ECOTOXICOLOGICAL STUDIES ON KUDUVAIYAR ESTUARY, SOUTHEAST COAST OF INDIA

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By

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CERTIFICATE

This is to certify that the thesis entitled **"ECOTOXICOLOGICAL STUDIES ON KUDUVAIYAR ESTUARY, SOUTHEAST COAST OF INDIA"** submitted to Bharathidasan University, Tiruchirappalli by **Mrs. B. GIJILET MARY (Reg. NO. BDU1910360068),** in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY IN ZOOLOGY**, is a record of research work done by her during the period of study under my guidance and supervision. I further certify that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title.

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I B. GIJILET MARY do hereby declare that this thesis entitled **"ECOTOXICOLOGICAL STUDIES ON KUDUVAIYAR ESTUARY, SOUTHEAST COAST OF INDIA"** is a genuine record of research work done by me under the scientific supervision of **Dr. M. SUKUMARAN,** Assistant Professor, Rajah Serfoji Govt. College (Autonomous), Thanjavur and has not previously formed the basis for the award of any Degree, Diploma, in associateship in any university.

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ABBREVIATIONS

ANOVA	Analysis of variance
BOD	Biological Oxygen Demand
CAT	Catalase
$CdCl_2$	Cadmium Chloride
COD	Chemical Oxygen Demand
DMRT	Duncan's Multiple Range Test
GPx	Glutathione Peroxidases
GR	Glutathione Reductase
GSH	Reduced Glutathione
GST	Glutathione S-Transferase
Hb	Haemoglobin
LC	Lethal Concentration
LCM	Lower Confidence Limits
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MDA	Malondialdehyde
PCV	Packed Cell Volume
r^2	R squared
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
UCM	Upper Confidence Limits

CHAPTER-I

INTRODUCTION

CHAPTER I

1. INTRODUCTION

India has a long coastline of 8,129 km and of this 6,000 km is rich in estuaries, creeks, brackish water, lagoons and lakes. The southeast coast of India is an important stretch of coastline, where many major rivers drain into the Bay of Bengal and they are also richer in marine fauna than the western coast of India. The Chennai coastal region is a distinctive example for numerous recreational and commercial activities that not only degrade the quality of coastal water but also pose a serious health hazard to marine biota and man (Tran *et al.,* 2002; Beiras *et al.,* 2003; Palanisamy *et al.,* 2006).

The coastal environment represents an eventual depository of wastewater discharges. Although the dynamic nature of marine environment allows rapid assimilation of such wastes via different processes (e.g. dilution, dispersal, oxidation, degradation, or sequestration into sediments), the capacity of other aquatic environment like lagoons to assimilation is restricted (Natesan and Seshan, 2010). In spite of the fact that metals occur in the ecosystem naturally by geogenic and lithogenic processes, the heavy metals of anthropogenic origin tend to be bioavailable and then toxic pollutants (Kaasalainen and Yli-Halla, 2003; Wuana and Okieimen, 2011; Abu Khatita *et al*., 2016). When entering the aquatic environment, a significant amount of such toxic elements could become directly or indirectly in contact with humans through food webs. In specific concentration, certain heavy metals are nutritionally essential for a healthy life.

Estuarine areas are complex and highly changing environments at the interface between freshwater and marine aquatic ecosystems. Despite their environmental variability, estuaries are usually characterized by high biomass due to their strong primary production, especially when compared with marine areas. These conditions enhance the development of heterotrophic populations such as filtering molluscs. Moreover, estuaries are of special interest in ecotoxicology because they are potentially the most exposed coastal areas regarding any source of pollution (Sun *et al*., 2012). In order to perform ecotoxicological studies, sampling strategy has to take into account the whole complexity of the estuarine ecosystem. Many environmental variables such as temperature, salinity, or suspended matter may have a great influence on physiological processes in invertebrate species.

1.1. Estuarine pollution

Our Earth is a blue planet. Water covers about seven-tenths of its surface, but most of this is salty sea water. Only 3% of Earth's water is freshwater and most of this freshwater is accessible as aquatic habitats such as rivers, lakes, ponds, dams (Bhandari, 2003). Freshwater is an estuary's lifeblood. Estuaries are semi-enclosed aquatic habitats, which have a free connection with the open sea and within which sea water is measurably diluted with freshwater from Land drainage (Pritchard, 1967).

Estuarine pollution may be defined as the human introduction to an estuary of any substances such as toxic chemicals (Heavy metals, pesticides, petroleum hydrocarbons, organotins, oil spills) and waste products that are

hazardous or potentially harmful to the estuarine ecosystem. This includes pollutants that are directly toxic to plants and animals, as well as materials that overload the estuaries capacity to assimilate wastes and thus deplete essential oxygen. Most of the estuaries themselves are bordered by dense population and industrial development that add significantly to the water pollution load.

Pollution of our aquatic bodies is among the greatest threats to our environmental health. Rivers are direct recipients of industrial wastes and municipal sewage. They also drain urban, suburban and rural areas where they collect more pollutants and litter. As they move towards the coast, they gather or join the waters of other streams. They eventually reach the sea usually via an estuary, the area where fresh and saltwater mingle. In addition to toxic chemicals and other pollutants and an abundance of oxygen-depleting organic matter, dense population are another problem for creates sewage.

The wedge of salt water characteristically underlying the layer of freshwater in an estuary tends to move sediments in an unusual way. As the denser salt water moves along the bottom toward the head of estuaries to replace water carried away freshwater surface flow, it takes with it some of the nutrients the river has brought downstream, thus dispersing them over a wide area in the estuary similarly, if the river is polluted, contaminants can be carried downstream in the fresh or brackish water surface layer, then back toward the head of the estuary after they settle out and into the deeper salt water. Valuable nutrient bearing sediments are also capable of carrying and dispersing a variety of dangerous pollutants, including pesticides and heavy

metals, such estuarine organisms are known to store and concentrate in their body tissues (Morrisey *et al*., 2003).

1.2. Heavy metal pollution in estuaries

Estuaries are shallow open systems influenced by river inflows, mixing with coastal ocean and exchanges across the sediment and atmosphere water interfaces. Estuaries are vital links in the life histories of more than two thirds of all our commercially important crab and mussels, most of which are filter feeding animals. Toxic substances commonly found in estuaries are heavy metals and insecticides. Some heavy metals such as mercury make their way to the plant's leaves where they either directly return to the water or enter the food chain, when plant-eating organisms consume the leaves or leaf particles called detritus. Heavy metal contamination leads to bioaccumulation of the food chain and food webs of estuaries. Normally such contaminants are transported from its sources through the river system and deposited downstream (Fang *et al*., 2001).

Since most of the metals could be mixed along with suspended solid in water and bottom sediment through sedimentation, the estuary is a potential sink for these pollutants for a long period of time. The presence of heavy metals in sediments can lead to greater environmental problems, when contaminated sediments are suspended and such metals are taken up by filter feeder crabs. Hence, consumption of such a kind of crab meat may form a significant pathway to metal contamination in the human being and eventually poses greater health risk (Abdullah *et al*., 2007).

One aspect of ecotoxicology is to find out how toxic metals vary over space and time in and between aquatic environments such as estuarine and marine environments. Biologists including Eco toxicologists have explained about this aspect and they have attempted to measure dissolved and / or sediment concentrations. There are three major categories of metal toxicity measured in attempts to compare differences in metal pollution in aquatic habitats over space and /or over time metal concentration in water, sediment and more recently in organisms such as crustaceans, clams and mussels (Rainbow, 2006).

Dissolved concentrations of trace metals usually vary over time, particularly for example in estuaries with differentiated inputs of river and sea at different periods of tides and differential freshwater inflows reasonably (Rainbow, 2002). The physico-chemistry of the medium (water) will affect the rate of uptake of a metal from solution by living organisms such as barnacles, amphipods, clams, mussels etc., in short, the bioavailability of metals in different aquatic habitats may vary even when their total dissolved concentrations are identical for example, the uptake rates of Zn and Cd by biota from solution typically increase will decrease salinity over salinity range found in an estuary (Rainbow, 1995a).

The second common measure of metals in aquatic habitats is their concentration in the local sediments. The concentration of metals in the sediments represents metals which have been accumulated over a period of time and any measurement made does have the same potential, as dissolved

concentrations to vary over short time periods. The physico-chemical characteristics of sediments greatly affect their ability to accumulate heavy metals, so that different sediments will reach different concentrations from identical dissolved sources of metals (Bryan, 1976).

Sediments high in organic carbon content will bind more metals than those with low organic carbon and sediments with particles of high surface area will also accumulate more metals than particles of low surface area. Thus muds (small particle size, high organic content) accumulate more metals than sands (large particle size, low organic content) sediments can also be a source of trophic metals in sediment ingesting animals like crab and the trophic availability of metals in ingested sediments will also vary with the physico-chemical characteristics of the sediments (Rainbow, 2007).

Biomonitors are the organisms which accumulate metals in their body organs or tissues, the accumulated metal concentration of which may be analysed to provide a relative measure of total amount of metal taken up by all routes by that organism (Phillips and Rainbow, 1994 and Abdullah *et al*., 2007). Aquatic organisms differ in their patterns of accumulation in different types of metals. Amphipods, crustaceans, barnacles, oyster, clams, polychaetes, and mussels are good examples of biomonitors of metal pollution (Al-Madfa *et al*., 1998; Abd Allah and Moustafa, 2002 and Otchere *et al*., 2003). The accumulated metal concentration of an aquatic invertebrate results from metal taken up from water or sediment and food, after subtraction of any metal that has been excreted (Szefer *et al*., 1990). As filter feeders, crabs

accumulate heavy metals in their tissues in proportion to the degree of estuarine environmental contamination. The relatively small increase in ambient metal concentration due to pollution will be reflected in measurable amounts then tissues. Therefore, they can be used as indicators of metal pollution in the estuarine environment.

1.3. Physiography of Kuduvaiyar estuary

Kuduvaiyar estuary is one of the estuaries in Tamil Nadu formed by one of the channel of Cauvery river, situated in Nagapattinam, a coastal district of Tamil Nadu , which is at Lat. 10° 76’ N, long 79° 84’ E/ 80 Km/away from Thanjavur. The river Cauvery rises at Talacauvery in Brahmagiri Range near Cherangala village of Kodagu (Coorg) district of Karnataka. The river Cauvery flows through Karnataka and Tamil Nadu states. The river branches into canals at Grand Anicut in Thanjavur district. One of the canals namely Vennar further divides into five channels. These are Paminiar, Koraiyar, Kilithangiyar, Maracakorayar and Valavanar. Pamaniyar and Koraiyar are joins together and extends for about 7 Km distance and joins Kuduvaiyar estuary, finally discharges their waters into the Bay of Bengal. The Kuduvaiyar estuary has a year-round connection with the sea and is subjected to sediment tides with maximum tidal amplitude of about 1m. The width of the estuary at the mouth is about 85m and the tidal flushing area extends to a distance of about 10km (Rajalakshmi and Sukumaran, 2020).

Nagapattinam district is bounded in the North by Karaikal in Puducherry state and Thiruvarur district, in the West and in the South and East by Bay of Bengal. Nagapattinam lies between Northern Latitude 10.7906 degrees and 79.8428 degrees East longitude. The district spreads over an area of 1205 sq.km.

Sandy beaches are vital coastal systems that occupy a significant part of the coastline. They are habitat for many inter-tidal flora and fauna and also are critical nesting habitats for marine turtles. About 41 river-estuarine systems have been recorded along this coastline with perennial and nonperennial flow. Among them, Adayar, Vellar, Coleroon, **Kuduvaiyar**, Agniyar, Tamirabarani, Pazhayar (Manakudy estuary) and Thengaipatinam are the major drainage systems have an estuarine characteristics with regular freshwater flow and tidal flushing (Mathur *et al*., 2014).

Such estuaries are endowed with special types of regulation and thereby contribute towards richness of the soil by organic matter. Point Calimere, located in the Coromandel Coast, is one among the 26 Ramsar sites in India. Ramsar sites are wetlands of international importance designated under the Ramsar convention. The Kuduvaiyar mangrove form a part of the Point Calimere Ramsar site and constitute the largest mangrove forest in Tamil Nadu state. The mangrove ecosystem consists of a shallow estuary, which is fed by the tributaries of Cauvery River. The freshwater discharge is controlled by barrage operations and there is no freshwater flow into the estuary for about 60% to 70% of the period of the year. The estuary has undergone some

geomorphic changes in the past twenty years. There has been a reduction in the average depth of water and the width of the mouth has reduced (Priya *et al*., 2014).

1.4. Ecological environment

The coastal tidal forest or mangrove ecosystem has a special association of plants and animals that develop in the intertidal zones of tropical coasts. The Kuduvaiyar estuary receives freshwater from the tributaries of river Cauvery namely Korayar and Paminiyar rivers; the inflows from other rivers such as Kilathingyar, Maraikakorayar, and Kandankurichiyar are insignificant. The fauna and flora are adapted to fluctuations in environmental conditions like water, salinity, temperature and nutrients and interact with the flow of freshwater as well as sea water.

Aquatic ecosystems are progressively coming under permanent pressure of anthropogenic pollutants and heavy metal contamination of aquatic ecosystems is a worldwide problem posing health hazards not only to the inhabitant organisms but also to the non-target populations including human beings through food chains and food webs (Jayakumar *et al.*, 2008). The aquatic environment is highly fragile, complex and diverse. It includes several distinct ecosystem types such as freshwater streams, lakes, ponds and rivers, estuaries, marine coastal and deep ocean waters that encompass many different biotic and abiotic components of unique characteristics. Coastal seas are characterized by highly productive and economically valuable ecosystems as

well as intense human activities capable of interfering with the system function and properties (Brooks, 1978).

Pollution is one of the challenging problems for environmental biologists, as hundreds of varieties of chemicals with potential harmful effects on biological organisms including human beings. These chemicals are being manufactured in large quantities and used for various purposes (Kavitha and Jagadeesan, 2003). Aquatic toxicology has been defined as the study of the effects of chemicals and other toxic agents on aquatic organisms with special emphasis on adverse or harmful effects. A toxicant is an agent that can produce an adverse response (effect) in a biological system, seriously damaging its structure or function or resulting in death. A toxicant or foreign substance (i.e., xenobiotic) may be introduced deliberately or accidentally into the aquatic ecosystem, impairing the quality of the water and making it unfavorable for aquatic life. Aquatic pollution means the introduction by humans, directly or indirectly, of substances or energy into the aquatic environment resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to aquatic activities including fishing, impairment of quality for use of water, and reduction of amenities modified from marine pollution (GESAMP,1993).

Among the various kinds of pollution, heavy metal pollution seems to be the most persistent one (Harvey *et al*., 1974). Heavy metals such as lead, mercury, copper and cadmium etc. have known for many years to produce toxic effects on the central nervous system (Margarat and Jagadeesan, 1999).

Their presence in the environment and at working place may pose various health hazards (Rao, 1997).

The contamination of the environment by toxic substances is linked both to industrialization and to agriculture. Pollution of aquatic environments originates from atmospheric input, land runoff and seepage through land as in the case of groundwater. A wide variety of pollutants namely, physical, chemical, biological and radiological have been identified as detrimental to human health and they can affect other living organisms in the ecosystems. Pollutants like pesticides, heavy metals, hydro-carbons are generated exclusively by man and so they are called anthropogenic substances. These substances when present in high concentration become toxic to the ecosystems (Pillai, 1985).

Metals are non-biodegradable and are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (More *et al*., 2003; Javed, 2003; Lewis *and Cohen*, 2004). Aquatic organisms have the ability to accumulate heavy metals from various sources including sediments, soil erosion and runoff, air depositions of dust and aerosol, and discharges of wastewater (Labonne *et al*., 2001; Goodwin *et al*., 2003). Therefore, accumulation of heavy metals in aquatic organisms can pose a long lasting effect on biogeochemical cycling in the ecosphere.

Heavy metals are being passed on into aqueous environments through industrial processes, sewage disposal, soil leaching and rainfall. The concentrations of these heavy metals are sub-lethal or lethal to aquatic

organisms when the duration of exposure to these metals are prolonged (Eisler and Gardener, 1973). It is well documented that effects of heavy metals are dependent upon the physical and chemical conditions of the environment, especially water salinity, hardness, pH and dissolved oxygen and can act synergistically. Freshwater is highly vulnerable to pollution, since they act as an immediate sink for the consequence of human activities always associated with danger of accidental discharge or criminal negligence (Vutukuru, 2005). Heavy metals form a dangerous group of potentially hazardous pollutants and additional concentrations of these metals accumulate in the aquatic ecosystem as a result of land based activities (Magar and Waghmare, 2010).

Urbanization and industrialization have resulted in the environmental degradation due to chemical and solid industrial wastes (heavy metal). One of the important problems therefore faced by industries is the treatment and disposal of such wastes. The aquatic bodies are exposed to high incidence of toxicity due to heavy metal contamination (Dhakad *et al*., 1997). Industrial processes involving the use of water are invariably, the essential source of pollution of metals. Fossil fuel combustion and cement production contribute to significant mobilization of metals. Electroplating processes and thermal power stations also generate large volumes of liquid waste containing metals. Domestic sewage is yet another source in the urban regions of the riverine system. The use of metal containing pesticides in agriculture and leaded petrol in motor vehicles results in atmospheric pollution by mercury and lead respectively. The fertilizers, fungicides and sludge release copper, zinc and

cadmium. Oil and coal particularly from some localities contain significant quantities of heavy metal. Oil is rich in vanadium, nickel, molybdenum and mercury (Nair, 1983).

The elements with atomic number greater than 20 are termed heavy metals excluding alkali metals, alkaline earth elements, lanthanides and actinides (Kopp and Kroner, 1972; Wolfe and Rice, 1972). Of all the elements present on the earth's crust H, C, N, O, Na, Mg, P, S, Cl and K constitute 99.9% of all living matter. In addition 14 elements viz B, F, Si, V, Cr, Mn, Zn, Fe, Co, Cu, Se, Mo, Sn and I are the essential trace elements including many heavy metals. Rest of the elements including heavy metals such as Hg, Pb, Cd, As, Cr not essential for the growth and development of organisms or their function in biological systems have not been established (Brooks, 1978).

The essential heavy metals have a specific role of play in living systems. Essential trace elements that exist naturally at background levels in the environment include chromium, cobalt, copper, iron, manganese, molybdenum, vanadium, strontium and zinc. However, non-essential heavy metals such as mercury, cadmium, arsenic, thallium and lead are toxic and tend to accumulate in living organisms (Wepener *et al*., 2001; Pourang *et al*., 2005; Ramakritinan *et al*., 2005; Kongchum *et al*., 2006; Ip *et al*., 2007; Shanthi and Gajendran, 2009).

Aquatic sediments act as a sink as well as a source for contaminants. Long-term addition of contaminants can lead to sedimental concentrations that can surpass water concentrations (Ridgway and Shimmield, 2002; Zaggia *et*

al., 2007; Zhang *et al*., 2006; Morillo *et al*., 2008). Fluxes of trace elements in estuaries and coastal waters are transported to the open ocean and the original composition of seawater is altered (Horsfall and Spiff, 2002; Muthuraj and Jayaprakash, 2007). Sediment associated metals pose a direct risk to detritus and deposit feeding benthic organisms, and may also represent long-term source of contamination to higher trophic levels (Mucha *et al*., 2005; Morillo *et al*., 2008; Twining *et al*., 2008).

Cadmium is ubiquitous and a potent environmental pollutant that can be found in the aquatic environment through anthropogenic sources. Cadmium is toxic to crab even at low concentrations (Yilmaz *et al*., 2004; Jarup and Akesson, 2009). Due to its long biological half-life and strong ability to accumulate in animal tissues, residual cadmium form a serious threat to the performance and survival of aquatic biota (Jones *et al*., 2001; Rashed, 2001a; Yilmaz *et al*., 2004; El-Naga *et al*., 2005; Seebaugh *et al*., 2005).

Zinc is the fourth most widely used metal in the world. Its major uses include galvanized steel for alloy production, and as an ingredient in rubber and paints. More concentrated sources of zinc to aquatic environments include urban runoff, mine drainage, municipal wastes and industrial effluents including zinc smelting, plastics and electroplating. Erosion of soil particles containing zinc is the overall source of zinc to the aquatic environment (USEPA, 1991a).

Copper is released from natural sources such as volcanoes, windblown dusts, decaying vegetation and forest fires. It is also used as a fungicide,

catalyst for organic reactions, pigment for ceramics, insecticides and preservative for cellulose materials (Khangarot and Rathore, 2003). Copper is generally used in the manufacture of chemicals, electric wires, cement, lime, plaster and concrete products, transport equipment, iron and steel products, petroleum and coal products (Baeyens, 1998). Copper enters the aquatic environment chiefly through leaching from paints on the hulls of boats and ships (Grosell *et al*., 2007; Singh and Turner, 2009). Due to persistence in the environment and tendency to accumulate in the biota, copper poses a potential hazard to environmental and human health. It is the most poisonous heavy metal when present in excess (Welsh *et al*., 2000; Grosell *et al*., 2002; De Boeck *et al*., 2006).

Lead is a naturally occurring non-essential heavy metal present in the earth's crust, rock, soil, and water. Sources of lead in marine environments include natural sources from rock weathering, riverbank and coastal erosion, and anthropogenic sources from urban and industrial emissions (Komarek *et al*., 2008; Kelly *et al*., 2009). Most waterborne lead is derived from mining and smelting, coal burning and cement manufacturing (WHO, 1995).

The most basic property of heavy metal is that they are bioavailable and are indestructible having toxic effects on living organisms when they exceed a certain concentration limit (MacFarlane and Burchett, 2000; Ridgway and Shimmield, 2002). Tolerance to heavy metals in metal accumulating organisms is linked to their ability to bind incoming metals, thereby controlling their intracellular availability. The binding of inappropriate metals

to metal-sensitive sites such as mitochondria is often interpreted as a failure of detoxification mechanisms and could be an indicator of metal-induced stress (Wallace *et al*., 2003; Ballesteros *et al*., 2009). The measurement of cellular and subcellular responses to chemical contaminants in sentinel organisms are used as bio-indicators from aquatic environments allowing early detection of biological effects as well as assessment of the extent of contamination of pollutants (Oost *et al*., 2003; Scott and Sloman, 2004).

Heavy metals deplete glutathione and protein bound sulfhydryl groups, resulting in enhanced production of Reactive Oxygen Species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radicals (Liu *et al*., 2009). The sequential reduction of oxygen leads to generation of superoxide anion and hydrogen peroxide (Monferran *et al*., 2008). Superoxide anion also rapidly reacts with nitric oxide, yielding yet another reactive species peroxynitrite. All of these ROS have the potential to trigger cellular death (Vieira *et al*., 2009). ROS are considered as crucial mediators for the metal-triggered tissue injuries and apoptosis (Xue *et al*., 2005; Gaitonde *et al*., 2006; Yokouchi *et al*., 2007; Tagawa *et al*., 2008; Liu *et al*., 2009).

To prevent oxidation induced damage, there must be effective antioxidant systems in the organisms. Some components of these systems involve reduced glutathione (GSH) and certain antioxidant enzymes including free radical scavenging enzymes, such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidases (GPx) and Glutathione Reductase (GR). Other associated enzymes are the Glyoxalase I (GI), Glyoxalase II (GII)

and Glutathione S-Transferase (GST). Under oxidative stress conditions, ROS can be reduced by GSH, with the related formation of the oxidized disulphide, oxidized glutathione (GSSG) (Hochachka and Lutz, 2001; Lushchak and Bagnyukova, 2007). Oxidative stress is also of ecological significance, particularly in the aquatic environment, which provides a sink for many pollutants that are capable of causing oxidative stress (Oliveira *et al*., 2010). Changes in the activity of enzymes and other biomarkers are the possible tools for aquatic toxicological research (Arellano *et al*., 2000).

1.5. Antioxidant defense

The continuous interaction of the animal physiological systems with free radicals, generated either indigenously or inhaled/ingested from exogenous sources leads to excess load of free radicals and causes cumulative damage of protein, lipid, DNA, carbohydrates and membrane, resulting in oxidative stress. Therefore, living creatures have evolved a highly complicated defense system with antioxidants, composed of enzymes and vitamins, against oxidative stress.

These defense systems are mainly classified as

(i) suppression of generation of ROS, (ii) scavenging of ROS, (iii) clearance, repairing and reconstitution of damage, and (iv) induction of antioxidant proteins and enzymes (Veena *et al*., 2007).

Antioxidants are substances that either directly or indirectly protects cells against the adverse effects of xenobiotics, drugs, carcinogens and toxic radical reactions (Janani *et al*., 2010). An antioxidant is a molecule capable of

slowing or preventing the oxidation of other molecules. In a biological system, they protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants terminate the chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. They are believed to play a role in preventing the development of chronic diseases like cancer, heart disease, stroke, AD, RA and cataracts (Chakraborty *et al*., 2009).

1.6. Enzymic antioxidants

Superoxide Dismutase (SOD)

SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense. SOD plays an important role in protecting cells against ROS by lowering the steady state level of $O_2^{\bullet -}$. In general, there are three types of SOD, containing Mn, Fe or Cu plus Zn as prosthetic metals (Corpas *et al*., 2006). SOD converts superoxide radicals to H_2O_2 and molecular oxygen, which, in turn, can be counteracted by catalase or GPx reactions, thereby reducing the level of cellular damage (Subash and Subramanian, 2009). SOD is widely distributed to protect such cells against the toxic effects of superoxide anion (Rao *et al*., 2009).

The enzyme SOD is considered the first-line defense because it catalyzes the first reaction in the system harvesting oxygen-free radicals. Therefore, SOD prevents the oxidation of biological molecules, performed by the same radicals or their derivatives (Sanchez-Venegas *et al*., 2009).

Catalase (CAT)

Catalase is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red blood cells and in the liver. CAT decomposes H_2O_2 and protects the tissue from highly toxic $OH^{\bullet}$. Therefore, the reduction in the activity of these enzymes may result in an accumulation of $O_2^{\bullet-}$ radicals and H_2O_2 (Deepak *et al*., 2007). Hence, catalase has been considered an important regulator of oxidative stress (Souza *et al.*, 2008).

Catalase, localized in peroxisomes, has two enzymatic activities, depending on the concentration of H_2O_2. If the concentration of H_2O_2 is high, catalase acts catalytically, that is, it removes H_2O_2 by forming H_2O and O_2 (catalytic reaction). However, at a low concentration of H_2O_2 and in the presence of a suitable hydrogen donor, eg., ethanol, methanol, phenol and others, catalase acts peroxidically, removing H_2O_2, but oxidizing its substrate (Turkseven *et al.*, 2005).

Glutathione peroxidase (GPx)

GPx is a seleno enzyme, two thirds of which (in liver) is present in the cytosol and one third in the mitochondria. It catalyzes the reaction of hydroxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of H_2O_2. Catalase and glutathione peroxidase activities were found to be decreased in the arthritic condition (Seufi *et al.*, 2009). GPx scavenges the highly reactive lipid hydroperoxide in the aqueous phase of cell membranes. Glutathione peroxidase catalyzes the reduction of

hydroperoxides, with glutathione (GSH) being oxidized to glutathione disulfide (GSSG). The latter is converted to glutathione by glutathione reductase in the presence of NADPH (Ayene *et al.,* 2008). Glutathione peroxidase reduces hydrogen peroxide to water, along with that the oxidation of GSH (Shieh *et al*., 2010).

Glutathione S-Transferase (GST)

GST comprises a multigene family of proteins involved in the metabolism of many disease-causing electrophonic substrates that protects the cells against oxidative stress, and is also useful in monitoring cellular induction (Sarkar *et al.,* 2010). It consists of a large family of GSH-utilizing enzymes that play an important role in the detoxification of xenobiotics in mammalian systems (Sohini and Rana, 2007; Tabrez and Ahmad, 2011).

GSTs are suspected to have an important protective function (Polit *et al.,* 2010). The GSTs are a supergene family of dimeric enzymes that catalyze the conjugation of GSH to a variety of electrophiles, including arene oxides, unsaturated carbonyls, organic halides and other substrates (eg., by-products of ROS activity). These enzymes are ubiquitously present in living organisms. A number of chemical agents, including some antioxidants, have cancer chemopreventive properties that can induce one or more GST isozymes (Bickers and Athar, 2006).

1.7. Non-enzymatic antioxidants

Reduced glutathione

Glutathione (GSH), a tripeptide, is the most abundant intracellular free thiol and an important antioxidant (Liu and Pravia, 2010). It is believed to scavenge ROS directly or act as a substrate for other antioxidative or repair enzymes (Gao *et al.*, 2009). This most abundant, non-protein antioxidant in the cells, plays a pivotal role in the defense against oxidative stress-induced cell injury and mitochondrial damage (Lakshmidevi and Anuradha, 2010).

GSH also preserves the cellular levels of active forms of vitamin C and vitamin E (Janani *et al.*, 2010). It is an important defense mechanism against potentially toxic hydrogen peroxide by glutathione peroxidase, which reduces hydrogen peroxide to water, and along with that, the oxidation of GSH (Shieh *et al.*, 2010). Glutathione in mammalian cells maintains the intracellular thiol redox status, and detoxifies exogenous and endogenous reactive molecules. Depletion of intracellular GSH predisposes cells to proapoptotic stimuli and can also activate apoptosis in the absence of such stimuli (Valko *et al.*, 2007).

Accumulation of heavy metals in tissues mainly depends upon concentration of metals in water and exposure period; although some other environmental factors such as salinity, pH, hardness and temperature play significant roles in metal accumulation (Chowdhury and Blust, 2002; Clearwater *et al.*, 2002; Blackmore and Wang, 2003). Sex, size, composition of food, molting and biochemical or physiological changes also affect accumulation of metals in tissues (Blackmore and Wang, 2002; Luoma and

Rainbow, 2005). Mussels and crab are specific indicators of different environmental compartments in relation to their habitat and food web position, and they exhibit different rates of bioaccumulation with respect to xenobiotics (De laTorre *et al*., 2000; Lee *et al*., 2000; Shin and Lam, 2001; Scaps, 2002; Subathra and Karuppasamy, 2008; Kord *et al*., 2010). Trophic transfer may be even more important than dissolved uptake in various aquatic organisms (Wang, 2002). For evaluation of an animal's potential for biomonitoring, Bioconcentration Factor (BCF) method is adequate and has been often used in studies with aquatic animals (Kahle and Zauke, 2003; Clason *et al*., 2004; Morley, 2010).

