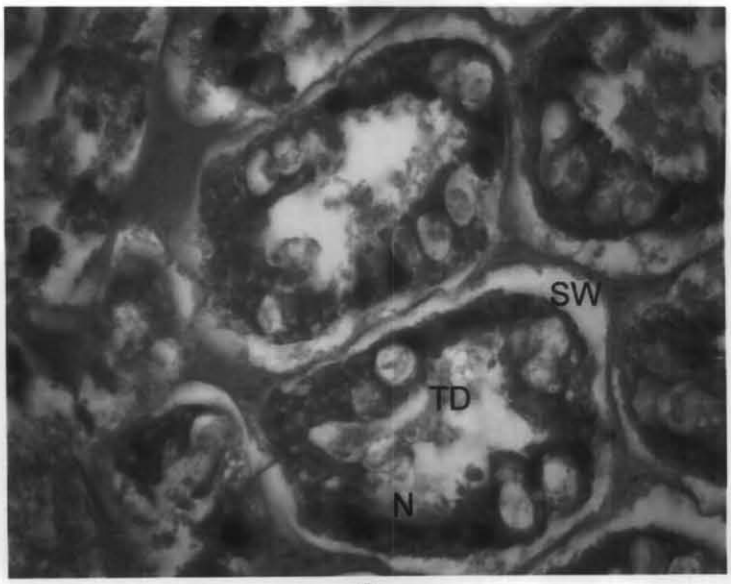


C

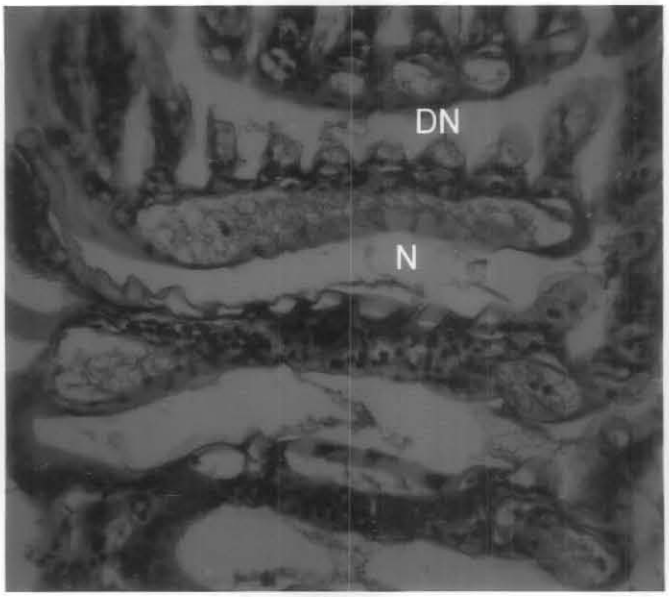
Plate 9. Changes in the gills of *Penaeus semisulcatus* exposed to cadmium

A. Cadmium induced alterations in the histoarchitecture of gills in shrimp. Degeneration and necrosis of secondary gill lamellae (DN) and necrosis (N) of the gill filament in the test shrimps exposed to 0.25 ppm of cadmium. X 40x

B. Cadmium induced alterations in the histoarchitecture of gills in shrimp. Malformation (ML), haemocytic infiltration (HI) and necrosis (N) of the gill lamellae in test shrimps exposed to 0.5 ppm cadmium. X 40x

C Cadmium induced alterations in the histoarchitecture of gills in shrimp. Haemocytic infiltration (HI) and necrosis (N) of the gill lamellae in test shrimps exposed to 2.8 ppm cadmium. X 40x

Plate. 9. Changes in the gills of *Penaeus semisulcatus* exposed to cadmium



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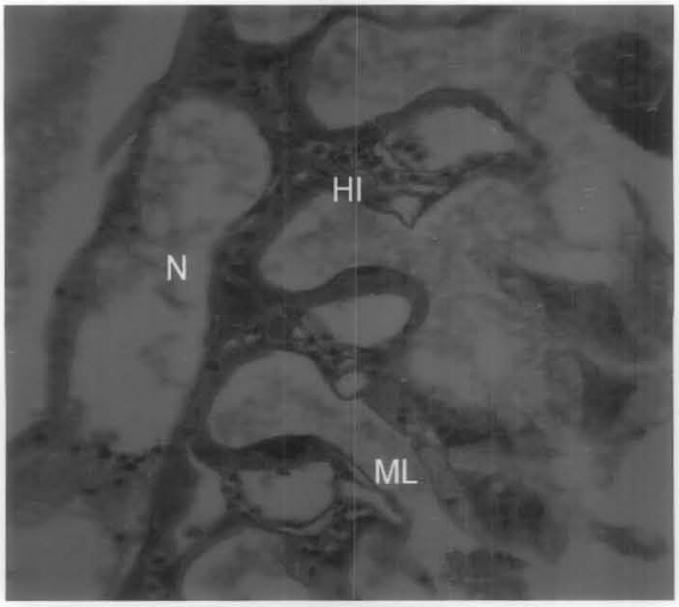

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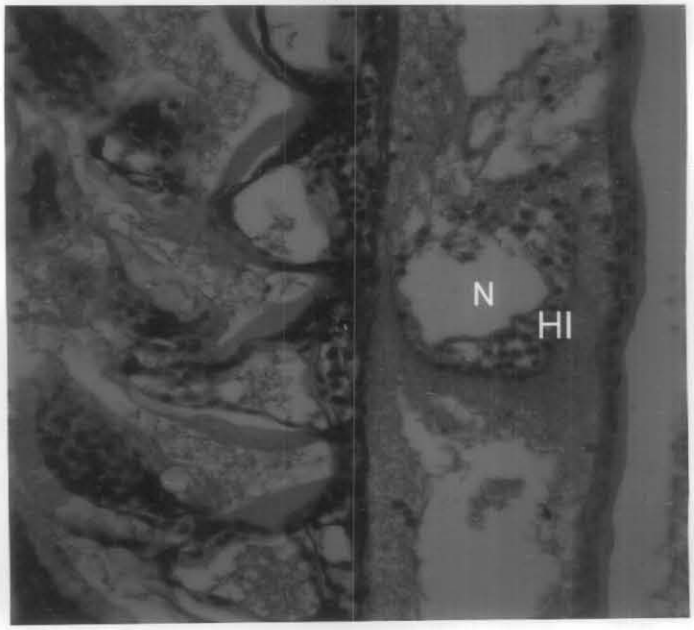


C

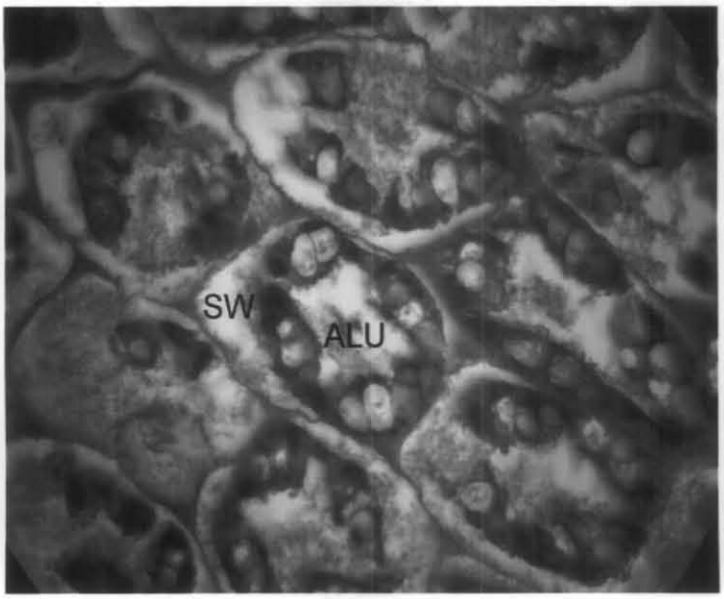
Plate 10. Changes in the hepatopancreas of *Penaeus semisulcatus* exposed to zinc

A. Zinc induced alterations in the histoarchitecture of hepatopancreas in shrimp. Abnormal lumen (ALU), slight swelling in the basal membrane ((SW) in the test shrimps when exposed to zinc at 0.5 ppm X 40x

B. Zinc induced alterations in the histoarchitecture of hepatopancreas in shrimp. Deformity in the architecture of the cellular organization leads to necrosis (N) and swelling (SW) in the test shrimps when exposed to 1.00 ppm zinc. X 40x

C. Zinc induced alterations in the histoarchitecture of hepatopancreas in shrimp. A total damage of the cells (TDC) are visible in the case of zinc exposure at 5.00 ppm. X 40x

Plate. 10. Changes in the hepatopancreas of *Peneaus semisulcatus* exposed to zinc



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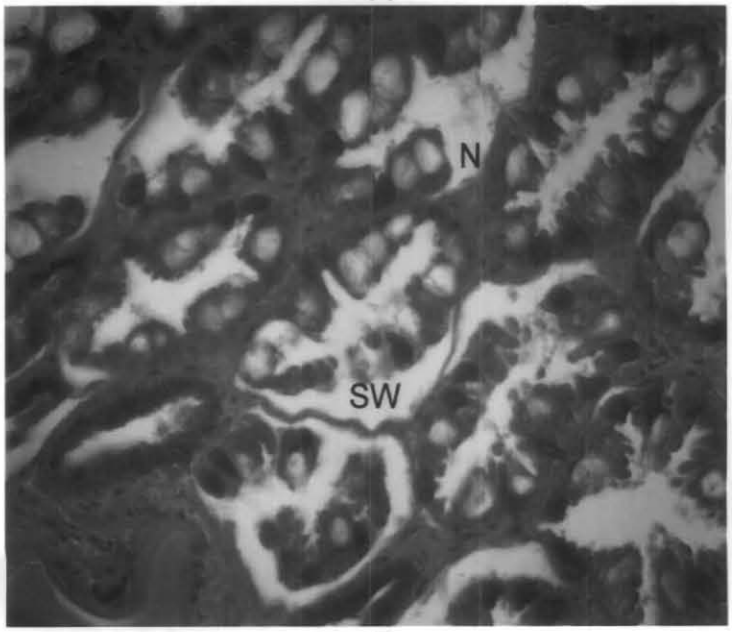

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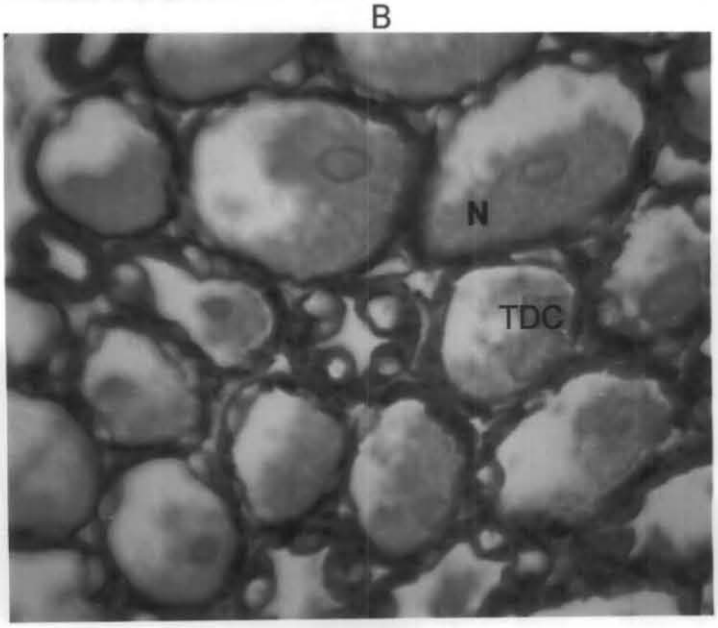


C

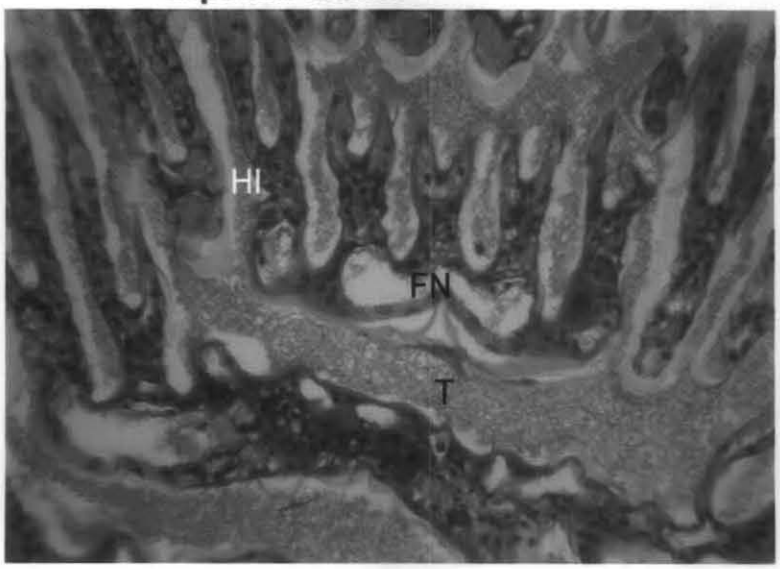
Plate 11. Changes in the gills of *Penaeus semisulcatus* exposed to zinc

A. Zinc induced alteration in the histoarchitecture of hepatopancreas in shrimp. Fusion and necrosis of the gill lamellae (FN), thickening of the basal membrane (T) and necrosis (N) of the gill lamellae in test shrimps exposed to 0.5 ppm zinc X 40x

B. Zinc induced alteration in the histoarchitecture of hepatopancreas in shrimp. Deformation at the tip of secondary gill lamellae (DF) and necrosis (N) of the gill lamellae in test shrimps exposed to 1.00 ppm zinc X 40x

C. Zinc induced alteration in the histoarchitecture of hepatopancreas in shrimp. Fusion of the lamellae (FL) in test shrimps exposed to 5.00 ppm zinc X 40x

Plate. 11. Changes in the gills of *Penaeus semisulcatus* exposed to zinc



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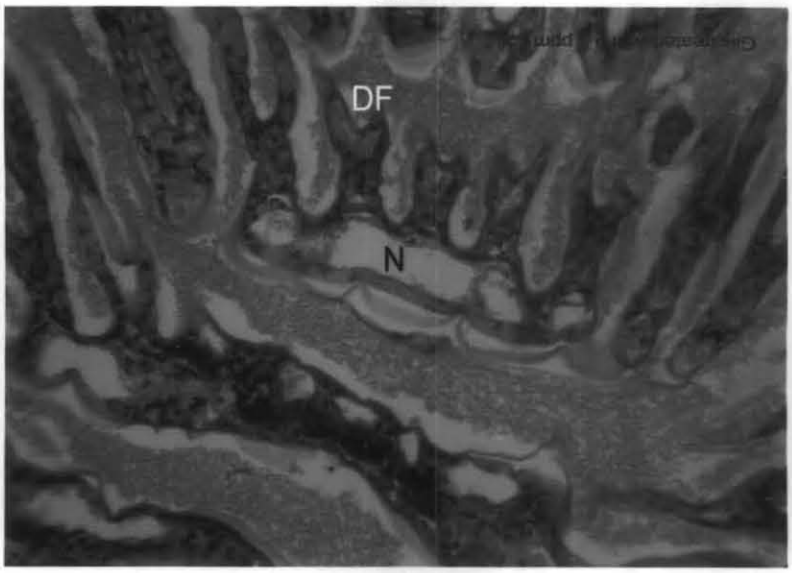

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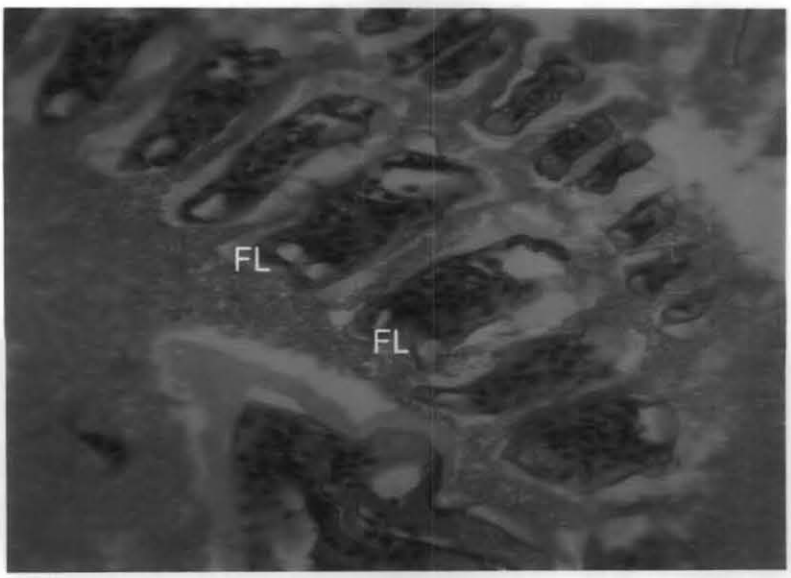


C

4.5.2. Electron Microscopy (TEM)

Copper

After 96 hrs exposure to 6.98 ppm copper the gills showed marked changes in the structure and integrity of the cells; the nuclear membrane damaged with the second nuclear membrane disintegrated (plate 12); the cell contain large number of vacuoles (plate 13) and in some cases granules in the vacuole (plate 14); the structure of mitochondria changed with the scattered cristae (plate 15); the nucleus wore a wavy structure. The other organelles were marginally affected by the treatment.

