Introduction
1. INTRODUCTION

Aquatic environment is continuously being contaminated with toxic chemicals by different sources like industries, pesticides, agricultural and domestic activities carrying different types of substances in different quantities. During the past few decades, rising trends of population explosion, development of modern technology, industrialization and modernized agricultural practices, have brought a dramatic increase in production and consumption of large variety of new synthetic chemicals and thereby high amount of pollutants were released into the aquatic environment.

Heavy metal pollution is the major problem in the aquatic environment because of the toxicity, persistence, tendency to accumulate in the organisms and undergo food chain amplification (Weis and Weis, 1977).

The effects of pollutants, both inorganic and organic, on the crustacean endocrine system represent a relatively unexplored one. The impact of heavy metals on endocrine - mediated events in crustaceans is the subject of a recent review (Fingerman et al., 1996). Weis (1987) reported that the effects of heavy metals on moulting and limb regeneration. They found that methyl-mercury and cadmium inhibit both processes in fiddler crab *Uca pugilator*, *U. pagnax*, and *U. minax*.

Heavy metal pollution and its impact on aquatic systems have been a matter of great concern in recent years. There is considerable
evidence that crustaceans, including *U. pugilator*. accumulate cadmium from both their environment and food (O'Hara, 1973; Jennings and Rainbow, 1979; Ahsanullah *et al*., 1984; Chou *et al*., 1987) but little is known about the impact of heavy metals on neurohormonally regulated physiological process in crustaceans. Weis (1976 and 1987) and co-workers (Weis *et al*., 1986) reported that mercury and cadmium exposure inhibits moulting hormone regulated process of *U. pugilator*. Reddy *et al*. (1994) reported cadmium exposure induced hyperglycemia in the crayfish, *Procambarus clarkii*.

Metal ions are serious pollutants especially in aquatic environment since they can be incorporated into food chains and concentrated by aquatic organisms to a level which affects their physiological state (Bryan, 1971). Physiological, histological and ultrastructural studies have been shown that heavy metal ions interfere with respiration and osmoregulation by disrupting the structure of the gill cells in fish and crustaceans (Eisler and Gardner, 1973; Jones, 1975).

It has been known for many years, the concentration of heavy metals is significantly higher in the marine biosphere (Waldichuk, 1974). Today additional quantities of heavy metals are being added to the estuarine and coastal regions from agriculture and industrial waste, hospitals, domestic sewage and from polluted, environment (Foyn, 1965; Ludwing and Storrs, 1970; Akefors, 1971; Frieberg *et al*., 1971; Nammalwar, 1984; Nammalwar *et al*., 1985). Some of the heavy metals are toxic even at
low doses to marine and estuarine organisms. Fish apparently can accumulate mercury and cadmium compounds more than other aquatic organisms either directly from sea water or indirectly through food chains (Bryan, 1976, 1979).

The impact of heavy metals on crustaceans is a matter of ongoing concern (Fingerman et al., 1996). The red swamp crayfish *P. clarkii*, has been extensively studied as bioindicator of the effects of pollutants and also because of its economic importance. Annually more than $10^7$ pounds of crayfish is being harvested and large proportion goes for human consumption (Hunter and Barr, 1991). The reports include studies of toxicity and accumulation effects on oxygen consumption, blood glucose, and enzyme activity (Mayans et al., 1986; Thorpe and Gloss, 1986; Ramo et al., 1987; Alikhan et al., 1990; Romero et al., 1990, Torreblanca et al., 1991, 1993; Martinez et al., 1993; Reddy and Fingerman, 1994; Reddy et al., 1994).

Contamination of the aquatic environment with cadmium is a matter of concern because this heavy metal can enter the food chain and as a result of bioaccumulation, cause serious health problems to human beings (Frieberg et al., 1973; Piscator, 1980). Large amounts of cadmium were used for electroplating and in the manufacture of pigments, plastic stabilizers and batteries (Eisler, 1985). Cadmium concentrations are highest near smelters and in urban industrialized areas (Hammos et al., 1978; Hutton, 1983).