To balance the ecosystem structure and functions several directives are being adopted over time to protect estuaries and coasts (Borja *et al*., 2008). The environmental quality standards rely on the concentrations of contaminants as quality objectives for comparing the state of sites (Crane, 2003). The ecological integrity is judged using water or sediment in toxicity tests. In other cases, concentrations of the contaminants are used to assess the ecological status of a location (Rodriguez *et al*., 2006; Tueros *et al*., 2009). The toxicity tests measure the integrated responses to the possible acute or chronic effects of contaminants, on these processes (Watts and Pascoe, 2000). Test species should be sensitive enough to respond to low levels of contaminants and must be available for use from field collection throughout the year. Additionally, if biological tests are to be ecologically relevant, the species should be widespread and easily available (Richardson and Martin,

1994). Toxicity is a relative property reflecting a chemical's potential to have a harmful effect on a living organism. Toxicity tests are, therefore, used to evaluate the adverse effects of a chemical on living organisms under standardized, reproducible conditions that permit comparison with other chemicals or species tested, and comparison of similar data from different laboratories (Nascimento *et al*., 2000).

Most commonly used stressor end points are variables related to growth performance (Becker *et al*., 2000; Breitholtz and Bengtsson, 2001; Sibly and Hone, 2002). The body size correlates with many ecological as well as life history traits and may thus influence the abundance of species as well as population structure and dynamics (Breitholtz and Bengtsson, 2001; Gaston *et al*., 2001). Growth rate has been frequently used as a measure of performance of the individual and is believed to be a more appropriate measure of toxicological effects (Jensen *et al*., 2001; Yang *et al*., 2002; Dahlhoff, 2004; Schamphelaere and Janssen, 2004). When toxicity tests are viewed within a legal context as needed to implement regulations, they are also accepted based on the ease and expense of performing them, the acquisition of irrefutable proof of harm and financial implications of the lost or threatened resource. Perhaps for those reasons, environmental risk assessment focuses on a simple and straightforward end point, lethality or survival (LC_{50} representing the lethal concentration to 50 percent of a population). This measurement represents a baseline for toxicity (Ankley *et al*., 2010).

1.8. Ecology of Estuarine crab

Crabs form an important part of the mangrove and coastal ecosystem. They feed on leaf litter and other organic matter and play an important role in recycling of nutrients. Their digging behavior results in changes of surface topography, distribution of particle size and degree of aeration in both ecosystems (Harshith *et al.*, 2016). The mangrove forests are characterized by higher productivity and unique ecosystems. Crabs, the most advanced members of the phylum Arthropoda are the dominant fauna of this ecosystem because they are morphologically, physiologically and behaviorally well-adapted to their environment. True crabs belonging to the suborder brachyuran of the order Decapoda under class Crustacea show the greatest size range of all arthropods. Brachyuran crabs are the reputed decapods which form the major components of crustacean fauna in Sind mangroves. Tirmizi and Ghani (1983) have reported 16 species of crabs from Sind mangroves.

Mangroves are unique as they support tropical coastal vegetation and they support genetically diverse groups of terrestrial and aquatic organisms. They are of great ecological and economic significance in the coastal region and also helps in enhancement of water quality (Kathiresan and Bingham, 2001; Kathiresan 2003). Among the various organisms found in the region, crustaceans make up the majority. These crustaceans exhibit remarkable adaptability which has led to their exceptional evolutionary history. Among the crustaceans decapod crabs are the most predominant with them being highly active animals with complex behavioral patterns (Pradnya *et al.*, 2011).

The crabs depend on the mangroves for survival and their litter has a significant role in the detritus formation. These moves around and live in burrow. This digging activity causes alterations in the surface topography, the grain size and facilitates the aeration of soil (Pradnya *et al.,* 2011). To a greater or lesser extent, the crabs are connected between land and sea due to the presence of mangrove forests. These forests provide better habitats, plant detritus and protection from the sun, which favor the presence and development of large populations of terrestrial and semi-terrestrial detrivorous decapods crustaceans (Crane, 1947; Mann, 1972).

Estuarine crabs (Crustacea: Decapoda: Brachyura) are important elements of almost all marine coastal ecosystems, especially in tropical latitudes, where there are represented by the highest number of species (diversity) playing a significant role in the organization of coastal ecosystems (Morton and Zeng, 1982; Morton and Morton, 1983; Coldrey, 1986).

Brachyuran crabs reach their greatest diversity in tropical and temperate regions of the world (Rahman *et al.,* 2008) play an important role in the food chain and marine ecosystem. According to Chhapgar, (1991) crabs play a significant role in the fishery wealth of many nations. It is often been reported that grapsoid crabs play an important ecological role in mangrove ecosystems (Lee, 1998). The removal and processing of mangrove leaves by crabs helps to trap the energy stored in these leaves within the mangal before the tide can carry them away (Skov and Hartnoll, 2002). Furthermore, their faecal material

potentially contributes to secondary production via a coprophagous food chain (Lee, 1997; Gillikin *et al.,* 2001).

1.9. The experimental animal (Estuarine crab *Sesarma quadratum*) (Latreille, 1802)

The class Malacostraca is divided into five orders, with over 10,000 species; the largest is Decapoda. This order also contains the biggest and most familiar crustaceans, such as shrimp, lobsters, crayfish, and crabs.

Anatomy

The name Decapoda means 10 legs, or five pairs of legs. Most decapod's first pair is modified as large claws, called chelipeds. In front of the main legs are three pairs of small appendages used in handling food. These are called maxillipeds, the maxillipeds and five pairs of legs are the appendages of the thorax. A carapace covers the thorax, the head and thorax together are called the cephalothorax. The head has a pair of compound eyes located on eye stalks. The abdomen is large and has six pairs of biramous appendages. The last pair called uropods, which is flattened and form a tail fan. Swimmerets or pleopods make up the first five pairs. This structure applies to all decapods except for the crab where the abdomen is reduced and folded beneath the cephalothorax, this is what gives the crab its short body form.

Diet and Feeding Habits

Most decapods are predators or scavengers, but some feed on algae, and the land crabs eat fruits and leaves. The chelipeds can be used for many different activities, depending on the species. Some crabs use one

large claw crushing and the small one for cutting. There are also filter feeders, such as the mole crabs, and fiddler crabs.

Respiration

Decapods have gills that project upward from near the base of the thoracic appendages and are enclosed within a protective gill chamber. The gill bailer creates a ventilating current by rapid sculling motion.

Reproduction

The reproductive organs are found in the dorsal part of the thorax. In the male there is a single pair of testes, the sperm ducts open at the base of the fifth pair of legs. In the female the oviducts open to the exterior at the base of the third pair of legs. During copulation the male assumes various positions astride the female and using the greatly modified anterior two pairs of pleopods, transfers spermatophores to the female gonopores or to a median seminal receptacle between the fourth or fifth pair of legs. Eggs are fertilized internally or on release from the oviduct. Eggs are attached to the pleopods.

Growth and Development

Most decapods hatch as a zoea, although some shrimp hatch as a nauplius. In the zoea all the thoracic appendages are present.

Aquatic ecotoxicology broadly focuses on how aquatic organisms interact with pollutants in their environment in order to determine environmental hazard and potential risks to humans. Research has produced increasing evidence on the pivotal role of aquatic invertebrates in the

assessment of the impact of pollutants on the environment. The present study aims to the use of estuarine crab *Sesarma quadratum* as an experimental test organism in aquatic ecotoxicology.

Grapsid Crab Genus *Sesarma*

The grapsid crab genus *Sesarma* (sensu lato) consists of more than 125 species in temperate and tropical regions of the world. Species occur in a variety of environments including intertidal marine, brackish water, freshwater, and terrestrial habitats. The genus was created by Say 1817 with *Ocypode reticulatus* (Say, 1817). Since the species assigned to the genus has been reassigned to no less than 20 other genera or subgenera.

Description

Carapace is decidedly broader than long, the dorsal surface moderately convex and uneven but generally naked and glabrous. The gastric region moderately convex and well defined; the oblique striae on either posterior lateral surface are strongly impressed but not furnished with hair. The front is broadly concave in the middle, the postfrontal ridge four-lobed, each lobe rounded. The external orbital angle is prominent and its outer margin arched, the second antero-lateral tooth is small and only shallowly separated from the former; the lateral border behind the second tooth is concave but again divergent near the posterior angle. The two oblique ridges on the dorsal surface of palm are similar to those of the former species; the ridge of upper surface of the movable finger is composed of 8-9 coarse tubercles, close to this row are about 10-12 smaller tubercles.

The merus of ambulatory legs is almost twice as long as broad; therefore, it is a little narrower than in *Parasesarma pictum* (Sesarma quadratum) by Sakai, 1939.

***Sesarma quadratum* (Fabricius) distribution (image capture from https://www.gbif.org/species/4382756)**

Fig 1.1: Distribution of the Experimental animal

OBJECTIVES

1.10. Objectives of the present study

The present work is designed to monitor the variability of heavy metals in aquatic mediums deriving from the seasonal cycle (Premonsoon, Monsoon and Post Monsoon) and the physical environment. Impact of heavy metal cadmium chloride on crab *Sesarma quadratum* was also investigated.

The main objectives of the present study are

- To monitor the physico-chemical changes in Kuduvaiyar estuary, Southeast coast of India.
- To determine the heavy metals concentrations in Kuduvaiyar estuarine water and crab.
- To find out the LC_{50} value through acute toxicity of cadmium chloride on the crab *Sesarma quadratum* .
- To estimate the amount of carbohydrate, protein and lipid content in cadmium chloride exposed crab, *Sesarma quadratum*.
- To study the chronic toxicity of cadmium chloride exposed crab, *Sesarma quadratum*
- To determine the immunological parameters of cadmium chloride exposed crab, *Sesarma quadratum*.
- To analysis the stress markers in cadmium chloride exposed *Sesarma quadratum*
- To investigate the enzymatic antioxidant in cadmium chloride exposed *Sesarma quadratum*
- To study the non-enzymatic antioxidant in cadmium chloride exposed *Sesarma quadratum*
- To monitor the histological changes in Gills and Hepatopancreas of cadmium chloride exposed *Sesarma quadratum*

CHAPTER-II

REVIEW OF LITERATURE

CHAPTER II

2. REVIEW OF LITERATURE

A lot of analytical work has been carried out on natural water bodies, both freshwater and marine water, throughout the globe and as such a voluminous literature is available on the subject. In view of the objective of the present research, a critical survey of literature was carried out to gather information on various relevant aspects such as physico-chemical features and heavy metals concentrations.

2.1. The International level of physico-chemical features

Seasonal variations in water temperature of various aquatic bodies have been recorded by Welch (1952), Hannan and Young (1974) and Harshey *et al*. (1982). Jolly and Chapman (1966) made a preliminary study on effects of pollution on Farmers Creeks and Cox's river with respect to temperature. pH variations in water are widely studied worldwide. Impact of addition of sewage and industrial effluents on pH levels has been observed by Oswald (1960) while Hannan and Young (1974) and Chapman and Kimstach (1992) recorded effects of industrial discharges on the pH level of water. Wanganeo (1984), Khalique and Afser (1995), Islam and Islam (1996) and Sithik *et al*. (2009) recorded changes in pH values with addition of sewage and agricultural effluents.

Dissolved oxygen, which is a parameter of primary importance in the aquatic ecosystem by virtue of its role in both chemical as well as biological reactions, has been recorded in various water bodies throughout

the world, Reid (1961), Ray *et al.* (1966) and Kara *et al.* (2004). The changes in dissolved oxygen levels in water with addition of domestic sewage, various industrial wastes and agricultural runoff have been investigated in different water bodies by Gonzalves and Joshi (1946), George *et al.* (1966), Jolly and Chapman (1966), De Smet and Evens (1972), Cairns *et al.* (1975), King (1981), Woodword (1984), Meybeck *et al.* (1992), Jameson and Rana (1996) and Jameel (1998) and Otieno (2008).

Mairs (1966) suggested total hardness to be a complex mixture of cations and anions while Cole (1975) recorded calcium and magnesium to account for most of the hardness. Thomson (1952), Chapman and Kimstach (1992) and Meybeck *et al.* (1992), investigated the impact of sewage and industrial effluents on the hardness values of water. Cadmium was found to be acutely toxic to rainbow trout in both soft and hard water.

Metals in water

Trace metals are considered to be major toxicants in contaminated water worldwide (Chi-Man and Jiu, 2006; Katsoyiannis and Katsoyiannis, 2006; Asonye *et al.*, 2007 and Yasuhiro *et al.*, 2007). Several studies have attempted assessing heavy metal pollution according to the distribution of particle size and to the relationship of its organic content (Hiraizumi *et al.*, 1978; Kristensen, 1982).

Heavy metal levels in many natural water bodies across the world have been investigated. Cooper *et al.* (1978) analyzed water quality of the river Tean Staff and found an increase in cadmium levels with addition of sewage.

Polprasert (1982) analyzed heavy metal levels in water of the Chao Phraya river estuary, Thailand and discussed their long term impact on the aquatic environment. Mart *et al.,* (1984) determined trace metal levels in the eastern Arctic ocean while Abaychi and Douabul (1985) determined trace metals in Shatt Al-Arab river, Iraq and indicated metal levels to be within the recommended limits.

Maroof *et al*. (1986) analyzed cadmium and zinc concentrations in drinking water supplies of Dhaka city, Bangladesh and highlighted the impact of addition of bleaching powder and pumping on zinc concentration. Jing and Wei-Wen (1988) analyzed concentrations of trace metals in the Qiantang-Jiang river and its estuary Southern China and found higher levels of metals with addition of industrial wastes. Pelig-Ba *et al*. (1991) analyzed trace metal concentrations in Borehole waters from the upper regions and the Accra plains of Ghana. Vazquez *et al*. (1998) analyzed dissolved metals in Alvarado lagoon,

Ozmen *et al*. (2004) conducted a preliminary study on heavy metal (Zn, Mn, Ni, Cu, Cr, Co and Pb) concentrations in surface water of Hazar lake and discussed the heavy metal pollution status of the lake. Emoyan *et al*. (2005) evaluated heavy metals loading of river Ijana, Nigeria and results indicated higher metal contents in winter season. Thari *et al*. (2005), in a multivariate analysis of heavy metal concentrations in soil, sediment and water in the region of Meknes (Central Morocco), compared the metal contents in water and sediment to suggest correlations between them. Abulude *et al*. (2006)

analyzed Fe, Cr, Cd, As, Ni, Co and Zn in drinking water samples in Akure, Nigeria. Adefemi *et al*. (2008) determined heavy metal (Zn, Pb, Mn, Fe, Cu,17 Co, Cr, Cd and Ni) contents in water from Ureje dam in south-western Nigeria to determine the water quality.

Impact of heavy metal inputs from various industries have been investigated in several studies. Huynh-Ngoc *et al*. (1988) determined cadmium levels in the river Rhone polluted by industrial wastes. Peerzada *et al*. (1990) studied distribution of heavy metals in Grove harbour, northern territory, Australia to find the impact of a bauxite treatment plant on the heavy metal status of water. Vazquez *et al*. (1993) investigated heavy metals to study the effects of industrial lead inputs into the San Andres lagoon, Tamaulipas, Mexico. They carried out a comparative study of several metals (Cd, Co, Cu, Fe, Mn, Ni, and Zn). Sah *et al*. (2000) conducted a study on assessment of heavy metal pollution of water in the Narayani river, Nepal contaminated by paper industry effluents. Sanayei *et al*. (2009) analyzed heavy metal levels in Zayandeh Rood river, Isfahan-Iran at seven sites to observe the influence of the industrial activities and dump of municipal waste on heavy metal concentrations in this region.

2.2. The National level physico-chemical features

Seasonal variations in water temperature of various aquatic bodies in India has been recorded by Rao (1955), Munawar (1970a), Qadri and Yousuf (1978), Swarup and Singh (1979), Goel *et al*. (1980), Patra and Nayak (1983), Bagde and Varma (1985a & b), Palharya and Malviya

(1988), Shyam Sunder (1988), Prakash (1990), Pandey *et al*. (1993), Kaur *et al*. (1995, 1996a & b and 1997), Kumar (1995a & b), Bath and Kaur (1999), Valarmathi *et al*. (2002), Singh and Mathur (2005), Chaurasia and Pandey (2007), Shyamala *et al*. (2008) and Pawar *et al*. (2009). Impact of contamination with industrial effluents on temperature of water in various aquatic systems have been recorded by Munawar (1970a & b), Mahadevan and Krishnaswamy (1983), Bisht (1986), Sarwar (1987), Palharya and Malviya (1988), Shah (1988), Adholia *et al*. (1991), Jindal and Kumar (1993), Bath (1996), Syal (1996) and Kumar and Sharma (1999) and that of contamination with sewage by Kaur *et al*. (2003).

Mohanty (1975) while studying physico-chemical features of the outer channel of the Chilka lake, recorded seasonal variations in pH of water. Seasonal variations in pH values have also been observed by Zutshi and Vass (1978) and Khan and Zutshi (1980), Sharma and Dhaneshwar (1986), Mishra (1988) for other water bodies. Impact of addition of sewage and industrial effluents on pH level has been observed by Singh *et al*. (1969), Seenayya (1971) and Kumar and Sharma (1979) while Trivedy and Goel (1986) and Sinha *et al*. (1991) recorded effects of industrial discharges on the pH level of water. Bansal and Khare (1990) and Mishra (1991) observed changes in pH due to addition of paper mill effluents. Singh *et al*. (1982), Sharma and Dhaneshwar (1986), Mishra (1988), Jindal and Kumar (1993), Bath (1996), Syal (1996), Narain and Chauhan (2000), Chaurasia and

Pandey (2007), Pawar *et al.* (2009) and Sithik *et al.* (2009) recorded changes in pH values with addition of sewage and agricultural effluents.

Dissolved oxygen has been recorded in various water bodies in India by Chakraboarty *et al.* (1959), Pahwa and Mahrotra (1996), Ray *et al.* (1966), Vasisht and Sharma (1975), Badola and Singh (1981), Bhowmick and Singh (1985), Shastree *et al.* (1991), Pandey *et al.* (1993) and Esmaeili and Johal (2005).

Paramasivam and Sreenivasan (1981) and Mishra (1991) carried out an investigation on effects of paper mill effluents on dissolved oxygen. The changes in dissolved oxygen levels in water with addition of domestic sewage, various industrial wastes and agricultural runoff have been investigated in different water bodies by Saxena *et al.* (1966), Mishra (1991), Kaur *et al.* (1995), Kumar (1995a & b and 1998), Bath (1996), Syal (1996), Narain and Chauhan (2000), Valarmathi *et al.* (2002), Kumar *et al.* (2003) and Prasanakumari *et al.* (2003). Sinha *et al.* (1991), Kaur *et al.* (1995 & 2003) and Syal (1996) investigated the impact of sewage and industrial effluents on the hardness values of water. Kannan (1991) provided standards depicting the nature of water on the basis of total hardness values.

Metals in water

There are several studies on heavy metal contamination of groundwater in different areas of India (Srikanth *et al*., 1993; Bhattacharya *et al*., 1997; Dixit *et al*., 2004; Bhattacharjee *et al*., 2005; Rajmohan and Elango,

2005; Ram and Singh, 2007; Lohani *et al*., 2008. Manivasakam (1984), Mishra (1990) and Kannan (1991) studied environmental pollution and suggested an increase in metal levels due to addition of effluents from industrial units.

Koul *et al*. (1988) studied trace metal concentrations in some Kashmir Himalayan lakes and discussed seasonal trends. Paul *et al*. (1994) analyzed trace metals and lanthanides in a tropical river environment and found higher metal levels in the summer season. Analytical studies on rivers in Punjab have been conducted by Dhillon and Kaur (1996), Kaur *et al*. (2000) and PSCST (2005). Meenakumari and Nair (1996) recorded levels of trace elements in the surface waters of Cochin harbor and discussed the impact of runoff during monsoon period on metal concentrations in water.

Agarwal and Gopal (1998) analyzed the physico-chemical characteristics and heavy metals in the Ganga River at various locations of Northern plains. Govindasamy *et al*. (1998) determined seasonal variation of heavy metals in water of Puducherry coast, Bay of Bengal and observed higher cadmium content in the post monsoon season. Khan *et al*. (1998) studied trace metals in Ganges-Brahmaputra-Meghna estuary and indicated higher metal levels in the winter season.

Kaushik *et al*. (1999) determined heavy metal levels in three lentic water bodies (Motijheel, Surajkund and Ranital) of Gwalior region. Seasonally lowest and highest values of metals were recorded during summer and rainy seasons respectively in all the three water bodies. Dash *et al*. (1999) assessed

lead specification in Rushikulya surface waters, east coast of India and recorded seasonal variations of dissolved and particulate fraction of lead in upper reaches of the estuary. Aggarwal *et al*. (2000), Lokhande and Sathe (2001) studied heavy metals in various water bodies and found an increase in metal content with addition of sewage and industrial effluents.

Mandal *et al*. (2006) recorded occurrences of various metals in surface water of river Ganga in West Bengal and discussed seasonal trends in concentration of metals. Buragohain *et al*. (2009) examined seasonal variations in metal contamination of groundwater in Dhemaji district, Assam. They recorded higher concentrations of all the metals in the dry season than in the wet season.

Prahalad and Seenayya (1989) analyzed zinc and lead levels in the Husain Sagar lake of Hyderabad polluted by industrial wastes and found metal levels to be within the permissible limits. Mhatre (1991) emphasized the need of bioindicators of heavy metals pollution and their possible use in biomonitoring. Garg *et al*. (1992) analyzed trace metals in river Ganges at Kanpur to study the impact of various industrial effluents.

Trivedy *et al*. (2002) analyzed heavy metal pollution in the river Krishna and Koyna in Maharashtra as a result of addition of domestic, industrial wastes and agricultural runoff.Gupta *et al*. (2002) recorded concentration of heavy metals in waters of the area around Mandideep industrial complex to study the impact of industrial wastes.

Borole and Patil (2004) reported the effect of waste water of the sugar industry on some heavy metals in a river in Maharashtra. Hussain and Hussain (2004) evaluated the impact of textile waste water on the drinking water and ground water quality of villages close to river Kothari, Rajasthan. Joseph and Chacko (2005) investigated the distribution of dissolved and particulate heavy metals in Chitrapuzha river in Kerala, contaminated with industrial wastes.

Sandwar and Tiwari (2006) recorded monthly variations in heavy metals concentration in Ganga river water in North Bihar region which receives wastes from Barauni-Mokama industrial complex. Gowd and Govil (2008) studied distribution of heavy metals in surface water contaminated with wastes from Ranipet industrial area in Tamil Nadu and discussed health hazards especially for the people working in the tannery industries. Kar *et al*. (2008) analyzed heavy metal pollution in surface water at different stations on the river Ganga (West Bengal) to study the impact of heavy metal loaded industrial waste water.

Begum *et al*. (2009a) analyzed water, plankton and sediment of river Cauvery to study the impact of fertilizers, agricultural ashes, industrial effluents and anthropogenic wastes on heavy metal concentrations in these components.

Studies on heavy metal contamination of various water bodies to assess the water quality has been done by Jain and shrivastava (1998) in Kolar reservoir, Bhopal (M.P.), by Rani and Reddy (2003) in Hussain Sagar lake, by

Chaudhary *et al*. (2004) in the river Yamuna at Faridabad, by Pandey *et al*. (2004) in river Pandu at Kanpur, by Jain *et al*. (2004) in a coastal aquifer of Andhra Pradesh, by Sinha (2004) in Sai river at Rae Bareli, by Anitha *et al*. (2005) in Mir Alam lake, Hyderabad, by Gupta *et al*. (2005) in the surface water of lower lake, Bhopal,by Rajappa *et al*. (2010) in the groundwater of Hakinaka Taluk and by Pandey *et al*. (2010) in the Ganga river at Varanasi.

2.3. Cadmium toxicity

Cadmium (Cd) is one of the most toxic heavy metals for humans; the main source of non- occupational exposure to Cd includes smoking, air, food and water contaminated by Cd (Nagata *et al.,* 2005). In addition, herbal medicine is another source of Cd. The World Health Organization (WHO) estimates that 4 billion people or 80 percent of the world population presently use herbal medicine (Naithani *et al.,* 2010). Several articles have reported the adverse effects of these herbal preparations due to the presence of high levels of heavy metals such as Cd, lead, chromium, nickel, etc. (Naithani *et al.,* 2010). Saeed *et al.* (2010) investigated twenty five herbal products. The results revealed that the concentrations of some heavy metals, including Cd, were far greater than the permissible limits proposed by the International Regulatory Authorities for herbal drugs. Acute or chronic exposure of Cd causes respiratory distress, lung, breast and endometrial cancers, cardiovascular disorders and endocrine dysfunction (Chang *et al.,* 2009; Nagata *et al.,* 2005).

In addition, Cd is a common inorganic contaminant of coastal sediments and waters due to anthropogenic pollution and natural sources

(Ivanina *et al.,* 2010; Sokolova *et al.,* 2004). It can be accumulated in aquatic animals (e.g. crabs, shrimps, oysters and mussels) after entering through different way such as respiratory tract, digestive tract, surface penetration etc. (Dailianis and Kaloyianni, 2004; Dailianis *et al.,* 2009; Ivanina *et al.,* 2010; Wang *et al.,* 2008; Zhao *et al.,* 1995). It is seriously harmful to the growth of aquatic life and survival, resulting in decline of their populations. At the same time, as aquatic food products, these animals exposed to Cd might threaten human health.

2.4. Cd accumulation and distribution in crabs

Cd in water can be absorbed by aquatic organisms via respiratory system, digestive system and body surface without significant excretion (Rainbow and White, 1989; van Hatton *et al.,* 1989). And we can get valuable information for evaluating the level of Cd pollution in water and sediments by assaying Cd concentration in crabs.

The difference of Cd accumulation and distribution in different tissues

Experiments have confirmed that Cd absorption and accumulation by crabs had obvious differences among the various body segments. Accumulated Cd was distributed to all organs with the highest proportions of body content being found in the exoskeleton, gills, hepatopancreas and so on. The first organ in which Cd accumulates is the exoskeleton. Cd has similar chemical properties to calcium (Ca), such as the same charge number, the similar ionic radius and electronic number. Therefore, the Cd in water can replace the Ca entering the body via exoskeletons (Jennings and Rainbow, 1979). The gill is a

respiratory organ for crabs. It plays an important role in the absorption and transport of heavy metals (Silvestre *et al.*, 2005) and is the target organ of Cd in water. The hepatopancreas are detoxifying organs in crabs which can change the toxic heavy metal into non-toxic compounds and reduce the toxicity of the heavy metal in the body. Thus the Cd concentration is higher in the hepatopancreas.

2.5. Factors influencing Cd accumulation and distribution

Due to the different treatment methods, the accumulation and distribution of Cd are different in different organs. When *Carcinus maenas* was exposed to seawater at a Cd dose of 10 ppm, the midgut gland contained absorbed 10% of the total Cd, while the exoskeleton contained. When Cd was absorbed from a food source, the midgut gland contained 16.9% of the absorbed Cd whereas the exoskeleton contained only 22.2% (Jennings and Rainbow, 1979). It can be inferred that in bath experiments, the exoskeleton was in direct contact with Cd and accumulated the most Cd; in feeding regimes, the exoskeleton had the lower proportion accumulation (Bjerregaard and Depledge, 2002). In addition, *Sinopotamon yangtsekiense* had the highest concentration of Cd in the exoskeleton after acute exposure (Silvestre *et al.*, 2005), while *Eriocheir sinensis* had highest Cd concentration in the gills after chronic exposure for 30 d adding the acute exposure for 3 d (Wang *et al.*, 2003).

The environment can also affect the absorption and accumulation of Cd. An increase in the Cd concentration in the environment will result in

increased Cd accumulation. Namely, the accumulation of Cd has an obvious dose-dependent relationship (Lan *et al.*, 2001; Wang *et al.*, 2003). Ca in the water environment will prevent the absorption and accumulation of Cd because it can form a competitive relationship with Cd. Therefore, accumulated Cd in the body will be less whenever the Ca concentration in water increases (Wright, 1977). Beltrame *et al.* (2010) reported that sex, habitat, and seasonality could influence heavy-metal concentrations in the burrowing crab (*Neohelice granulata*) from a coastal lagoon in Argentina.

The influence of Cd on the enzyme activity in crabs

Small amounts of Cd can be detoxified into non-toxic substances by metallothionein in the organism (van Hatton *et al.*, 1989). Excessive Cd will damage the body, however, as it will combine with protein molecules having sulphur, hydroxyl and amino groups, and restrain some enzyme system activity. In addition, the affinity of Cd with sulfhydryl group is stronger than zinc (Zn), it can replace the enzyme-bond Zn and cause the enzyme to lose its function (Müller and Ohnesorge, 1982).

The influence of Cd on antioxidant enzymes system in crabs

One of the mechanisms for Cd toxicity to animals is the oxidative damage. On one hand, Cd can cause the body to produce excessive active oxygen. On other hand, it can change the expression and vitality of antioxidant enzymes. Antioxidant enzymes mainly include the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione enzyme turn sulfur (GST), etc. They can effectively scavenge active oxygen in the

body and avoid oxidative damage to the body (Lan *et al.,* 2010). Numerous studies have been published on the influence of Cd on antioxidant enzymes in terrestrial creatures, while reports about crabs are rare. In one study the Cd concentration was 0.025 mg/L and 0.05 mg/L in water, and SOD, CAT and GPX activities in *Charybdis japonica* could be stimulated after 0.5 d, and then reduced during the experimental period (Pan and Zhang, 2006). When crabs (*S. yangtsekiense*) were exposed with a dose range of 7.25-116.00 mg/L for 24, 48, 72 and 96 h, the activities of SOD, CAT and GPX increased initially and decreased subsequently (Wang *et al.*, 2008). After Immersing the juvenile crab *E. sinensis* in 2.0 mg/L water, the activities of SOD, CAT and GPX in hepatopancreas were all initially decreased, and then recovered to some degree during the duration of the study (Liu *et al.,* 2003). This showed that low concentration of Cd stimulated antioxidant enzymes activity while high concentration inhibited antioxidant enzymes activity.

The influence of Cd on metabolic enzymes in crabs

Glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) are the important aminotransferase in the protein metabolism. Low concentration of Cd stimulated the activity of GPT and GOT in *Scylla serrata* while high Cd concentrations showed apparent inhibition. The results showed the obvious dose-effect relations (Tang *et al.,* 2000). This may be because the green gland is an excretory organ with strong detoxification (Zhao *et al.,* 1995). GPT activity in serum of *E. sinensis* increased with increasing Cd concentration after poisoning. That might be

because tissues were damaged and the enzyme released into serum (Lu *et al.*, 1989). Lactic dehydrogenase (LDH) plays an important role in carbohydrate metabolism. The crab *Uca pugilator* were immersed in 2.0 mg/L water for 24 h, 48 h, LDH activity reduced in hepatopancreas and that is opposite in the abdominal muscles (Devi *et al.*,1993). Alkaline phosphatase is a kind of low-specific phosphomonoesterase which plays an important role in nucleic acid, protein and lipid metabolism. The influence of Cd on enzymatic activity in *S. serrata* also exhibited a dose-effect relationship that was similar to that observed above (Tang *et al.*, 2000).