The gill cells treated with 0.7 ppm copper (sublethal concentration) also showed marked structural changes - a clear damaged nuclei (Plate 16), distorted endoplasmic reticulum (plate 17) number of organelles in the cell reduced with damage to mitochondria, many of which retain their saucer shape with considerably fewer cristae (plate 18); damage to apical cells (plate 19); and increase in the number of vacuoles.

The hepatopancreas of the shrimp when exposed to lethal concentration (6.98 ppm) of copper showed structural deformity of mitochondria (plate 20), increased number of vacuoles (plate 21), disfigured mitochondria (plate 22), scattered and broken endoplasmic reticulum and fewer cell organelles leading to whole cell damage (plate 23).

Hepatopancreas of shrimps exposed to 0.7 ppm of copper for 14 days resulted in broken cell membrane (plate 24), damaged second nuclear membrane (plate 25), condensed nucleus (plate 26) and disturbed cytoplasm with numerous vacuoles in the cells (plate 27).

Cadmium

Gills of shrimps exposed to 2.8 ppm cadmium showed whole cell destruction (plate 28), damaged nucleus with condensation due to water loss; disintegration of the outer membrane of nucleus (plate 29); swollen mitochondria and scattered cristae (plate 30) increase in the number of vacuoles and damaged and scattered endoplasmic reticulum (plate 31).

Shrimps exposed to 0.25 ppm cadmium showed distortion of endoplasmic reticulum (plate 32); fewer swollen mitochondria with a clear disorientation of outer membrane (plate 33); loss of integrity in cuticle and the region just underlying it (plate 34); degenerated mitochondria, fewer cellular organelles and structural deformation of the nuclei (plate 35) in the gills.

The hepatopancreas of shrimps exposed to 2.8 ppm of cadmium observed many nuclei had lost their typical elliptical shape due to extensive swelling of the nuclear membrane. (plate 36). The integrity of the cell lost its characteristic inclusions, scattered endoplasmic reticulum, vacuoles, shrunken and condensed nucleus (plate 37). Damage in nucleus forming aggregations and empty space in the cell (plate 38) Mitochondria were destroyed with scattered cristae (plate 39).

In the case of shrimps treated with 0.25 ppm cadmium, electron dense bodies (plate 40), disfigured and disintegrated endoplasmic reticulum and vacuoles (plate 41); damaged mitochondria (plate 42) and formation of vacuoles attached to nuclear membrane (plate 43) in hepatopancreas.

Shrimps exposed to lethal concentration of 5 ppm zinc for 4 days showed numerous vesicles in the gills (plate 44), fewer organelles in the cell (plate 45), disintegrated cytoplasm and damaged outer nuclear membrane, broken endoplasmic reticulum (plate 46) and damage to mitochondria (plate 47).

Zinc

In shrimps exposed to 0.5 ppm zinc, the gill cells showed few disoriented microtubles (plate 48), vacuole formation (plate 49), granular deposits in the cuticle (plate 50), and there were also cells with less cell components and slightly disoriented mitochondria (plate 51).

Exposure to 5 ppm zinc resulted in granular formation (plate 52), mitochondrial swelling (plate 53), vacuoles formations (plate 54) and shrunken nucleus (plate 55) in the cells of hepatopancreas.

Shrimps exposed to 0.5 ppm zinc showed electron dense body (plate 56), slight damage to mitochondria (plate 57), breakage in cell wall (plate 58) and the wavy appearance of condensed nucleus (plate 59) in the hepatopancreas.

EFFECT OF COPPER ON GILLS AT 6.98 ppm

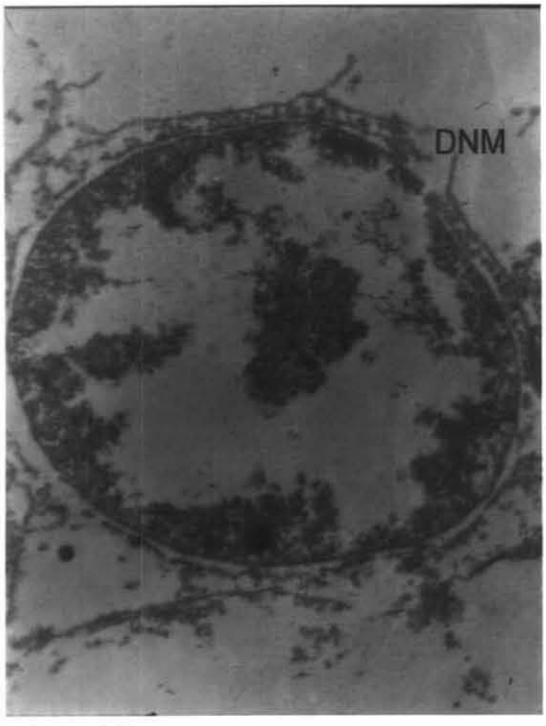


plate 12

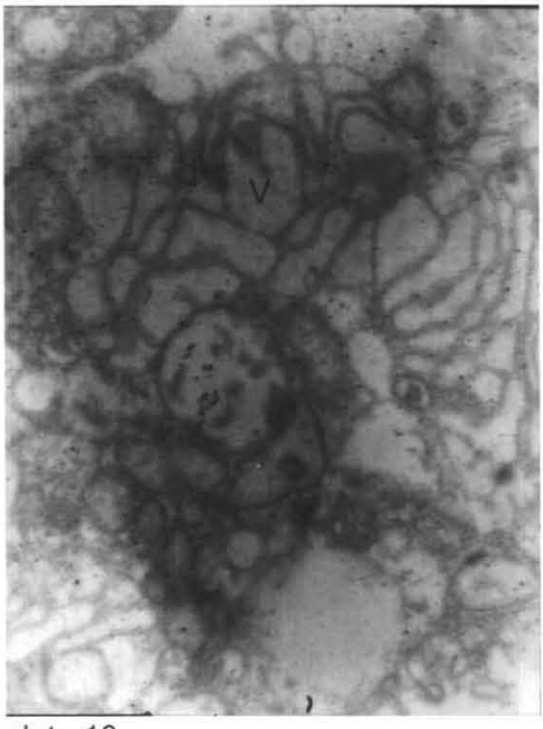


plate 13

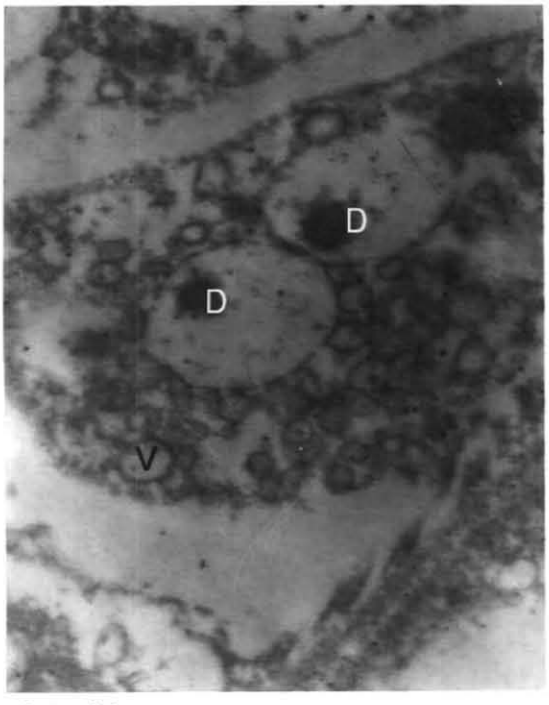


plate 14

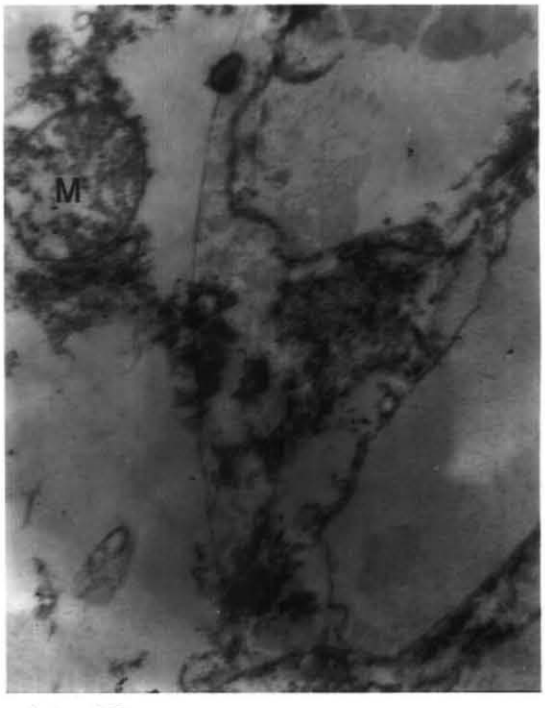


plate 15

Plate 12 Damaged nuclear membrane (DNM) X 6,000
Plate 13. Numerous vacuoles (V) X 12,000
Plate 14. Deposition of copper in vacuoles (D) and vacuoles (V) X 12,000
Plate 15. Disrupted mitochondria (M) X 10,000