Several studies indicated that the effects of cadmium on physiological and metabolic processes in crayfish (Mayans et al., 1986; Ramo

Cadmium has a deleterious effect on most, if not all marine species tested (Gardner and Yevich, 1969; Gardner and Yevich, 1970; Eisler et al., 1972; Eisler and Gardner, 1973; Newman and MacLean, 1974). Cadmium contamination in the food can be a major source of toxicity to man and indeed long-term exposures to cadmium in humans has been implicated as contributing to a painful and crippling disease called "itai-itai" (Eisler, 1971). Cadmium occurs in estuarine water and sediments after receipt of industrial wastes and this has resulted in cadmium accumulation in sea foods (Holmes et al., 1974).

Vernberg et al. (1977) investigated the sublethal concentrations of cadmium and their effects on the adult shrimp *Palaemonetes pugio* under static and tidal conditions. Adult shrimps were exposed to cadmium at 50 μg/l. The shrimp was highly tolerant to cadmium and after exposure to 23 mg/l the mortality rate was only 10%. The shrimps kept at lowest salinity level (5‰), the cadmium body burden reached 40 mg/kg, there was inhibition of moulting. An investigation on the effects of cadmium on respiratory rate was inconclusive because of considerable variation.
The effects of cadmium on three species of shrimps, *Penaeus duorarum*, *Palaemonetes pugio* and *P. vulgaris* was reported by Nimmo et al. (1977), and sublethal dose has histological effects and blackening and damage to gill filaments. The effect on the gill occurred with exposure to cadmium concentrations approaching the LC$_{50}$ which, with an exposure duration of 96 hr, was 0.76 mg/l for *P. vulgaris* and 3.5 mg/l for *P. duorarum*.

While, there is considerable evidence that crustaceans including the fiddler crab, *U. pugilator*, accumulate cadmium from both their environment and food (O'Hara, 1973; Jennings and Rainbow, 1979; Ahsanullah et al., 1984; Chou et al., 1987), little is known about the physiological and biochemical effects of sublethal concentrations of cadmium on crustaceans. Weis (1976) and Weis et al. (1986, 1987) were aimed at elucidating the effects of cadmium and showed that this heavy metal inhibits moulting and limb regeneration in the fiddler crab, *U. pugilator*.

The available information regarding bioaccumulation of heavy metals in aquatic organisms are scanty and contradictory. Studies on the accumulation of heavy metals in different organs of an organism are important in order to understand not only the extent of environmental pollution but also the role of metal ions in the metabolic processes.

Mercury is a systemic poison. The toxicity of mercury was known as early as 16th century as mercury has been used in medicine, agriculture and industries. Mercury released into the environment is converted into
methyl-mercury by anaerobic bacteria and then carried through food chains especially by fish to human beings (Clarkson, 1997).

Mercury uptake was faster in organic form than inorganic form though both were initially linear (Boudou and Ribeyre, 1984). All the organisms readily accumulate phenyl-mercury acetate (PMA), however, the uptake was related to the length of exposure and concentration with the absorbed PMA largely converted into inorganic mercury (Backstrom, 1969). The uptake nature of organic mercury both alkyl and aryl in an amphipod was approximately three times more than inorganic form (Zubarik and O’Connor, 1977).

Victor et al. (1986) reported the toxicity of mercury, cadmium on oocyte differentiation in the vitellogenesis of teleost Lepidosophichthys thermalis. This exposure causes several lesions in the ovary. There was also impairment of vitellogenesis in the cadmium treated fishes. Mercury produced more lytic changes in the oolemma.

Higher concentration of mercury appear to occur in the crustaceans either when they pass large quantities of water through their respiratory surfaces or bioaccumulated through food chain. There is considerable evidence to indicate that mercury can cause structural damage to gill epithelia and kidney tubules. In 1985, Sarojini and Victor reported toxicity of mercury on the ovaries of the Caridian prawn, C. rajadhari. In this species they have reported changes in the colour of the ovary, swelling of oocytes, increased vacuolization, degeneration of oolemma and ovarian
stroma. Subsequently oocytes lost their characteristic shape. The degenerative changes of oocytes have become more generalised after 20 days exposure with fusion of adjacent oocytes and extensive necrosis. The process of degeneration advanced with more nuclear pycnosis and cytolysis of oocytes after 30 days of exposure. Meanwhile degenerated ovary is hypertrophied with haemocytes.