The influence of Cd on the ultrastructure of crabs

Studies concerning the influence of Cd on the ultrastructure of crabs have appeared in the past few years. The published studies have focused on the destruction of membrane systems and morphological changes of cells, Cd can accelerate cellar lipid peroxidation and cause the accumulation of lipid peroxides. These free radicals and their reaction products, peroxides, can often cause various biological macromolecules, including DNA, to change structures and properties through chemical reactions, such as hydrogen abstraction, oxidation sulfhydryl and carbon chain destruction. Cadmium (Cd) can also decompose the unsaturated fatty acid into malondialdehyde (MAD) by peroxiding and cause biological macromolecules to crosslink into abnormal macromolecules which degrade membrane structure and alter the membrane permeability (Shukla *et al.*, 1989).

The crab *E. sinensis* were exposed to Cd, many changes appeared in the R-cell in hepatopancreas, organelles such as mitochondrial damage, endoplasmic reticulum expansion, and thinning of the cytoplasmic matrix (Lan *et al.,* 2001). Cd can partly disintegrate the mitochondrial cristae of neurosecretory cells in *E. sinensis* (Mingshen *et al.,* 2008). Whenever injected into the crab *S. yangtsekiense*, Cd resulted in damage to the membrane structure, among the organelles the mitochondria was damaged first, which suggested that mitochondria was a sensitive organelle to Cd that could be used to show the level of damage caused by Cd (Lan *et al.*, 2002).

Studies regarding the effects of Cd on ovarian development in crabs have been conducted since the 1990s. The majority of experiments showed that Cd inhibited ovarian growth, reduced hatch rates of the fertilized eggs and led to embryonic deformity. Lee *et al.* (1996) documented that Cd deformed eyespots, reduced hatching success, and inhibited growth of oocytes of *Callinectes sapidus*.

CHAPTER-III

MATERIALS AND METHODS

CHAPTER III

3. MATERIALS AND METHODS

3.1. Location of Sample Collection

Water samples and adult *Sesarma quadratum* crabs were collected from Kuduvaiyar estuary, which is situated in the South east coast, near Nagapattinam, Tamil Nadu, India (Station : Lat. 10° 45' N, Long. 79° 96' E. The Kuduvaiyar river is a tributary of the major river, 'Cauvery' of South India.

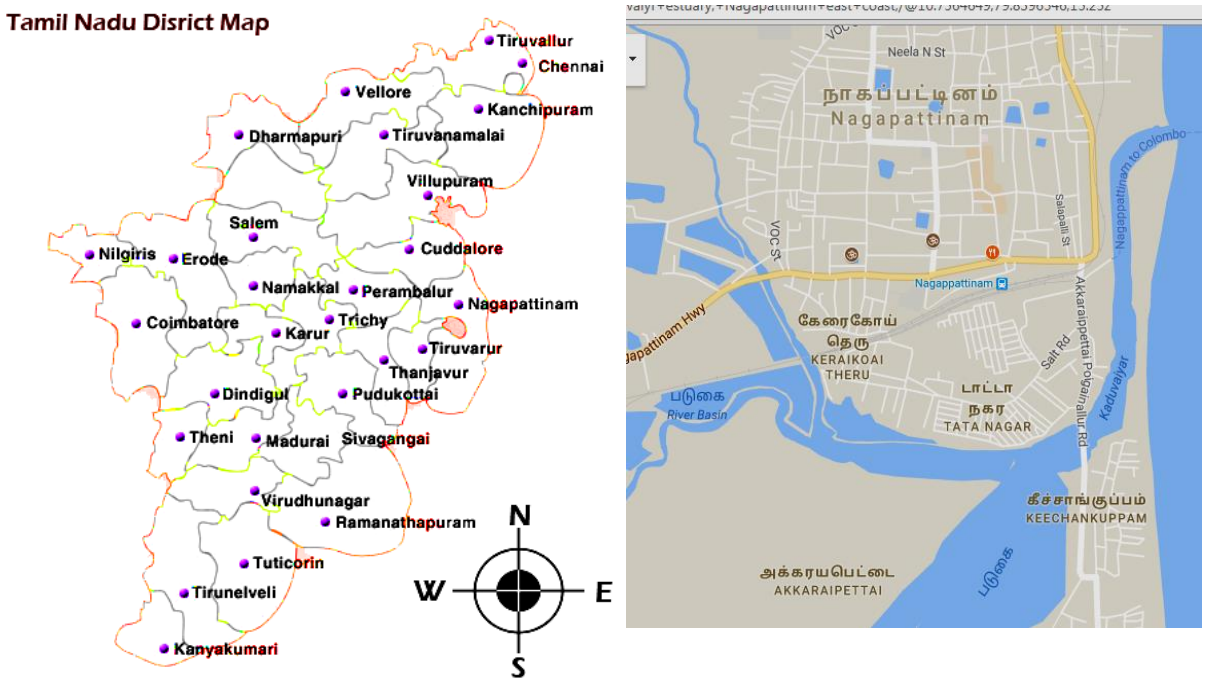


Fig 3.1: Shows the location of sample collection

Study area

The study was carried out in Kuduvaiyar estuary at Nagapattinam district in TamilNadu located along the Southeast coast of India. The Kuduvaiyar estuary is formed by the tributaries of Cauvery River and opens into an estuary on the South East coast of India. The three distinct seasons were,pre-monsoon, monsoon and post-monsoon periods. The samples were collected from five different points in the same site and were mixed together to prepare an integrated sample. The heavy metals cadmium, chromium, copper, lead and zinc were analyzed in water and crabs.

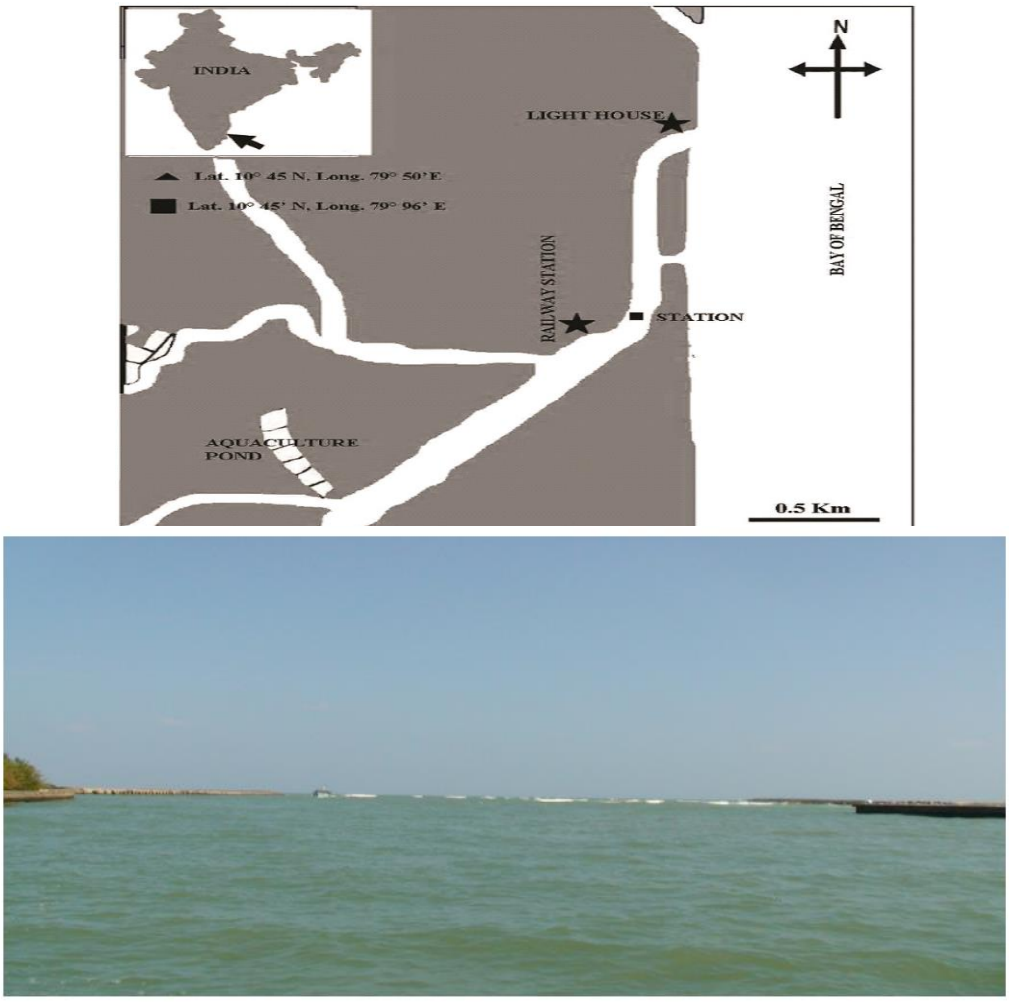


Fig 3.2: Shows the Kuduvaiyar Estuary

3.2. Collection and acclimation of experimental Crab

Sesarma quadratum crabs were collected from Kuduvaiyar estuary in the South East coast, near Nagapattinam, Tamil Nadu, India .The crabs were collected by hand picking with the help of the local crab collectors. The crabs were acclimatized under laboratory conditions for 7 days prior to the start of this experiment. Glass aquaria of 10 litre capacity, marine water were used in this experiment. Fresh air was supplied to each aquaria through an air pump fitted with a capillary system.

3.3. SESARMA QUADRATUM

CLASSIFICATION OF *Sesarma quadratum*

Sesarma quadratum (Born, 1778)

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Infraorder: Brachyura

Family: Sesarmidae

Genus: *Sesarma*

Species: *quadratum*

Fig 3.3: Shows the study animal of crab *Sesarma quadratum*.

3.4. Physico-chemical parameters

The methods used for the analysis of various physico-chemical parameters were the same as given in Standard Methods for the examination of water (APHA, 1967, 1980; APHA-AWWA-WPCF, 1976); Golterman *et al.*,(1978) and National Environmental Engineering Research Institute (NEERI, 1986).

Measurement of temperature

This was carried out in-situ at the site of sample collection using a mobile thermometer. This was done by dipping the thermometer into the sample and recording the stable reading

pH

The pH was determined using ELICO-LI 127 pH meter. The pH of the water sample was directly determined with the electrode while the pH of the

sediment sample was determined by preparing (1:5, sediment: water) suspension in distilled water. The contents were stirred well and allowed to settle and supernatant was used to check pH.

Electrical Conductivity (EC)

To determine the soluble salts in the sediments, it is preferable to keep the sediment measured at the field condition and also measured its conductivity, to get the true picture. For this purpose saturated sediment water extract was prepared. Sediment extract is first prepared by taking 20 gms of sediment into a clean 100 ml beaker, add 50 ml of conductivity water then suspension is stirred for 30 minutes and allowed the suspension filtered by using ordinary filter paper. EC of the water samples and sediment suspension was measured by using ELICO EC-TDS meter (CM 183, "Make"-India) where electrodes directly dipped into the respective solutions for the direct display of result on a digital scale. It was reported in micro Siemens (μS). The clear supernatant used for pH was also used for EC measurement

Dissolved Oxygen (DO)

Dissolved oxygen content of the water samples was measured by using Winkler's method (modified azide method). The sample was collected in 300 ml bottle and DO was fixed on site by using 1 ml each of Manganous sulphate and Alkaline-iodide-azide. The precipitate formed was dissolved in the laboratory by using sulphuric acid and titrated with 0.025N sodium thiosulphate ($Na_2S_2O_3$) solution to a pale straw colour , added a few drops of

starch solution and continue titration for the first disappearance of blue colour.

$$DO\ (mg/L) = \frac{A \times N \times 8 \times 1000}{\text{Volume of Sample}}$$

A=Total volume of $Na_2S_2O_3$ req. N=Normality of $Na_2S_2O_3$ and 8= Eq. Wt. of Oxygen.

Chemical Oxygen Demand (COD)

COD determination was carried out with dichromate reflux method with the addition of 10 ml of 0.25 N potassium dichromate ($K_2Cr_2O_7$) and 30ml H_2SO_4+Ag_2SO_4 reagent in 20 ml diluted sample. The mixture was refluxed for 2h and was cooled to room temperature. The solution was then diluted to 150 ml by using distilled water and excess $K_2Cr_2O_7$ remained was titrated with Ferrous Ammonium Sulphate (FAS) using a ferroin indicator.

$$COD\ (mg/L) = \frac{(A- B) \times N \times 1000 \times 8}{\text{Volume of Sample}}$$

Where, A= volume of FAS used for blank(ml); B= volume of FAS used for sample(ml); N= Normality of FAS; 8= equivalent weight of oxygen.

Biological Oxygen Demand

The dilution method was followed to determine the BOD after three days at 27 ˚C. For the same dilution water was prepared with the addition of nutrients namely phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride. The diluted sample was transferred to BOD bottles of 300 ml

capacity. After determining initial dissolved oxygen (DO), final DO was estimated from the bottles kept for incubation period for three days.

Alkalinity of Water sample

100 ml of water sample was mixed with 2-3 drops of phenolphthalein. The development of pink colour to the solution indicated the presence of alkalinity and was then titrated with 0.02 H_2SO_4 till the colour disappeared.

$$\text{Alkalinity} = \frac{\text{Volume of } H_2SO_4 \times N \times 50 \times 1000}{\text{Volume of Sample}}$$

Where N=Normality of H_2SO_4; 50 =volume of sample

Salinity

Salinity (PSU) was determined by Mohr Knudsen argentometric titration method, using standard solution of silver nitrate ($AgNO_3$) and Potassium chromate as an indicator to form silver halides, presence of excess silver ions lead to the formation of red silver chromate (reddish brown colour) the endpoint of titration)

$V1N1=V2N2$, $N2=V1N1/V2$

Where V1=Volume of $AgNO_3$; V2=Volume of sample; N1=Normality of $AgNO_3$; N2=Normality of sample.

Total Hardness of Water sample

The total hardness of the water samples was determined by EDTA (Ethylenediaminetetraacetic Acid) titration method, where 50 ml of well mixed sample was mixed with 1-2 ml of Ammonia buffer solution pH 10 and

a pinch of Eriochrome black-T indicator. The contents were then titrated with0.01N EDTA till wine red solution changes to blue.

$$\text{Hardness(mg/L)} = \frac{C \times D \times 1000}{\text{volume of Sample}}$$

Where C=volume of EDTA used for titration, D= mg of $CaCO_3$ equivalent to 1ml of EDTA solution.

Estimation of Ammonia

Ammonia present in the water sample was quantified spectrophotometrically after treating with Nessler's reagent. The quantity of the ammonia in the sample was estimated from a standard graph and expressed in mg/l. Anhydrous ammonium chloride was used to prepare standard ammonium solution.

Estimation of Nitrate

Hundred ml of water sample was taken in a conical flask and added 2 ml of naphthylamine and 2 ml of H_2SO_4 solution. The content was mixed thoroughly. Development of pink colour indicated the presence of nitrate. The colour intensity of the solution was read out in the Spectronic-21 at 530 nm against blank. The amount of nitrate was estimated by comparing the reading of the test solution with the reading of the known volume standard solution.

Estimation of Chlorides

Five drops of potassium chromate indicator were added to 50ml of sample and titrated with silver nitrate solution till the sample turned brick red. The chlorides are expressed in mg/l.

Estimation of Sulphates

Filtered the water sample through Whatman filter paper and 50ml of filtered water sample was taken into a conical flask containing not more than 10mg/ml sulphate. Added 0.15gm of barium chloride and mixed for 30 minutes using a magnetic stir. Measured the absorbance in a Photoelectric colorimeter against distilled water blank at 420nm and compared with the standard curve. The sulphates are expressed in mg/l.

Estimation of Fluoride

Added sequentially to the 5ml volumetric flasks, 3.5ml of THF solution, 0.1ml of working solution, 1.3ml of sample and 0.1ml of NaH_2PO_4-NaOH Buffer. Mix the content of the flask thoroughly. After 10min, measure the absorbance at 600nm using THF-water (7:3 v/v) solution as the reference. The fluoride is expressed in mg/l.

3.5. Heavy metal analysis in water samples

Bioconcentration of heavy metals (Cd, Cu, Cr, Pb, Ni, Hg, Zn) in water samples collected in different seasons were measured by pre concentration methodology described by Abdullah *et al.* (2007). Estuarine water samples were filtered through a 0.45 µm membrane filter before being acidified with conc. Nitric acid in plastic bottles. The levels of metals concentrated in water were expressed as μgL^{-1}.

Estimation of Sodium

The estimation for sodium was carried out using Systronics mediflame 127 – flame photometer.

Preparation of stock solutions

Sodium stock solution was prepared by dissolving 2.542g NaCl in 1 liter of distilled water. It contains 1mg Na per ml. Stock solution was diluted to give four solutions containing 10, 5, 2.5 and 1 ppm of sodium ions.

Procedure

For sodium the flame intensity corresponding to the concentration of stock solution was noted using appropriate filters. The results were plotted in a graph. The flame intensity of the sample was noted. The concentration of sodium was calculated from the graph.

3.6. LC_{50} determination

After acclimation, healthy crabs of *Sesarma quadratum* were chosen for the LC_{50} determination of cadmium by static renewable bioassay. Crabs were not fed during the experimental period. Various concentrations (5, 10, 15, 20 and 25µg/L) of the test solutions were prepared from cadmium chloride stock solutions. A group of 10 laboratory acclimatized crabs of a particular species having the same weight, size and age were introduced into each test concentration of cadmium. Triplicates and appropriate controls were maintained for each concentration. LC_{50} values were calculated as per Speeman-Kerber's methods (1931). Toxicity tests were conducted in accordance with the method recommended by Sprague (1973). Median lethal concentrations of 96 h were calculated by Finney's (1971) probit analysis using SPSS Ver.20 Log_{10} Base calculation.

3.7. Subacute toxicity

Chronic toxicity tests were carried out with the sub-lethal concentrations of cadmium chloride based on the 96 hour LC_{50} value. Crabs divided into four groups including group I served as control, group II to IV cadmium chloride (12µg/L) exposed for 10, 20 and 30 days experiment.After the end of the experiment, haemolymph and separation of haemocytes and preparation of serum, gills, muscles and hepatopancreas collected for various biochemical parameters.

3.8. Preparation of Tissue Homogenate

Crabs exposed to sublethal concentrations of cadmium chloride were sacrificed and the gills, muscles and hepatopancreas were dissected out, washed with ice-cold physiological saline. The required amount was weighed and homogenized using a Teflon homogenizer. Tissue homogenate was prepared in 0.1 M Tris HCl buffer (pH 7.4) and used for the estimation of various biochemical parameters, antioxidant enzymes activity and oxidative stress markers were measured in freshly prepared samples.

3.9. Biochemical analysis

Estimation of total protein

Protein was estimated by the method of Lowry *et al.* (1951).

Reagents

1. Alkaline copper reagent:

Solution A: 2% sodium carbonate in 0.1N NaOH.

Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate.

50ml of solution A was mixed with 1ml of solution B just before use.

2. Folin's phenol reagent (commercial reagent, 1:2 dilution)
3. Bovine serum albumin (BSA).

Procedure To 0.1ml sample, 0.9ml of water and 4.5ml of alkaline copper reagent were added and kept at room temperature for 10min. Then 0.5 ml of Folin's phenol reagent was added and the color developed was read after 20min at 640 nm.

The level of protein was expressed as mg/gm tissues.

Estimation of total lipids

Total lipids in tissues were estimated by the method of Folch *et al* (1957).

Reagents:

1. Chloroform: methanol mixture, 2:1 ratio

Procedure:

Known volume of 10% homogenate was taken and to the 10ml of chloroform, methanol mixture (2:1 v/v) was added. This was kept at room temperature overnight. At the end of this period, further 5ml of chloroform: methanol mixture was added. This was centrifuged. Three layers were formed and the lower chloroform layer was collected. This layer was taken in a preweighed vial and evaporated in the oven at 40^{o}C. When the organic mixture was evaporated, the weight was taken. The weight of lipids was then calculated.

Total lipids were expressed as mg/gm tissue.

Estimation of Carbohydrates

Total carbohydrates and free glucose were determined by a modified procedure of the perchloric acid/anthrone method Carroll *et al.,* (1956) and Mendel *et al*., (1954). In this method, hot ethanol was used to extract soluble sugars from a ground sample of tissues. The supernatant was poured into a flask for later sugar determination. The remaining residue was washed two times with 52% perchloric acid to solubilize and extract carbohydrates. Which is determined by the anthrone method.

Estimation of total carbohydrate

Carbohydrate estimated by the method of Hedge and Hofreiter (1962) method

Reagents

1. 2.5N HCl
2. Anthrone Reagent: Dissolve 200mg anthrone in 100ml of ice cold 95% H_2SO_4.
3. Standard glucose: 100mg/100ml distilled water

Procedure

Known volume of homogenate hydrolysed by boiling water bath for three hours with 5ml of 2.5N HCl and cool. Neutralize it with solid sodium carbonate until the effervescence ceases. The solution is made up to 100ml and centrifuge and takes 0.5ml for analysis. 4ml of anthrone reagent was added and heated for eight minutes in a boiling water bath and cooled rapidly and read at 630nm.

The values were expressed as mg/g wet weight

3.10. Immunological parameters of cadmium exposed crab

Total haemocyte count

The total number of haemocytes (THC) in haemolymph was determined by use of a haemocytometer. Haemocytes in a mixture of 20 µL of diluted haemolymph and trypan blue (Sigma, T-6164; 0.05% trypan blue in TBS; 50 mM Tris; 370 mM NaCl; pH 8.4), were enumerated by use of a haemocytometer under a light microscope at a magnification of 40×10.

Phenoloxidase assay

Activity of phenoloxidase in haemolymph plasma was assessed by use of the procedure of Asokan *et al*. (1997). Briefly, 100 lL of plasma were mixed with an equal volume of Tris buffered saline (TBS; 50 mM Tris; 370 mM NaCl; pH 8.4) and incubated for 15 min at 22 C. After incubation, 2 mL of 1 mg mL1 L-DOPA (3,4-Dihydroxy-L-phenylalanine, Sigma Chemicals) was added and further incubated for 5 min at 22 °C. All incubations were performed in the dark. After incubation, the optical density (O.D) of samples was determined at a wavelength of 460 nm in a Shimadzu UV-1700 spectrophotometer. Values were compared to a reagent blank that contained 200 lL TBS and 2 mL L-DOPA. Protein content of plasma was by the method of Lowry *et al.* (1951). The PO activity was expressed as unit mg protein/L min/L.

Phagocytosis

Phagocytosis assays were performed on monolayers of yeast cells as targets (Thiagarajan *et al.,* 2006). A suspension of haemolymph (50 lL) was spread on a glass slide and the haemocytes were allowed to adhere to the plate

for 20 min at 25^{o} C. After 20 min, the monolayers were gently washed with TBS, to remove unattached haemocytes and overlaid with 50 lL of 0.5% yeast cells and the glass slides were further incubated for 15 min at 25^{o} C. After rinsing with filtered seawater, the slides were fixed with 2.5% glutaraldehyde for 5 min. Monolayers were washed with TBS, overlaid with a cover slip, and observed using phase optics of a Carl Zeiss Axioskop 2 plus microscope. Replicates were made for each crab, and three counts of approximately 200 haemocytes were made for each replicate. Results were expressed as percentage of phagocytic haemocytes

Lysosomal membrane stability

Haemolymph (100 lL) was pipetted into 0.5 mL a centrifuge tube and aliquots (10 lL) of 0.33% neutral red (Sigma) solution in TBS was added to each tube and the tube was incubated for 1 h at 10^{o} C. Tubes were then centrifuged 200g for 5 min and washed twice in TBS. Aliquots (100 lL) of 1% acetic acid in 50% ethanol were added to all tubes. Tubes were covered with foil, incubated for 15 min at 20 °C and then the amount of neutral red in the medium determined at 550 nm. The results were expressed as O.D per mg^{-1} mL^{-1} haemocyte protein.

3.11. Metabolic activity and oxidative stress

Estimation of Malondialdehyde (MDA/LPO)

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978).

Reagents:

1. Trichloroacetic acid : 15%

2. Thiobarbituric acid : 0.375%
3. Hydrochloric acid : 0.25N
4. TCA-TBA-HCl reagent : Mix the reagents 1,2,3 in the ratio of 1:1:1.
5. Stock standard : 4.8mM of 1,1',3,3'-tetra methoxy propane.
6. Working standard : Stock solution was diluted to get 48nM/ml.

Procedure:

The tissue sample was combined with 2.0ml of TCA-TBA-HCl reagent and mixed thoroughly. The solution was heated for 15minutes in a boiling water bath. The flocculants centrifuged at 1000 ×g for 10 minutes. The absorbance of the sample was read at 535nm against a blank without sample.

Values were expressed as nmole of MDA formed/mg protein in tissues.

Assay of Superoxide dismutase (SOD)

Superoxide dismutase activity was determined by the procedure of Kakkar *et al* (1984).

Reagents:

1. Sodium pyrophosphate buffer: (0.025 M; pH 8.3)
2. Phenazine metho sulphate : 186 micromol/L
3. Nitroblue tetrazolium : 300 micromol/L
4. NADH : 780 micromol/L
5. KCN : 1mM
6. Glacial acetic acid
7. n-butanol

8. Chloroform

9. Ethanol

Procedure: 0.5ml of tissue sample was diluted to 1ml with water. Then 2.5ml of ethanol and 1.5ml chloroform (all reagents chilled) were added. This mixture was shaken for one minute at 4ºC and then centrifuged. The enzyme activity in the supernatant was determined.

The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.01ml of phenazine methosulphate, 0.3ml of nitroblue tetrazolium, 0.2ml of NADH, appropriately diluted enzyme preparation, 0.01ml of KCN and water in a total volume of 3ml. Reaction was initiated by the addition of NADH. After incubation at 30ºC for 90 seconds, the reaction was stopped by the addition of 1ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4ml of n-butanol. The intensity of the chromogen in the n-butanol layer was measured at 560 nm against butanol blank. A system devoid of enzymes served as control.

Enzyme activity was expressed as U/mg protein in tissues.

Assay of Catalase (CAT)

The activity of catalase was assayed by the method of Beers and Sizer (1952).

Reagents:

1. Phosphate buffer : 50 mM/L, pH: 7.0

2. Hydrogen peroxide : 0.059 M/L in phosphate buffer pH: 7.0.

Procedure:

1.9 ml of distilled water and 1ml of the hydrogen peroxide reagent, as substrate was added and incubated for 4-5minutes. Added 0.1 ml of tissue sample and recorded the decrease in absorbance for 2-3minutes at 240nm.

Enzyme activity was expressed as U/mg protein in tissues.

Assay of Glutathione peroxidase (GPx)

The activity of mitochondrial glutathione peroxidase was assayed by the method of Rotruck *et al* (1973).

Reagents:

1. 0.32M Phosphate buffer, pH: 7.0
2. 0.8mM EDTA
3. 10mM Sodium azide
4. 3mM Reduced glutathione
5. 2.5mM H_2O_2
6. 10% TCA
7. 0.3 M Disodium hydrogen phosphate
8. DTNB solution (40mg of DTNB in 100ml of 1% sodium citrate)
9. Reduced glutathione

Procedure:

The reaction mixture consisted of 0.2ml each of EDTA, sodium azide, H_2O_2, 0.4ml of phosphate buffer; 0.1ml of tissue sample was incubated at 37^{o}C. The reaction was arrested by the addition of 0.5ml of TCA and the tubes were centrifuged at 2000 rpm. To 0.5ml of supernatant, 4ml of disodium hydrogen

phosphate and 0.5ml DTNB were added and the colour developed was read at 420nm immediately.

The activity of GP_X was expressed as U/mg protein in tissues.

Assay of glutathione-s-transferase (GST):

GST was assayed by the method of Habig *et al* (1974).

Reagents:

1. Phosphate buffer : 0.3M, pH 6.5
2. Reduced glutathione (GSH) : 30mM
3. 1-chloro-2, 4-dinitrobenzene (CDNB) : 30mM

Procedure:

The reaction mixture contained 1.0 ml of phosphate buffer, 0.01ml of CDNB, 0.1 ml of sample and 0.7 ml of distilled water. This mixture was preincubated at 37^{o}C for 5 minutes, and then the reaction was started by the addition of 0.1 ml of GSH. The change in the absorbance was read at 340 nm for 5minutes and the reaction mixture without the enzyme was used as the blank.

The enzyme activity was expressed as μmoles CDNB conjugated/ min/ mg protein.

3.12. Histopathological studies

Histological studies of gill and hepatopancreas were carried out by the method of (Ochei and Kolhatkar, 2000). The various steps involved in the preparation of tissues for histopathological studies were fixation, dehydration, clearing, impregnation, embedding, section cutting, staining and mounting

Fixation

In order to avoid tissue autolysis by the autosomal enzymes and to preserve the physical and chemical structure, a bit of tissue from the organ was cut and fixed in Bouin's fluid immediately after removal from the animal body. Bouin's fluid was the commonly used fixative prepared by mixing the following chemicals.

1. Picric acid (saturated) - 75 ml
2. Formaldehyde (40%) - 25 ml
3. Glacial acetic acid - 5 ml

The tissues were fixed in Bouin's fluid for about 24 hrs. The tissues were taken out and washed in tap water to remove excess of picric acid.

Dehydration

The term dehydration means the removal of water from the tissues using ethanol of varying grades. The tissues were kept in the following grades of alcohol each for an hour.

1. 30% alcohol
2. 40% alcohol
3. 70% alcohol
4. 100% alcohol

Clearing

Dealcoholization or replacement of alcohol from the tissues with a clearing agent is called clearing. Xylol was used as the clearing agent. The tissues were kept in the clearing agent till they became transparent and impregnated with xylol.

Impregnation

In this process, the clearing agent xylol was replaced by paraffin wax. The tissues were taken out of xylol and were kept in a molten paraffin embedding bath, which consists of metal posts filled with molten paraffin and maintained at about 50°C. The tissues were given three changes in the molten wax at half an hour intervals.