EFFECT OF COPPER ON GILL AT 0.7 ppm

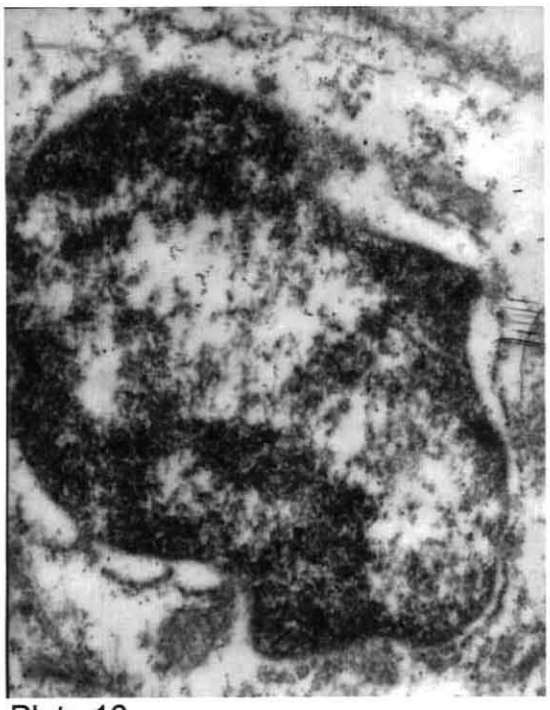


Plate 16

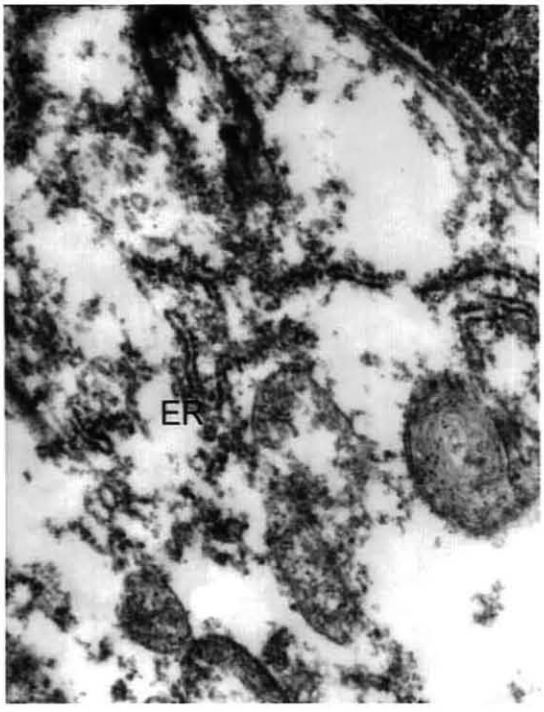


Plate 17

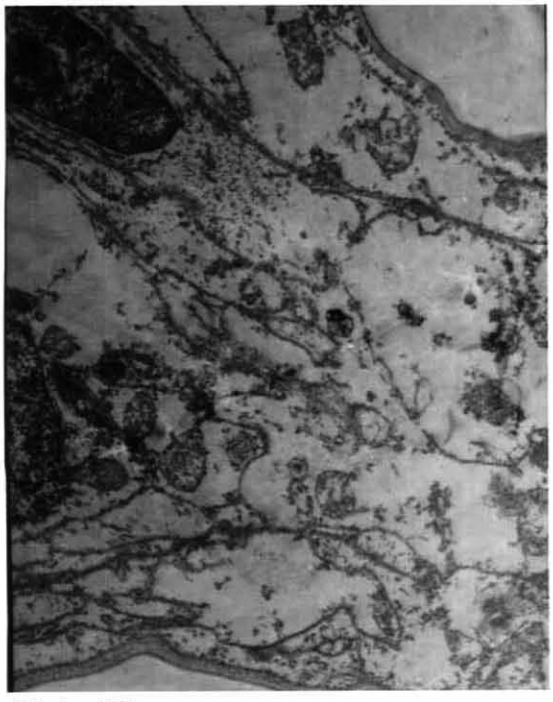


Plate 18

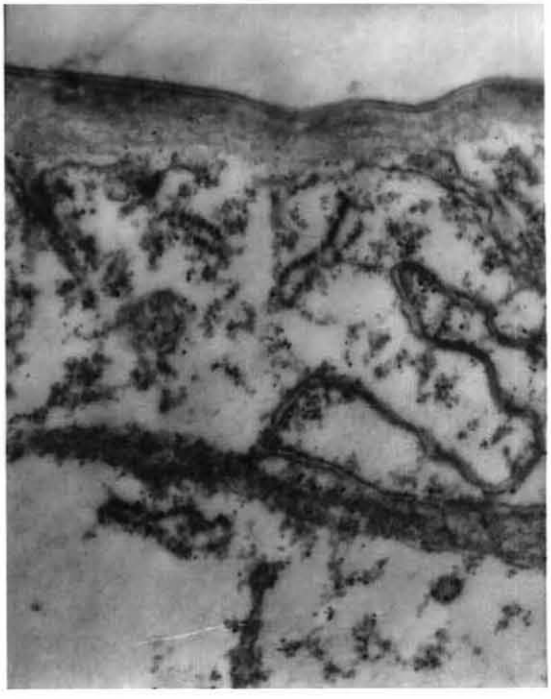


Plate 19

Plate. 16. Damaged nucleus X 25,000

Plate. 17. Distorted endoplasmic reticulum (ER) X 25,000

Plate. 18. Fewer organelles in the cell X 8,000

Plate. 19. Apical cell damage X 15,000

EFFECT OF COPPER ON HEPATOPANCREAS AT 6.98 ppm

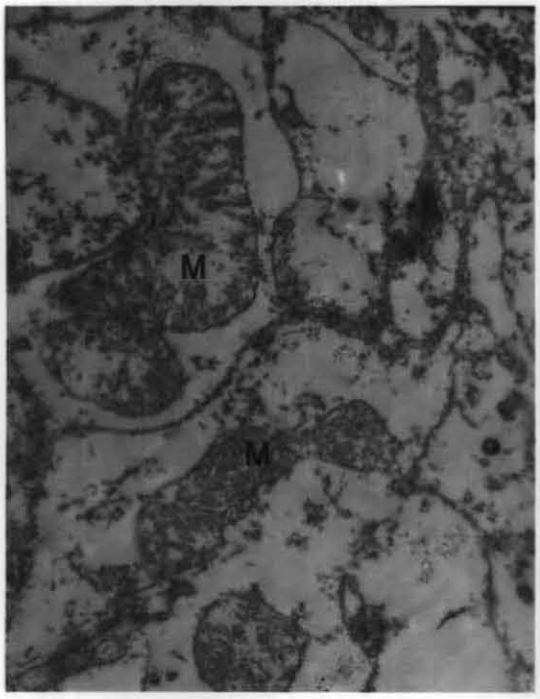


Plate 20

Plate 21

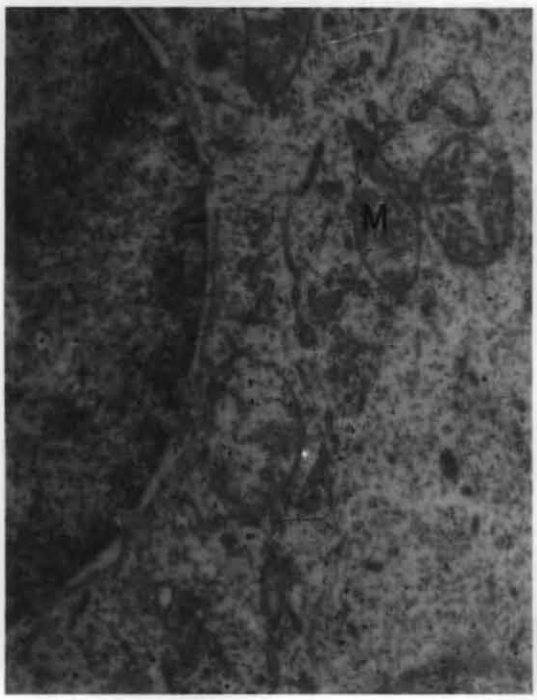


Plate 22

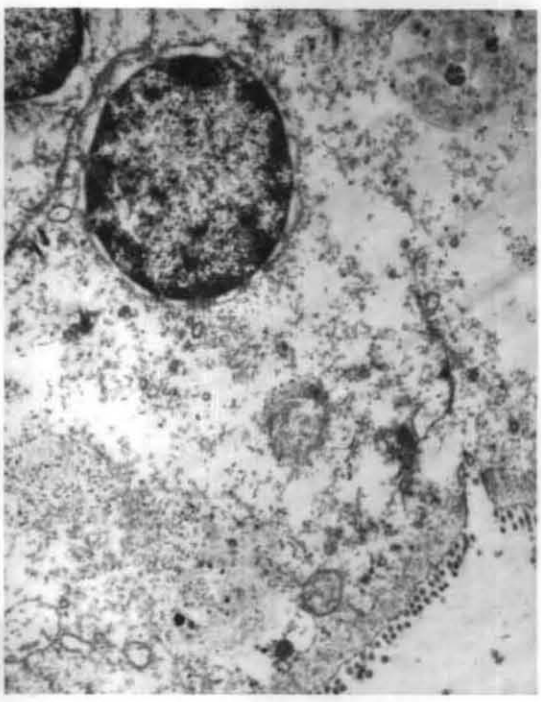


Plate 23

Plate. 20. Damaged mitochrondria X 15,000
Plate. 21. Vacuoles (V) in the cell X 20,000
Plate. 22. Pear shaped mitochondria (M) X 17,000
Plate. 23. Whole cell damage X 8,000

EFFECT OF COPPER ON HEPATOPANCREAS AT 0.7 ppm

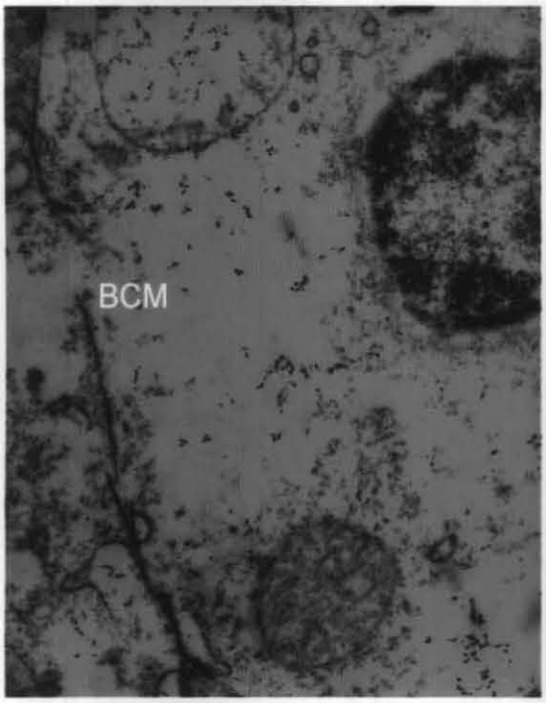
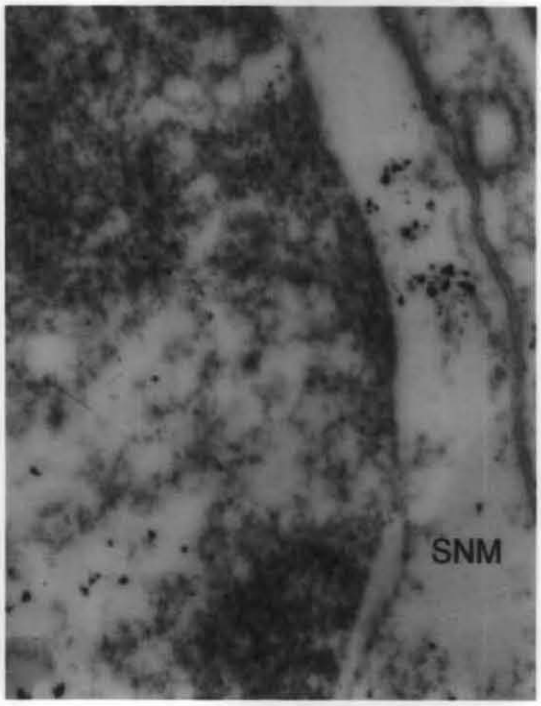


Plate 24



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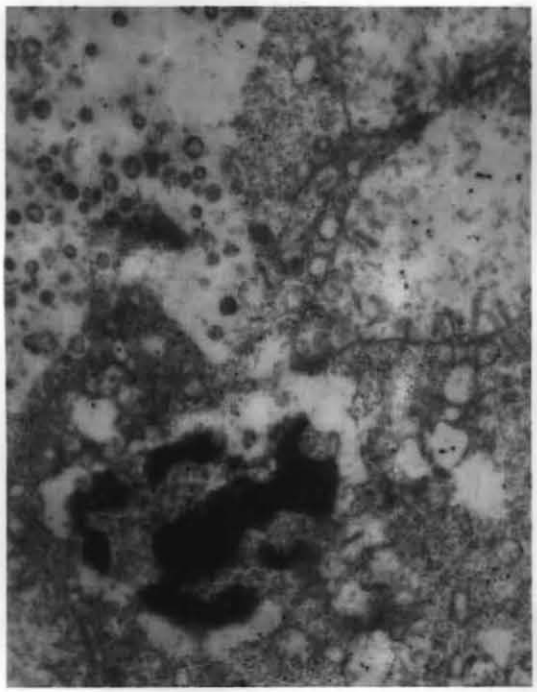


Plate 26

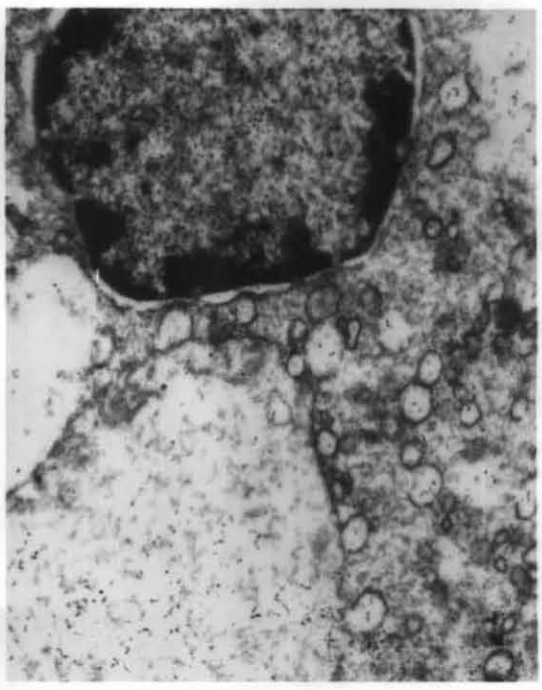


Plate 27

Plate. 24. Breakage in the cell membrane (BCM) X 12,000
Plate. 25. Swelling of the nuclear membrane (SNM) X 50,000
Plate. 26. Condensed nucleus and its damage X 17,000
Plate. 27. Numerous vacuoles X 12,000

EFFECT OF CADMIUM ON GILLS AT 2.8 ppm

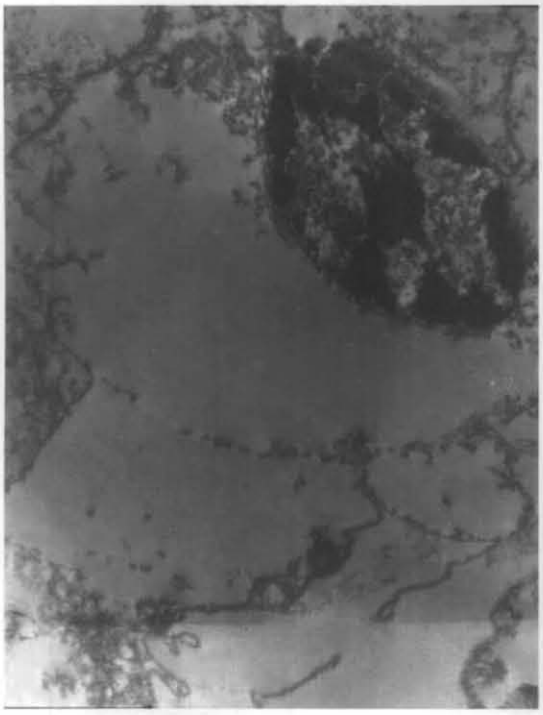
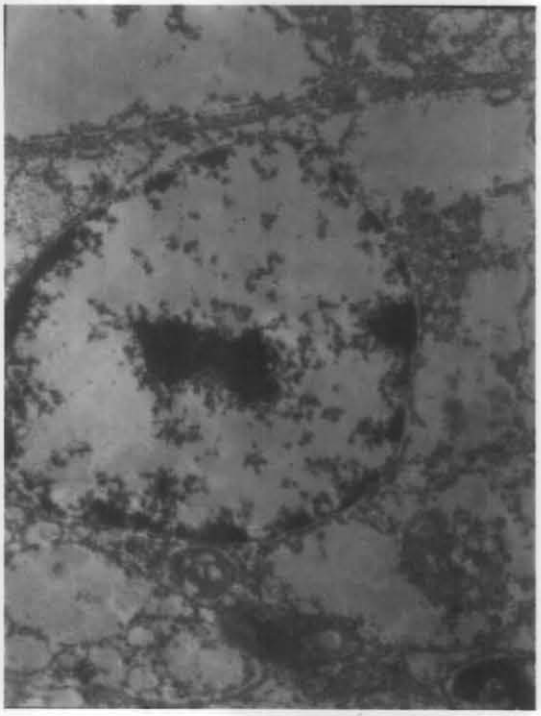


Plate. 28



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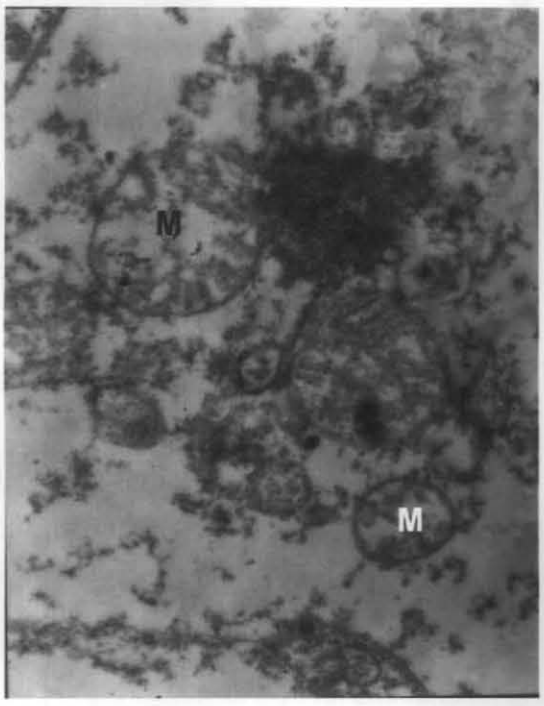

Plate 30

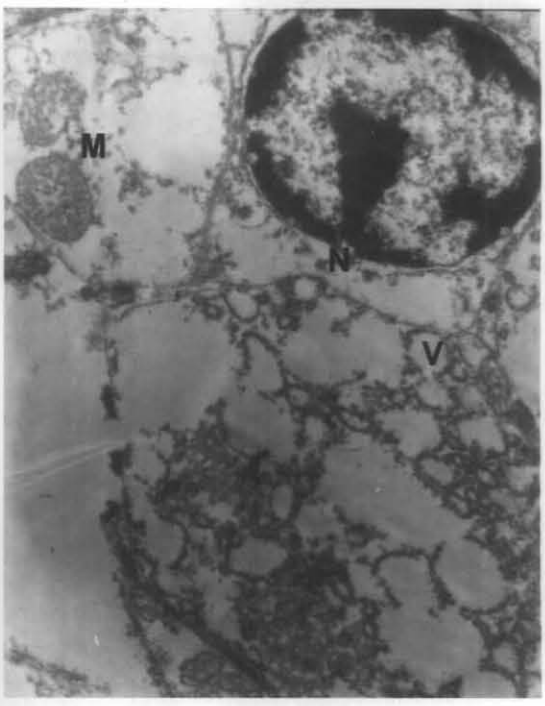

Plate. 31

Plate. 28. Whole cell destruction X 5,000

Plate. 29. Damaged Nucleus X 5,000

Plate. 30. Swelling of mitochondria (M) X 10,000

Plate. 31. Mitochondrial damage (M), Nuclear membrane damage (N), Vacuoles (V) X 5,000