Mercury pollution in the aquatic environment has been reported from many parts of the world and higher concentration of mercury have been found in many species of fishes (Miettinen et al., 1972). If concentration of mercury exceeds a permissible limit i.e. 0.01 mg/l (Central Board for the preventive and pollution, India) causes toxic effect. Elevated mercury concentration in fish have been reported in areas remote from direct sources of mercury contamination (Spry and Wiener, 1991; Wiener and Spry, 1996). The mercury concentration in fishes frequently exceeded limits of maximum concentration for human consumption (Ome and Omnr, 1988). Backstrom (1969) found that the uptake by fish of various forms of mercury compounds, the methyl mercury is rapidly absorbed compared with phenyl-mercury and inorganic mercury, but the difference in the uptake between methyl mercury and other mercury compounds were less pronounced.

The major objectives of aquatic toxicological studies in the laboratory was to identify the mechanism of toxicity and to predict 'safe' contaminant concentrations in the environment (Johnson and Bargman,
Acute toxicity bioassay is the first step of such probe in the aquatic toxicology (Sprague, 1970). The toxicology of heavy metals to a particular organism is usually expressed in terms of LD$_{50}$ (Lethal Dose). This value represents the amount of poison per unit weight which will kill 50% of the particular population of the animal species employed for the tests and this in turn represents the median tolerance limit (TLm) (Finney, 1971). The relationship between the dose of a compound and its toxicity is the central theme in toxicology. Since the selected test animal's aquatic lethality is expressed in terms of lethal concentration (LC$_{50}$) and the poison used in water is expressed as parts per million (ppm).

The term bioaccumulation indicates that organisms take-up chemical to a greater concentration than that is found in their environment or their food. Heavy metals are known pollutants which inflict disorders in aquatic ecosystem (Kaviraj and Konar, 1982) and their accumulated concentration are significantly higher in the aquatic biosphere (Waldichuk, 1974). The capacity of bioaccumulation of potentially toxic trace metals in the tissue of organism in excess of ambient levels are well known (Kureishy et al., 1981; Patel et al., 1985) and thus the excess level tend to cause deleterious effect on organism. Heavy metal uptake and concentration in the food chains, especially those terminating in the human beings have renewed interest largely due to several instances of human intoxication (Frieberg et al., 1971). Further, it may be difficult to generalise the uptake and accumulation of metals in aquatic organisms (Lyla and Ajmalkhan, 1996; Barber and Sharma, 1998).
The distribution of metal compound among the various organ system is dependent on its transport across the cell membranes from the interstitial fluid as well as on the varying affinities and inherit in the cell membrane. A number of factors such as the metal concentration, the speciation of metals in water, the pH of water (Holdson et al., 1978), period of exposure, volume of water, rate of flow, temperature, salinity (Hutchinson, 1974) and dissolved oxygen and activity of the animal (Doudoroff and Katz, 1953) are found responsible for the accumulation and modulation of the metal concentration in animal tissues (Everall et al., 1989; Sayer et al., 1989). The heavy metals being non-biodegradable, primarily necessitate knowledge on their uptake, distribution and persistence in tissues. The ratio between bioaccumulation and exposure concentration with periods of exposure has been shown in a number of studies (Pragatheeswaran, 1987; Giles, 1988; Sayer et al., 1989; Vincent and Ambrose, 1994; Barber and Sharma, 1998).

Urbanization and industrialization cause serious deterioration of aquatic environment and pose threat to the aquatic life (Kimura, 1988; Chua et al., 1989). Accumulation of industrial effluents in water bodies have become a major concern (FAO, 1986). Mercury forms one of the important components in these effluents and cause serious damage to the life systems (Moore and Ramamoorthy, 1983).