Embedding

The paraffin wax used for embedding should be fresh and stable in its optimum melting point at about 56°C to 58°C. A clear glass plate was smeared with glycerine. L-shape mould was placed on it to form a rectangular cavity. The paraffin wax was poured into the mould and air bubbles if any were removed by using a hot needle. Tissues were placed in the paraffin and oriented with the surface to be sectioned. Then the tissues were pressed gently towards the glass plate to make it settle uniformly with a metal pressing rod and allowed the wax to settle and solidify at room temperature. The paraffin block was kept in cold water for cooling.

Section cutting

Section cutting was done with a microtome. The excess of paraffin around the tissues was removed through trimming by leaving 0.5 cm around the tissues. Then the block was attached to the gently heated object holds. Additional support was given by some extra wax, which was applied at the sides of the block alone. To produce uniform sections, the microtome knife was adjusted to the proper angle in the knife holder with only the cutting edge

contact with the paraffin block. The tissues were cut at the size range of 5 - 7 micron thickness.

Flattening and mounting of sections

This was carried out in a tissue-floatation warm water bath. The sections were spread on a warm water bath after they were detached from the knife with the help of a hair brush. Dust free clean sides were coated with egg albumin over the whole surface. Required section was spread on the clean side and kept at room temperature.

Staining

The sections were stained as follows:

Deparaffinization with xylol for five minutes.

Dehydration through descending grades of ethyl alcohol as mentioned below

100% absolute alcohol	-	2 minutes
90% absolute alcohol	-	1 minute
70% absolute alcohol	-	1 minute
50% absolute alcohol	-	1 minute

Stain with Ehrlich's Hemetoxylin for 15-20 minutes, Wash in tap water and blowing for 10 minutes then Rinse in the distilled water and Stain with Eosin.

The Dehydration process again with ascending grades of alcohol as given below.

70% absolute alcohol	-	2 minutes
90% absolute alcohol	-	2 minutes
100% absolute alcohol	-	1 minute

Mounting

DPX mountant was applied uniformly over a slide and stained sections were overlaid carefully on the mountant and covered with coverslip.

Observation

Slides were observed under microscope using high magnification objectives and photomicrographs were taken to find out histological changes in comparison with the control tissues.

3.14. Statistical analysis

The results were analyzed by SPSS Software ver. 25. Values were expressed as Mean ± SD for three experiments in each group and significant differences between mean values were determined by One Way Analysis of Variance (ANOVA) followed by Tukey HSD test. $P< 0.05$ was considered significant. $*P<0.05$, $**P<0.001$ statistically significant and NS= Non significant ($P>0.05$) compared with Group I (Control).

CHAPTER-IV

RESULTS

CHAPTER IV

4. RESULTS

The crab fishery in India is fast developing, and there is a vast scope for the crab meat, both national and international markets. Crabs rank third next to the shrimps and lobsters for their esteemed seafood delicacy and also the value of fishery they support (Mohammed Saved and Rajeev Rahavan, 2001). Physico-chemical characteristics of seawater play a crucial role for productive marine ecosystems and fisheries activities. The distribution of pollutants within the aquatic environment is governed by complex processes of material exchange affected by various natural and anthropogenic activities. This physicochemical approach, essential for the qualitative and quantitative assessment of pollutants in the various compartments of the environment (water and organisms) was used in this study to contribute to the assessment of the health status of the Marine ecosystem of Kuduvaiyar estuary at Nagapattinam.

4.1. Physico-chemical and Heavy metals parameters studied in the Kuduvaiyar estuary

The concentration of water quality parameters like temperature, pH, electrical conductivity, dissolved oxygen, biological oxygen demand, chemical oxygen demand, total alkalinity, salinity and total hardness were done triplicate in the laboratory as per the standard methods. Surface water temperature varied with the seasons as lowest in monsoon months (25.11°C) and highest during pre-monsoon (29.13°C). pH varied with the seasons as lowest in monsoon months (7.9) and highest during pre-monsoon (8.7) while

post monsoon pH was 8.3. Highest EC was recorded in Post monsoon (459Mscm$^{-1)}$, lowest during monsoon (376Mscm^{-1}) while pre-monsoon 412Mscm^{-1}. Minimum dissolved oxygen was recorded during monsoon months (5.2mg/L), Maximum dissolved oxygen were recorded during pre-monsoon and post monsoon months (5.9 and 5.3mg/L respectively). COD was lowest in pre-monsoon months (22.10mg/L) and highest during postmonsoon (23.91mg/L) while monsoon COD was 23.13mg/L. BOD was lowest in monsoon months (2.34mg/L) and highest during pre-monsoon (4.57mg/L) while post monsoon BOD was 3.72mg/L.

Alkalinity varied with the seasons as lowest in monsoon months (118.37mg/L) and highest during pre-monsoon (119.25mg/L) while post monsoon Alkalinity was 118.95mg/L. Salinity was low during monsoon (23.18 PSU) and high during pre-monsoon (25.08PSU) while post monsoon salinity was 24.79PSU. The hardness was lowest in monsoon months (1637mg/L) and highest during pre-monsoon (1727mg/L) while post monsoon hardness was 1703mg/L (Table 4.1 and fig 4.1- 4.3).

The ammonium was lowest in monsoon months (0.45mg/L) and highest during pre-monsoon (0.62mg/L) while post monsoon hardness was 0.58mg/L. The nitrate was lowest in monsoon months (32.67mg/L) and highest during pre-monsoon (38.57mg/L) while post monsoon hardness was 35.92mg/L. The sodium was lowest in monsoon months (97.51mg/L) and highest during pre-monsoon (132.49mg/L) while post monsoon hardness was 125.74mg/L. The chloride was lowest in monsoon months (213.47mg/L) and highest during pre-

monsoon (302.28mg/L) while post monsoon hardness was 286.51mg/L. The sulphate was lowest in monsoon months (73.21mg/L) and highest during pre-monsoon (86.92mg/L) while post monsoon hardness was 80.65mg/L. The fluoride was lowest in monsoon months (0.54mg/L) and highest during pre-monsoon (0.65mg/L) while post monsoon hardness was 0.59mg/L (Table 4.1 and fig 4.3-4.4).

In pre-monsoon, the maximum level of cadmium (0.017mg/L) was observed followed by chromium (0.007mg/L), copper (0.004mg/L),lead (0.09mg/L), mercury (0.00005mg/L), nickel (0.03mg/L) and zinc (0.05mg/L) . In monsoon, the minimum level of cadmium (0.011mg/L) was observed followed by chromium(0.005mg/L), copper(0.002mg/L), lead (0.05mg/L), mercury (0.00002mg/L), nickel (0.01mg/L) and zinc (0.02mg/L). In post monsoon, level of cadmium (0.015mg/L) was observed followed by chromium (0.006mg/L), copper (0.003mg/L), lead (0.07mg/L), mercury (0.00003mg/L), nickel (0.02mg/L) and zinc (0.04mg/L), (Table 4.1 and Fig. 4.5- 4.6).

Among the various seasons, the maximum level of cadmium was observed in pre monsoon.

Table 4.1: Physico-chemical and heavy metal analysis of Kuduvaiyar estuary, East coast of Tamil Nadu (2019-2020)

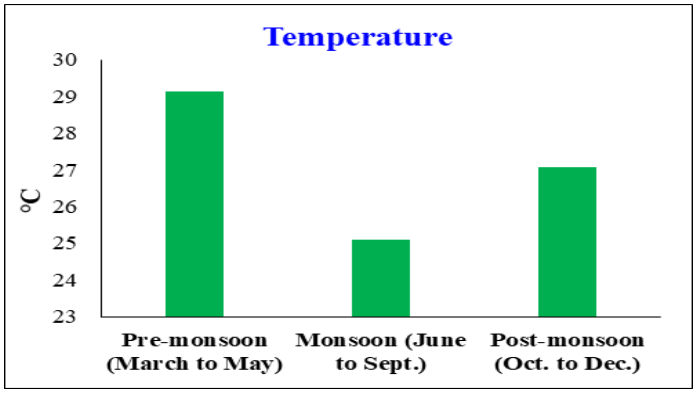
Parameters	Season			* Fisheries and aquatic life (1996) Std.
	Pre-monsoon (March to May)	Monsoon (June to Sept.)	Post-monsoon (Oct. to Dec.)	
Temperature (°C)	29.13	25.11	27.07	-
pH	8.7	7.9	8.3	6.0-9.0
EC (MS CM^{-1})	412	376	459	-
DO (mg/L)	5.9	5.2	5.3	4.0-6.0
COD (mg/L)	22.10	23.13	23.91	-
BOD (mg/L O_2)	4.57	2.34	3.72	3
Alkalinity (mg/L)	119.25	118.37	118.95	-
Salinity (PSU)	25.08	23.18	24.79	-
Hardness (mg/L)	1727	1637	1703	-
Ammonium (mg/L)	0.62	0.45	0.58	0.5
Nitrate (mg/L)	38.57	32.67	35.92	40
Sodium (mg/L)	132.49	97.51	125.74	120
Chloride (mg/L)	302.28	213.47	286.51	300
Sulphate (mg/L)	86.92	73.21	80.65	100
Fluoride (mg/L)	0.65	0.54	0.59	0.75
Heavy metals (Trace elements)				
Cadmium (mg/L)	**0.017**	**0.011**	**0.015**	0.005
Chromium (mg/L)	0.007	0.005	0.006	0.02-0.005
Copper (mg/L)	0.004	0.002	0.003	0.001
Lead (mg/L)	0.09	0.05	0.07	0.1
Mercury (mg/L)	0.00005	0.00002	0.00003	0.00001
Nickel (mg/L)	0.03	0.01	0.02	0.01
Zinc (mg/L)	0.05	0.02	0.04	0.01

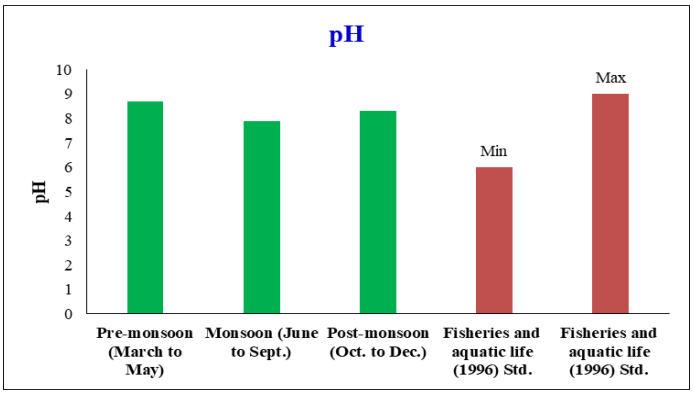
* Maximum allowable concentrations of selected water quality variables for Fisheries and aquatic life uses.

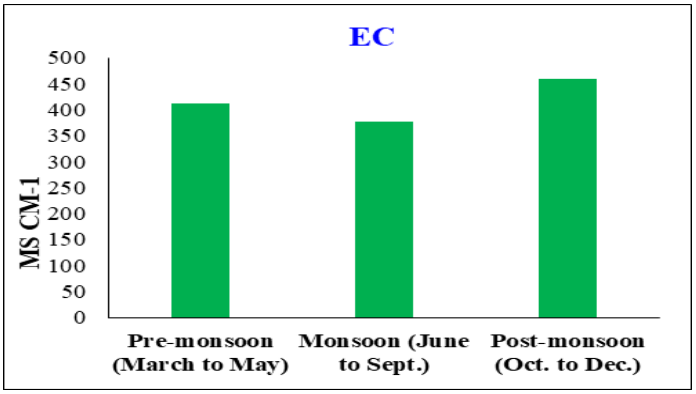
Table 4.2: Correlation matrix (n = 3) of Physico-Chemical parameters in Kuduvaiyar estuary , East coast of Tamil Nadu (2019-2020)

Parameters	Temperature	pH	EC	DO	COD	BOD	Alkalinity	Salinity	Hardness	Ammonium	Nitrate	Sodium	Chloride	Sulphate	Fluoride
Temperature	1														
pH	0.999	1													
EC	0.419	0.432	1												
DO	0.929	0.924	0.056	1											
COD	-0.579	-0.567	0.497	-0.838	1										
BOD	0.988	0.990	0.551	0.864	-0.450	1									
Alkalinity	0.980	0.983	0.588	0.840	-0.409	0.998	1								
Salinity	0.922	0.928	0.737	0.716	-0.219	0.970	0.980	1							
Hardness	0.961	0.965	0.652	0.793	-0.333	0.991	0.996	0.993	1						
Ammonium	0.952	0.956	0.677	0.772	-0.301	0.987	0.993	0.996	0.999	1					
Nitrate	0.997	0.998	0.484	0.900	-0.518	0.996	0.992	0.948	0.979	0.971	1				
Sodium	0.937	0.942	0.708	0.743	-0.259	0.979	0.987	0.999	0.996	0.999	0.960	1			
Chloride	0.932	0.937	0.719	0.733	-0.244	0.975	0.984	0.999	0.995	0.998	0.955	0.999	1		
Sulphate	0.997	0.998	0.476	0.904	-0.526	0.996	0.991	0.945	0.977	0.969	0.999	0.957	0.953	1	
Fluoride	0.999	0.998	0.384	0.943	-0.609	0.982	0.972	0.907	0.950	0.939	0.993	0.923	0.917	0.994	1

Correlation matrix was described for relationship between the physico-chemical parameters







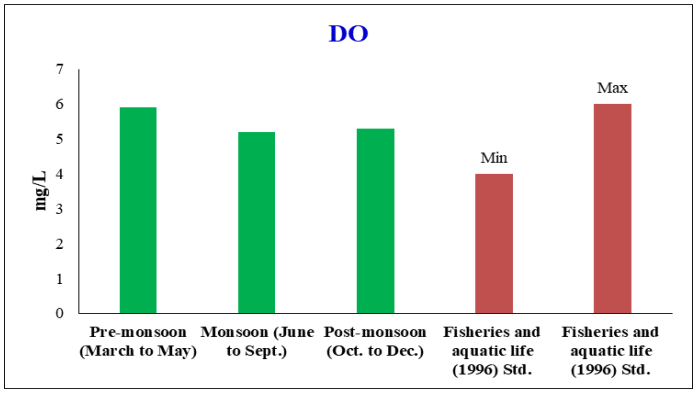
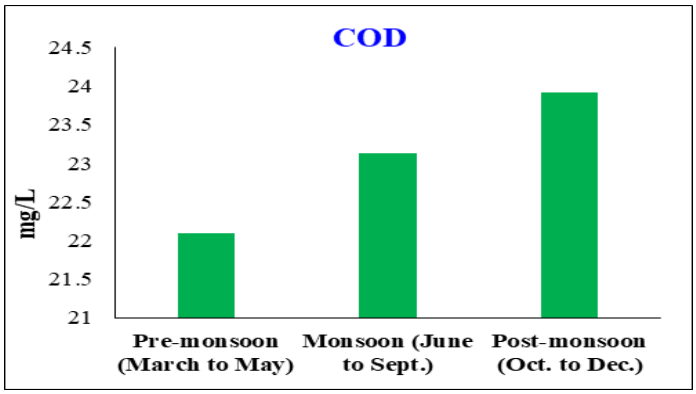
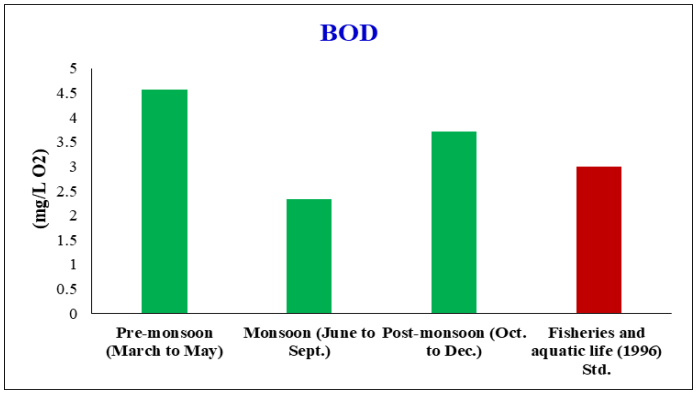
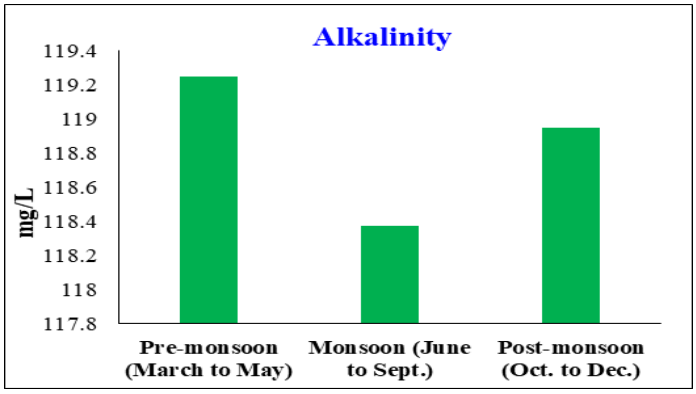


Fig. 4.1: Seasonal variations in physico-chemical parameters of Kuduvaiyar estuary, East coast of Tamil Nadu (2019-2020)







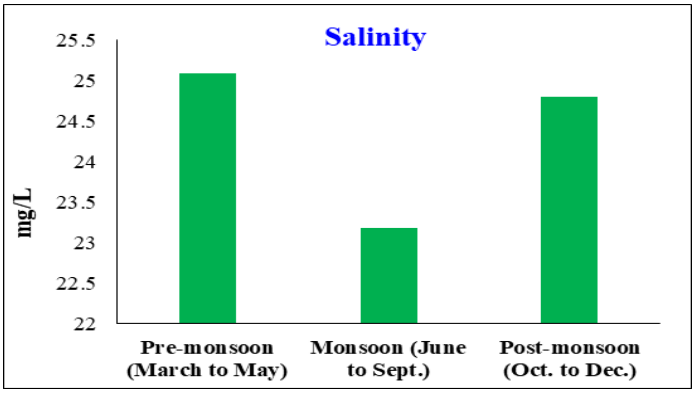
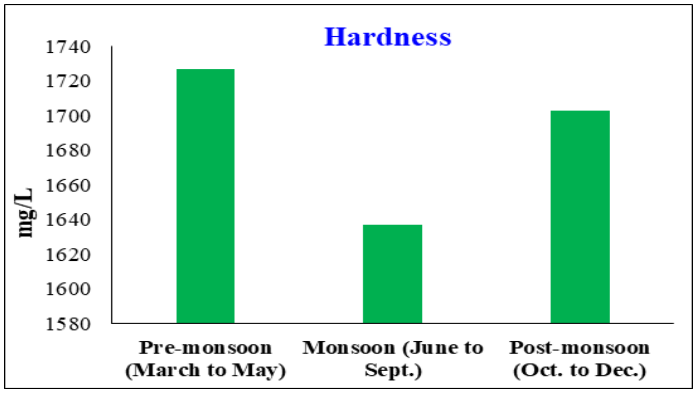
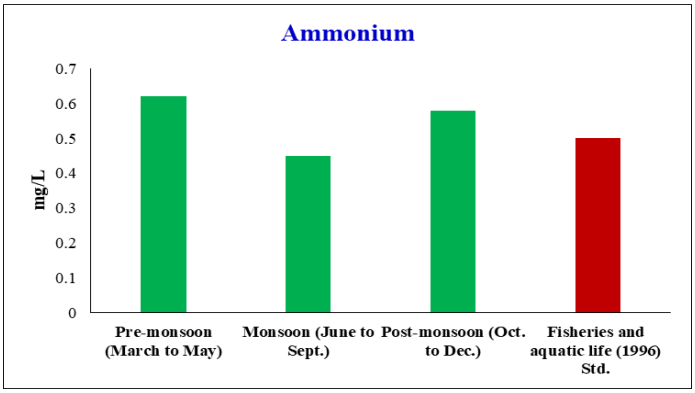


Fig. 4.2: Seasonal variations in physico-chemical parameters of Kuduvaiyar estuary, East coast of Tamil Nadu (2019-2020)





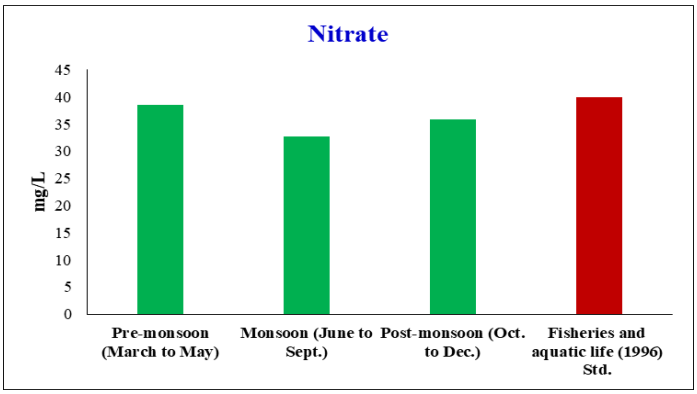
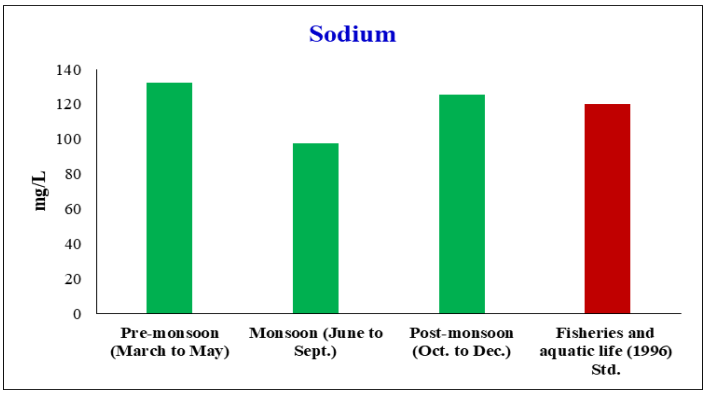
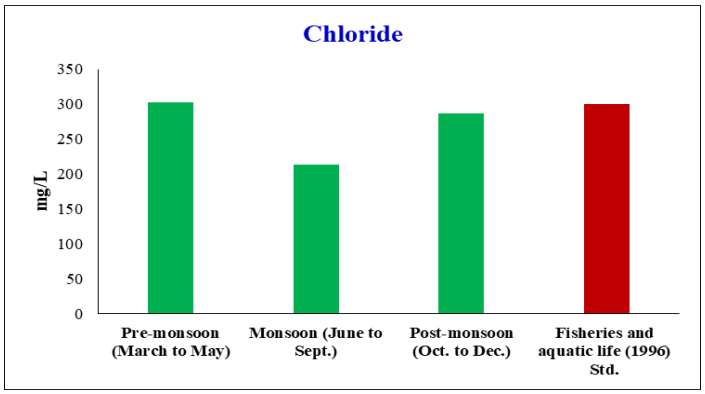
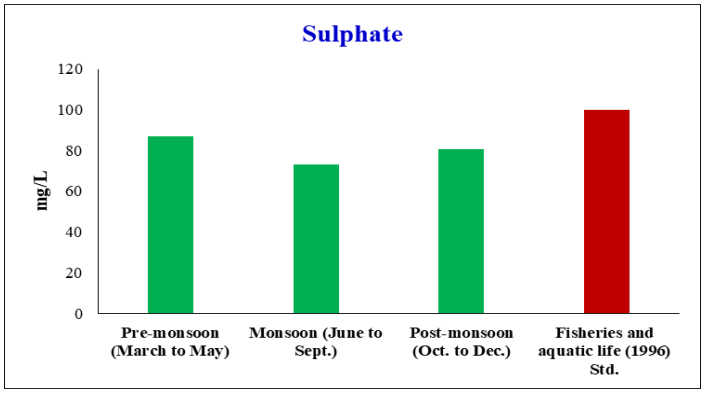


Fig. 4.3: Seasonal variations in physico-chemical parameters of Kuduvaiyar estuary, East coast of Tamil Nadu (2019-2020)







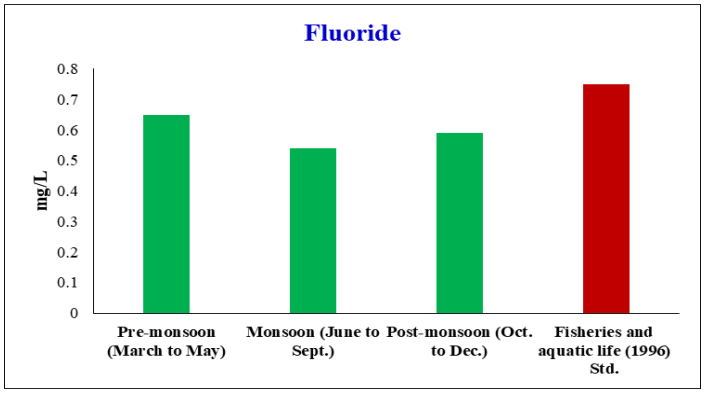


Fig. 4.4: Seasonal variations in physico-chemical parameters of Kuduvaiyar estuary, East coast of Tamil Nadu (2019-2020)

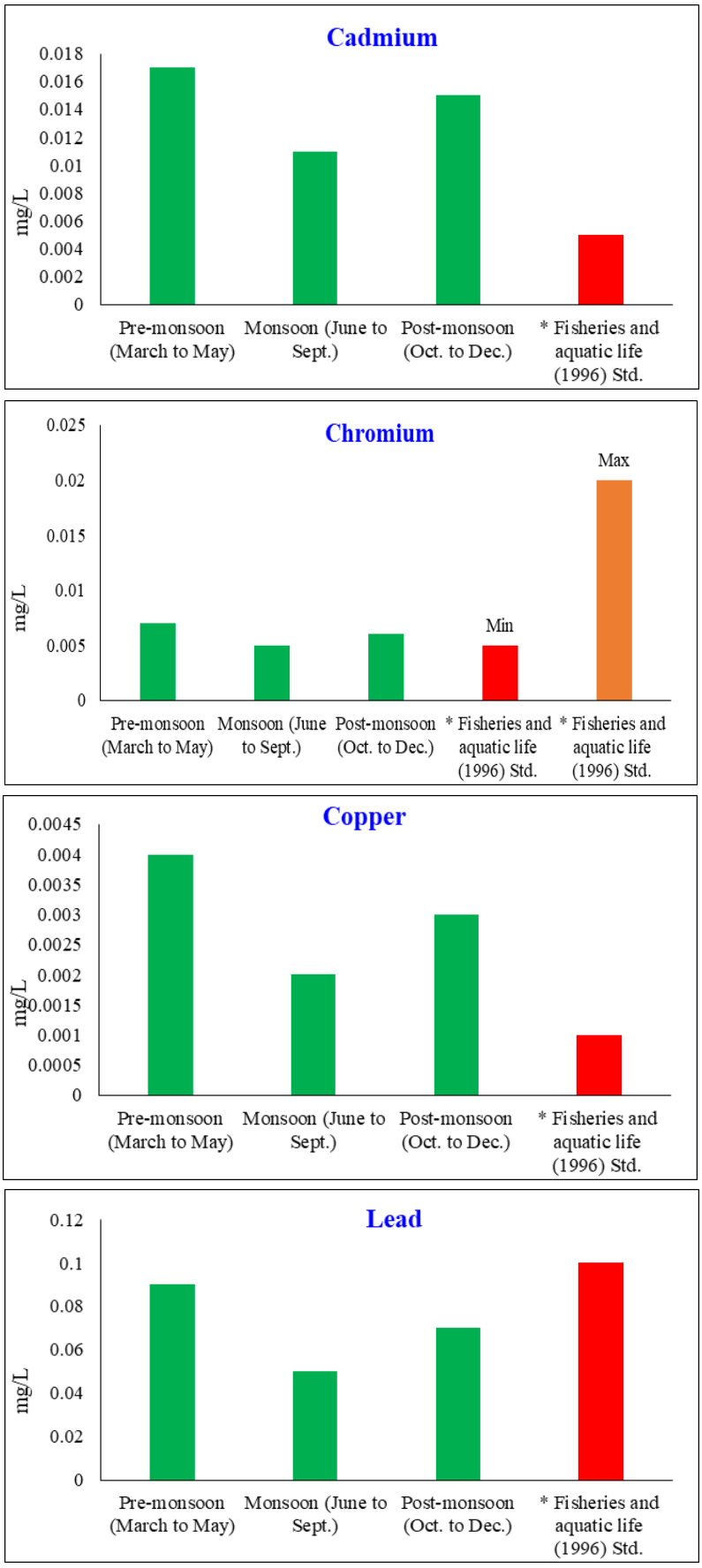
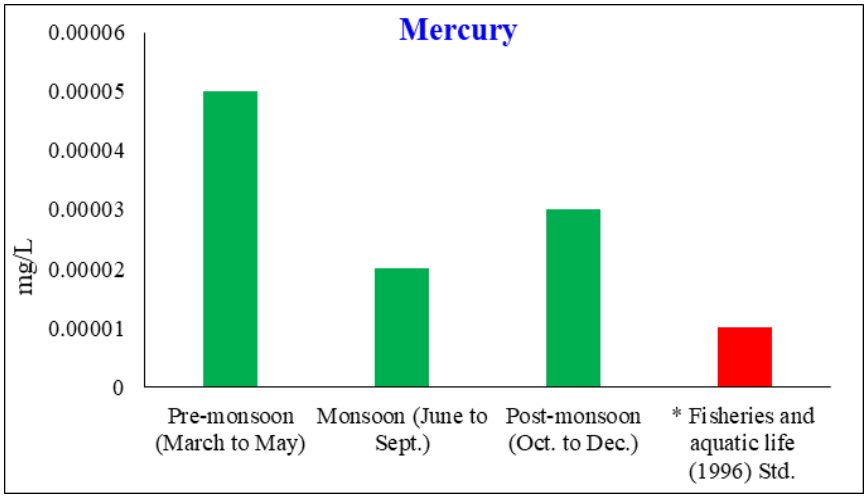
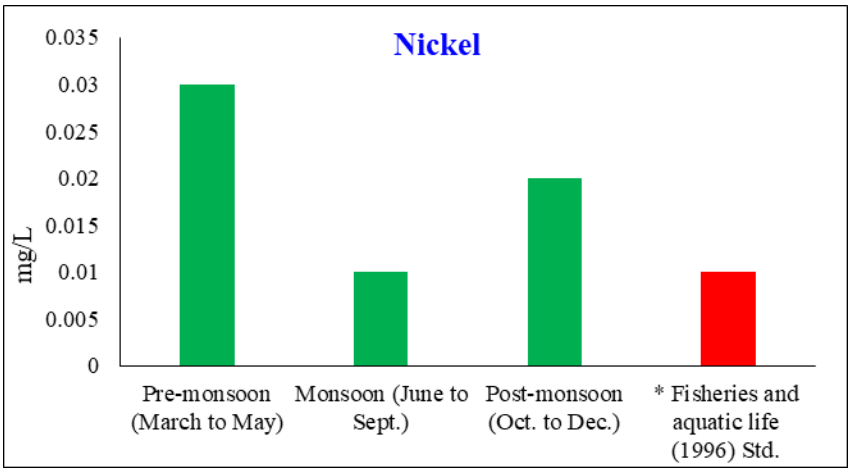


Fig. 4.5: Seasonal variations of heavy metals distribution in Kuduvaiyar estuary, East coast of Tamil Nadu (2019-2020)





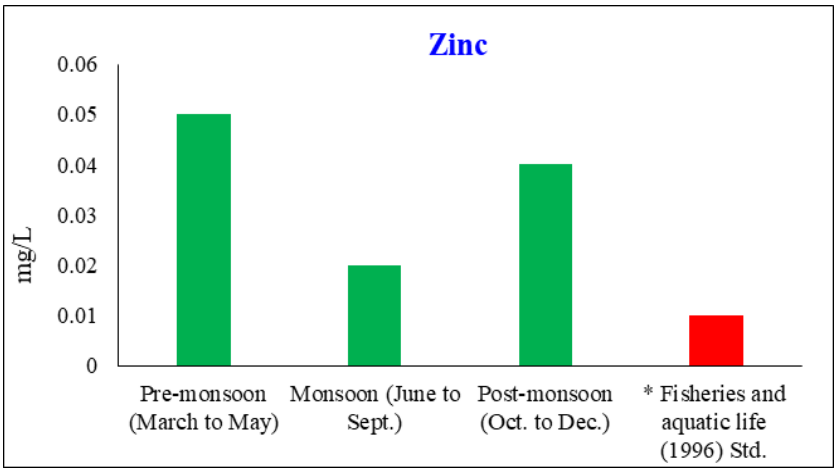


Fig. 4.6: Seasonal variations of heavy metals distribution in Kuduvaiyar estuary, East coast of Tamil Nadu (2019-2020)

4.2. Acute toxicity studies of cadmium on *Sesarma quadratum*

To determine the LC_{50} value graphs were plotted between % mortality and log concentrations of toxicant. The concentrations obtained by drawing a perpendicular line against 50% mortality and calculated their antilog value. In the present investigation, LC_{50} of *Sesarma quadratum* at 96h was 12.30µg/L for cadmium. Results of present studies (Table 4.3 and figure 4.7), clearly indicated that the rate of mortality for any fixed time increased with increase in concentration and for a particular concentration with increase in exposure time and a regular mode of toxicant due to accumulation up to dangerous level leading to death. Table-4.3 shows the relation between the cadmium concentration and the mortality rate of *Sesarma quadratum*. The results obtained for 96 hour toxicity experiments of cadmium crabs were estimated by Finney's probit analysis method.