EFFECT OF CADMIUM ON GILLS AT 0.25 ppm

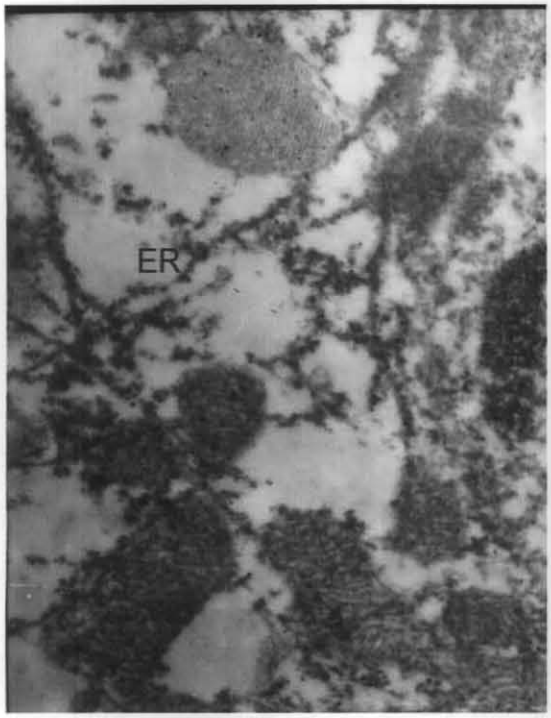

Plate 32

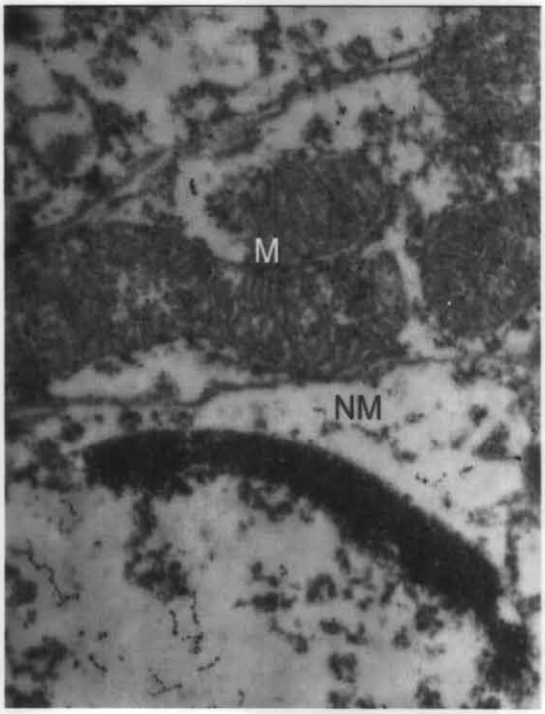

Plate 33

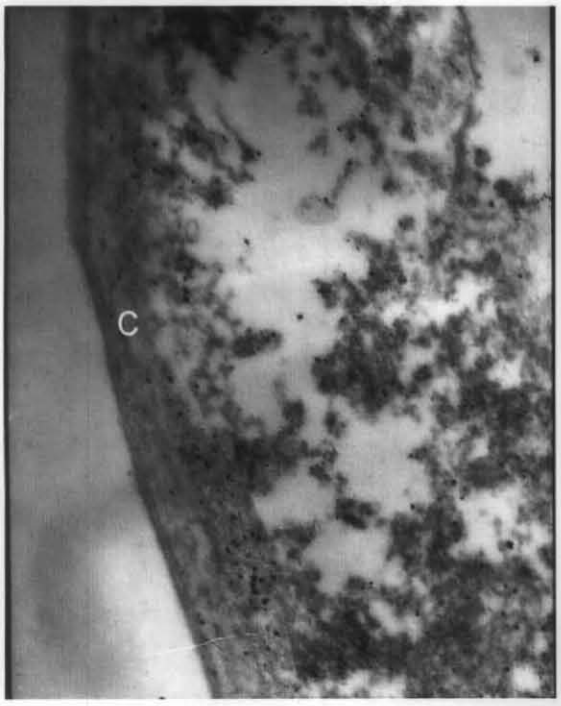

Plate 34

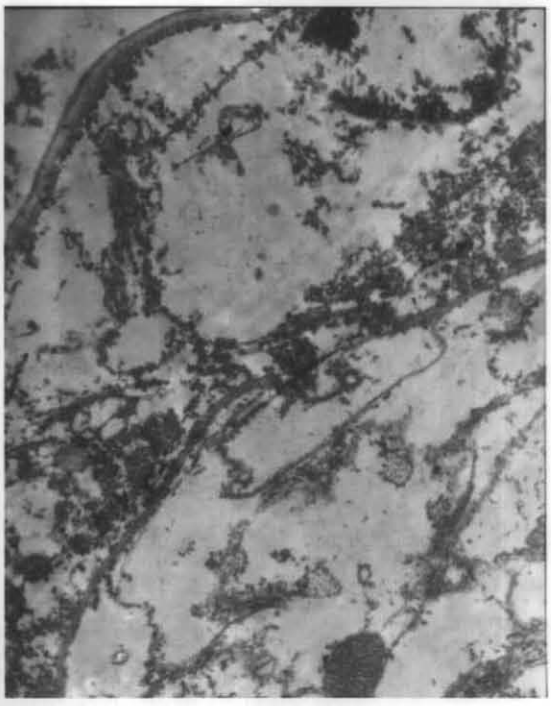

Plate 35

Plate 32. Endoplasmic reticulum (ER) distorted X 25,000

Plate 33. Damages in mitochondrial membrane (M) and nuclear membrane (NM) x 25,000

Plate 34. Damage in cuticle (C) X 35,000

Plate 35. Whole cell damage X 10,000

EEFECT OF CADMIUM ON HEPATOPANCREAS AT 2.8 ppm

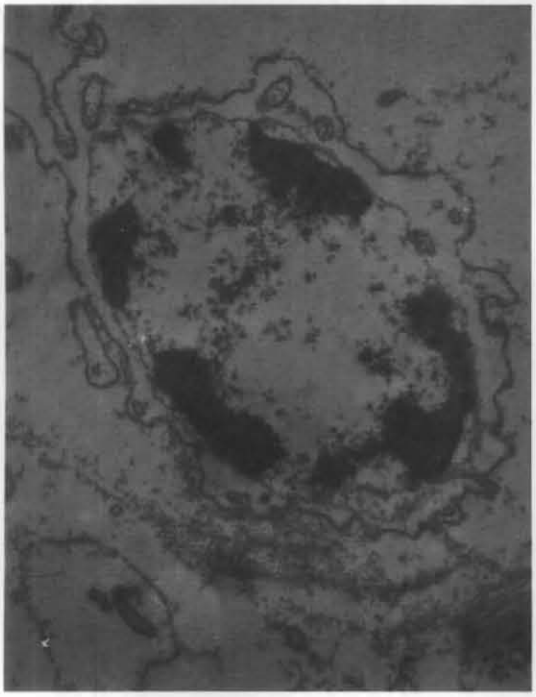

Plate 36

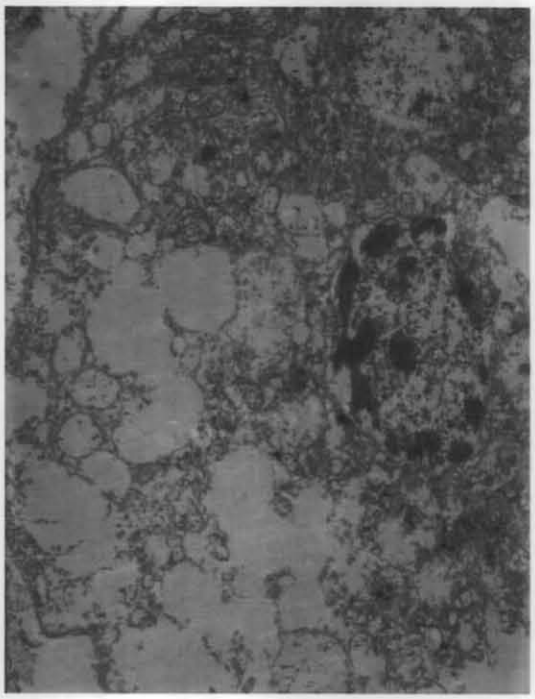

Plate 37

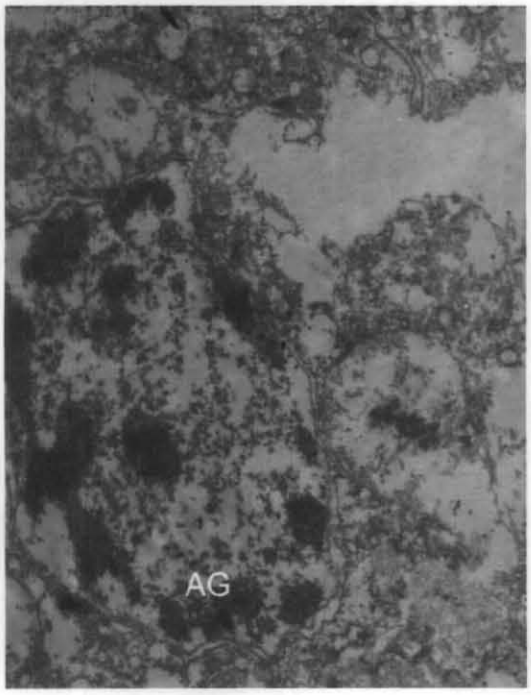

Plate 38

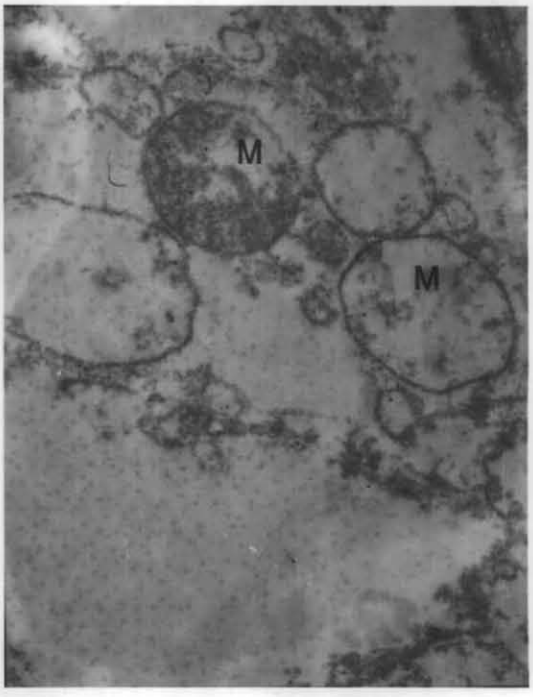

Plate 39

Plate. 36. Swelling of nuclear membrane X 6,000
Plate. 37. Whole cell damage X 5000
Plate. 38. Aggregations in nucleus (AG) and empty space in the cell X 5,000
Plate. 39. Mitochondrial damage (M) X 8,000