Use of biological indicator organisms to define areas of heavy metal pollution appears more significant in recent years (Kumaraguru et al.,
1992). Mercury was found to be taken up directly (no food chain required) from the water by gold fish (Kim et al., 1977). Nammalwar (1985) reported the bioaccumulation of mercury and cadmium in different tissues of *Liza macrolepis* from polluted water. Ribeyre and Boudou (1984) examined uptake of mercury over time into specific organs of rainbow trout. Barber and Sharma (1998) observed cadmium residue in different tissues of *Labio rohita*, *Channa punctatus* and *L. reticulatus* after subacute exposure. Rao et al. (1998) reported the uptake of zinc, lead and cadmium in different organs of *Macrobrachium rufae* inhabiting in a stream polluted by industrial effluent.

Thaker and Haritos (1989) reported that cadmium exposure affects enzyme activity in the hepatopancreas of a shrimp, *Callianassa tyrhenena* and also producing *in vivo*, increase in glutathione-S-transferase activity and esterase activity. Meyer et al. (1991) found in another species of crayfish *Astacus astacus*, that after cadmium or lead exposure, the activity of three enzymes in the gills and hepatopancreas, namely succinic dehydrogenase, NADPH - cytochrome P450 reductase, glutathione S-transferase, decrease. Later, Torreblanca et al. (1991) using *P. clarkii* demonstrated that cadmium exposure affects the chemical composition of hepatopancreas, gills, and muscles. Torreblanca et al. (1992), using *P. clarkii*, found that mercury exposure produces composition changes similar to those produced by cadmium.
The glycolytic enzyme lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate with the concomitant oxidation of NADH. Lactate is the end product of anaerobic carbohydrate metabolism in crustaceans. Fingerman et al. (1996) reported the effects of heavy metals on the biochemistry and physiology in *U. pugilator* and also investigated the effects of enzymes involved in anaerobic carbohydrate metabolism in crustaceans.

The heavy metal cadmium and aromatic hydrocarbon naphthalene have different modes of action on colour changes and blood glucose levels in the fiddler crab, *U. pugilator* (Reddy et al., 1996). Both of these pollutants exert similar physiological effects on colour changes preventing black pigment dispersion and the blood glucose concentration (producing hyperglycemia), but their actions on the neuroendocrine system was different. Naphthalene inhibits release of the neurohormones that regulate these phenomena, whereas cadmium inhibits synthesis of these neurohormones. Naqvi and Howell (1993), using *P. clarkii*, reported that cadmium exposure reduced fecundity and hatching success.

The use of histopathological techniques is a promising area of research in aquatic toxicology as it gives the real picture of the effects imposed and the involvement of the heavy metals in either disturbing or destroying the vital organs of living organism. Histopathological studies were also useful in evaluating the pollution potential of pesticides, since trace amount of these chemicals which do not bring animal mortality over
a given period, were capable of producing considerable organ damage (Kumar and Pant, 1984). Several workers have reported that degenerative changes in selected tissues in response to pollution by various toxicants (Eller, 1971; Bhattacharya et al., 1975; Anees, 1978; Dubale and Shah, 1979; Goel and Garg, 1980; Ram and Sathyanesan, 1987; Banerjee and Bhattacharya, 1997). Despite voluminous information available on the histopathological changes caused by the heavy metals, the mode of action on the vital organs is still not fully understood.

From the above literature review, it is evident that the information regarding toxicity on brackish water crabs are rare, particularly on the study related to heavy metals such as cadmium and mercury. Hence this study was done.

The study includes:

1. Morphology and histological studies of female reproductive system of *U. annulipes*.

2. Assessment of LC$_{50}$ value of cadmium and mercury.

3. Effects of cadmium and mercury on histopathological changes in ovary, spermatheca, hepatopancreas, brain and thoracic ganglia.

4. Effects of cadmium and mercury on the quantitative biochemical changes of proteins, carbohydrates and lipids.
5. Effects of cadmium and mercury on the LDH activity in the ovary, spermatheca, hepatopancreas, muscle, gill and haemolymph.

6. Effects of cadmium and mercury on the SDH activity in the ovary, spermatheca, hepatopancreas, muscle, gill and haemolymph.

7. Effects of cadmium and mercury on the acid and alkaline phosphatase activity in the ovary, spermatheca, hepatopancreas, muscle, gill and haemolymph.

8. Bioaccumulation of cadmium and mercury in the ovary, spermatheca, hepatopancreas, muscle, gill and haemolymph.