The observed percentage of mortality in *Sesarma quadratum* exposed to different concentration of cadmium is given in table 4.4. Figure 4.7 showed the probit line graph of the cadmium toxicity data and probit kill vs log concentrations. The 96-hr LC_{50} study observed that *Sesarma quadratum* was significantly more susceptible to cadmium toxicity. Present 96 hours of crabs mortality were concentration (µg/L) of cadmium-dependent $R^2 = 0.9592$.

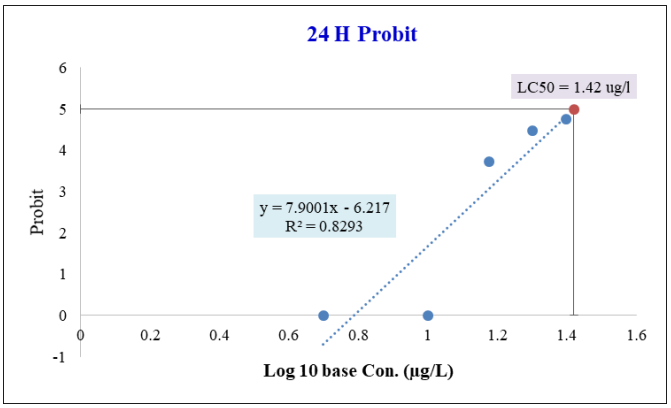
Table 4.3: Toxicity of cadmium against crab as *Sesarma quadratum*

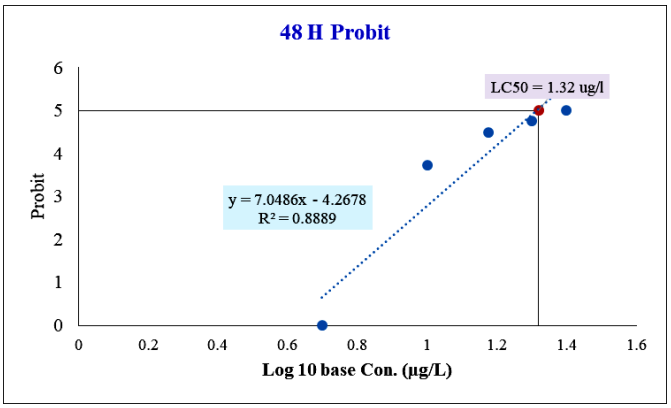
Concentration (µg/L)	Log Con. (µg/L)	# of exposed crab	24 hours			48 hours			72 hours			96 hours		
			# of mortality	% of mortality	Probit	# of mortality	% of mortality	Probit	# of mortality	% of mortality	Probit	# of mortality	% of mortality	Probit
5	0.69	10	0	0	0	0	0	0	0	0	0	0	0	0
10	1.00	10	0	0	0	1	10	3.72	1	10	3.72	3	30	4.48
15	1.17	10	1	10	3.72	3	30	4.48	4	40	4.75	7	70	5.52
20	1.30	10	3	30	4.48	4	40	4.75	7	70	5.52	9	90	6.28
25	1.39	10	4	40	4.75	5	50	5	9	90	6.28	10	100	7.33
Control	0	10	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

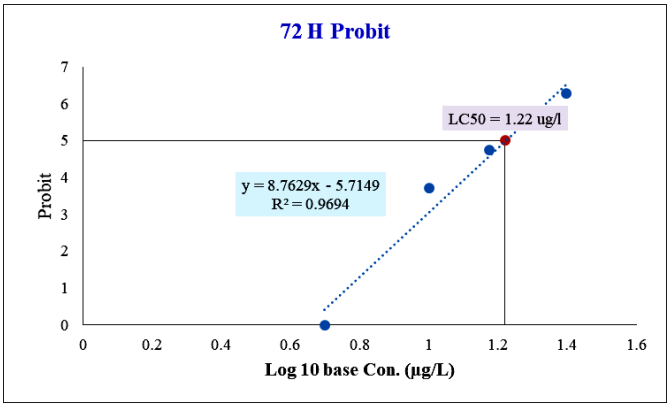
Table 4.4: LC_{50} value of cadmium against crab as *Sesarma quadratum*

Hours	LC_{50} µg/L (LCL - UCL)	Regression equation	r^2 value	Chi-Square (Df = 3)	
				X^2 value	Sig.
24	26.58 (21.35 - 195.88)	y = 7.9001x - 6.217	0.8293	0.316	0.957
48	23.58 (17.82 - 68.18)	y = 7.0486x - 4.267	0.8889	0.290	0.962
72	16.24 (13.23 - 19.46)	y = 8.7629x - 5.714	0.9694	0.067	0.995
96	12.30 (9.57 - 14.58)	y = 10.072x - 6.505	0.9592	0.379	0.945

Data analyzed by SPSS ver. 20, Regression equation and r^2 value was calculated by log. Concentration vs probit value, LCL: 95% of Lower Confidence Limits, UCL: 95% of Upper Confidence Limits. Since the significance level is $P > 0.050$, no heterogeneity factor is used in the calculation of confidence limits.







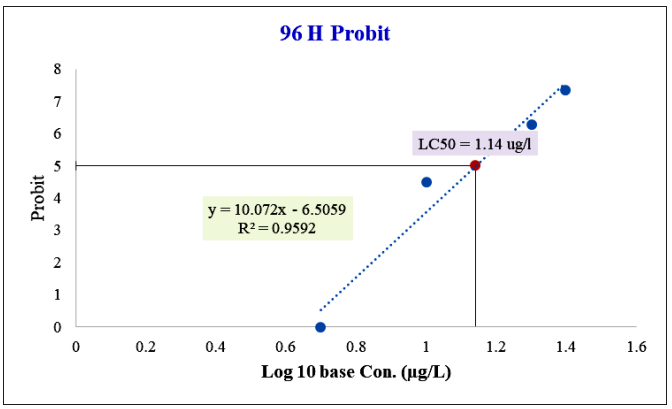


Fig. 4.7: Graphs show the toxicity of cadmium against crab as *Sesarma quadratum* (determination of LC50)

4.3. Biochemical response in subacute concentrations of cadmium exposure in *Sesarma quadratum*

The biochemical analysis is also known as composition of some fundamental elements like protein, lipids and carbohydrates etc. In the present study, Protein, lipids and carbohydrates were investigated in gills, muscles and hepatopancreas of control and experimental crabs as *Sesarma quadratum*. Protein, lipids and carbohydrate content were significantly decreased in gills, muscles and hepatopancreas of *Sesarma quadratum*. The decreased carbohydrate, protein and lipid contents directly proportional to the duration of the cadmium exposure.

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significantly decreased, the protein content in gills (7.68±0.51mg/gm), muscles (15.23±1.02mg/gm), and hepatopancreas (11.25±0.81mg/gm) as compared to control were observed. In 20 days exposure of $CdCl_2$ also significantly decreased of protein content in gills (5.76±0.61mg/gm), muscles (12.71±0.94mg/gm), and hepatopancreas (8.64±0.84mg/gm) as compared to control were observed. In 30 days exposure of $CdCl_2$ also significant decrease of protein content in gills (3.95±0.53mg/gm), muscles(9.17±0.71mg/gm) and hepatopancreas (5.30±0.92mg/gm) as compared to control were observed (Table 4.5 and Fig. 4.8). Among the various duration of exposure of $CdCl_2$, 30 days exposure of $CdCl_2$ has significant alterations were observed.

Table 4.5: Effect of cadmium exposure (12µg/L) on protein content in crab, *Sesarma quadratum*

Protein (mg/100gm)	Group I (Control)	Cadmium exposed 12µg/L		
		Group II (10^{th} day)	Group III (20^{th} day)	Group IV (30^{th} day)
Gills	9.29±1.76	7.68±0.51*	5.76±0.61**	3.95±0.53***
Muscles	18.44±1.24	15.23±1.02*	12.71±0.94**	9.17±0.71***
Hepatopancreas	14.57±0.95	11.25±0.81*	8.64±0.84**	5.30±0.92***

Values were expressed as Mean ± SD for three experiments in each group and significant differences between mean values were determined by One Way Analysis of Variance (ANOVA) followed by Tukey HSD test. $P<0.05$ was considered significant. *$P<0.05$, **$P<0.01$, ***$P<0.001$ statistically significant and NS= Non significant ($P>0.05$) compared with Group I (Control).

In the present study protein level of Muscles > Hepatopancreas > Gills in control and experimental crab were found. The levels of protein were found to be significantly decreased in cadmium chloride exposed crab comparison with control crabs in muscles, hepatopancreas and gills.

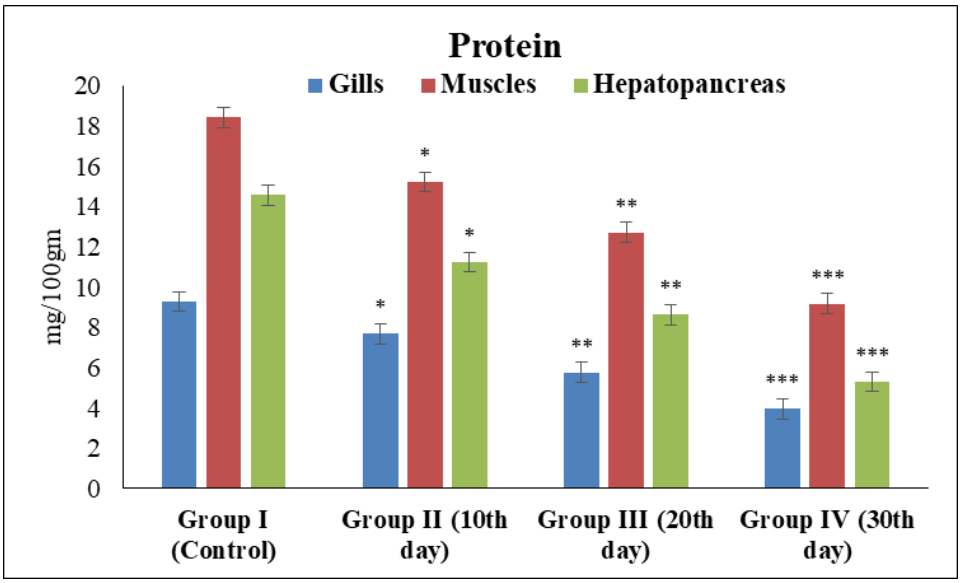


Fig. 4.8: Effect of cadmium exposure (12µg/L) on protein content in crab, *Sesarma quadratum*

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum*, significantly decreased of lipid content in gills (5.74±0.21mg/gm), muscles (3.31±0.31mg/gm) and hepatopancreas(13.24±1.17mg/gm) as compared to control were observed. In 20 days exposure of $CdCl_2$ also significantly decreased of lipid content in gills(3.74±0.24mg/gm),muscles (2.04±0.15mg/gm) and hepatopancreas (9.95±1.15mg/gm), as compared to control were observed. In 30 days exposure of $CdCl_2$ also significant decrease of lipids content in gills (2.15±0.21mg/gm), muscles (1.37±0.17mg/gm and hepatopancreas (6.49±1.05mg/gm),) as compared to control were observed (Table 4.6 and Fig. 4.9). Among the various duration of exposure of $CdCl_2$, 30 days exposure of $CdCl_2$ has significant alterations were observed.

Table 4.6: Effect of cadmium exposure (12µg/L) on lipids content in crab, *Sesarma quadratum*

Lipids (mg/100gm)	Group I (Control)	Cadmium exposed 12µg/L		
		Group II (10^{th} day)	Group III (20^{th} day)	Group IV (30^{th} day)
Gills	6.79±0.38	5.74±0.21*	3.74±0.24**	2.15±0.21***
Muscles	5.24±0.29	3.31±0.31*	2.04±0.15**	1.37±0.17***
Hepatopancreas	16.09±1.32	13.24±1.17*	9.95±1.15**	6.49±1.05***

Values were expressed as Mean ± SD for three experiments in each group and significant differences between mean values were determined by One Way Analysis of Variance (ANOVA) followed by Tukey HSD test. $P<0.05$ was considered significant. *$P<0.05$, **$P<0.01$, ***$P<0.001$ statistically significant and NS= Non significant ($P>0.05$) compared with Group I (Control).

In the present study lipids level of Hepatopancreas > Gills > Muscles in control and experimental crab were found. The levels of lipids were found to be significantly decreased in cadmium chloride exposed crab comparison with control crabs in hepatopancreas, gills and muscle tissue.

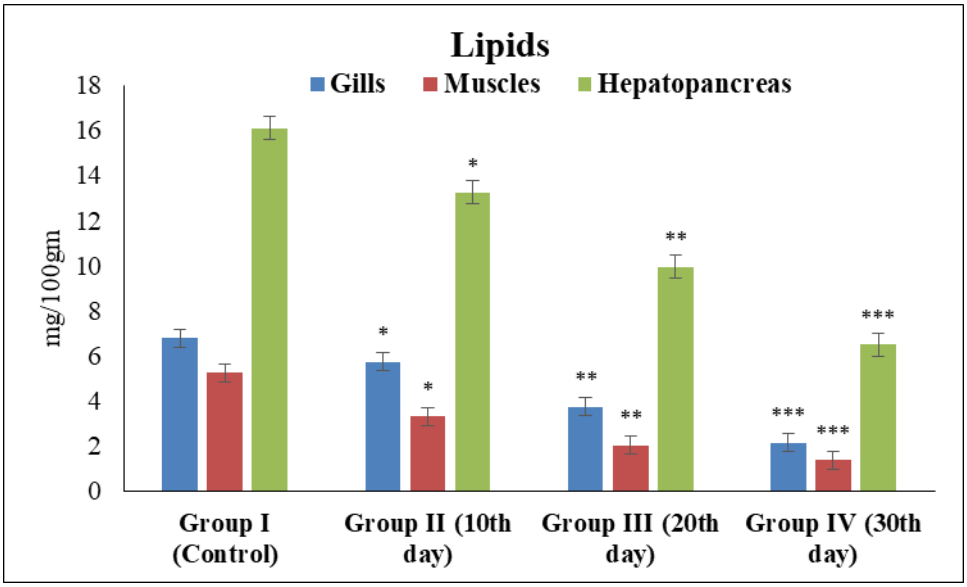


Fig. 4.9: Effect of cadmium exposure (12µg/L) on lipids content in crab, *Sesarma quadratum*

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significantly decreased of carbohydrate content in gills (0.97±0.11mg/gm), muscles(1.52±0.12mg/gm) and hepatopancreas (1.53±0.09mg/gm), as compared to control were observed. In 20 days exposure of $CdCl_2$ also significant decreased of carbohydrate content in hepatopancreas (1.16±0.13mg/gm), muscles (0.89±0.12mg/gm) and gills (0.71±0.18mg/gm) as compared to control were observed. In 30 days exposure of $CdCl_2$ also significant decreased of carbohydrate content hepatopancreas (0.94±0.08mg/gm), muscles (0.61±0.10mg/gm) and gills (0.54±0.09mg/gm) as compared to control were observed (Table 4.7 and Fig. 4.10). Among the various duration of exposure of $CdCl_2$, 30 days exposure of $CdCl_2$ has significant alterations were observed.

Table 4.7: Effect of cadmium exposure (12µg/L) on carbohydrate content in crab, *Sesarma quadratum*

Carbohydrate (mg/100gm)	Group I (Control)	Cadmium exposed 12µg/L		
		Group II (10th day)	Group III (20th day)	Group IV (30th day)
Gills	1.44±0.14	0.97±0.11*	0.71±0.18**	0.54±0.09***
Muscles	1.74±0.17	1.52±0.12*	0.89±0.12**	0.61±0.10***
Hepatopancreas	2.79±0.19	1.53±0.09*	1.16±0.13**	0.94±0.08***

Values were expressed as Mean ± SD for three experiments in each group and significant differences between mean values were determined by One Way Analysis of Variance (ANOVA) followed by Tukey HSD test. $P<0.05$ was considered significant. *$P<0.05$, **$P<0.01$, ***$P<0.001$ statistically significant and NS= Non significant ($P>0.05$) compared with Group I (Control).

In the present study Carbohydrate level of Hepatopancreas > Muscles > Gills in control and experimental crab were found. The levels of Carbohydrate were found to be significantly decreased in cadmium chloride exposed crab comparison with control crabs in hepatopancreas, muscles and gill tissue.

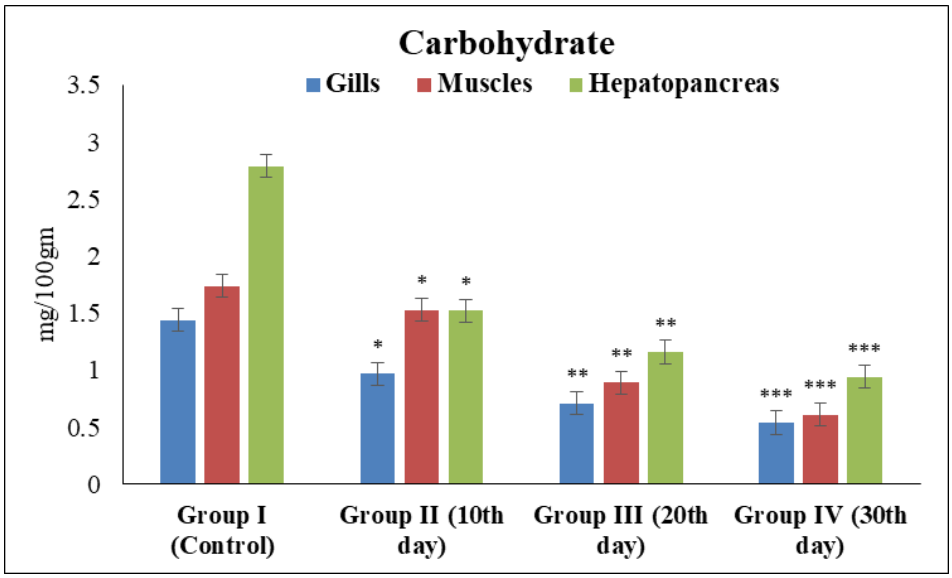


Table 4.10: Effect of cadmium exposure (12µg/L) on carbohydrate content in crab, Sesarma quadratum

4.4. Immunological parameters in subacute concentrations of cadmium chloride exposure in *Sesarma quadratum*

Immune systems of crustaceans include both cellular and noncellular defense responses and circulating haemocytes are part of the defense against potential pathogens. Since this is an early internal defense by circulating haemocytes against pathogens, decrease in total haemocyte count (THC) or phagocytic activity (PA) due to the exposure of contaminants can impair the defensive response of the host against pathogens. In the present study to investigate the immunological parameters as total haemocyte count, phenoloxidase assay, phagocytosis and lysosomal membrane stability. The altered level of total haemocyte count, phenoloxidase assay, phagocytosis and lysosomal membrane stability were observed. The phenoloxidase activity was directly proportional to the duration of the cadmium chloride exposure.

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of haemocyte count (4.9±0.09mg/gm), phagocytosis (13.18±1.83mg/gm) and lysosomal membrane stability (5.29±0.11mg/gm) while phenoloxidase assay was significantly increased (0.012±0.002mg/gm) as compared to control were observed. In 20 days exposure of $CdCl_2$ on *Sesarma quadratum* significantly decreased of haemocyte count (3.1±0.08mg/gm), phagocytosis (9.81±1.58mg/gm) and lysosomal membrane stability (3.65±0.09mg/gm) while phenoloxidase assay was significant increased (0.017±0.002mg/gm) as compared to control were observed. In 30 days exposure of $CdCl_2$ on *Sesarma quadratum* significant

decrease of haemocyte count (2.3±0.08mg/gm), phagocytosis (5.39±1.35mg/gm) and lysosomal membrane stability (2.17±0.010mg/gm) while phenoloxidase assay was significantly increased (0.025±0.003mg/gm) as compared to control were observed (Table 4.8 and Fig. 4.11). Among the various duration of exposure of $CdCl_2$, 30 days exposure of $CdCl_2$ has significant alterations were observed.

Table 4.8: Effect of cadmium exposure (12µg/L) on immunological parameters in crab, *Sesarma quadratum*

Immunological parameters	Group I (Control)	Cadmium exposed 12µg/L		
		Group II (10^{th} day)	Group III (20^{th} day)	Group IV (30^{th} day)
Total haemocyte count (No. x 10^6 mL/L)	6.7±0.12	4.9±0.09*	3.1±0.08**	2.3±0.08***
Phenoloxidase assay (mg protein/L	0.006±0.001	0.012±0.002*	0.017±0.002**	0.025±0.003***
Phagocytosis (%)	22.53±1.76	13.18±1.83*	9.81±1.58**	5.39±1.35***
Lysosomal membrane stability (mg mL protein/L	6.52±0.15	5.29±0.11*	3.65±0.09**	2.17±0.010***

Values were expressed as Mean ± SD for three experiments in each group and significant differences between mean values were determined by One Way Analysis of Variance (ANOVA) followed by Tukey HSD test. $P<0.05$ was considered significant. *$P<0.05$, **$P<0.01$, ***$P<0.001$ statistically significant and NS= Non significant ($P>0.05$) compared with Group I (Control).

The levels of total haemocyte count, lysosomal membrane stability and phagocytosis were significantly decreased while phenoloxidase was significantly increased in cadmium chloride exposed crab comparison with control crabs.

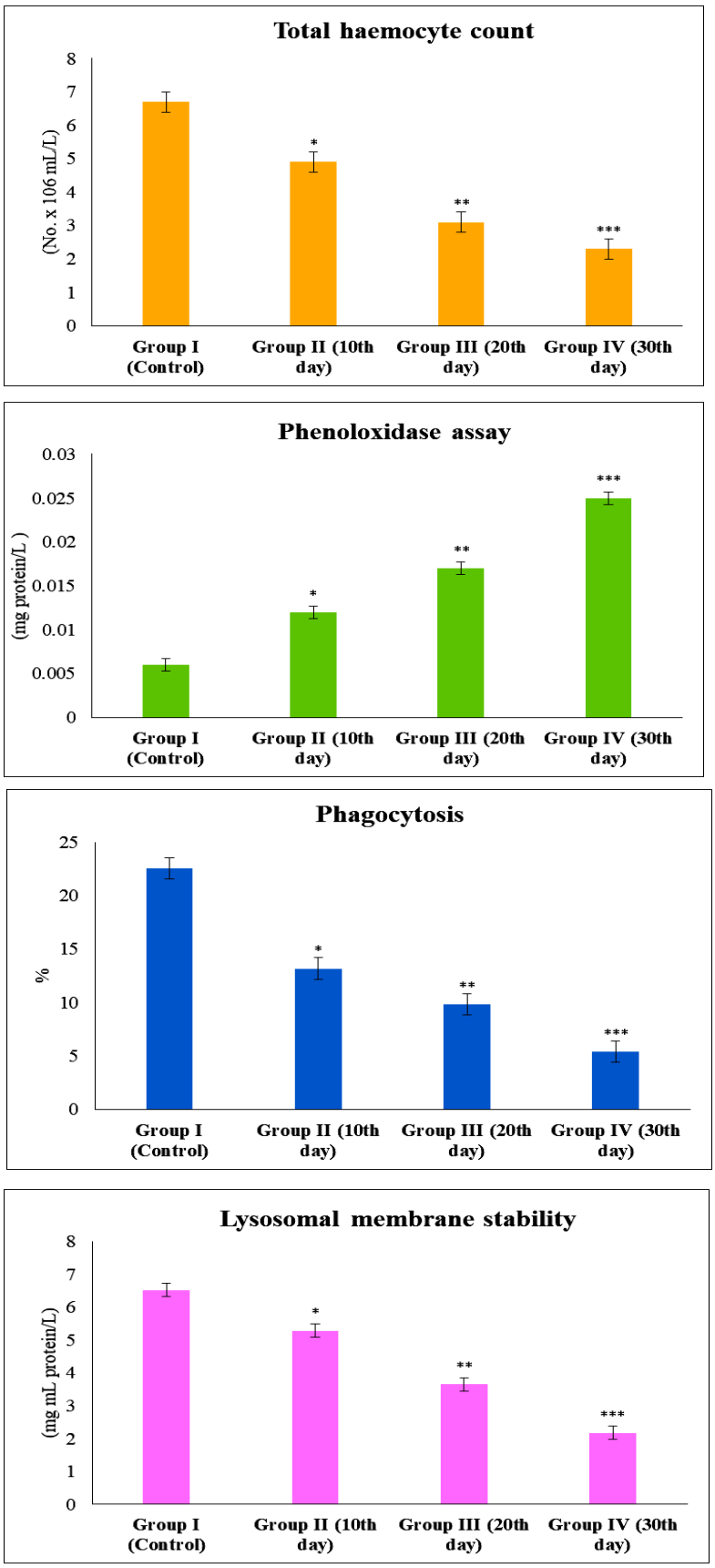


Fig. 4.11: Effect of cadmium exposure (12µg/L) on immunological parameters in crab, *Sesarma quadratum*

4.5. Stress markers in subacute concentrations of cadmium chloride exposure in *Sesarma quadratum*

Lipid peroxidation induced by metals at sub-lethal levels, which alter the physiological and biochemical characteristics of biological systems. To counter the detrimental effects of the prooxidant activity of metals, a group of antioxidant enzyme systems function in the organisms. The present study was performed to investigate lipid peroxidation product formation due to the exposure to effects of the metal namely cadmium at sub-lethal concentrations and the biological response through protective antioxidant enzyme activity in the marine crab, *Sesarma quadratum.* Variations in the activity of antioxidant enzymes have been proposed as biomarkers of pollutant mediated oxidative stress. Because oxidative stress responses are directly associated with cellular function, they may give a clear indication of the environment's local pollution status. In the present study to investigate the content of Malondialdehyde (MDA) and antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S- transferase (GST). The data indicated that SOD, CAT, GPx and GST were expressed in *Sesarma quadratum* due to metal stress.

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significant increase of MDA content in serum (1.72±0.09nmole of MDA formed/mg protein in tissues), gills (11.27±1.43nmole of MDA formed/mg protein in tissues), hepatopancreas (15.32±1.83nmole of MDA formed/mg protein in tissues) and muscles (9.19±0.81nmole of MDA formed/mg protein in tissues) as compared to control were observed.

In the observation, on 20 days exposure of $CdCl_2$ on *Sesarma quadratum* significantly increased of MDA content in serum (1.96±0.05nmole of MDA formed/mg protein in tissues), gills (16.71±1.69nmole of MDA formed/mg protein in tissues), hepatopancreas (19.74±1.53nmole of MDA formed/mg protein

in tissues) and muscles (13.27±1.08nmole of MDA formed/mg protein in tissues) as compared to control were observed.

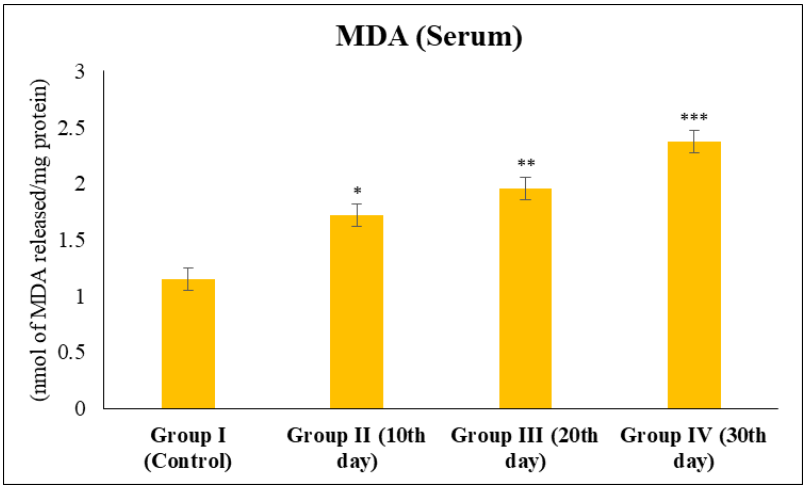
In the observation, on 30 days exposure of $CdCl_2$ on *Sesarma quadratum* significant increase of MDA content in serum (2.37±0.05nmole of MDA formed/mg protein in tissues), gills (19.84±1.53nmole of MDA formed/mg protein in tissues), hepatopancreas (23.64±1.27nmole of MDA formed/mg protein in tissues) and muscles (17.64±1.01nmole of MDA formed/mg protein in tissues) as compared to control were observed. Among the 10, 20 and 30 days exposure of $CdCl_2$, MDA content in *Sesarma quadratum* was significantly increased in 30 days exposure compared to 10 and 20 days exposure (Table 4.9 and Fig. 4. 12). Among the various duration of exposure of $CdCl_2$, 30 days exposure of $CdCl_2$ has significant alterations were observed.