EFFECT OF CADMUIM ON HEPATOPANCREAS AT 0.25 ppm

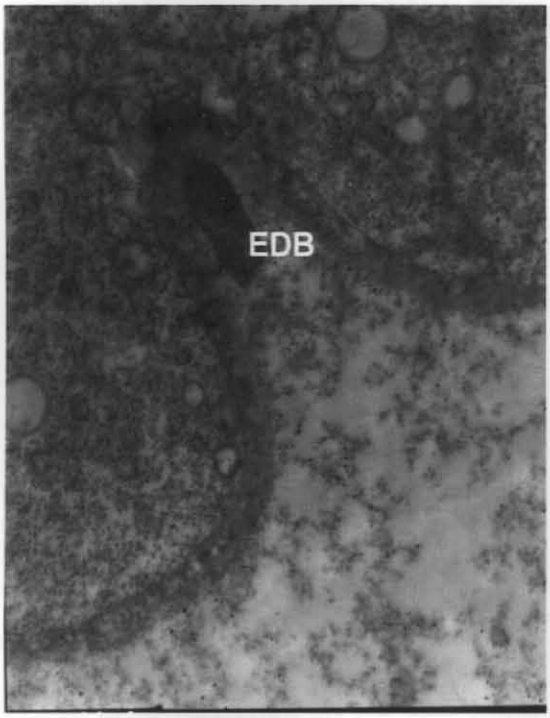

Plate 40

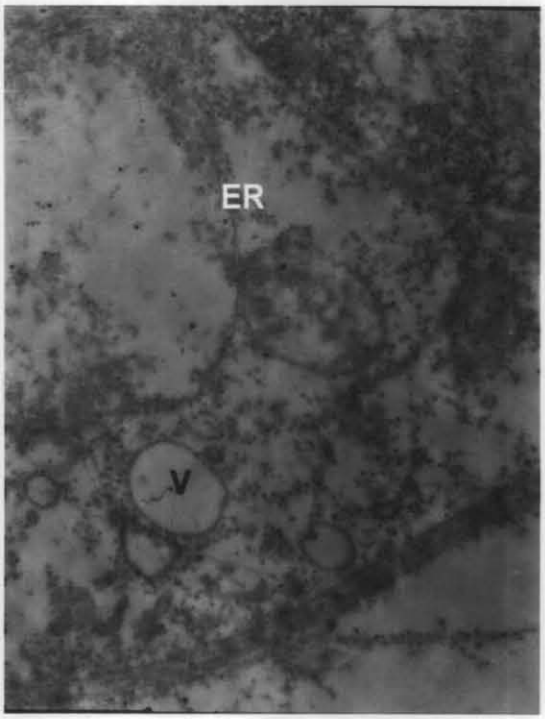

Plate 41

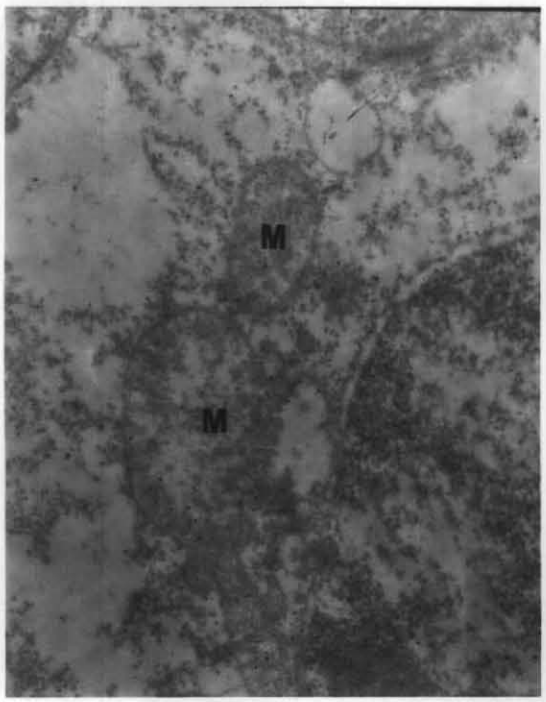

Plate 42

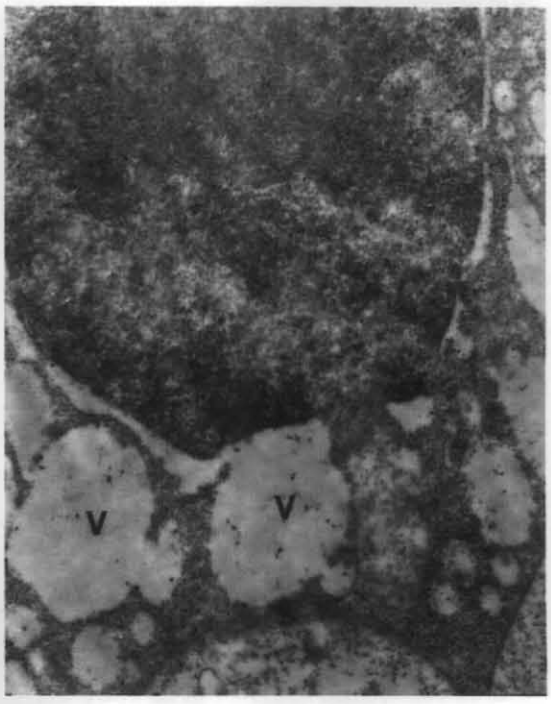

Plate 43

Plate. 40. Electron dense body (EDB) X 10,000

Plate. 41. Disturbed endoplasmic reticulum (ER) and vacuoles X12,000

Plate. 42. Damage in mitochondria (M) X 10,000

Plate. 43. Vacuoles attached to nuclear membrane (V) X 8,000

EFFECT OF ZINC ON GILLS AT 5.00 ppm

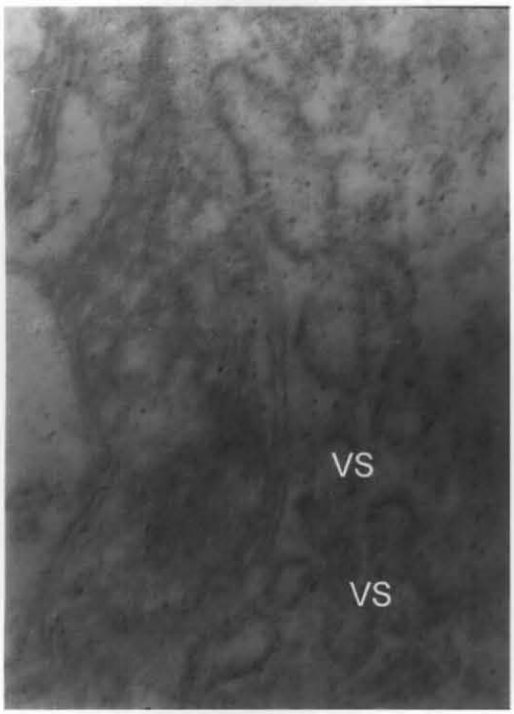

Plate 44

Plate 45

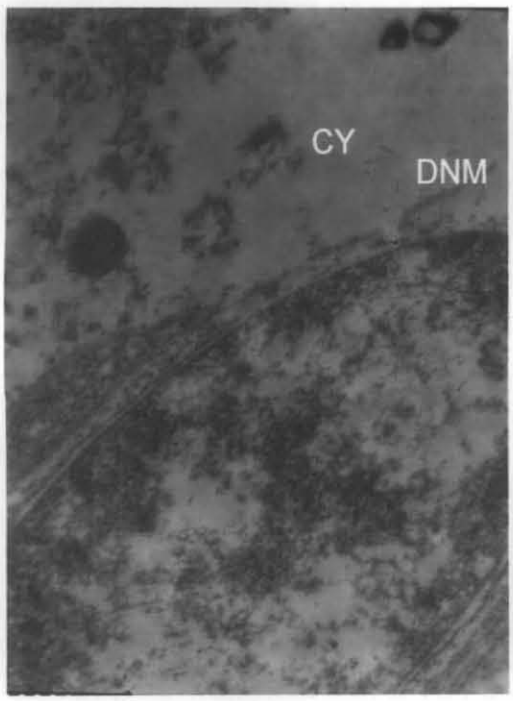

Plate 46

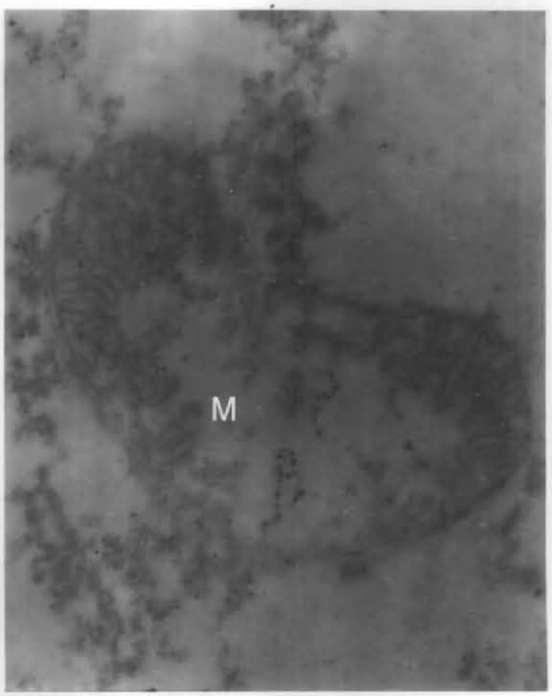

Plate 47

Plate. 44. Numerous vesicles (VS) X 20,000
Plate. 45. Whole cell damage X 6,000
Plate. 46. Disintigrated cytoplasm (CY) and damage in nuclear membrane (DNM) X 8,000
Plate. 47. Disrupted mitochondria (M) X 20,000

EFFECT OF ZINC ON GILLS AT 0.5 ppm

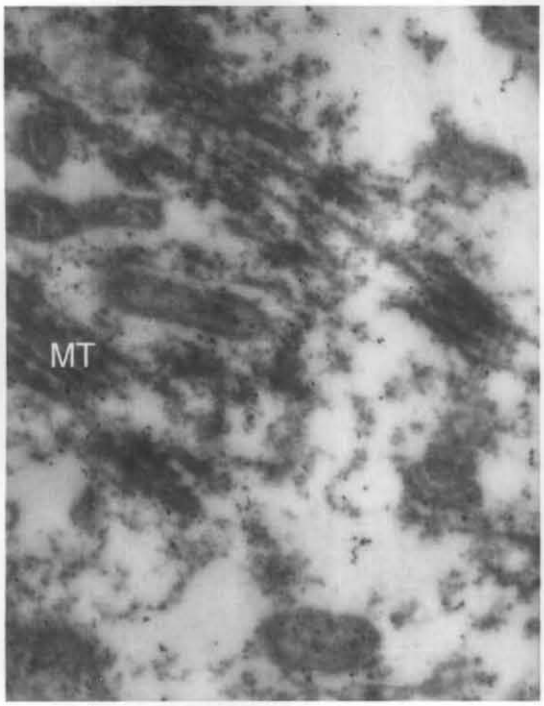

Plate 48

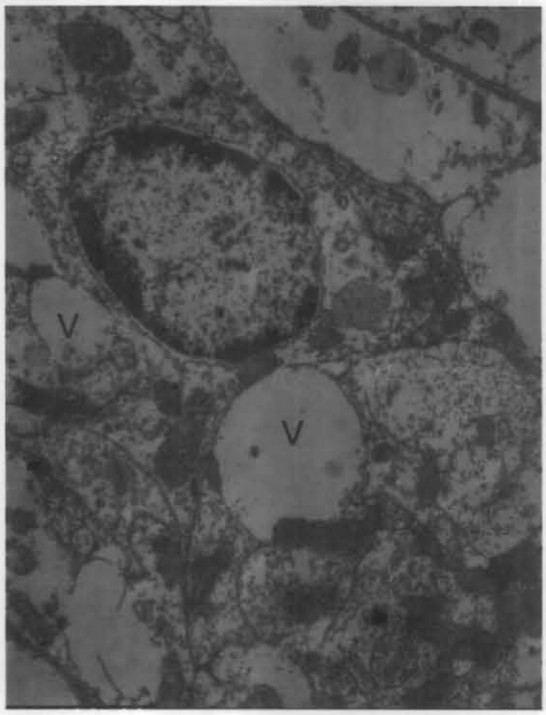

Plate 49

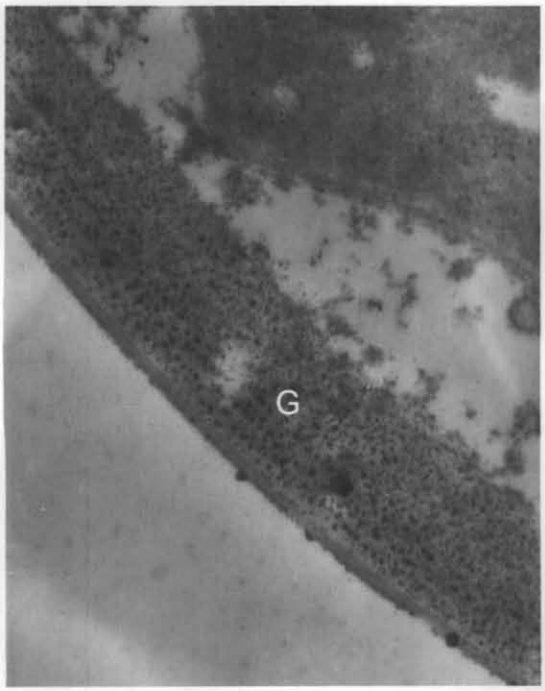

Plate 50

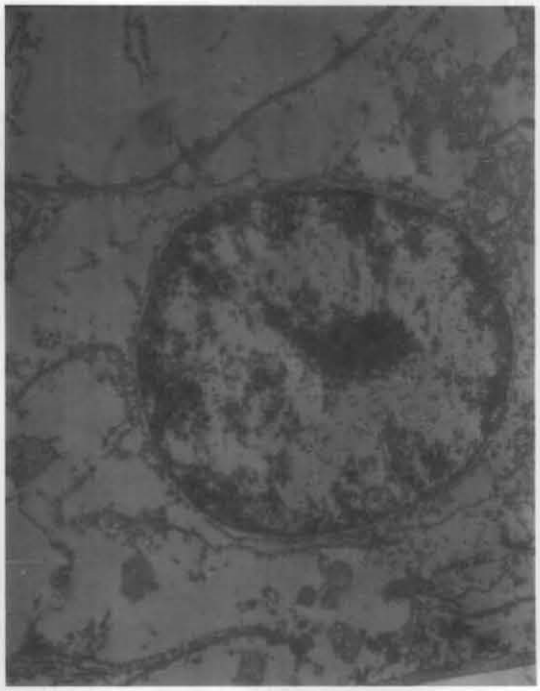

Plate 51

Plate. 48. Microtubules (MT) X 30,000
Plate. 49. Cell with vacuoles (V) X 6,000
Plate. 50. Granules (G) in the cuticle of the gill cell X 30,000
Plate. 51. Cells with less cell components X 8,000

EFFECT OF ZINC ON HEPATOPANCREAS AT 5 ppm

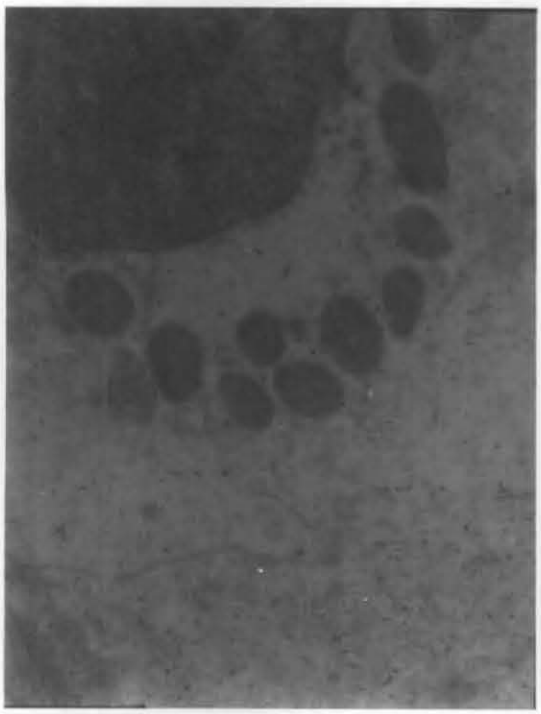

Plate 52

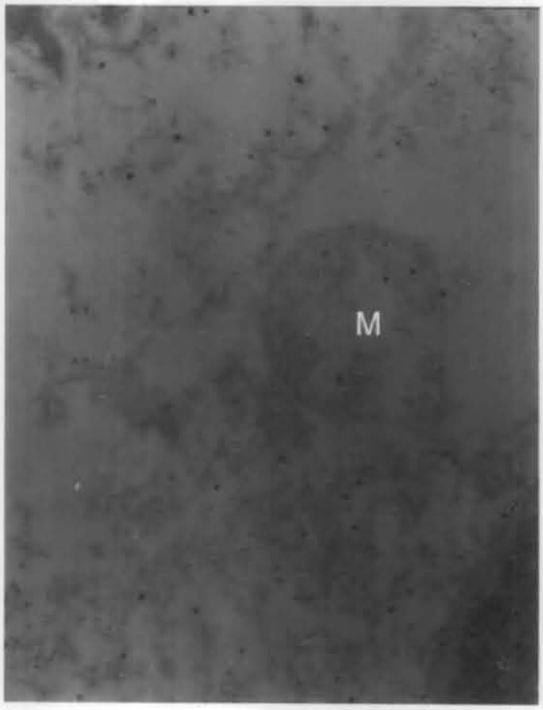

Plate 53

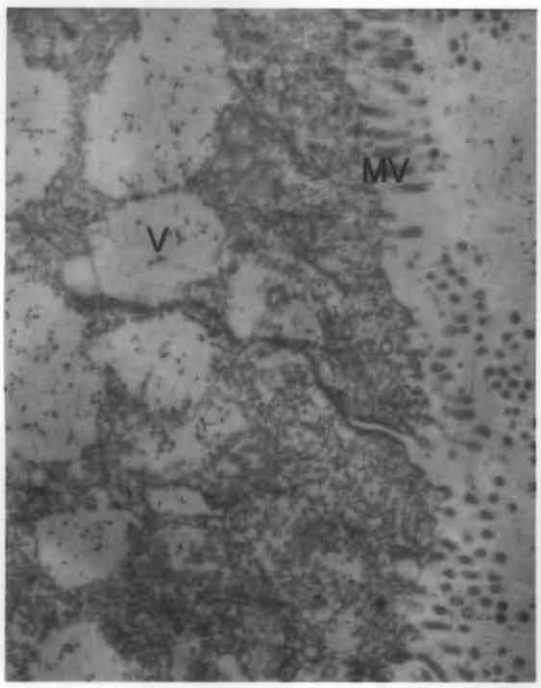

Plate 54

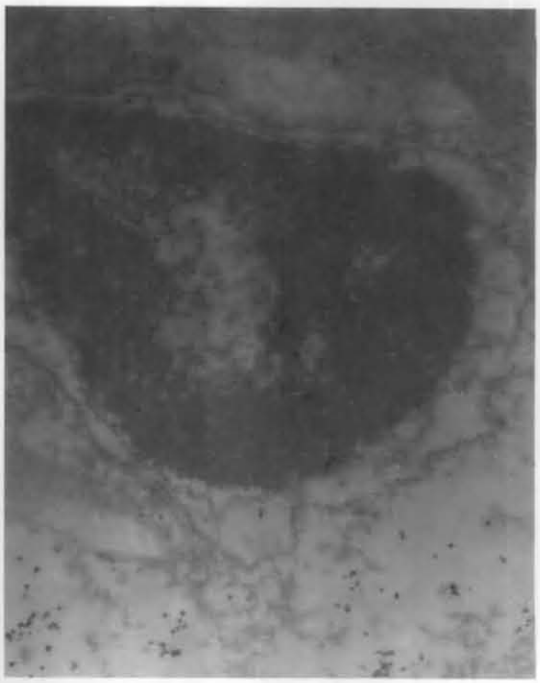

Plate 55

Plate. 52. Granular formation X 8,000
Plate. 53. Damaged mitochondria X 15,000
Plate. 54. Vacuole (V) and microvilli X 6,000
Plate. 55. Shrunken nucleus X 8,000