Table 4.9: Effect of cadmium exposure (12µg/L) on MDA content in crab, *Sesarma quadratum*

MDA	Group I (Control)	Cadmium exposed 12µg/L		
		Group II (10^{th} day)	Group III (20^{th} day)	Group IV (30^{th} day)
Serum (nmol of MDA released/mg protein)	1.15±0.07	1.72±0.09*	1.96±0.05**	2.37±0.05***
Gills (nmol of MDA released/mg protein)	8.43±1.56	11.27±1.43*	16.71±1.69**	19.84±1.53***
Hepatopancreas (nmol of MDA released/mg protein)	9.54±1.15	15.32±1.83*	19.74±1.53**	23.64±1.27***
Muscles (nmol of MDA released/mg protein)	7.29±0.94	9.19±0.81*	13.27±1.08**	17.64±1.01***

Values were expressed as Mean ± SD for three experiments in each group and significant differences between mean values were determined by One Way Analysis of Variance (ANOVA) followed by Tukey HSD test. $P< 0.05$ was considered significant. *$P<0.05$, **$P<0.01$, ***$P<0.001$ statistically significant and NS= Non significant ($P>0.05$) compared with Group I (Control).

In the present study MDA level of Hepatopancreas > Gills > Muscles > Serum in control and experimental crab were found. The levels of MDA were found to be significantly increased in cadmium chloride exposed crab comparison with control crabs in muscles, hepatopancreas, gills and serum.



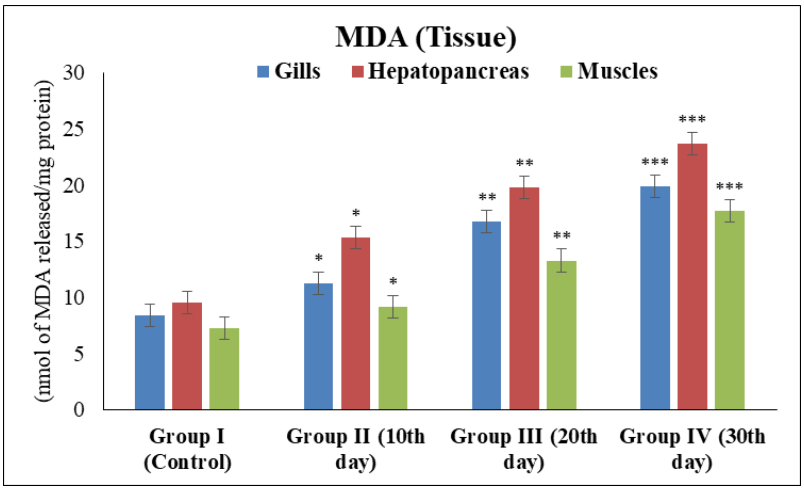


Fig. 4.12: Effect of cadmium exposure (12µg/L) on MDA content in crab, *Sesarma quadratum*

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significantly decreased of SOD content in serum (0.23±0.04mg protein/L), gills (0.42±0.09U/mg protein), hepatopancreas (0.74±0.18U/mg protein) and muscles (0.45±0.05U/mg protein) as compared to control were observed.

In the observation, on 20 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of SOD content in serum (0.18±0.04mg protein/L), gills (0.26±0.05U/mg protein), hepatopancreas (0.53±0.15U/mg

protein) and muscles (0.31±0.05U/mg protein) as compared to control were observed.

In the observation, on 30 days exposure of $CdCl_2$ on *Sesarma quadratum* significantly decreased of SOD content in serum (0.09±0.03mg protein/L), gills (0.21±0.05U/mg protein), hepatopancreas (0.39±0.11U/mg protein) and muscles (0.19±0.04U/mg protein) as compared to control were observed. Among the 10, 20 and 30 days exposure of $CdCl_2$, SOD content in *Sesarma quadratum* was significantly decreased in 30 days exposure compared to 10 and 20 days exposure (Table 4.10 and Fig. 4. 13). Among the various duration of exposure of $CdCl_2$, 30 days exposure of CdCl2 has significant decreased activities were observed.

Table 4.10: Effect of cadmium exposure (12µg/L) on superoxide dismutase (SOD) activity in crab, *Sesarma quadratum*

SOD	Group I (Control)	Cadmium exposed 12µg/L		
		Group II (10th day)	Group III (20th day)	Group IV (30th day)
Serum (mg protein/L)	0.49±0.05	0.23±0.04*	0.18±0.04**	0.09±0.03***
Gills (U/mg protein)	0.65±0.09	0.42±0.09*	0.26±0.05**	0.21±0.05***
Hepatopancreas (U/mg protein)	1.19±0.21	0.74±0.18*	0.53±0.15**	0.39±0.11***
Muscles (U/mg protein)	0.96±0.09	0.45±0.05*	0.31±0.05**	0.19±0.04***

Values were expressed as Mean ± SD for three experiments in each group and significant differences between mean values were determined by One Way Analysis of Variance (ANOVA) followed by Tukey HSD test. $P< 0.05$ was considered significant. *$P<0.05$, **$P<0.01$, ***$P<0.001$ statistically significant and NS= Non significant ($P>0.05$) compared with Group I (Control).

In the present study SOD level of Hepatopancreas > Muscles > Gills > Serum in control and experimental crabs were found.The levels of SOD were found to be significantly decreased in cadmium chloride exposed crab comparison with control crabs in muscles, hepatopancreas, gills and serum.

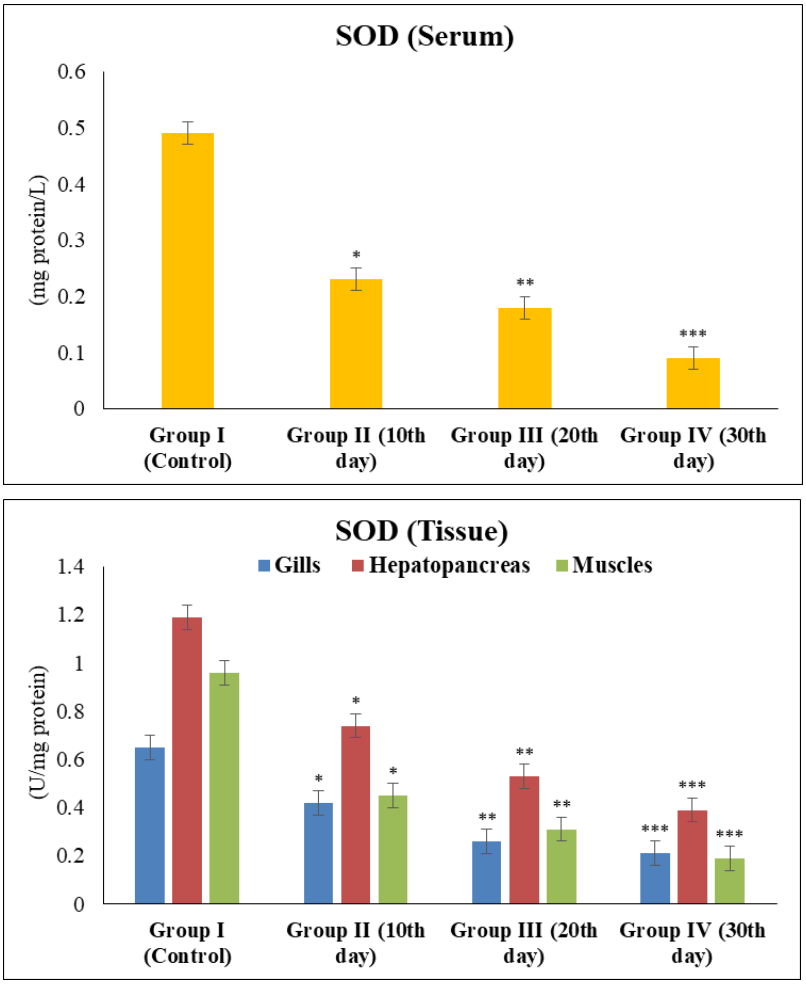


Fig. 4.13: Effect of cadmium exposure (12µg/L) on superoxide dismutase (SOD) activity in crab, *Sesarma quadratum*

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significantly decreased of catalase content in serum (97.43±4.97mg protein/L), gills (152.11±3.41µmol of H_2O_2 consumed/min/mg protein), hepatopancreas (267.89±8.47µmol of H_2O_2 consumed/min/mg protein) and muscles (218.59±7.21µmol of H_2O_2 consumed/min/mg protein) as compared to control were observed.

In the observation, on 20 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of catalase content in serum (69.85±4.82mg protein/L), gills (125.76±3.74µmol of H_2O_2 consumed/min/mg protein), hepatopancreas (215.01±9.05µmol of H_2O_2 consumed/min/mg protein) and

muscles (186.61±7.65µmol of H_2O_2 consumed/min/mg protein) as compared to control were observed.

In the observation, on 30 days exposure of $CdCl_2$ on *Sesarma quadratum* significantly decreased of catalase content in serum (51.74±3.18mg protein/L), gill (113.76±3.28µmol of H_2O_2 consumed/min/mg protein), hepatopancreas (179.87±9.81µmol of H_2O_2 consumed/min/mg protein) and muscles (154.37±6.43µmol of H_2O_2 consumed/min/mg protein) as compared to control were observed. Among the 10, 20 and 30 days exposure of $CdCl_2$, catalase content in *Sesarma quadratum* was significantly decreased in 30 days exposure compared to 10 and 20 days exposure (Table 4.11 and Fig. 4. 14). Among the various duration of exposure of $CdCl_2$, 30 days exposure of $CdCl_2$ has significant decreased activities were observed.

Table 4.11: Effect of cadmium exposure (12µg/L) on catalase (CAT) activity in crab, *Sesarma quadratum*

Cat.	Group I (Control)	Cadmium exposed 12µg/L		
		Group II (10th day)	Group III (20th day)	Group IV (30th day)
Serum (mg protein/L)	125.74±5.13	97.43±4.97*	69.85±4.82**	51.74±3.18***
Gills (µmol of H_2O_2 consumed/min/mg protein)	180.93±3.18	152.11±3.41*	125.76±3.74**	113.76±3.28***
Hepatopancreas (µmol of H_2O_2 consumed/min/mg protein)	351.74±11.36	267.89±8.47*	215.01±9.05**	179.87±9.81***
Muscles (µmol of H_2O_2 consumed/min/mg protein)	295.73±7.34	218.59±7.21*	186.61±7.65**	154.37±6.43***

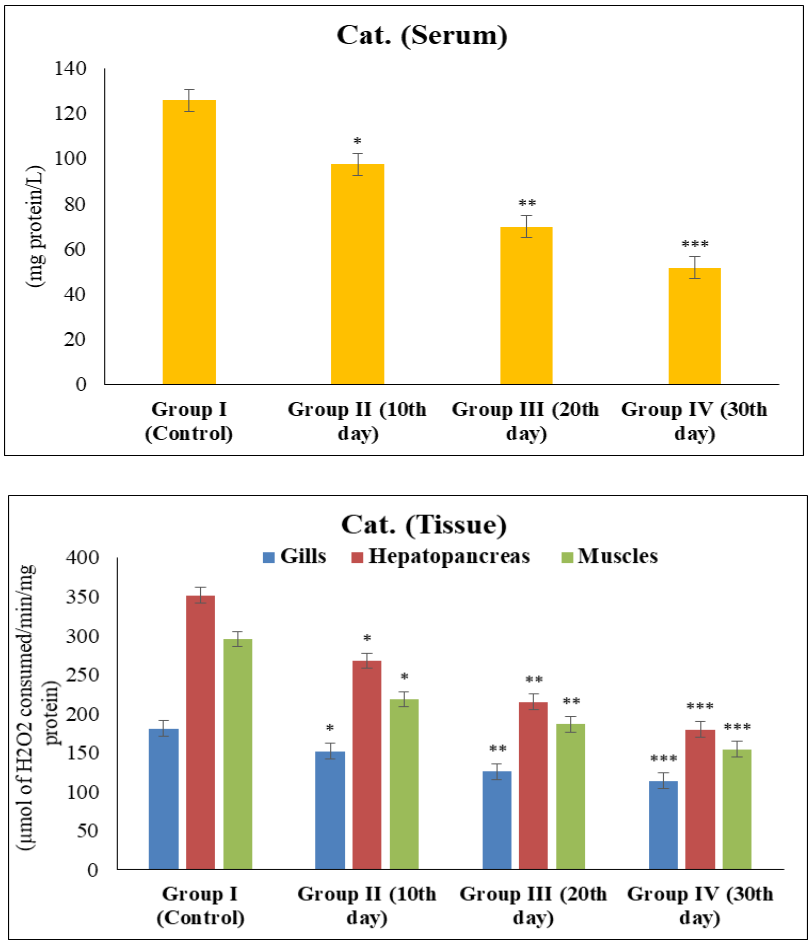


Fig. 4.14: Effect of cadmium exposure (12µg/L) on catalase (CAT) activity in crab, *Sesarma quadratum*

Values were expressed as Mean ± SD for three experiments in each group and significant differences between mean values were determined by One Way Analysis of Variance (ANOVA) followed by Tukey HSD test. $P< 0.05$ was considered significant. *$P<0.05$, **$P<0.01$, ***$P<0.001$ statistically significant and NS= Non significant ($P>0.05$) compared with Group I (Control).

In the present study Cat. level of Hepatopancreas > Muscles > Gills > Serum in control and experimental crab were found. The levels of Catalase were found to be significantly decreased in cadmium chloride exposed crab comparison with control crabs in muscles, hepatopancreas, gills and serum.

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of GPx content in serum (296.85±13.69µ mol GSH oxidized/min mg protein/L), gills (232.48±4.79µ mol GSH oxidized/min mg protein/L), hepatopancreas (229.47±9.53µ mol GSH oxidized/min mg protein/L) and muscles (209.48±6.21µ mol GSH oxidized/min mg protein/L) as compared to control were observed.

In the observation on 20 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of GPx content in serum (180.49±13.97µ mol GSH oxidized/min mg protein/L), gills (196.74±4.52µ mol GSH oxidized/min mg protein/L), hepatopancreas (185.63±7.45µ mol GSH oxidized/min mg protein/L) and muscles (173.69±5.82µ mol GSH oxidized/min mg protein/L) as compared to control were observed.

In the observation, on 30 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of GPx content in serum (143.19±11.82µ mol GSH oxidized/min mg protein/L), gills (152.47±4.76µ mol GSH oxidized/min mg protein/L), hepatopancreas (153.21±7.39µ mol GSH oxidized/min mg protein/L) and muscles (131.28±5.93µ mol GSH oxidized/min mg protein/L) as compared to control were observed. Among the 10, 20 and 30 days exposure of $CdCl_2$, GPx content in *Sesarma quadratum* was significantly decreased in 30 days exposure compared to 10 and 20 days exposure (Table 4.12 and Fig. 4. 15). Among the various duration of exposure of $CdCl_2$, 30 days exposure of $CdCl_2$ has significant decreased activities were observed.

Table 4.12: Effect of cadmium exposure (12µg/L) on Glutathione peroxidase (GPx) activity in crab, *Sesarma quadratum*

GPx	Group I (Control)	Cadmium exposed 12µg/L		
		Group II (10th day)	Group III (20th day)	Group IV (30th day)
Serum (µ mol GSH oxidized/min mg protein/L)	321.53±15.74	296.85±13.69*	180.49±13.97**	143.19±11.82***
Gills (µ mol GSH oxidized/min mg protein)	317.83±4.56	232.48±4.79*	196.74±4.52**	152.47±4.76***
Hepatopancreas (µ mol GSH oxidized/min mg protein)	313.52±8.76	229.47±9.53*	185.63±7.45**	153.21±7.39***
Muscles (µ mol GSH oxidized/min mg protein)	256.81±5.97	209.48±6.21*	173.69±5.82**	131.28±5.93***

Values were expressed as Mean ± SD for three experiments in each group and significant differences between mean values were determined by One Way Analysis of Variance (ANOVA) followed by Tukey HSD test. $P< 0.05$ was considered significant. *$P<0.05$, **$P<0.01$, ***$P<0.001$ statistically significant and NS= Non significant ($P>0.05$) compared with Group I (Control).

In the present study GPx level of Serum > Gills > Hepatopancreas > Muscles in control and experimental crab were found. The levels of GPx were found to be significantly decreased in cadmium chloride exposed crab comparison with control crabs in muscles, hepatopancreas, gills and serum.

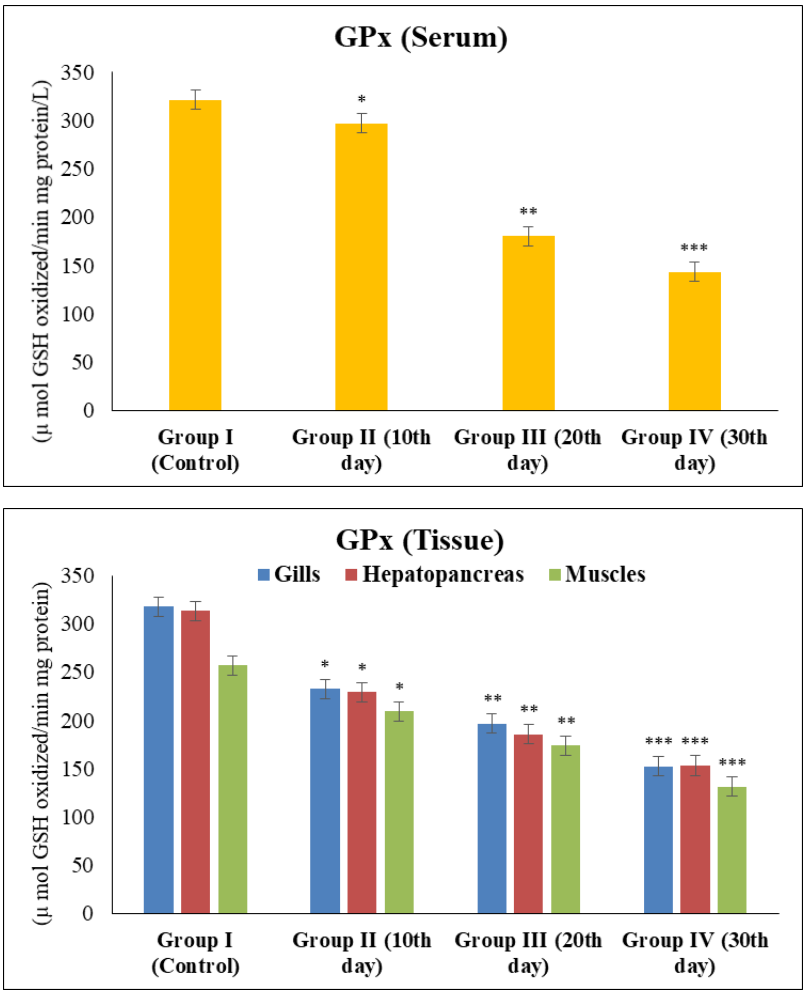


Fig. 4.15: Effect of cadmium exposure (12µg/L) on Glutathione peroxidase (GPx) activity in crab, *Sesarma quadratum*

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of GSH content in serum (4.23±0.17µg.mg protein/L), gills (3.94±0.17µg.mg protein), hepatopancreas (5.12±0.51µg.mg protein) and muscles (4.31±0.64µg.mg protein) as compared to control were observed.

In the observation, on 20 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of GSH content in serum (2.78±0.19µg.mg protein/L), gills (1.67±0.21µg.mg protein), hepatopancreas (3.21±0.54µg.mg

protein) and muscles (2.94±0.56µg.mg protein) as compared to control were observed.

In the observation, on 30 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of GSH content in serum (1.65±0.21µg.mg protein/L), gills (0.83±0.19µg.mg protein), hepatopancreas (1.32±0.49µg.mg protein) and muscles (1.34±0.43µg.mg protein) as compared to control were observed. Among the 10, 20 and 30 days exposure of $CdCl_2$, GSH content in *Sesarma quadratum* was significantly decreased in 30 days exposure compared to 10 and 20 days exposure (Table 4.13 and Fig. 4.16). Among the various duration of exposure of $CdCl_2$, 30 days exposure of $CdCl_2$ has significant decreased content was observed.

Table 4.13: Effect of cadmium exposure (12µg/L) on Glutathione (GSH) content in crab, *Sesarma quadratum*

GSH	Group I (Control)	Cadmium exposed 12µg/L		
		Group II (10th day)	Group III (20th day)	Group IV (30th day)
Serum (µg.mg protein/L)	6.97±0.21	4.23±0.17*	2.78±0.19**	1.65±0.21***
Gills (µg/mg protein)	5.28±0.25	3.94±0.17*	1.67±0.21**	0.83±0.19***
Hepatopancreas (µg/mg protein)	7.47±0.74	5.12±0.51*	3.21±0.54**	1.32±0.49***
Muscles (µg/mg protein)	6.73±0.41	4.31±0.64*	2.94±0.56**	1.34±0.43***

Values were expressed as Mean ± SD for three experiments in each group and significant differences between mean values were determined by One Way Analysis of Variance (ANOVA) followed by Tukey HSD test. $P<0.05$ was considered significant. *$P<0.05$, **$P<0.01$, ***$P<0.001$ statistically significant and NS= Non-significant ($P>0.05$) compared with Group I (Control).

In the present study GSH level of Hepatopancreas > Serum > Muscles > Gills in control and experimental crab were found. The levels of GSH were found to be significantly decreased in cadmium chloride exposed crab comparison with control crabs in muscles, hepatopancreas, gills and serum.

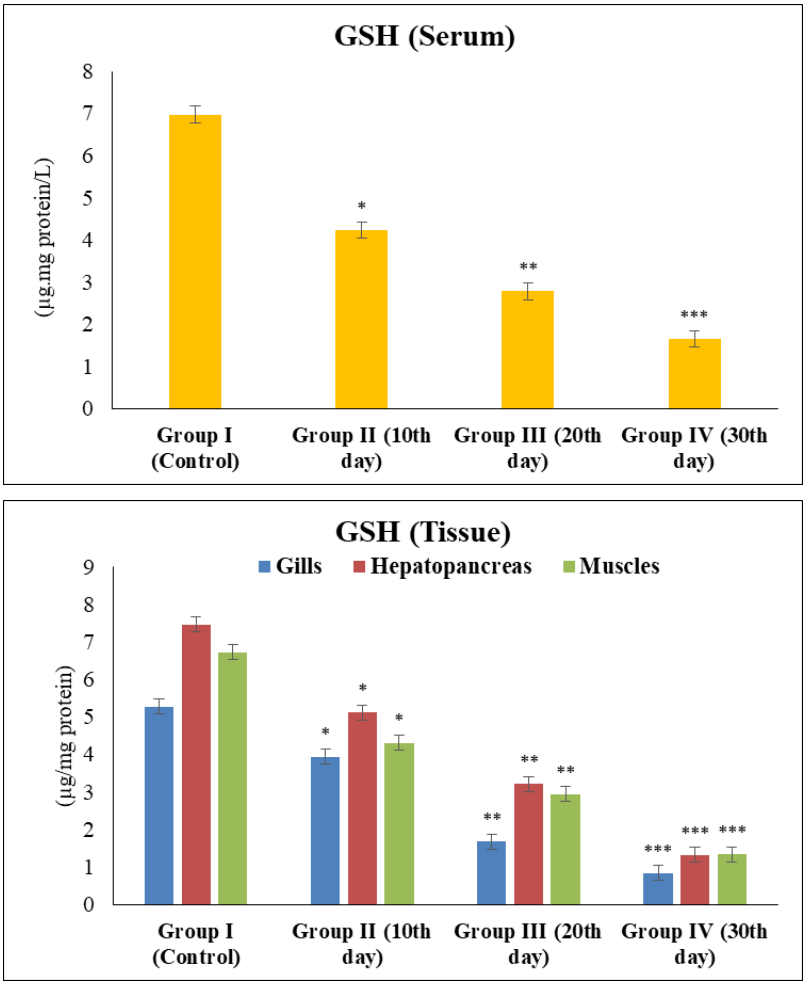


Table 4.16: Effect of cadmium exposure (12µg/L) on Glutathione (GSH) content in crab, *Sesarma quadratum*

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significant increase of GST content in serum (15.94±0.42mg protein/L), gills (32.19±2.17U/mg protein), hepatopancreas (53.18±1.96U/mg protein) and muscles (47.63±1.29U/mg protein) as compared to control were observed.

In the observation, on 20 days exposure of $CdCl_2$ on *Sesarma quadratum* significant increase of GST content in serum (21.79±0.35mg protein/L), gills (43.96±2.95U/mg protein), hepatopancreas (72.19±2.28U/mg

protein) and muscles (62.01±1.95U/mg protein) as compared to control were observed.

In the observation, on 30 days exposure of $CdCl_2$ on *Sesarma quadratum* significant increase of GST content in serum (24.61±0.31mg protein/L), gills (37.65±1.74U/mg protein), hepatopancreas (81.43±2.11U/mg protein) and muscles (70.85±1.78U/mg protein) as compared to control were observed. Among the 10, 20 and 30 days exposure of $CdCl_2$, GST content in *Sesarma quadratum* was significantly increased in 30 days exposure compared to 10 and 20 days exposure (Table 4.14 and Fig. 4.17). Among the various duration of exposure of $CdCl_2$, 30 days exposure of $CdCl_2$ has significant decreased activities were observed.

Table 4.14: Effect of cadmium exposure (12µg/L) on Glutathione – S-transferase (GST) activity in crab, *Sesarma quadratum*

GST	Group I (Control)	Cadmium exposed 12µg/L		
		Group II (10th day)	Group III (20th day)	Group IV (30th day)
Serum (mg protein/L)	11.29±0.31	15.94±0.42*	21.79±0.35**	24.61±0.31***
Gills (U/mg protein)	20.74±2.69	32.19±2.17*	43.96±2.95**	37.65±1.74***
Hepatopancreas (U/mg protein)	42.97±2.19	53.18±1.96*	72.19±2.28**	81.43±2.11***
Muscles (U/mg protein)	31.18±1.48	47.63±1.29*	62.01±1.95**	70.85±1.78***

Values were expressed as Mean ± SD for three experiments in each group and significant differences between mean values were determined by One Way Analysis of Variance (ANOVA) followed by Tukey HSD test. $P< 0.05$ was considered significant. *$P<0.05$, **$P<0.01$, ***$P<0.001$ statistically significant and NS= Non-significant ($P>0.05$) compared with Group I (Control).

In the present study GST level of Hepatopancreas > Muscles > Gills > Serum in control and experimental crab were found. The levels of GST were found to be significantly increased in cadmium chloride exposed crab comparison with control crabs in muscles, hepatopancreas, gills and serum.

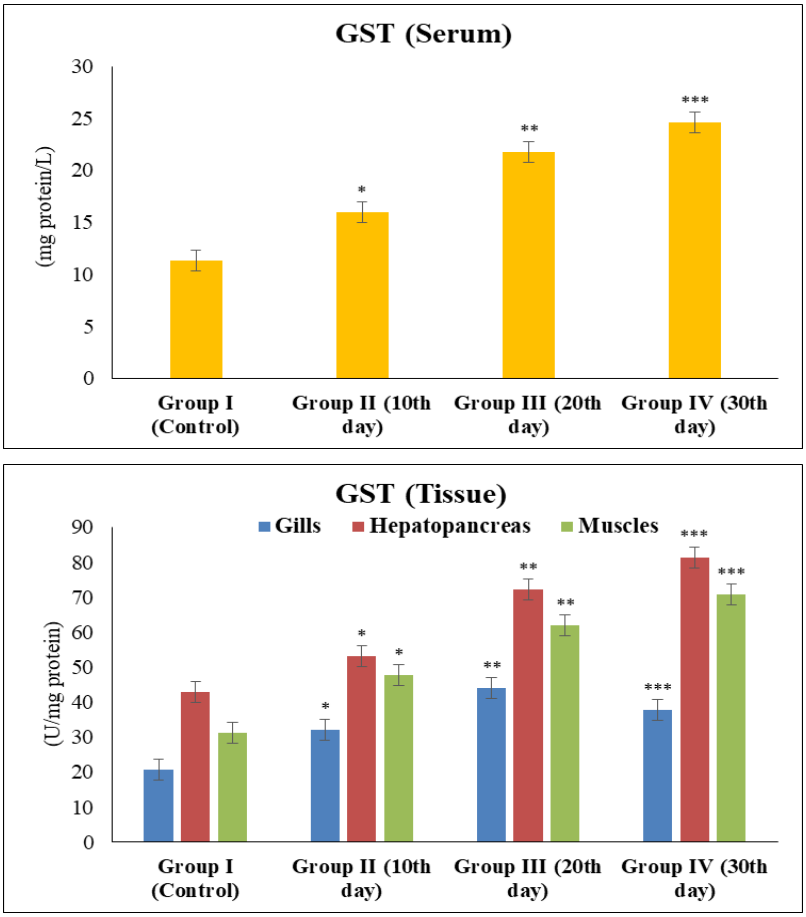


Fig. 4.17: Effect of cadmium exposure (12µg/L) on Glutathione –S-transferase (GST) activity in crab, *Sesarma quadratum*

4.6. Histological observation in subacute concentrations of cadmium chloride exposure in *Sesarma quadratum*

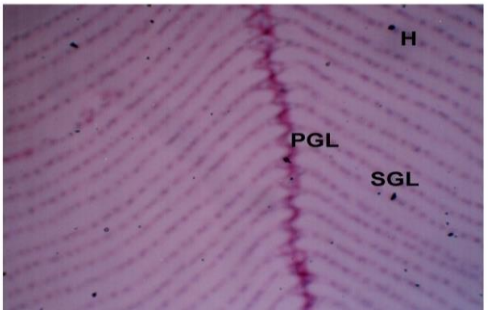
Histopathological studies help in understanding the stress caused to the animal exposed to pollution. This is a universal method for the assessment of the impact of xenobiotics on the tissues of animals. The ability of any tissue to regulate its normal physiological function is extensively related to its structural integrity. Any damage to the tissues usually results in altered and frequently abnormal metabolic activities. Hence there is a need to study the histopathological changes in gills and hepatopancreas of *Sesarma quadratum*

in response to cadmium exposure. Moderate alterations were observed in the gills followed by hepatopancreas of *Sesarma quadratum* (Plate 4.1 and 4.2).

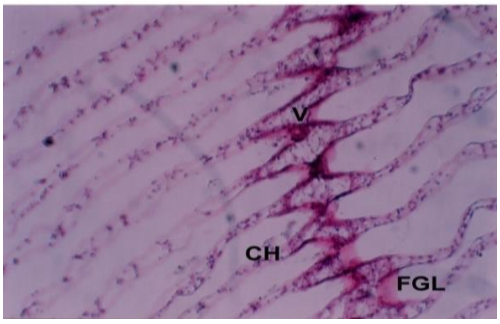
Histology of gills

The gills of *Sesarma quadratum* are formed of a number of lamellae or broad flattened plates arranged serially in pairs along a control gill stem. The central axis of gill tissue is the primary gill lamellae (PGL) and it further divides into secondary gill lamellae (SGL) or filaments. The control gill exhibits a thin layer of cuticle covering the entire outer surface. Underlying the cuticle is a continuous layer of epithelial cells; at irregular intervals pillar cells join the lamellae. The distal part of the lamella is expanded. The epithelial cells of the lamellae are continued as the lining of the gill stem and large connective tissue cells compose the chief support of the gill stem (Plate 4.1 Group I (Control)).

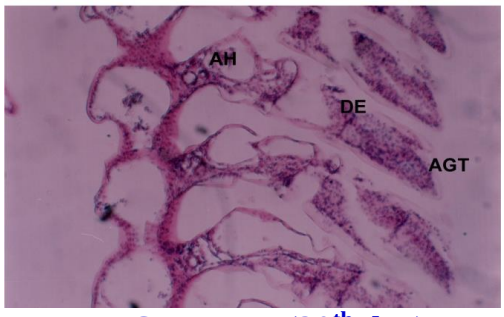
In the observation of gills in 10 days exposure of cadmium showed the following changes were seen as congestion of haemocytes and fusion of gill lamellae (Plate 4.1 Group II-10 day's exposure). In the observation of gills in 20 days exposure of cadmium showed the following changes were seen as abnormal gill tips, accumulation of haemocytes and disintegration of the epithelium (Plate 4.1 Group II-20 days exposure). In the observation of gills in 30 days exposure of cadmium showed the following changes were seen as an accumulation of haemocytes and disintegration of the epithelium (Plate 4.1 Group III-30 days exposure).



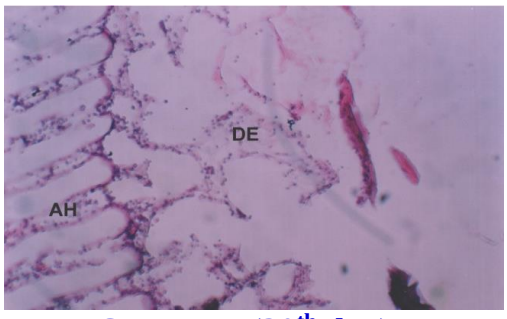
Group I (Control)



Group II (10th day)



Group III (20th day)



Group IV (30th day)

Note: H –Haemocyte; RGL – Ruptured Gill Lamellae; PGL – Primary Gill Lamellae; SGL – Secondary Gill Lamellae; CH – Congestion of Haemocytes; FGL – Fusion of Gill Lamellae; AGT– Abnormal Gill Tips; AH – Accumulation of Haemocytes; DE – Disintegration of Epithelium; AH – Accumulation of Haemocytes; DE – Disintegration of Epithelium.

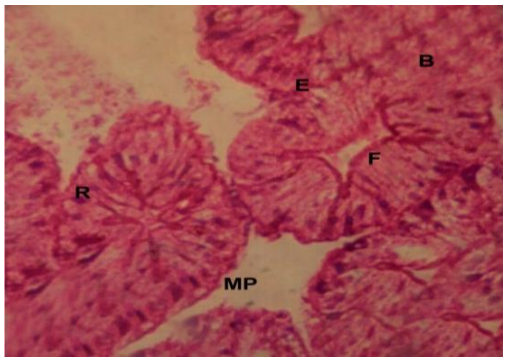
Plate 4.1: Effect of cadmium exposure (12µg/L) on Histopathological structure of Gills of *Sesarma quadratum*

Histology of Hepatopancreas

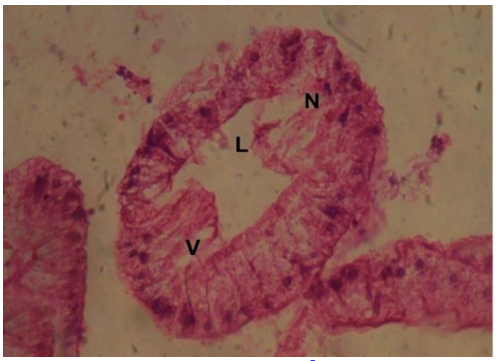
In the control crab the yellowish-brown tissue of the hepatopancreas occupied much of the cephalothoracic cavity. The histology of control crab exhibited the well-organized glandular tubular structure. Histologically, the tubules consisted of an epithelium composed of four cell types E-cells (embryonic), the F-cells (fibrillar), the B cells (blister like), and the R-cells (resorptive). The E-cells, which were generally among the smallest of the hepatopancreatic cell types, were undifferentiated polyhedral cells. They had high nucleo-cytoplasmic ratio, and were concentrated in the distal tip of the tubules, which is the area of proliferation. The F-cells, which appeared striated due to extensively developed rough endoplasmic reticulum, were tall columnar epithelial cells with basally situated nuclei. They are secretory in function and present in the mediodistal, and medioproximal portions of the tubules.The B-cells, which are secretory and excretory in function and have a single large vacuole with compressed basal nuclei, were the largest of hepatopancreatic cell types seen mainly in the proximal areas of the tubules. The R-cells, the most abundant of the four cell types, had multi-vacuolated cytoplasm and are storage in nature. They were seen in the mediodistal and proximal areas of the tubules (Plate 4.2 Group I (Control)).

In the observation of hepatopancreas in 10 days exposure of cadmium showed the following changes were seen as mild alteration in lumen, vacuole and necrosis (Plate 4.2 Group II-10 day's exposure). In the observation of hepatopancreas in 20 days exposure of cadmium showed the following

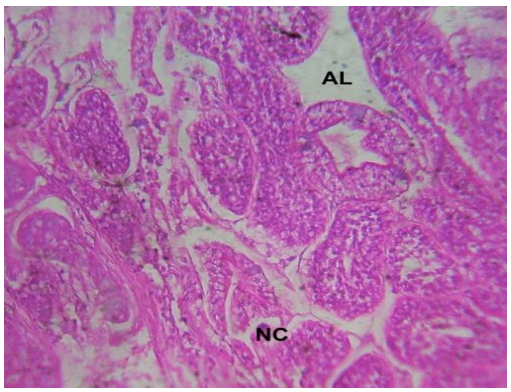
changes were seen as abnormal lumen and necrotic cells (Plate 4.2 Group III- 20 days exposure). In the observation of hepatopancreas in 30 days exposure of cadmium showed the following changes were seen as tissue debris and necrotic cells (Plate 4.2 Group IV- 30 days exposure).



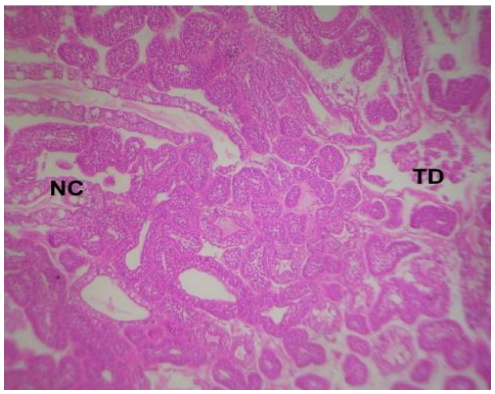
Group I (Control)



Group II (10th day)



Group III (20th day)



Group IV (30th day)

Note: E – Embryonic Cells; F – Fibrillar Cells; B – Secretory Cells; MP – Peritrophic Membrane; R – Absorptive Cells; TD – Tissue Debris; NC – Necrotic Cells; AL – Abnormal Lumen; NC – Necrotic Cells; L – Lumen; V – Vacuole; N – Necrosis.

Plate 4.2: Effect of cadmium exposure (12µg/L) on Histopathological structure of Hepatopancreas of *Sesarma quadratum*

CHAPTER-V

DISCUSSION

CHAPTER V

5. DISCUSSION

Estuaries are semi-enclosed coastal bodies of water which have a free connection with the open sea and within which sea water measurably diluted with freshwater from land drainage (Pritchard, 1967; Cross and Williams, 1981 and Nixon *et al*., 2004). Estuaries may be classified in different ways. At the simplest level, there are two types of estuaries-river mouth estuaries and lagoons estuaries. Estuaries and then associated wetlands serve as storm buffers that absorb wave energy and rising tidal waters during storms.

The estuarine environment is a complex blend of continuously changing habitats. Unlike freshwater bodies like lakes and rivers, estuaries can produce a wide range in the values of physical and chemical parameters that will be recorded and frequent changes occur in these values both with tidal and meteorological events. In streams, rivers and lakes, water quality parameters are more likely to fluctuate within a well-defined range largely determined by rainfall and season and these values are often homogeneous throughout the water body (Bouillon *et al*., 2001). In an estuary, in contrast these parameters can change abruptly in time and space depending on the study locations (sampling sites) and may or may not reflect general conditions throughout the estuary.

Two key phenomena that control physical and chemical parameters are tidal oscillation and stratification (vertical and horizontal) (Chugh, 1961; Quasim and Gopinathan 1969; Murthy and Henry 1983; Lanzoni and

Seminara 1998; Sadhuram *et al*., 2005 Meenakshi Chatterjee *et al*., 2013) tidal oscillations are the net transport for water (as well as sediments and contaminants) out of an estuary with tidal flow and river flow. Stratification is layering of the estuary generally associated with the inflow of dense salt water at depth and the outflow of more buoyant freshwater at the surface. Layering can also occur when seasonal heating causes a sharp differential or thermocline (interface where temperature changes rapidly with depth) so that the warm surface layer is isolated from the cold bottom layer.

Biological processes such as primary production and decomposition can modify the physico-chemical conditions which the biological inter–relationship such as reproduction, recruitment and predator–prey cycles can modify the community structure. The latter in turn can have further consequences for the modification of physico-chemical characteristics of estuaries (De Jonge *et al*., 2002).

Physico-chemical parameters

Seasonal variations of different environmental features in the estuarine system which is chiefly controlled by the spectacular region of the rainfall during monsoon. Thus making the year divisible as mentioned earlier into three distinct seasons namely pre monsoon (March to May), monsoon (June to September) and post monsoon (October to December).

Monthly variations in physico-chemical parameters viz., atmospheric and surface water temperature, pH, EC, dissolved oxygen (DO), chemical oxygen demand (COD), biological oxygen demand (BOD), alkalinity, salinity,

hardness, ammonium, nitrate, sodium, chloride, sulphate and fluoride in Kuduvaiyar estuarine waters were recorded for a period of 2019 to 2020.

Seasonal variations of physical characters in Kuduvaiyar estuary

The light penetration into the water column depends upon the clarity of water, depth and the nature of sediment as observed by Ouasim, (1978). One of the changing conditions brought about by the monsoon in tropical estuaries was found to be as a factor of light (Ouasim, 1978) and it is very interesting to examine the fluctuation in the other environmental factors (Biological) which are controlled by the light penetration in the water. The seasonal pattern recorded in the present study is in conformity with the earlier reports from Cochin backwaters (Ouasim *et al*., 1968 and 1969 and Gupta *et al*., 1980), Netravati Gurupur estuary (Bhat and Gupta, 1980), Ashtamudi estuary (Nair *et al*., 1984), Vellar estuary (Thangaraj, 1984) and Coleroon estuary (Prabha Devi, 1986).

Statistical analysis revealed that a positive correlation was observed for light penetration with gross and net primary production. Therefore, it may be inferred that the influence of light on biotic factors are appreciable. However, Yoo and Lee (1980) have not stressed the importance of light as a limiting factor in determining the diatom standing crop even though the correlation was statistically significant. The light penetration depth was high during summer which possessed clear water and shallow bottom, during monsoon due to high turbidity caused by heavy soil erosion from the surrounding mangrove swamp area and clay nature of the substratum.

In the present study, the variation in temperature, pH, salinity, dissolved oxygen, total hardness, total alkalinity, electrical conductivity, dissolved oxygen (DO), biological oxygen demand (BOD) and chemical oxygen demand (COD) were done triplicate in the laboratory as per the standard methods.

Temperature is a universal factor of importance in the study of aquatic ecosystems. There are documents that measure the effect of temperature on the growth of phytoplankton in the laboratory (Hulburt and Guillard, 1968). But, there are few evidence to show the importance of effects of temperature on the production, abundance and distribution of organisms in the shallow ecosystems. Veliea (1984) suggested that temperature may be more important as a covariate with other factors than an independent factor.

In the present study, the variation in atmospheric temperature is found to be associated with the time of collection of water samples and the rainfall during the period. The surface water temperature closely followed the atmospheric temperature. In Kuduvaiyar estuary, the highest atmospheric temperature (29.13°C) was recorded during pre-monsoon while lowest atmospheric temperature in the monsoon (25.11°C) and post monsoon (27.07°C). Similar findings were also recorded by Ravichelvan *et al*. (2015) and Eshwaralal and Angadi (2002).

The variations in water and atmospheric temperature were very narrow and sometimes no such variation was observed. Day (1951) suggested that in shallow estuaries with wide intertidal flats, solar radiation and evaporation causes marked temperature changes. Throughout the study period, the higher

atmospheric and water temperature was observed during summer and pre-monsoon and lower temperature during monsoon and post-monsoon periods.

Quasim and Sengupta (1981) reported from Mandovi and Zuari estuaries of Goa that seasonal changes of temperature in water of mid depth of the six stations should increase thereafter at some stations. Bhat and Gupta (1980) and Gupta *et al*. (1980) from Ashtamudi estuary recorded higher water temperature during pre-monsoon and lower temperature during monsoon period. Thus the present findings favor the earlier reports on the fluctuations of water temperature on the estuaries.

pH refers to a scale of intensity of acidity or alkalinity. This is regarded as a measure of concentration of H^+ ions in water. It is one of the important indicators of water quality and is of great importance to living systems because both cell structure and function can be affected by even small changes in pH (Weber and Stun, 1963). Low pH in water is caused by acids, acid-generated salts, and dissolved carbon dioxide. Low pH values give water acidic, acidic-sour taste and kill small microorganisms. High pH is from carbonates, bicarbonates, hydroxides, phosphates, silicates and borates and a very high value of pH makes water strongly alkaline and bitter in taste making it unfit for human consumption. The acidic (low pH) groundwater is corrosive and can dissolve metals, especially copper from pipes and pumps. The corrosion can shorten the economic life of plumbing and hot water cylinders and in some cases, the dissolved metals in the water may cause illness (Banerji, 1999 and Mahesha *et al*., 2004).

The pH of the water showed alkaline range throughout the period of study. Seasonal average values of pH were always towards the alkaline condition in the study area. The pH varied with the seasons as lowest in monsoon months (7.9) and highest during pre-monsoon (8.7) while post monsoon pH was 8.3. Our findings are in concordance with Mohan Raj *et al.* (2013) and Ravichelvan *et al*. (2015).

The pH was higher during the summer season while it was low during the monsoon period due to the uptake of CO_2 by the photosynthesizing organisms, especially phytoplankton and planktonic cyanobacteria from the seawater could have increased the pH level during the summer season (Subramanian and Mahadevan, 1999). The low pH observed during the monsoon season is due to the influence of freshwater influx and dilution of seawater, reduction of salinity and temperature and decomposition of organic matter (Kannan, 1980).

The highest value may be due to the Industrial effluent because it contained many chemicals, salts and dissolved solids (Mishra and Saksena, 1993). Higher EC indicates the presence of a high amount of dissolved inorganic substances in ionized form (Murhekar, 2011). Domestic Effluent also showed moderate to high value of EC. Electrical Conductance in monsoon was found below detection limit because it is diluted water and in diluted water presence of ions and chemicals is in minute quantity so; EC is found below the detection limit. In the present study, highest EC was recorded

during post monsoon (459Mscm^{-1}) and lowest during monsoon (376Mscm^{-1}) while Pre monsoon EC was 412Mscm^{-1}.

Oxygen dissolved in water is usually referred to as dissolved oxygen. It is an index of physical and biological processes going on in water. Dissolved oxygen is also an important factor for the dissolution of inorganic substances in water (NEERI, 1998). Generally, unpolluted surface waters are more saturated with dissolved oxygen than ground waters due to natural aeration. A rapid fall of dissolved oxygen level in drinking waters is one of the first indications of organic pollution.

DO is one of the most important parameters to check the water quality. The presence of oxygen is essential for the survival of aquatic life in water. The DO level in water depends on the physical, chemical and biological activities in the water body. Generally variation in concentration of DO is largely governed by photosynthesis, respiration, mineralization and decomposition activities in water (Chanu and Devi, 2008). Good potable water should have the level of saturated DO at 7 – 9 mg/L at 30^0C. As the DO level falls, undesirable odour, taste and colour appear which reduce the acceptability of water (Garg *et al*, 2007). Increase in DO is related to decrease in temperature or decrease in DO is related to higher temperature as solubility of O_2 decreases with increase in temperature (Dwivedi and Sonar, 2004).

Oxygen is dissolved in most waters in varying concentrations. The solubility of oxygen depends on temperature, pressure and salinity of water. The low DO values may be due to discharge of waste water into the water

body demanding more oxygen and it indicates deterioration of water quality (Achuthan Nair *et al*, 2005).

The present investigation showed minimum dissolved oxygen was recorded during monsoon months (5.2mg/L), maximum dissolved oxygen were recorded during pre-monsoon and post monsoon months (5.9 and 5.3mg/L respectively). Similar observations were drawn by Ravichelvan *et al*. (2015) and Sahu *et al*., (2000).

Chemical Oxygen Demand (COD) is a measure of pollution in aquatic ecosystems. It estimates the carbonaceous factor of organic matter. In the present study COD was lowest in pre-monsoon months (22.10mg/L) and highest during monsoon (23.13mg/L) and post monsoon (23.91mg/L).

Biological Oxygen Demand (BOD) is the amount of oxygen required by the living organisms engaged in the utilization and ultimate destruction or stabilization of organic water. It is a very important indicator of the pollution status of a water body. In the conducted experiments, BOD was high in Domestic and Industrial Effluent due to high organic load and excessive growth of total microorganisms (Kandhasamy and Santhaguru, 1994). In the present study, BOD was lowest in monsoon months (2.34mg/L) and highest during pre-monsoon (4.57mg/L) while post monsoon BOD was 3.72mg/L.

This may be as a result of escape of organic matter (organic) into the river mostly from faecal waste deposition by the surrounding urban area and human settlements. Control Sample revealed BOD value below detection limit, because it was distilled water and had no organic load. Highest COD

values were found in Domestic Effluent which may be due to the incessant inflow of sewages from urban areas (Mishra *et al*., 1990).

The alkalinity of water is its capacity to neutralize acids. Alkalinity of water is a measure of weak acid present in it and of the cations balanced against them (Singh *et al*., 2010). Total alkalinity is the total concentration of bases in water, usually bicarbonates and carbonates (Ouyang *et al.*, 2006). In the present study, alkalinity varied with the seasons as lowest in monsoon months (118.37mg/L) and highest during pre-monsoon (119.25mg/L) while post monsoon alkalinity was 118.95mg/L. Total alkalinity depends on the concentration of the substance which would raise the pH of the water. High levels of alkalinity indicate the presence of strongly alkaline industrial waste water and sewage in the estuary (Safari *et al.*, 2012). The degradation of plants, living organisms and organic waste in the estuary might also be one of the reasons for increase in carbonate and bicarbonate levels; it shows an increase in alkalinity value (Wang *et al.*, 2006). Our results agree with the earlier report (Mohan Raj *et al.*, 2013).

Salinity is considered to be the prime factor among the most important environmental parameters that control the dynamic situation of the estuarine environment. Salinity at both stations showed high values during the summer season due to less rainfall, decreased freshwater inflow and rise in temperature of the estuary and low during the monsoon season due to high rainfall and land runoff. In the present study, salinity was low during monsoon (23.18 PSU) and high during pre-monsoon (25.08PSU) while post monsoon salinity was

24.79PSU. Minimum salinity was recorded during monsoon and slowly built up during post-monsoon and attained its maximum during summer.

Salinity is one of the important factors which profoundly influence the abundance and distribution of the fauna and flora in the estuarine environment which in turn depends on the inflow of freshwater and the prevailing temperature. During the monsoon season low salinity was recorded due to heavy rainfall and large quantities of freshwater inflow. Thus, the variations in salinity were mainly influenced by the rainfall and entry of freshwater (Sasinayar and Gowda, 1999).

However, the freshwater inflow into the estuary and constant evaporation of the estuarine water together with mixing of sea water into the estuary are the important factors that influenced the distribution of salinity in Kuduvaiyar estuary. The present observation of high salinity during post-monsoon and low salinity during monsoon periods is in conformity with the earlier reports from different estuaries of India (Ramamirtham and Jayaraman, 1983; Michael, 1970; Sreenivasan and Pillai, 1972; Haridas *et al*., 1973; Santhanam *et al*., 1975; Thangaraj *et al*., 1984 and Nair *et al*., 1984).

The capacity of water to absorb oxygen depends upon temperature and salinity (Kinne, 1964). The increasing tendency of oxygen at stations perhaps related to the freshwater flow in the river during the rainy season. The dissolved oxygen concentration was associated with phytoplankton density. In general, high concentrations of dissolved oxygen always tend to result in

higher gross and net primary production during summer and pre-monsoon period.

Total hardness is the parameter of water quality used to describe the effect of dissolved minerals (mostly Ca and Mg), determining suitability of water for domestic, industrial and drinking purposes attributed to presence of bicarbonates, sulphates, chloride and nitrates of calcium and magnesium. High values of hardness are probably due to regular addition of large quantities of detergents used by the nearby residential localities into lakes which drain into estuaries. In the present study, hardness was lowest in monsoon months (1637mg/L) and highest during pre-monsoon (1727mg/L) while post monsoon hardness was 1703mg/L. Present finding is in agree with Mohan Raj *et al.* (2013).

Seasonal variations in the chemical characters in Kuduvaiyar estuary

The marine environment, as a complex system, is mainly influenced by various physical, chemical and biological processes. The open ocean is more stable compared to the near shore waters, where the interaction with the terrestrial zone is more effective in bringing about variations in different physico-chemical parameters. Hence a thorough knowledge of hydrography is indispensable to estimate the quality of the environment and its influence on biological fertility. Physical parameters play an important role in the biochemistry of the water body. Subtle changes in physical conditions can have profound effects on the water quality of the study system, which may in turn affect the spatial

and temporal distribution of nutrients and biological communities. Additionally, changes in physical ocean process can affect weather patterns and climatic variability (Poonam and Rahul, 2012).

The quality of water in any ecosystem provides significant information about the available resources for supporting life in that ecosystem. Good quality of water resources depends on a large number of physico-chemical parameters and biological characteristics. So, monitoring of these parameters is essential to identify the magnitude and source of any pollution load (Thirupathaiah *et al*. 2012). The present study involves the analysis of water described by its physico-chemical parameters as ammonium, nitrate, sodium, chloride, sulphate and fluoride.

The ammonium was lowest in monsoon months (0.45mg/L) and highest during pre-monsoon (0.62mg/L) while post monsoon hardness was 0.58mg/L. The nitrate was lowest in monsoon months (32.67mg/L) and highest during pre-monsoon (38.57mg/L) while post monsoon 35.92mg/L. The sodium was lowest in monsoon months (97.51mg/L) and highest during pre-monsoon (132.49mg/L) while post monsoon 125.74mg/L. The chloride was lowest in monsoon months (213.47mg/L) and highest during pre-monsoon (302.28mg/L) while post monsoon 286.51mg/L. The sulphate was lowest in monsoon months (73.21mg/L) and highest during pre-monsoon (86.92mg/L) while post monsoon 80.65mg/L. The fluoride was lowest in monsoon months (0.54mg/L) and highest during pre-monsoon (0.65mg/L) while postmonsoon 0.59mg/L .

Ammonia is toxic to aquatic organisms, even in very low concentrations. It may be contributed by the natural materials such as proteins, peptides, nucleic acids, urea and the compounds in the form of azide, hydrazine, hydroxylamine, nitrate, nitrite, oxime and semicarbazone (Jameel, 1998). Decomposition from the organic matter in the sediments could also lead to increase in its concentration (Shrihari and Venkatesa, 1994). Even though the exact reason is not correctly known, it is interesting to note that in contrast to nitrate and ammonia was more during the pre-monsoon period. The relatively low concentration during the monsoon can be attributed to intense photosynthesis by algae, which removes ammonia in water bodies (John Hargreaves and Craig Tucker, 2004).

Chloride in the form of chloride (Cl-) ion is one of the major inorganic anions in water and wastewater. Chloride exists in all natural waters; the concentrations vary very widely and reach a maximum in sea water. In the present study chloride level within the limit in post monsoon and monsoon while slightly increased in pre-monsoon as compared with Fisheries and aquatic life standard. This difference could result from the no negligible contribution of chemicals and in particular in the waste water discharged at these sites. High chloride concentrations in the water may also be associated with the presence of sodium in drinking water. Elevated concentration levels of sodium may have an adverse health effect on normal healthy persons. Along the sea coast chloride may be present in high concentration because of

leakage of salt water into the sewage system. It also may be increased by industrial processes.

Fluorine is presented in aquatic ecosystems as fluoride (F^-). However, in volcanic emissions, marine aerosols and weathering of minerals some of its natural sources are present (Camargo, 2003). Its extremity is used in the production of some industrial products such as, fertilizers, graphite, semiconductors, and alumina electrolysis (El-Said and Draz, 2010). High levels of fluoride in ecosystems are harmful for aquatic organisms, animals and particularly humans. In the present study fluoride level in pre-monsoon, Post- monsoon and monsoon within the limit as compared with Fisheries and aquatic life standards.

Sodium is the most abundant cation in seawater. Sodium is a soft, silvery-white, highly reactive metal that is never found in nature in an uncombined state. Sodium, an alkali-metal element, has a strong tendency to exist in the ionic form (Kelly et al., 2012). In biological systems and even in solids such as sodium chloride, sodium remains distinctly separate as the sodium ion. Sodium is the most abundant of the alkali elements and constitutes 2.6 percent of the Earth's crust. Compounds of sodium are widely distributed in nature. Weathering of salt deposits and contact of water with igneous rock provide natural sources of sodium. Toxic symptoms of sodium poisoning include general involvement of the central nervous system with increase in sensitivity, muscle twitching, tremors, cerebral and pulmonary oedema, and stupor. (WHO, 1996). In the present study fluoride level in pre-monsoon, Post-

monsoon and monsoon within the limit as compared with Fisheries and aquatic life standards.

Nitrate content is an excellent parameter to judge organic pollution and it represents the highest oxidized form of nitrogen and a vital nutrient for growth, reproduction and the survival of organisms. Nitrate level less than 0.5ml/l will not pollute the water bodies. Under normal conditions nitrate content in the water surface occurs in trace amounts but the value is enhanced by the inputs of other sources (Bilger and Atkinson, 1997). The presence of nitrates in the water samples is suggestive of some bacterial action and bacterial growth. These findings support the observations (Majumder *et al.*, 2006).

The results of the study concluded that fresh water contaminant influx from the adjacent river; rainy seasons and industrial wastes are the main source of chemical contaminant in this estuary.

Heavy metal content of water

In recent years, pollution of marine environments by heavy metal has become a national and international problem. Then there was ‘Itai itai’ disease caused by the consumption of foods contaminated by cadmium from Niigata in Japan. The term heavy metal is a loose one. It includes transition metals like cadmium, chromium, copper, lead, mercury, nickel and zinc. Of these, the most toxic metallic pollutants are mercury, lead, zinc and copper. Heavy metals occur naturally in the marine environment. In addition, these heavy metals enter the aquatic systems by direct discharges via industrial and urban

effluents, surface runoff and indirectly from aerial fallout (Nammalwar, 1983).

In India, use of heavy metal fungicides in agriculture is increasing as seed-dressing. agents. Antifouling properties of mercury compounds are yet used in pulp mills, industrial and domestic sewage wastes from various sources are now a threat to the survival of fishes and other organisms. The common feature of these metals is that they are all relatively toxic even at fairly low concentrations and are readily concentrated by aquatic organisms and plants. The seriousness of heavy metal contamination is further compounded by the fact that they are generally water-soluble, non-degradable, vigorous oxidizing agents and are strongly bonded to many biochemicals inhibiting their functions (Nammalwar, 1983).

The present study pre-monsoon, maximum level of cadmium (0.017mg/L) was observed followed by chromium (0.007mg/L), copper (0.004mg/L), lead (0.09mg/L), mercury (0.00005mg/L), nickel (0.03mg/L) and zinc (0.05mg/L). In monsoon, the level of cadmium (0.011mg/L) was observed followed by chromium (0.005mg/L), copper (0.002mg/L), lead (0.05mg/L), mercury (0.00002mg/L), nickel (0.01mg/L) and zinc (0.02mg/L). In post monsoon, the lowest level of cadmium (0.015mg/L) was observed followed by chromium (0.006mg/L), copper (0.003mg/L), lead (0.07mg/L), mercury (0.00003mg/L), nickel (0.02mg/L) and zinc (0.04mg/L).

Trace elements are found in natural water bodies at varying concentrations. The most potentially dangerous of these elements are heavy

metals. Body levels of essential metals such as copper, chromium, nickel and zinc can be regulated by some decapod crustaceans at concentration below a threshold level. Accumulation of these metals only begins after the organisms are faced with high concentration in the surrounding medium (Rainbow and White, 1989), but body levels of nonessential metals such as cadmium and lead were not found to be regulated by crustacean (Pastor, 1988). Heavy metal concentrations in aquatic ecosystems are usually monitored by measuring its concentration in water, sediments and biota (Camusso *et al*., 1995). Water is an important sink for various pollutants such as heavy metals (Eimers *et al*., 2001; Ikem *et al*., 2003) and also plays an important role in the assessment of heavy metal contamination (Clements and Newman, 2002).