EFFECT OF ZINC ON HEPATOPANCREAS AT 0.5ppm

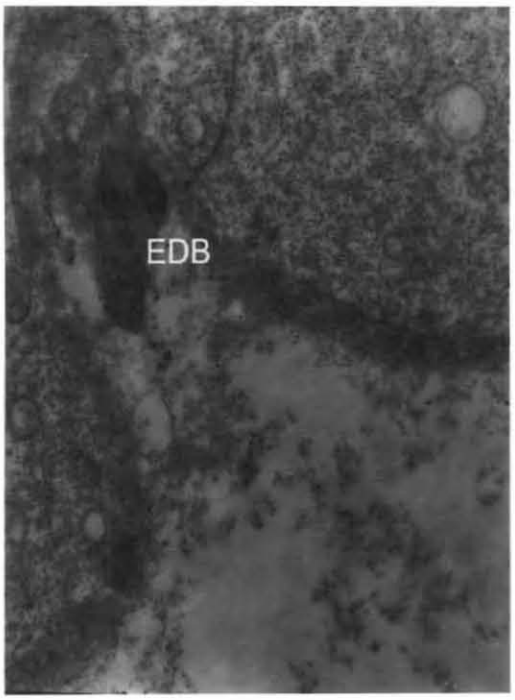

Plate 56

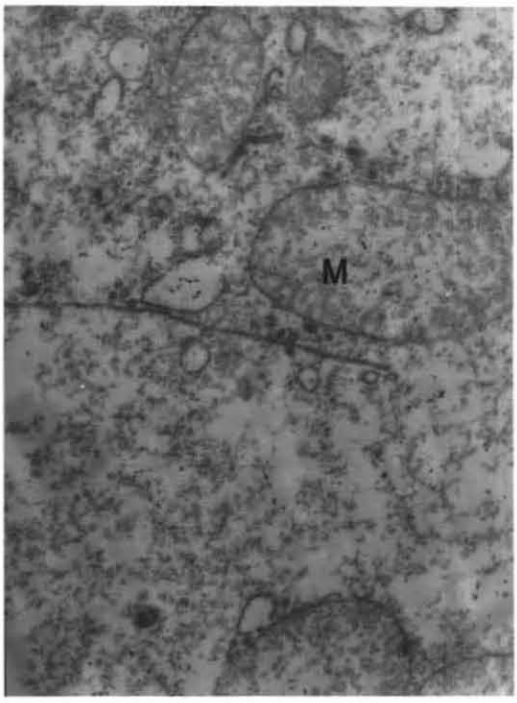

Plate 57

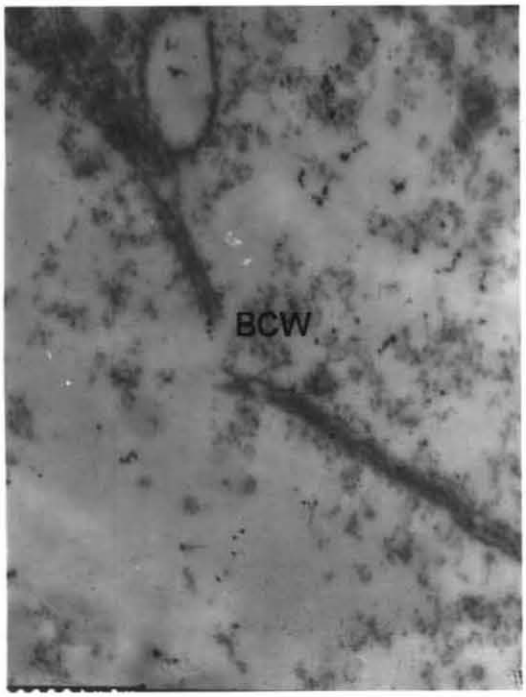

Plate 58

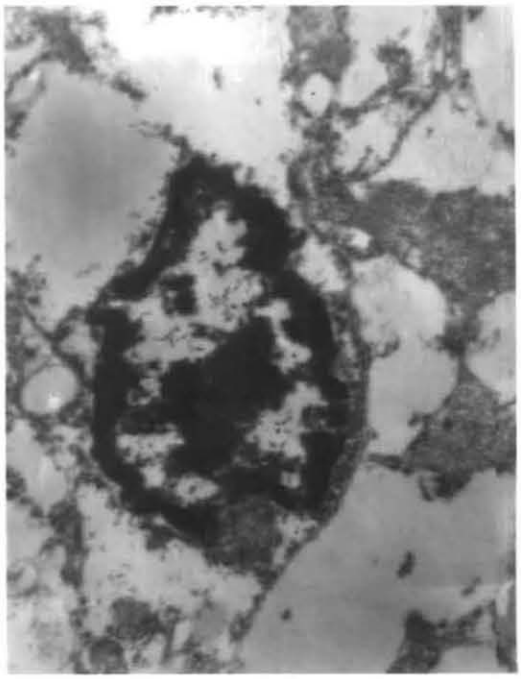

Plate 59

Plate. 56. Electron dense body (EDB) X 10,000
Plate. 57. Slight damages in mitochondria (M) X 12,000
Plate. 58. Breakage of cell wall (BCW) X 30,000
Plate. 59. Wavy appearance of nucleus X 6,000

DISCUSSION

5. DISCUSSION

5.1. Bioassay studies

Acute toxicity studies have been performed to determine the effects of metals on freshwater and marine fishes and shellfishes (Sprague, 1969, 1973, 1976). From the present static bioassay study, the 96 h LC_{50} of *P. semisulcatus* for copper, zinc and cadmium was determined as 6.98 ppm Cu/l, 5.0 ppm Zn/l and 2.8 ppm Cd/l respectively.

The results of the present study suggests that the toxicity of the three metals to *Penaeus semisulcatus* is in the order Cd> Zn> Cu. Copper being a component of haemolymph protein, haemocyanin and involvement of zinc in the metabolism of proteins and nucleic acids, these two are essential metals at optimum concentrations, whereas, cadmium is a non-essential metal and its toxic effect is more pronounced when compared to copper and zinc. The same pattern of toxicity (Cd> Cu> Zn) was reported by Vijayaraman (1993) in *Macrobrachium malcolmsonii*.

5.1.1. Copper

In *Crangon crangon*, 96 h LC_{50} was 1.9 ppm Cu/l (Portmann and Wilson, 1971). For *Penaeus merguiensis*, 96 h LC_{50} for copper was found to be 6.1 ppm (Denton and Burdon-Jones, 1982) and for *P. indicus*, 120 h LC_{50} was 0.3 ppm (Mary Carmel *et al.*, 1983). Sivadasan *et al.* (1986) reported a 96 h LC_{50} of 2.25 ppm for *Metapenaeus dobsoni*. In *Penaeus japonicus*, 96 h LC_{50} was 2.05 ppm when reared in seawater whereas in dilute seawater (27 ppt) it was 1.2 ppm. (Bambang *et al.*, 1994).

Among the freshwater prawns *Macrobrachium rude* showed an LC_{50} of 0.018 ppm (Vijayaraman and Geraldine, 1992) and for *M. malcolmsonii* it was 0.955 ppm (Vijayaraman, 1993).

From the present study, it can be concluded that *Penaeus semisulcatus* exhibits a better tolerance to copper than *Penaeus merguensis*, *Crangon crangon*, *Penaeus japonicus*, and *Metapenaeus dobsoni*. The least tolerance elicited by the freshwater prawns is due to the lower salinity which is a major factor determining the toxicity of heavy metals (Vijayaraman, 1993).

5.1.2. Zinc

Viswanathan and Manisseri (1993) found that the 96 h LC_{50} for Zinc to *P. indicus* was 1.67 ppm. In *Metapenaeus dobsoni*, the 96 h LC_{50} was 1.7 ppm (Sivadasan *et al.*, 1986). In the freshwater prawns, *Macrobrachium rude* and *M. malcolmsonii*, the 96 h LC_{50} was 3.025 and 2.284 respectively (Vijayaraman and Geraldine, 1992; Vijayaraman, 1993).

The present study reveals that *Penaeus semisulcatus* has the propensity to tolerate higher concentration of zinc when compared to *P. indicus*, *Metapenaeus dobsoni*, *Macrobrachium rude* and *M. malcolmsonii*. A possible reason for this higher tolerance must be the efficiency of this shrimp to regulate this metal if the ambient level of zinc is high.

5.1.3. Cadmium

In *Penaeus duorarum*, the 96 h LC_{50} was 4.6 ppm (Bahner and Nimmo, 1975). Ahsanullah (1976) reported a high value of 6.6 ppm as the 96 h LC_{50} of cadmium in *Palaemon spp*. Bambang *et al.* (1995) found that in *Penaeus japonicus*, the 96 h LC_{50} was 3.5 ppm. In the common Indian marine crab, *Scylla serrata*, the 96 hr LC_{50} was found to be 6.61 ppm (Krishnaraja *et al.*, 1987).

Among the freshwater prawns, 96 h LC_{50} of cadmium in *Palaemonetes vulgaris* and *Macrobrachium malcolmsonii* were, 0.76 ppm and 0.628 ppm respectively (Nimmo *et al.*, 1977; Vijayaraman, 1993).

In the present study, *P. semisulcatus* showed very low tolerance to cadmium when compared to other marine shrimps.

Thus the result of the present study suggests that *Penaeus semisulcatus* can tolerate relatively higher concentrations of copper and zinc when compared with other shrimps mentioned above, however, in case the of cadmium it showed a low tolerance than other marine shrimps.

5.2. Morphological and behavioural changes

5.2.1. Morphological changes

Penaeus duorarum, *Palaemonetes pugio* and *P. vulgaris* exposed to cadmium in acute and subacute tests developed blackened foci or melanised lamellae (Nimmo *et al.*, 1977). Melanisation of gills, carapace, telson and pareiopods was observed in *Macrobrachium malcolmsonii*, exposed to copper, cadmium, chromium and zinc individually as well as in combination (Vijayaraman, 1993). Blackened gills were observed in *Penaeus japonicus* exposed to copper (Soegianto *et al.*, 1999). Viswanathan and Manisseri (1993) observed blackening in *Penaeus indicus* exposed to zinc and it resulted in blackening of the gills. In the present study also melanisation was observed in body parts of *P. semisulcatus*.

The ultrastructural studies of gills of *Penaeus duorarum*, exposed to cadmium revealed that cell death leads to blackening of the gills (Couch, 1977). Experimental removal of the sinus gland of *Palaemon serratus*, was found to result in darkening of the carapace (Knowles, 1959). The sinus gland, which is

endocrine in function, controls and regulates body pigmentation in crustaceans (Gorbman and Bern, 1974). Neurosecretory hormones secreted by sinus gland have a significant role in the regulation and dispersion of the chromatophores in crustaceans (Ranga Rao and Fingerman, 1983).

The black pigmentation in various body parts seen in *P. semisulcatus* could be attributed to either cell death or disruption in the functioning of the sinus gland. Thus, the exposure of shrimps to copper, cadmium and zinc may adversely affect the sinus gland, which is involved in the hormonal control of colour change in crustaceans.

5.2.2. Behavioural changes

Studies on the behavioural aspects of heavy metal toxicity in shrimps or prawns are scanty. Vijayaraman (1993) who studied the effect of copper, cadmium, chromium and zinc on *Macrobrachium malcolmsonii* reported behavioural responses like a gradual cessation of activity, paralysis due to impairment of muscular movements and erratic swimming. In the present study also, *P. semisulcatus* exhibited similar behavioural responses, which include erratic swimming and reduced response to stimuli, which may be due to the impairment of respiratory and endocrine system of the animal under heavy metal exposure.

5.3. Bioaccumulation

5.3.1. Copper

Copper is an essential trace element for cell growth and differentiation. The concentration of this metal can govern diverse metabolic pathways and physiological processes (Ghidalia, 1985).

In *Penaeus semisulcatus*, the highest amount of copper was accumulated in hepatopancreas and accumulation in the other tissues was in the following order: hepatopancreas > gills > tail > carapace > muscle in all the exposures. This is in conformity with the findings in many other decapod crustaceans (Bryan, 1968; Peerzada, *et al.*, 1992) including *Cambarus bartoni* (Alikhan *et al.*, 1990), *Carcinus maenas* (Truchot and Rtal, 1998) *Penaeus japonicus* (Soegianto *et al.*, 1999) freshwater prawn, *Macrobrachium malcolmsonii* (Vijayaraman, 1993) crabs *Potamonautes perlatus* (Snyman *et al.*, 2002) and *Portunus pelagicus* (Al-Mohanna and Subrahmanyam, 2001).

The copper content in the experimental shrimps increased significantly, with increase in the concentration of metal in the medium and the duration of exposure. The increase was only marginal in the tail, carapace and muscle tissues but sharper in hepatopancreas and gills. A similar pattern has been reported on exposure of copper in *Cambarus bartoni* (Alikhan *et al.*, 1990), *Carcinus maenas* (Trichot and Rtal, 1998) *Macrobrachium malcolmsonii* (Vijayaraman, 1993) *P. monodon* (Vogt and Quinitio, 1994) and *Penaeus japonicus* (Soegianto *et al.*, 1999).

The significant increase in copper concentration in all the tissues of *P. semisulcatus* exposed to 6.98 ppm for four days and 0.7 and 1.4 ppm Cu/l for 14 days suggests that, the bioaccumulation of copper occurs through the circulation of haemolymph, and the high exchange surface of the gills with the external medium, constitute the entry site of the metal and act as a transient store for accumulated metal as proposed by Martin and Rainbow (1998).

The hepatopancreas performs a central role in metabolism, storage and detoxification of a number of metals similar to the liver of vertebrate counterparts (Rainbow and Scott, 1979; Dall and Moriarty, 1983). Copper is primarily accumulated and deposited in the hepatopancreas, which is a major storage organ in decapods (Brown, 1982; Rainbow, 1988, 1990, 1992) and levels of

copper in the digestive gland are affected by environmental conditions (Darmono and Denton, 1990).

In the sublethal studies with 0.7 ppm copper in the medium for 14 days, the hepatopancreas of *P. semisulcatus* accumulated significantly higher quantity of copper than that of the lethal concentration. As the lethal study was conducted only for 96 h, the bioavailability of copper might not have crossed the threshold level required for inducing the bioaccumulation process. Studies on the strategies of copper accumulation by decapods over a wide range of concentrations under defined physio-chemical conditions in the laboratory indicate that the concentration of copper in the body is regulated to a constant level till copper bioavailability reaches high threshold level, when regulation breaks down and net accumulation begins (White and Rainbow, 1982; Amiard *et al.*, 1985; Rainbow, 1985, 1988,1990, 1992, 1995a,b, 1997a,b). The accumulation of copper in the carapace of *P. semisulcatus* seems to be a mechanism to counteract the metal stress in which moulting could be considered as a detoxifying mechanism accelerating the output of Cu accumulated in the carapace as observed in crab larvae (Lopez Greco *et al.* 2000).

In the sublethal study with two concentrations (0.7 and 1.4 ppm) in *P. semisulcatus*, the bioaccumulation of copper in hepatopancreas showed a dose dependent response. This shows that the environment copper concentration greatly influences the accumulation in the hepatopancreas compared to the other organs as highlighted by Rainbow (1985, 1988, 1990, 1992, 1995a, b, 1997a, b).

5.3.2. Cadmium

Cadmium in decapods is preferentially accumulated in the digestive gland; the pattern of accumulation, however differ among species. In *P. semisulcatus*, accumulation of this metal was highest in the hepatopancreas followed by gills and other organs. This is consistent with reports in other decapods (Eisler, *et al.*, 1972; Eisler, 1973, 1981; Nimmo *et al.*, 1977; Jennings and Rainbow, 1979;

Jennings *et al.*, 1979; Ray *et al.*, 1980; Davies *et al.*, 1981; Papathanassiou and King, 1983; 1986; White and Rainbow, 1986; Vijayaraman, 1993; Vijayram and Geraldine, 1996). This non essential metal could be sequestered and progressively accumulated in the hepatopancreas, perhaps as granules or bound to metal binding proteins (Wright, 1977a, b; Bjerregaard, 1990).

The increase in the accumulation of cadmium based on dosage and /or duration of exposure observed in the present study is a trend similar to that recorded with other crustaceans such as *Palaemon serratus* (Pappathanassiou and King, 1986) *Palaemon elegans* (White and Rainbow, 1986) *Carciuns maenas* (Wright 1977a,b; Jennings *et al.*, 1979; Amiard-Triquet *et al.*, 1986) *Uca pugilator* (O'Hara, 1973), *Callinassa australiensis* (Ahsanullah *et al.*, 1984), *Callianassa tyrrhena* (Thaker and Hariotis, 1989, 1993) and in freshwater prawn, *Macrobrachium malcolmsonii* (Vijayaraman, 1993).

Cadmium is found to be accumulated in the gills, hepatopancreas, muscle and exoskeleton of *Penaeus semisulcatus*, with hepatopancreas and gills being the organs with highest concentrations. In the lobster, *Homarus americanus* cadmium is accumulated to a higher concentration in the gills than muscle, exoskeleton and viscera (Chavez-Crooker, 2002).

There is no evidence to suggest that any decapod regulates the body cadmium concentrations to a constant level by balancing uptake and excretion (White and Rainbow, 1982; Napierska and Radlowska, 1998). Indeed the crab *Carcinus maenas* (Wright, 1977a; Jennings and Rainbow, 1979; Rainbow, 1985), the prawn *Palaemon elegans* (White and Rainbow, 1982; 1986) and the shrimp *Crangon crangon* (Dethlefsen, 1978; Amiard *et al.*, 1985) show increasing body concentrations of cadmium with increased exposure to dissolved cadmium as observed in *Penaeus semisulcatus*. In this context, it is of interest to note that both penaeids and palaemonids can concentrate cadmium in their tissues in ambient conditions (White and Rainbow, 1982; Napierska and Radlowska, 1998). Earlier studies revealed that metallothioneins bind the excess cadmium absorbed

from the ambient medium and store it in a detoxified form particularly in the hepatopancreas. In *Cancer pagurus* (Overnell and Trewhella, 1979; Overnell, 1982, 1984, 1986) *Palaemon elegans* (White and Rainbow, 1984) *Homarus americanus* (Engel and Brouwer, 1984) *Carcinus maenas* (Wong and Rainbow, 1986) *Callianassa tyrrhena* (Thaker and Haritos, 1989, 1993) *Callinectes sapidus* (Syring *et al.*, 1992; Brouwer, *et al.*, 1994, 2000, 2002) and in fish, *Sarotherodon mossambicus* (Ramalingam, 1988, 2003). *Onchorynchus mykiss* (Kammann, 1995) *Carcinus maenas* (Pedersen *et al.*, 1998) and *Onchorynchus spp* (Gehrig *et al.*, 2000) detoxification mechanisms by metallothioneins are reported.

5.3.3. Zinc

Zinc is associated with the activity of nearly 100 enzymes involved in protein, carbohydrate, lipid and nucleic acid metabolism (Martin *et al.*, 1977; Elinder, 1986; Berry, 1997). At excessive concentrations, however, this essential metal becomes toxic to organisms (Phillips, 1980).

The accumulation of zinc in *P. semisulcatus* increased with increase in the concentration of Zn in the test medium. Both in the lethal and sublethal exposures, the pattern of accumulation is hepatopancreas > gills > tail > muscle > exoskeleton.

Tissue concentrations of zinc corresponds to the seawater concentrations (Bryan *et al.*, 1986). According to Al-Mohanna and Nott (1985, 1986, 1987), decapods respond to zinc concentrations in the ambient environment differentially. Regulatory responses of zinc were reported in *Homarus vulgaris* (Bryan, 1964; 1968; 1976), *Carcinus maenas* (Wright, 1976, 1978) *Pandalus montagui* (Ray *et al.*, 1980) and *Palaemon elegans* (White and Rainbow, 1982, 1984) to control their internal concentration of this metal. The results of the present study in *P. semisulcatus* indicates breakdown of the internal regulatory mechanism after a threshold level leading to bioaccumulation of zinc in various tissues. Decapod crustaceans regulate the body concentration of zinc to an approximately constant level over a wide range of ambient zinc bioavailability. If

the rate of zinc uptake is beyond the physiological control of the net accumulation of absorbed zinc begins (Bryan, 1964; 1976; (White and Rainbow, 1982; Amiard *et al.*, 1985; Rainbow, 1985).

Rainbow (1987) reported a very high concentration of zinc in species of crustaceans like barnacles in which this metal is stored in the form of zinc phosphates. Al-Mohanna (1986, 1987) also reported the presence of zinc as zinc phosphates in *P. semisulcatus*.

5.4. Physio - Biochemical studies

5.4.1. Respiratory studies

In *P. semisulcatus*, an increase in oxygen consumption was observed while exposed to lethal concentrations of copper, cadmium and zinc. This is in agreement with the results reported in scallop, *Argopectin irradians* (Nelson *et al.*, 1976), lobster, *Homarus americanus* (Thurberg *et al.*, 1977), *Crassostrea virginica* (Engel and Fowler, 1979) and in *Palaemon serratus* (Papathanassiou, 1983) when exposed to metals.

In contrast to the above observations, a decrease in oxygen consumption was reported in *Eurypanopeus depressus* (Collier *et al.*, 1973), *Carcinus maenas* and *Cancer irroratus* (Thurberg *et al.*, 1973), *Uca pugilator* (Vernberg and Vernberg, 1972), *Palaemonetes vulgaris* and *Ilyoplax gangetica* (Reish, 1978), *Scylla serrata*. (Narayanan 1989) and in *Macrobrachium malcolmsonii* (Vijayaraman, 1993).

Kinne (1960) reported that the initial increase in oxygen consumption when aquatic animals are exposed to heavy metals is an "overshoot" response. The increase in oxygen consumption seen in the present study might result from toxic stress-induced mobilization of metabolic reserves to meet immediate

energy requirements. It appears that shrimps exposed to heavy metals had to expend more energy to mitigate the toxic impact of heavy metals present in the medium.

5.4.2. Biochemical

5.4.2.1. Carbohydrate

Carbohydrate is the first and the most efficient source of energy. Almost all animals use carbohydrate as respiratory fuel. They serve as an important source of energy for vital activities in the form of glucose and glycogen (Berry, 1997).

Hepatopancreas is a storage tissue and is actively involved in metabolism in crustaceans (Chang and O' Connor, 1983). In the present study, *P. semisulcatus* exposed to copper, cadmium and zinc showed a marked reduction in the carbohydrate level of hepatopancreas, gills and muscle.

Inside the tissues of animals subjected to toxic stress, a reduction in carbohydrate content occurs due to various physiological changes like elevation of AMP level, triggering the activity of phosphorylase A to effect glycogenolysis. As a result of glycogenolysis there occurs a reduction in glycogen reserve and ultimately a drop in total carbohydrate level results. This has been demonstrated in *Macrobrachium lamarrei* (Omkar and Shukla, 1985), *Macrobrachium malcolmsonii* (Vijayaraman, 1993), *Penaeus indicus* (Srinivasalu Reddy *et al.*, 1985, 1986), *Scylla tranquibarica* (Anbarasu, 1993), *Barytelphusa guerini* (Fingerman, *et al.*, 1981) *Scylla serrata* (Kulkarni and kulkarni, 1989; Srinivasaulu Reddy and Bhagyalakshmi, 1994) *Homarus americanus* (Chavez-Crooker, 2002), *Achatina fulica* (Indra, 1998), *Mytilus edulis* (Dezwan and Zandec, 1972), *Sarotherodon mossambica* (Koundinya and Ramamurthi, 1979; Ramalingam, 1988, 1989, 1990, 2003) *Oreochromis mossambicus* (Balasubramanian, *et al.*, 1999) *Spiralotelphusa hydrosdroma* (Sakundala, 1992)

Therapon jarbua (Selvakumar, 1981) and *Carassius auratus* (Gargiulo *et al.*, 1996).

The possible reason for a reduction in the carbohydrate levels can be attributed to the utilization of glycogen reserve for meeting the additional energy requirements caused by the toxicant exposure.

5.4.2.2. Protein

Proteins occupy a key position in the general body growth and in the repair of wear and tear of the cells with the help of aminoacids. A general reduction in the protein content of animal leads to a reduction in growth and also its metabolic activity in the cell (Berry, 1997).

In the present study, *Penaeus semisulcatus* showed a marked reduction of protein in hepatopancreas, gills and muscle when exposed to lethal and sublethal concentration of copper, cadmium and zinc.

Depletion of proteins due to various toxicants like heavy metal and pesticides has been reported in *Oncorhynchus kisutch* (Mc Leay and Brown, 1974), *Sarotherodon mossambicus* (Ramalingam and Ramalingam, 1982), *Mugil cephalus* (Mihelic *et al.*, 1999), *Metapenaeus monoceros* (Vijayalakshmi and Ramana Rao, 1985), *Penaeus indicus* (Srinivasalu Reddy *et al.*, 1986), *Scylla serrata* (Narayanan, 1989), *Spiralotelphusa hydrodroma* (Sakundala, 1992) and *Macrobrachium malcolmsonii* (Vijayaraman, 1993).

The primary energy sources are carbohydrates and lipids. Proteins will be used to meet the energy requirements only when the carbohydrate and lipid sources are exhausted (Berry, 1997). In the present study, as there is a marked decrease in the carbohydrate and lipid levels, it can be presumed that these primary sources are exhausted and so the proteins are used to meet the energy requirements. Reduction of DNA and RNA was reported in killi fishes (Jackim *et*

al., 1970), *Macrobrachium kistensis* (Nagabhusanam *et al.*, 1987), *Scylla serrata* (Narayanan, 1989) and *Macrobrachium malcolmsonii* (Vijayaraman, 1993), *Penaeus monodon* (Reddy and Ramamurthy, 1997) when exposed to heavy metals and pesticides. The reduced RNA content implies reduced protein content. The heavy metals can bind to certain sites on the nucleic acids, thus disrupting the normal function (Eichron, 1975). As nucleic acids are involved in protein synthesis, a disruption in their normal function can affect the protein synthesis. Therefore, heavy metal exposure can adversely affect the protein synthesis ultimately leading to a reduction in the protein content of the animal.The marked reduction in protein content observed in the present study, can be attributed to two reasons: (1) for meeting the energy requirements when the primary energy sources like carbohydrate and lipids got exhausted; (2) the protein synthesis was affected due to the disruption in the functioning of nucleic acids brought about the binding of metal ions to certain sites on them.

5.4.2.3. Lipid

Lipids serve as a tremendously important, energy rich fuel in higher animals and plants, since large magnitude can be stored in cells in the form of triglycerols (Gur and Harwood, 1991).

In the presence of heavy metal and pesticides, a reduction in lipid has been observed in *Penaeus indicus* (Srinivasulu Reddy *et al.*, 1985) and *Macrobrachium malcolmsonii* (Vijayaraman, 1993). In the present study lipid content in various tissues showed a significant reduction. As the carbohydrate source of energy was exhausted, the lipids might have been broken down as an alternate fuel source. This can be a possible explanation for the reduction in lipid content observed in the present study.

5.5. Histopathology

Heavy metals are known to affect the structure and function of cellular components leading to impairment of vital functions of many marine organisms (Papathanasiou and king, 1983; Papathanasiou, 1985; Baticados, *et al.*, 1987; Vogt, 1987; Baticodos and Tendencia, 1991; Vijayaraman, 1993; Manisseri and Menon, 1995; Marinescu *et al.*, 1997; Soentigo *et al.*, 1999; Bhavan and Geraldine, 2000; Cheng *et al.*, 2000). It is in this context that histological and ultrastructural alterations are employed as effective indices of physiological and biochemical changes caused by copper, cadmium and zinc induced stress at the lethal levels and sublethal levels of exposure. These biological indices provide insight into cellular injuries before any irreversible alteration occurs.

The two vital organs most affected by environmental contamination are hepatopancreas and the gills. Gills perform osmoregulation and respiration while, hepatopancreas has secretory, excretory, absorptive and digestive functions. These organs have been identified as target organs for studying heavy metal pollution, pesticide pollution and other ecotoxicants because critical histopathological and ultrastructural alterations occur in these organs at very early stages of exposure.

5.5.1. Gills

In aquatic organisms, the gills represent a vital organ, since it plays an important role in the exchange of respiratory gases and regulate the osmotic and ionic balance. Toxic substances will cause damage to gill tissues (Vijayaraman, 1993; Lignot *et al.*, 2000).

The notable alteration in the histoarchitecture of the gills of *P. semisulcatus* include accumulation of haemocytes, necrosis, swelling, fusion of the lamellae lifting of the laminar epithelium, deformities and fusion and necrosis. Similar changes were noticed in *Callinectes sapidus* (Copeland and Fitzjaarel,

1968), *Penaeus duorarum* (Couch, 1977), *P. duorarum* and *Palaemonetes elegans* (Nimmo *et al.*, 1977), *Macrobrachium sp.* and *Caridina sp.* (Ghate and Mulherkar, 1979), *Cancer irroratus* (Greig *et al.*, 1982), *Scylla serrata* (Narayanan, 1989), *Macrobrachium idae* (Victor *et al.*, 1990), *Macrobrachium malcolmsonii* (Vijayaraman, 1993), *Carcinus maenas* (Hebel *et al.*, 1999), *Penaeus japonicus* (Soegianto *et* al., 1999), *Macrobrachium malcolmsonii* (Bhavan and Geraldine, 2000) when exposed to heavy metals and endosulfan. Chandy and Kolwalkar (1984) also reported same alterations in gill histology of *Charybdis lucifera* and *Scylla serrata* due to crude oil.

Ultrastructural changes observed in the gills of *P. semisulcatus* were damaged nuclear membrane, increase in number of vacuoles, deposition of metals in the vacuoles, disrupted mitochondria, damaged nucleus, distorted endoplasmic reticulum, apical cell damage, swelling of mitochondria, damage in mitochondrial membrane, scattered microtubules, numerous vesicles and disintegrated cytoplasm. Similar changes in gills were reported in *Palaemon serratus* (Papathanassiou and King, 1983), *Penaeus duorarum*, *Palaemontes pugio* and *P. vulgaris* (Nimmo *et al.*, 1977), *Palaemon serratus* (Papathanassiou and King, 1983; Papathanassiou, 1985), *Jaera nordmanii* (Bubel, 1976), *Carcinus maenas* (Lawson *et al.*, 1985), *Macrobrachium malcolmsonii* (Vijayaraman, 1993), *Carcinus maenas* (Hebel *et al.*, 1999), *Penaeus japonicus* (Soegianto *et* al., 1999) when exposed to heavy metals and pesticides.

In the gills of *Penaeus semisulcatus* the most affected organelles were the mitochondria, with damage in cristae, breakage in bounding membranes and swelling. Mitochondria with closely packed cristae have high metabolic activity (Berry, 1997). The disruption of mitochondrial membranes reduces the ability of mitochondria to synthesize ATP and leads to an increase in permeability which might account for their swollen appearance (Bubel, 1976).

The increase in the intracellular vesicles helps in the transportation of metals from external medium to haemolymph (Papathanassiou and king, 1983;

Papathanassiou, 1985; Vijayaraman, 1993; Soegianto *et al.*, 1999). The presence of large number of vesicles can be attributed to the transportation of metal to haemolymph in *P. semisulcatus*.

Damage of the endoplasmic reticulum suggests that an energy deficiency occurred within the cells due to inhibition of protein synthesis by the reduced number of ribosomal particles. The invagination of the apical membrane and the associated pinocytic vesicles are probably involved in the salt absorbing mechanism (Copeland and Fitzjaarel, 1968). Damages to the cuticle, when exposed to toxicants suggest that the first part of the gill to be affected is this outer protective covering. The structural integrity of the cuticle is affected by pollutant exposure (Papathanassiou, 1985).

Thus metals had an effect on the fine structure of the gill cells of *P. semisulcatus* and affect several functions such as enzymatic activities, absorption and transportation of salts, active ion uptake and protein synthesis (Papathanassiou, 1985; Soegianto, *et* al., 1999).

Disoriented microtubules were observed when the shrimps were exposed to metals. This suggests that the cytoskeletal system is impaired by the exposure of the pollutants to the shrimps (Sandborn *et al.*, 1964).

Since exposure to copper, cadmium and zinc results in structural damages in the gills of *Penaeus semisulcatus*, it is likely to interfere with the diverse physiological functions of this vital organ. Several studies have reported that metal exposure to this organ can disrupt respiratory processes (Depledge, 1984; 1989; Boitel and Truchot, 1989; Spicer and Weber, 1991, 1992; Nonnotte *et al.*, 1993; Vijayaraman, 1993; Bhavan and Geraldine, 2000).

5.5.2. Hepatopancreas

The hepatopancreas of crustaceans have 4 groups of epithelial cells namely; E-, R-, F- and B- cells and formed by a sequence of cellular

differentiation (Dall, 1967; Miyawaki *et al.*, 1961, 1984; Miyakawi and Tanoue, 1962; Davis and Burnett, 1964; Bunt, 1968; Gibson and Barker, 1979; Dall and Moriarty, 1983; Chambers, 1992; Vogt, 1994; Marinescu *et al.*, 1997; Stainer *et al.*, 1968; Hopkin and Nott, 1979; Erri Babu *et al.*, 1982; Robinson and Dillaman, 1985; Al-Mohanna, *et al.*, 1985; Al-Mohanna and Nott, 1986, 1987; 1989; Vogt, 1987; Caceci *et* al., 1988). The structural details of various cellular differentiation in hepatopancreas of *P. semisulcatus* observed in the present study was in conformity with the description given in the literature.

The notable changes in *P. semisulcatus* exposed to copper, cadmium and zinc were necrotic tubule, tissue debris, swelling and necrosis of hepatopancratic cells and abnormal lumen. The observed changes are similar to that reported in *Penaeus vannamei* (Lightner *et al.*, 1996), *Penaeus stylirostris*, *P. vannamei* and *P. monodon* (Lightner *et al.*, 1982; Bautisa *et al.*, 1994), *Penaeus indicus* (Viswanathan and Manisseri, 1993), *Macrobrachium malcolmsonii* (Vijayaraman, 1993), *Metapenaeus dobsoni* (Manisseri and Menon, 1995), *Astacus leptodactylus* (Marinescu *et al.*, 1997) and in *Macrobrachium malcolmsonii* (Bhavan and Geraldine, 2000) when exposed to metals and pesticides.

Ultrastructural changes observed in the study includes, mitochondrial damage, increase in number of vacuoles, breakage of cell membrane, swelling of nuclear membrane, condensation of nucleus, presence of electron dense bodies, disrupted endoplasmic reticulum and presence of granules. Similar changes were noticed in *Penaeus stylirostris* and *P. vannamei* (Lightner, *et al.*, 1982; Bautisa, *et al.*, 1994), *Penaeus monodon* (Vogt *et al.*, 1985; Baticados, *et al.*, 1987; Baticodos and Tendencia, 1991; Vogt and Quininto, 1994), *Macrobrachium malcolmsonii* (Vijayaraman, 1993), *Metapenaeus dobsoni* (Manisseri and Menon, 1995) *Penaeus vannamei* (Lightner *et al.*, 1996) and *Eriochier sinensis* (Cheng *et al.*, 2000) when exposed to metals and pesticides.

The nuclear damage observed in all the metal treatments in *P. semisulcatus* included distortion of nuclei, damage to nuclear membrane, overall shrinkage and condensation of nuclear material resulting in the nuclei losing its characteristic shape. The metals can interact with the nuclear proteins, resulting in the alteration of the complex structure of chromatin or the catalytic activity of the enzymes involved in DNA and RNA metabolism. This may induce the depolymerisation, favor hydrolysis of RNA, affect the correct replication and transcription of DNA and alter the fidelity of the translation of RNAs during the process of protein synthesis at the ribosomal level and reduction in the protein content of the organ (Eichron, 1975). The decrease in protein content of the tissue as mentioned earlier is probably caused by the damage in the normal function of nucleus due to metals.

The endoplasmic reticulum showed an extensive damage leading to disruption and disintegration in the present study. Various functions have been suggested for the endoplasmic reticulum, including the mechanical support of the cytoplasm, protein synthesis, glycogen storage, steroid synthesis, intracellular transport of metabolic products and cellular impulse conduction (De Robertis and De Robertis, 1980; Berry, 1997). The pollutants reduce the rate of protein synthesis by reducing the rate of RNA synthesis, influencing the attachment of polyribosomes to the endoplasmic reticulum and potentially damaging the ribosomes themselves (Viarengo and Noh, 1993). The structural damages of this organelle exposed to heavy metals leads to the non-functioning of this organ.

The mitochondria transfer the chemical energy of the metabolites of the cell into high-energy phosphate bond of Adinosine triphosphate (ATP). Thus they are the "power house" of the cell that provides the energy for many vital cellular functions viz. biosynthesis of cell material, active transport etc. (Berry, 1997). *P. semisulcatus* exposed to Cu, Cd and Zn showed deformities, including disfigured, swollen mitochondria, losing its typical saucer shape and loss of its integrity which inevitably disrupt in its vital functions like ATP synthesis.

Penaeus semisulcatus exposed to copper, cadmium and zinc showed merging of vesicles or vacuoles containing electron dense bodies. This is a method by which metal rich bodies are transported to the R-cells for further sequestration and elimination (Al Mohanna and Nott, 1987). The presence of these bodies was also observed in ***Metapenaeus dobsoni*** (Manisseri and Menon, 1995). The R- cells of the hepatopancreas of *Penaeus semisulcatus* can take up particulate material from the haemolymph by pinocytosis at the basal membrane and store in vacuoles and vesicles as large dense bodies (Al Mohanna and Nott, 1987).

The lysosomal-vesicular systems are considered to be the major degradative systems within the cell. Lysosomes in the hepatopancreas and excretory organs can accumulate large quantities of metals. In *Penaeus monodon*, metals acquired from water were directed into the hepatopancreas for detoxification and stored as granules. The granules observed in the present study in hepatopancreas may be a process by which metals are stored and eliminated. (George, 1982; Vogt and Quininto, 1994).

Thus, exposure of *Penaeus semisulcatus* to lethal and sublethal concentrations of copper, cadmium and zinc caused ultrastructural alterations in both gill and hepatopancreas. The functions of the organs are severely impaired due to the toxicity.

6. SUMMARY

The present investigation is an attempt to study the lethal and sublethal effects of three heavy metals, namely, copper, cadmium and zinc individually on the commercially important Green tiger prawn, *Penaeus semisulcatus*.

1. Acute toxicity bioassays were conducted for copper, cadmium and zinc. The LC_{50} 96 h was copper: 6.98 ppm, cadmium: 2.8 ppm and zinc: 5.00 ppm. The degree of toxicity of the three metals were in the order Cd > Zn > Cu.

2. Based on the LC_{50} values, two sublethal concentrations namely, copper (0.7 ppm and 1.4 ppm), cadmium (0.25 ppm and 0.5 ppm) and zinc (0.5 ppm and 1.00 ppm) were selected for most of the investigations.

3. Bioaccumulation studies revealed that the accumulation of metals occur in the hepatopancreas, gills, muscle, tail and carapace. The results obtained suggest that the shrimps could not regulate the accumulation of Cu, Cd and Zn. The bioaccumulation in all the organs/body parts were found to be dose dependent in the case of sublethal concentration, with the highest concentration in hepatopancreas.

4. In the physio-biochemical studies, an increase in the respiration rate was found in shrimps exposed to lethal concentrations of copper, cadmium and zinc. The biochemical components, namely, carbohydrate, protein and lipid were found to reduce during the various phases of metal exposure. This reduction varied according to the essentiality of the metal in the biochemical aspects of the animal. The changes in the proximate components is due to the variation in the enzymes concerned with the tissue energy generation; like glycogen degradation, increase in the sugar pool by phosphorylase, formation of lactic acid due to increase in LDH;

inhibition of protein synthesis by the alteration in the DNA and RNA content. Increase in the free fatty acid content due to changes in the synthesis and mobilization of lipids.

5. Histopathological studies exhibited the changes in the structural integrity of the cells of gills and hepatopancreas. In all the lethal exposures, blackening of the gill lamellae and exoskeleton was noticed. Ultrastructural studies revealed changes in the cell organelles, especially in mitochondria, endoplasmic reticulum and nucleus.

Future research should focus on the molecular basis of heavy metal toxicity and on the role of metallothionein and other enzymes that can sequester the metals in cellular functions and in detoxification processes.

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8. APPENDICES

Appendix 1. Summary of ANOVA between the metals and bioaccumulation of metals in various tissues at lethal concentration studies

1a. hepatopancreas

Source of Variation	df	MS	F	P-value
Between metals	2	241507.1	36.06	< 0.05
error	6	6696.892		
Total	8			

1b. gills

Source of Variation	df	MS	F	P-value
Between metals	2	17985.5	28.27	< 0.05
error	6	635.99		
Total	8			

1c. muscle

Source of Variation	df	MS	F	P-value
Between metals	2	2223.76	318.62	< 0.05
error	6	6.97		
Total	8			

1d. tail

Source of Variation	df	MS	F	P-value
Between metals	2	5796.20	2808.02	< 0.05
error	6	2.06		
Total	8			

1e. exoskeleton

Source of Variation	df	MS	F	P-value
Between metals	2	840.84	134.88	< 0.05
error	6	6.23		
Total	8			

1f. whole animal

Source of Variation	df	MS	F	P-value
Between metals	2	28428.71	351.04	<0.05
error	6	80.98		
Total	8			

Appendix 2. Summary of ANOVA between the metals and bioaccumulation of metals in various tissues at sublethal (10% of lethal concentration) studies.

2a. hepatopancreas

Source of Variation	df	MS	F	P-value
Between metals	2	5251779	8564.77	< 0.05
error	6	613.18		
Total	8			

2b. gills

Source of Variation	df	MS	F	P-value
Between metals	2	8223.5	221.49	< 0.05
error	6	37.12		
Total	8			

2c. muscle

Source of Variation	df	MS	F	P-value
Between metals	2	2883.26	525.81	< 0.05
error	6	5.48		
Total	8			

2d. tail

Source of Variation	df	MS	F	P-value
Between metals	2	5432.64	107.74	< 0.05
error	6	50.42		
Total				

2e. exoskeleton

Source of Variation	df	MS	F	P-value
Between metals	2	214.42	22.67	< 0.05
error	6	9.45		
Total	8			

2f. whole animal

Source of Variation	df	MS	F	P-value
Between metals	2	4572.62	247.61	< 0.05
error	6	18.46		
Total	8			

Appendix 3. Summary of ANOVA between the metals and bioaccumulation of metals in various tissues at Sublethal levels (20% of lethal concentration) studies

3a. hepatopancreas

Source of Variation	*df*	*MS*	*F*	*P-value*
Between metals	2	23280766	27168	< 0.05
error	6	856.91		
Total	8			

3b. gills

Source of Variation	df	MS	F	P-value
Between metals	2	16453.19	286.78	< 0.05
error	6	57.37		
Total	8			

3c. muscle

Source of Variation	df	MS	F	P-value
Between metals	2	5409.47	395.66	< 0.05
error	6	13.67		
Total	8			

3d. tail

Source of Variation	df	MS	F	P-value
Between metals	2	10332.7	392.75	< 0.05
error	6	26.30		
Total	8			

3e. exoskeleton

Source of Variation	df	MS	F	P-value
Between metals	2	1167.46	177.76	< 0.05
error	6	6.56		
Total	8			

3f. whole animal

Source of Variation	df	MS	F	P-value
Between metals	2	20181.89	691.97	< 0.05
error	6	29.16		
Total	8			

Appendix 4. Summary of ANOVA between variations of biochemical composition of different organs when exposed to lethal concentration of copper, cadmium and zinc.

4a. protein in hepatopancreas

Source of Variation	df	MS	F	P-value
Between metals	2	3.22	8.67	< 0.05
error	6	0.37		
Total	8			

4b. carbohydrate in hepatopancreas

Source of Variation	df	MS	F	P-value
Between metals	2	0.43	14.24	< 0.05
error	6	0.03		
Total	8			

4c. lipid in hepatopancreas

Source of Variation	df	MS	F	P-value
Between metals	2	7.55	3.70	>0.05
error	6	2.04		
Total	8			

4d. protein in gills

Source of Variation	df	MS	F	P-value
Between metals	2	0.16	0.12	> 0.05
error	6	1.37		
Total	8			

4e. carbohydrate in gills

Source of Variation	df	MS	F	P-value
Between metals	2	0.01	4.784	> 0.05
error	6	0.003		
Total	8			

4f. lipid in gills

Source of Variation	df	MS	F	P-value
Between metals	2	0.028	3.154	> 0.05
error	6	0.009		
Total	8			

4g. protein in muscle

Source of Variation	df	MS	F	P-value
Between metals	2	7.27	5.19	< 0.05
error	6	1.39		
Total	8			

4h. carbohydrate in muscle

Source of Variation	df	MS	F	P-value
Between metals	2	0.081	2.33	> 0.05
error	6	0.034		
Total	8			

4i. lipid in muscle

Source of Variation	df	MS	F	P-value
Between metals	2	0.11	0.71	> 0.05
error	6	0.15		
Total	8			

Appendix 5. Summary of ANOVA between variations of biochemical composition of different organs when exposed to 10% of LC_{50} levels of copper, cadmium and zinc.

5a. protein of the hepatopancreas

Source of Variation	df	MS	F	P-value
Between metals	2	2.51	3.48	> 0.05
error	6	0.72		
Total	8			

5b. carbohydrate in hepatopancreas

Source of Variation	df	MS	F	P-value
Between metals	2	0.48	11.78	< 0.05
error	6	0.04		
Total	8			

5c. lipid in hepatopancreas

Source of Variation	df	MS	F	P-value
Between metals	2	1.66	3.56	> 0.05
error	6	0.47		
Total	8			

5d. protein in gills

Source of Variation	df	MS	F	P-value
Between metals	2	10.41	26.15	< 0.05
error	6	0.40		
Total	8			

5e. carbohydrate in gills

Source of Variation	df	MS	F	P-value
Between metals	2	0.04	13.27	< 0.05
error	6	0.00		
Total	8			

5f. lipid in gills

Source of Variation	df	MS	F	P-value
Between metals	2	0.02	3.40	> 0.05
error	6	0.01		
Total	8			

5g. protein in muscle

Source of Variation	df	MS	F	P-value
Between metals	2	6.57	11.28	< 0.05
error	6	0.58		
Total	8			

5h. carbohydrate in muscle

Source of Variation	df	MS	F	P-value
Between metals	2	0.01	1.37	> 0.05
error	6	0.01		
Total	8			

5i. lipid in muscle

Source of Variation	df	MS	F	P-value
Between metals	2	1.80	2.55	> 0.05
error	6	0.71		
Total	8			

Appendix 6. Summary of ANOVA between variations of biochemical composition of different organs when exposed to 20% LC_{50} levels of copper, cadmium and zinc.

6a. protein in hepatopancreas

Source of Variation	df	MS	F	P-value
Between metals	2	8.22	13.34	< 0.05
error	6	0.62		
Total	8			

6b. carbohydrate in hepatopancreas

Source of Variation	df	MS	F	P-value
Between metals	2	1.11	27.95	< 0.05
error	6	0.04		
Total	8			

6c. lipid in hepatopancreas

Source of Variation	df	MS	F	P-value
Between metals	2	2.59	4.58	>0.05
error	6	0.57		
Total	8			

6d. protein in gills

Source of Variation	df	MS	F	P-value
Between metals	2	12.22	56.02	< 0.05
error	6	0.22		
Total	8			

6e. carbohydrate in gills

Source of Variation	df	MS	F	P-value
Between metals	2	0.08	1.84	>0.05
error	6	0.05		
Total	8			

6f. lipid in gills

Source of Variation	df	MS	F	P-value
Between metals	2	0.01	1.85	>0.05
error	6	0.00		
Total	8			

6g. protein in muscle

Source of Variation	df	MS	F	P-value
Between metals	2	10.55	16.63	< 0.05
error	6	0.63		
Total	8			

6h. carbohydrate in muscle

Source of Variation	df	MS	F	P-value
Between metals	2	0.03	7.90	< 0.05
error	6	0.00		
Total	8			

6i. Lipid in muscle

Source of Variation	df	MS	F	P-value
Between metals	2	0.25	2.87	>0.05
error	6	0.09		
Total	8			