The marine pollution is mainly contributed by industrial discharges, urban storm-water runoff, municipal wastes, agricultural drainages and other anthropogenic waste can results in heavy metal, pesticides, aliphatic and aromatic compounds, phthalate esters, nutrients, and organic waste pollutants in marine environments. These pollutants cause undesirable changes in the physico-chemical or biological factors of the ecosystem, which in turn directly or indirectly affect the ecological balance of the environment. Among these innumerable contaminants, pollution by heavy metals in aquatic environment has become a global phenomenon because of its toxicity and persistence for several decades in the aquatic environment (Cheung and Wang, 2008). In the current study to investigate the metal pollutants present in the Kuduvaiyar estuary.

Acute toxicity studies of Cadmium chloride on crab *Sesarma quadratum*

Acute toxicity caused by various toxicants on marine animals can be evaluated by quantitative parameters like survival or mortality of test animals and sensitivity of different crabs species to toxicants. Toxicity in crab is the culmination of a series of events involving various physical, chemical and biological processes. Toxicity studies measure a response of an organism to biologically active substances (Spear, 1981) and are useful in determining water quality. The wide variation in sensitivity of different species to different heavy metals depends on various factors like age, sex, weight, physical stage of the animal and presence or absence of enzyme system that can detoxify the pollutants (Nagaratnamma and Ramamurti, 1981).

Toxicity tests are important in assessing the response of organisms exposed to pollutants compared to a control. Bioassay tests have been used to evaluate the toxicity level of contaminants for aquatic organisms. There have been many types of toxicity assays employed to characterize the potential effects of all types of toxicants and validity of these tests are standardized and established by ISO or OECD (Nunes *et al*., 2008). The biological responses induced by toxicants are different among living organisms and it depends on their sensitivity to toxicants. The practice of an array of bioassays involving many indicator species at different trophic levels is a highly effective and vital method for analyzing environmental threats to the aquatic ecosystem.

Thus, an attempt was made in the current study to evaluate the 96 hours LC_{50} values of Cadmium chloride on marine crab *Sesarma quadratum*.

Cadmium is a common inorganic contaminant of coastal sediments and waters due to anthropogenic pollution and natural sources (Ivanina *et al*., 2008, 2010). Cd in water can be absorbed by aquatic organisms via respiratory system, digestive system and body surface without significant excretion (Rainbow & White, 1989).

In the present investigation, they were analyzed by SPSS 20 to obtain a number of cumulative mortality and lethal concentrations. LC_{50} of *Sesarma quadratum* at 96hr was 12.30mg/L for cadmium. The mortality at any fixed time, increased with the increase in concentration and for a particular concentration mortality increased with the increase in exposure time due to accumulation of toxicants to a dangerous level leading to death.

Biochemical response in subacute concentrations of cadmium chloride exposure in *Sesarma quadratum*

Measurement of biochemical parameter is commonly used as a diagnostic tool in aquatic toxicology and bio monitoring. Biochemical biomarkers have been used in order to prevent irreversible damage in whole organisms, communities and ecosystems (López-Barea and Pueyo, 1998). The impact of a number of contaminants on aquatic ecosystems can be assessed by the measurement of their external levels in the surrounding water or sediments, or by determining some biochemical parameters in bivalves and other organisms that respond specially to the degree and type of contamination (Nicholson and Lam, 2005).

Biochemical approach has been advocated to provide an early warning of potentially harmful changes in stressed bivalves. Analysis of chemical substances in tissues and body fluids, toxic metabolites, enzyme activities and other biochemical variables have frequently been used in documenting the toxin interaction with biological systems. Tissues reflect the physiological state of an animal because they are the products of intermediate metabolism (Artacho *et al.,* 2007).

Proteins are the most abundant intracellular macromolecules and constitute over half the dry weight of most organisms. They occupy a central position in the architecture and functioning of living matter. They are intimately connected with all phases of chemical and Physical activity that constitutes the life of the cell. Therefore they are essential to cell structure and cell function. The interplay between enzymatic and non-enzymatic proteins governs metabolic harmony (Lehinger, 1984). They are also involved in major physiological events to maintain the homeostasis of the cell. Therefore, the assessment of the protein content can be considered as a diagnostic tool to determine the physiological process of the cell (Kapil and Ragothaman, 1999).

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decrease of protein content in muscles (15.23±1.02 mg/gm), hepatopancreas (11.25±0.81mg/gm) and gills (7.68±0.51mg/gm) as compared to control were observed. In 20 days exposure of $CdCl_2$ also significant decrease of protein content in muscles (12.71±0.94mg/gm), hepatopancreas (8.64±0.84mg/gm) and gills (5.76±0.61mg/gm) as compared

to control were observed. In 30 days exposure of $CdCl_2$ also significant decreased of protein content muscles (9.17±0.71mg/gm), hepatopancreas (5.30±0.92mg/gm) and gills (3.95±0.53mg/gm) as compared to control were observed.

In the observation, on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decrease of lipid content in hepatopancreas (13.24±1.17mg/gm), gills (5.74±0.21mg/gm) and muscles (3.31±0.31mg/gm) as compared to control were observed. In 20 days exposure of $CdCl_2$ also significant decrease of lipid content in hepatopancreas (9.95±1.15mg/gm), gills (3.74±0.24mg/gm) and muscles (2.04±0.15mg/gm) as compared to control were observed. In 30 days exposure of $CdCl_2$ also significant decrease of lipid content hepatopancreas (6.49±1.05mg/gm), gills (2.15±0.21mg/gm) and muscles (1.37±0.17mg/gm) as compared to control were observed.

In the observation on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decrease of carbohydrate content in hepatopancreas (1.53±0.09mg/gm), muscles (1.52±0.12mg/gm) and gills (0.97±0.11mg/gm) as compared to control were observed. In 20 days exposure of $CdCl_2$ also significant decrease of carbohydrate content in hepatopancreas (1.16±0.13mg/gm), muscles (0.89±0.12mg/gm) and gills (0.71±0.18mg/gm) as compared to control were observed. In 30 days exposure of $CdCl_2$ also significant decrease of carbohydrate content in hepatopancreas (0.94±0.08mg/gm), muscles (0.61±0.10mg/gm) and gills (0.54±0.09mg/gm) as compared to control were observed.

Protein content was significantly decreased in cadmium chloride exposed crab than control crab due to the higher toxicity of mercuric chloride. When an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demand and hence the protein level is depleted. The depletion of total protein content may be due to breakdown of protein into free amino acid (Neff, 1985).

A plausible explanation for such depletion of the protein levels in the tissues of test crabs might be due to the enhanced proteolytic activity in these organs under heavy metal stress. The depletion in the tissue protein might be due to the diversification of energy to accomplish the impending energy demands when the animals are under toxic stress. The decreased protein concentrations might also be attributed to the necrosis of cells and consequent impairment in protein synthesis machinery. Metal causes adverse effects on the lysosomal membrane as stabilizers and release nucleases and proteases thereby affecting the RNA and protein metabolism. Subsequently, the lower rate of protein synthesis depends on the reduced availability of ATP or an increased activity of proteolytic lysosomal enzymes (Jana and Choudhari 1984).

The depletion of total protein suggests an increased proteolysis and possible utilization of the products of their degradation for metabolic purpose. The decreased protein level during exposure to pollutants may be due to increased catabolism and decreased anabolism of proteins as reported in freshwater bivalve *Parrysia corrugata* (Deshmukh and Lomte 1998). Heavy

metal-induced biochemical alteration in rohu and catla has been reported by Neha *et al*., (2004) and Ilavazhahan *et al*., (2012), In addition to this impairment of protein metabolism on metal exposure has also been reported (Shoba Rani *et al*., 2001; De Smet and Blust, 2007).

Carbohydrates form an important biochemical constituent of an animal tissue which acts as primary and immediate source of energy. Heavy metal-induced biochemical alteration in aquatic organism has been reported by Neha *et al*., (2004) and Ilavazhahan *et al*., (2012). In addition to this impaired carbohydrate metabolism on metal exposure has also been reported (Sunita Rani *et al*., 2015; De Smet and Blust, 2007).

The depletion of carbohydrate content may be due to its rapid utilization to meet the reduced energetic efficiency under the impact of Hg and Zn. Carbohydrate metabolism is broadly divided into anaerobic glycolysis in which breakdown of glucose or glycogen occurs and the aerobic citric acid cycle in which oxidation of acetyl co-A into ATP and CO_2 occurs (Nelson and Cox 2005).

As a consequence of hypoxia, the metabolic pathway is shifted from aerobiosis to anaerobiosis and a strong suppression of the specific activities of enzymes involved in citric acid cycle. These conditions might have depleted the carbohydrate levels in the crab exposed to Hg in order to meet the increased energy demands as carbohydrates form the major source of energy under stressful conditions (Hochachka and Somero, 1984). Carbohydrate metabolism is not considered to be a major energy source in crab (Walton and

Cowey, 1982), but its importance increases during hypoxia because of activation of anaerobic glycolysis. This may explain the observed depletion of the carbohydrate levels in test crab during the later stages of exposure as a result of increased demand of these molecules to provide energy for the cellular biochemical processes under hypoxic conditions induced by Hg.

Lipids are important indicators of health and normal metabolic state of any animal. Lipids constitute the rich alternate energy reserves and its calorific value is twice as that of carbohydrates and proteins and the mobilisation of lipid reserves may be due to the high energy demands to counter the toxic stress (Reddy and Rao, 1989).

The concentrations of the lipids decreased in all the tissues significantly with the progress of exposure period irrespective of exposure concentrations. Lipid depletion in tissues may be due to its utilization for energy during detoxification mechanism, stress and starvation. The decrease in lipid content may be linked with the oxidation of polyunsaturated fatty acids found in aquatic tissues into aldehydes, free fatty acids, ketones, and peroxides (Horner, 1992; Mahboob *et al*., 2004). The mechanism of decrease in lipids might be due to its conversion into carbohydrates and proteins during metal stress. Significant decrease in lipid has been noted in muscles, liver, gills and the whole body of *Oreochromis mossambicus* exposed to metals (Sunita Rani *et al*., 2015).

Pollutants induce cellular machinery that has altered the levels of biochemical constituents in crabs. The potency of toxicity of the compounds

differs in relation to the tissues, biomolecules and duration of exposure. Pollutants are responsible for cellular disorganization of the internal tissues system that in turn affects storage metabolism (Somaraj *et al*., 2005).

The alterations in the biochemical constituents of crab during exposure to toxicants imposes stress that leads to rapid mobilization of energy yielding biomolecules to generate energy required by the stressed animals to combat the erratic and rapid movements and high respiratory rates (Saravanan *et al*., 2003).

Immunological parameters in subacute concentrations of cadmium chloride exposure in *Sesarma quadratum*

The immune system of crab is physiologically similar to that of higher vertebrates, despite certain differences. In contrast to higher vertebrates, fish are free-living organisms from early embryonic stages of life and depend on their innate immune system for survival (Rombout *et al*., 2005). In recent years, the increasing consumer concern about the residues of antibiotics, hormones, growth promoters, are the danger of development of antibiotic resistant strains has led to the use of immunostimulants in aquaculture. By definition, immunostimulants are substances that can enhance the nonspecific defense mechanisms as well as specific immune response if the treatment is followed by infection or vaccination (Anderson, 1995). Many natural and synthetic substances have been reported that potentiate the fish immune system and increase disease resistance (Logambal and Michael, 1997, Hemapriya, 1997, Venkatalakshmi and Michael ,2000, Logambal *et al,* 2000,

and Sudhakaran et al., 2006). The search for new immunostimulants continues as an attempt to improve intensive crab farming.

Nonspecific immunity is a fundamental defense mechanism in aquatic animals. In addition, it plays a key role in the acquired immune response and homeostasis through a system of receptor proteins. These receptor proteins identify molecular patterns that are typical of pathogenic microorganisms, including polysaccharides, lipopolysaccharide (LPS), peptidoglycan bacterial DNA, viral RNA and other molecules that are not normally on the surface of multicellular organisms. This response is divided into physical barriers and cellular and humoral immune responses. These immunological parameters include growth inhibitors, lytic enzymes, the classic complement pathways, the alternative and lectin pathway, agglutinins and precipitins (opsonins and primary lectins), antibodies, cytokines, chemokines and antibacterial peptides. Various internal and external factors can influence innate immune response parameters. Temperature changes, stress management and density may have suppressive effects on this type of responses, while several food additives and immunostimulants can enhance their efficiency (Magnadottir, 2006, 2010).

In the observation on 10 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of haemocyte count (4.9±0.09mg/gm), phagocytosis (13.18±1.83mg/gm) and lysosomal membrane stability (5.29±0.11mg/gm) while phenoloxidase assay was significant increased (0.012±0.002mg/gm) as compared to control were observed. In 20 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of haemocyte

count (3.1±0.08mg/gm), phagocytosis (9.81±1.58mg/gm) and lysosomal membrane stability (3.65±0.09mg/gm) while phenoloxidase assay was significant increased (0.017±0.002mg/gm) as compared to control were observed. In 30 days exposure of $CdCl_2$ on *Sesarma quadratum* significant decreased of haemocyte count (2.3±0.08mg/gm), phagocytosis (5.39±1.35mg/gm) and lysosomal membrane stability (2.17±0.010mg/gm) while phenoloxidase assay was significant increased (0.025±0.003mg/gm) as compared to control were observed.

Phagocytosis is one of the most important processes in poikilothermic animals because it is the process that is least influenced by temperature (Blazer, 1991; Lange and Magnadottir, 2003; Magnadottir et al., 2005). The main cells involved in phagocytosis in fish are neutrophils and macrophages (Secombes and Fletcher, 1992). These cells remove bacteria mainly by the production of reactive oxygen species during a respiratory burst. In addition, neutrophils possess myeloperoxidase in their cytoplasmic granules, which in the presence of halide and hydrogen peroxide kills bacteria by halogenation of the bacterial cell wall. Moreover, these cells have lysozymes and other hydrolytic enzymes in their lysosomes (Fischer *et al.*, 2006). Similarly, macrophages can produce nitric oxide in mammals and can be as potent as antibacterial agents, peroxynitrites and hydroxyl groups (Secombes and Fletcher, 1992).

Lysozyme is a bacteriolytic enzyme that is widely distributed throughout the body and is part of the nonspecific defence mechanisms in

most animals. In salmonids, lysozyme has been detected in serum, secretions, mucous membranes and tissues rich in leukocytes, mainly the kidney and intestine (Grinde *et al*., 1988; Lie *et al*., 1989). Apparently, the main sources of lysozyme are monocytes/ macrophages and neutrophils. However, recent studies have detected this enzyme in the granules of the eosinophilic granular cells of the intestine (Sveinbjornsson *et al*., 1996). The bactericidal action of this enzyme involves the hydrolysis of the peptidoglycan of bacterial cell walls resulting in cell lysis. Lysozyme was initially associated with the defence against Gram-positive bacteria, but has been found to lyse Gram-negative bacteria as well. Furthermore, this enzyme is known to trigger an opsonin of the complement system and phagocytic cells (Magnadottir, 2006).

Stress markers in subacute concentrations of cadmium chloride exposure in *Sesarma quadratum*

All aerobic organisms produce free radicals as a side product during the reduction of molecular oxygen by mitochondria (Siman-Tov *et al.* 2011). Free radicals play an essential role in maintaining the physiological conditions of the body. Oxidative stress is induced by excess accumulation of the reactive oxygen and nitrogen species (RONS) that can be able to damage basic components for cell function and survival. Oxidative stress is defined as the condition occurring when the physiological balance between oxidants and antioxidants is disrupted in favor of the former with potential damage for the organism (Vignini 2011). One of the mechanisms of heavy metal toxicity is the contaminant-stimulated production of reactive oxygen and nitrogen species' (ROS and RNS), which leads to oxidative damage/stress. Therefore, antioxidant defense indices and/ or

products of cellular oxidative damage, such as proteins, lipids and nucleic acids (Dovzhenko *et al*. 2005; Valavanidis *et al*. 2006; Belcheva *et al*. 2011) and the ability of cells to counteract the effects of ROS and RNS (Regoli, 2000) serve as pollution biomarkers for assessing the quality of the aquatic environments. Among cell damaging processes caused by ROS, lipid peroxidation in biological membranes has received much attention. Laboratory and field investigations have demonstrated the usefulness of lipid peroxidation products as indicators of pollution-induced cellular damage (Dovzhenko *et al*. 2005; Belcheva *et al*. 2011).

Malondialdehyde (MDA) is the major aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid. MDA, a secondary product of lipid peroxidation, is used as an indicator of tissue damage by a series of chain reactions (Ray and Husain, 2002). The study of lipid peroxidation is attracting much attention in recent years due to its role in disease process membrane lipids are particularly susceptible to lipid peroxidation due to the presence of polyunsaturated fatty acids. It has been implicated in the pathogenesis of a number of diseases and clinical conditions. Experimental and clinical evidence suggests that aldehyde products of lipid peroxidation can also act as bioactive molecules in physiological and pathological conditions. It is now generally accepted that lipid peroxidation and its product play an important role in liver, kidney, heart and brain toxicity (Lakshmi *et al.,* 2005).

In the present study, the MDA content in serum and different body tissues viz. gills, hepatopancreas and muscles of 10, 20 and 30days of cadmium treated *Sesarma quadratum were* investigated. Among the 10, 20 and 30 days exposure of $CdCl_2$, MDA content in *Sesarma quadratum* was

significantly increased in 30 days exposure compared to 10 and 20 days exposure.

The mechanisms of the toxicity of Cd were not completely understood by Stohs and Bagchi (1995), and the antioxidant enzymatic responses observed in organisms due to Cd exposure may depend on the species, metal concentration, and duration of exposure. Cd readily interacts with sulfhydryl groups of amino acids, proteins, and enzymes. Differences in the antioxidant response between the hepatopancreas and gills may be related to different physiological functions of these tissues. The hepatopancreas involved in digestion to neutralize large amounts of toxicants that are ingested by the organism, and is the major site of metal accumulation (Pipe *et al*., 1999). In contrast, the gills are located in the ventilated mantle cavity and directly interact with the marine environment. Furthermore, as the gill epithelium is very thin, gills are frequent target organs of environmental pollutants (Rajalakshmi and Mohandas, 2005).

In the present study, the SOD activity in serum and different body tissues viz. gills, hepatopancreas and muscles of 10, 20 and 30days of cadmium treated *Sesarma quadratum* investigated. Among the 10, 20 and 30 days exposure of $CdCl_2$, SOD content in *Sesarma quadratum* was significantly decreased in 30 days exposure compared to 10 and 20 days exposure.

SOD catalyzes the dismutation of two superoxide anions ($O^{\bullet}_2 -$ and $^{\bullet}OH$) to molecular oxygen and hydrogen peroxide (Fridovich, 1998). GPx and CAT catalyze the conversion of hydrogen peroxide to water. MDA is regarded as a

useful biomarker for measuring the level of oxidative stress (Nesto *et al.*, 2007). Thus GPx is able to reduce tissue injury by removing H_2O_2 (Almeida *et al.,* 2007).

In the present study, the catalase activity in serum and different body tissues viz. gills, hepatopancreas and muscles of 10, 20 and 30days of cadmium treated *Sesarma quadratum* investigated. Among the 10, 20 and 30 days exposure of $CdCl_2$, catalase content in *Sesarma quadratum* was significantly decreased in 30 days exposure compared to 10 and 20 days exposure.

In the present study, the GPx activity in serum and different body tissues viz. gills, hepatopancreas and muscles of 10, 20 and 30days of cadmium treated *Sesarma quadratum* investigated. Among the 10, 20 and 30 days exposure of $CdCl_2$, GPx content in *Sesarma quadratum* was significantly decreased in 30 days exposure compared to 10 and 20 days exposure.

In the present study, the GSH content in serum and different body tissues viz. gills, hepatopancreas and muscles of 10, 20 and 30days of cadmium treated *Sesarma quadratum* investigated. Among the 10, 20 and 30 days exposure of $CdCl_2$, GSH content in *Sesarma quadratum* was significantly decreased in 30 days exposure compared to 10 and 20 days exposure.

In the present study, the GST activity in serum and different body tissues viz. gills, hepatopancreas and muscles of 10, 20 and 30days of cadmium treated *Sesarma quadratum* investigated. Among the 10, 20 and 30

days exposure of $CdCl_2$, GST content in *Sesarma quadratum* was significantly decreased in 30 days exposure compared to 10 and 20 days exposure.

Our results are in accordance with other studies, concerning the induction of peroxidative processes in crabs exposed to metals (Viarengo *et al*., 1990). Moreover, the results of the present study is in accordance with previous studies, regarding the possible ability of heavy metals, such as zinc and cadmium to induce ROS production and oxidative damage to macromolecules, such as DNA (Regoli, 2000), proteins and lipids (Livingstone, 2001).

Histological observation in subacute concentrations of cadmium chloride exposure in *Sesarma quadratum*

Histopathological investigations have long been recognized to be reliable biomarkers of stress in organisms (Van der Oost, Beyer and Vermeulen, 2003). Histopathological changes have been widely used as biomarkers in the evaluation of the health of organisms exposed to contaminants, both in the laboratory and field studies. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, hepatopancreas, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the aquatic animals (Gernhoferer *et al*., 2001). Hence there is a need to study the histopathological changes in the gill and hepatopancreas of *Sesarma quadratum* in response to cadmium exposure.

The lifting of the epithelium, oedema, epithelial necrosis, fusion of adjacent secondary lamellae and haemorrhage at primary lamellae were observed in the gills of the crab examined 10, 20 and 30 days of exposure. Epithelial necrosis and rupture of gill epithelium are direct deleterious effects of the irritants. The animal's defense responses are excessive mucus secretion. Lifting of the epithelium, lamellar fusion and club shaped lamellae could be protective in that it diminishes the amount of vulnerable gill surface area (Richmonds and Dutta, 1989).

The histopathological changes of gills can result in hypoxia, respiratory failure problems with ionic and acid-base balance (Alazemi *et al*., 1996). Similar observations were made by Victor *et al*. (1985) in gill pathology and haemocyte responses in the *Macrobrachium idea* exposed to mercuric chloride. Patil and Kaliwal (1989) also observed that the degree of damage to gill tissue increases according to the concentration and period of exposure of zinc in *Macrobrachium hendersoni*. Changes in the gill surfaces and increased mucus production are consistent with observed histological effects such as hyperplasia, necrosis and lamellar aneurysms in the exposed crab with response to sub lethal concentrations of cadmium.

In the present study, the hepatopancreas showed changes in the F and B cells exposed to cadmium in 10 days showed cells were found clumped and intercellular spaces invisible in the medium concentration, and a general degeneration, loss of tubules structures, vacuolation and star shape of lumen and necrosis of cells in 30 days exposure of cadmium in *Sesarma quadratum*.

The star shape of the lumen was partially lost due to morphological changes of the tubular epithelial cells, because some cells decreased in height from a normal columnar height to a low cuboidal form. In the present study, one of the most evident changes is a proliferation of B-cells in the different days of cadmium exposure in crabs, indicating a high rate of excretion from the hepatopancreas. The accumulation and elimination of the xenobiotic entering the hepatopancreatic tubules are perhaps affected by a large number of F-cells converting into B-cells.

Previous studies on the hepatopancreas at different biological levels such as the structure, development, physiology, metabolism, and biochemistry concluded that this digestive organ possesses several functions, including absorption, digestion, storage, and secretion (Caceci *et al*., 1988). Krishnamoorthy and Subramonian (1996) also reported changes such as elongation of hepatopancreatic cells, and shrunken cells in *Macrobrachium lamarrg* exposed to low (0.0065 ppm), and high (0.02l5ppm) concentrations of copper. Destructive and deteriorative changes in the hepatopancreas and gills were observed in *Penaeus indicus* exposed to Zn at a low concentration of 100 ppb (Viswanathan and Manisseri, l995). The noted histopathological changes in the hepatopancreas may be due to the accumulation of the pesticides since this organ is the center of storage, metabolism and detoxification.

Abnormal infiltration of hemocytes in the interstitial sinuses noted in the hepatopancreas of test animals suggest that the mechanism of cellular/ host defense was in operation to neutralize the tissue damage caused by cadmium

and since hemocytes are the most important form of cellular defense in crustaceans (Bodhipaksha and Weeks-Perkins, 1994). The formation of necrotic hepatopancreatic tubules recorded in test crabs indicates the fact that the distortion, disintegration and death of cells occurred in the hepatopancreas of *Sesarma quadratum* exposed to the highest sublethal concentration of cadmium. Therefore cadmium toxicity affects the normal integrity and causes tissue damage in the body tissues of *Sesarma quadratum*.

CHAPTER-VI

SUMMARY AND CONCLUSION

CHAPTER VI

6. SUMMARY AND CONCLUSION

6.1 SUMMARY

Estuarine areas are complex and highly changing environments at the interface between freshwater and marine aquatic ecosystems. Despite their environmental variability, estuaries are usually characterized by high biomass due to their strong primary production, especially when compared with marine areas. These conditions enhance the development of heterotrophic populations. Moreover, estuaries are of special interest in ecotoxicology because they are potentially the most exposed coastal areas regarding any source of pollution. Worldwide chronic contamination of estuaries by inorganic toxicants (e.g., heavy metals), hydrocarbons, or persistent organic pollutants and its effect on estuarine organisms have retained much attention to date. Seasonal changes in the nature and level of contaminants found in these areas may occur, and some toxic substances may be present at higher levels at some critical periods for estuarine organisms.

Crustaceans are useful as integrating sentinels of exposure to certain contaminants. The crab (*Sesarma quadratum*) is a useful sentinel, due to its biological and ecological characteristics. Crabs are important in the estuarine food web because they are not only predators, but also scavengers and prey themselves. Also, they are economically important as food for humans. The purpose of this study was to biomonitoring heavy metals Hg, Cd, Cr, Cu, Ni, Pb and Zn on water associated with crab from Kuduvaiyar estuary is situated

in the Southeast coast, near Nagapattinam, India. The environmental quality of a stretch of Kuduvaiyar estuary and the coastal water of the East coast of Tamil Nadu, assessed by the use of physicochemical parameters. In the present study to assess the Ecotoxicological studies in estuarine crab *Sesarma quadratum* in Kuduvaiyar estuary, Southeast coast of India.

From the experimental studies, the following were obtained

Hydro-biological studies:

The analysis carried out in hydrobiological parameters (temperature, pH, electrical conductivity, dissolved oxygen, biological oxygen demand, chemical oxygen demand, total alkalinity, salinity and total hardness) allowed to make a diagnostic about the physico-chemical health of this estuarine ecosystem. They revealed a real disturbance of the physical characteristics of this ecosystem and particularly at the site of Kuduvaiyar estuary at Nagapattinam.

Hydro-chemical studies:

Water samples were collected from different seasons (Pre-monsoon, monsoon and post monsoon) and analyzed varied chemical parameters as ammonium, nitrate, sodium, chloride, sulphate and fluoride. Among the various seasons, the maximum level observed in pre-monsoon is followed by ammonium, nitrate, sodium, chloride, sulphate and fluoride. Results of the study concluded that chemical characteristics of this ecosystem has distressed at the site of Kuduvaiyar estuary at Nagapattinam.

Heavy metals analysis

Heavy metals such as cadmium, chromium, copper, lead, mercury, nickel and zinc in water were investigated in different seasons and significant alterations were observed.

Toxicity bioassays: LC_{50} / 96h determination.

Crab samples were exposed to different concentrations (5, 10, 15, 20 and 25μg/L) of toxicants as cadmium for 96h and their cumulative mortality was calculated in 12 hours of intervals. Results were analysed by SPSS 20 to obtain a number of cumulative mortality and lethal concentrations. LC_{50} of *Sesarma quadratum* at 96hr was 12.30mg/L for cadmium was observed. The acute toxicity values obtained in this study strongly suggest negative effects on survival as Cadmium concentration increased. The cause for mortality is due to the toxicity of metal toxicants and it depends primarily on duration of exposure and concentration combinations.

Biochemical studies

Protein, carbohydrate and lipids were investigated in cadmium exposure tissues of gills, hepatopancreas and foot muscle of crab as *Sesarma quadratum*. Protein, carbohydrate and lipid content were significantly decreased in gills, hepatopancreas and muscle of *Sesarma quadratum*. The decreased protein, carbohydrate and lipid content depends the duration of the cadmium exposure.

Immunological parameters

The present study was to investigate the immunological parameters such as total haemocytes, phagocytosis, phenoloxidase and lysosomal membrane stability. To reduce the level of total haemocyte count and the immune functions were observed. Phenoloxidase activity was directly proportional to the duration of the cadmium chloride exposure.

Oxidative stress markers

Oxidative stress markers as Malondialdehyde (MDA) highly increased during the experimental period, antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S- transferase (GST) evaluated in cadmium exposed to *Sesarma quadratum*. Significant alterations were observed in oxidative stress markers, which are useful indicators to understand the physiological state of crab under stress when exposed to cadmium toxicants.

Histological studies

Histopathological changes in gills and hepatopancreas of *Sesarma quadratum* examined in response to cadmium exposure. Moderate alterations were observed in the gills followed by hepatopancreas of *Sesarma quadratum* Therefore cadmium toxicity affects the normal integrity and causes tissue damage in the body tissues of *Sesarma quadratum*.

6.2 CONCLUSION

Overall studies indicated that accumulation of heavy metals in water and organs of *Sesarma quadratum* from Kuduvaiyar estuary, East coast of Tamil Nadu slightly higher than the standard values. The experimental data presented in this report demonstrate that exposure of the crab *S. quadratum* to Cd causes drastic changes in biochemical, immunological, antioxidant and stress related parameters. Considering all of the biomarkers investigated in this study, it was concluded that the detoxification system of *S. quadratum* is interactive and complex. The detoxification of Cd metal in organs of *Sesarma quadratum* produced the free radicals of oxygen as a Cd mediated toxicity and stress in *S. quadratum*. The implication of this finding is to reduce the consumption of *Sesarma quadratum* from the coastal water of Kuduvaiyar estuary helps to avoid the various health hazards induced by heavy metals.

The following suggestions to maintain the health of the Kuduvaiyar estuary:

1. To stop the discharges of waste from the fish/prawn export factories.

2. Over- dumping of spoiled fishes and meat waste should be prohibited.

3. The improper recycling of the industrial effluents and domestic waste water mix with the sea water should be avoided.

4. Reduce the use of chemical fertilizers and pesticides and to increase the use of biofertilizers.

5. Scientific monitoring of heavy metal concentration in water and crustaceans must be undertaken periodically throughout the year.

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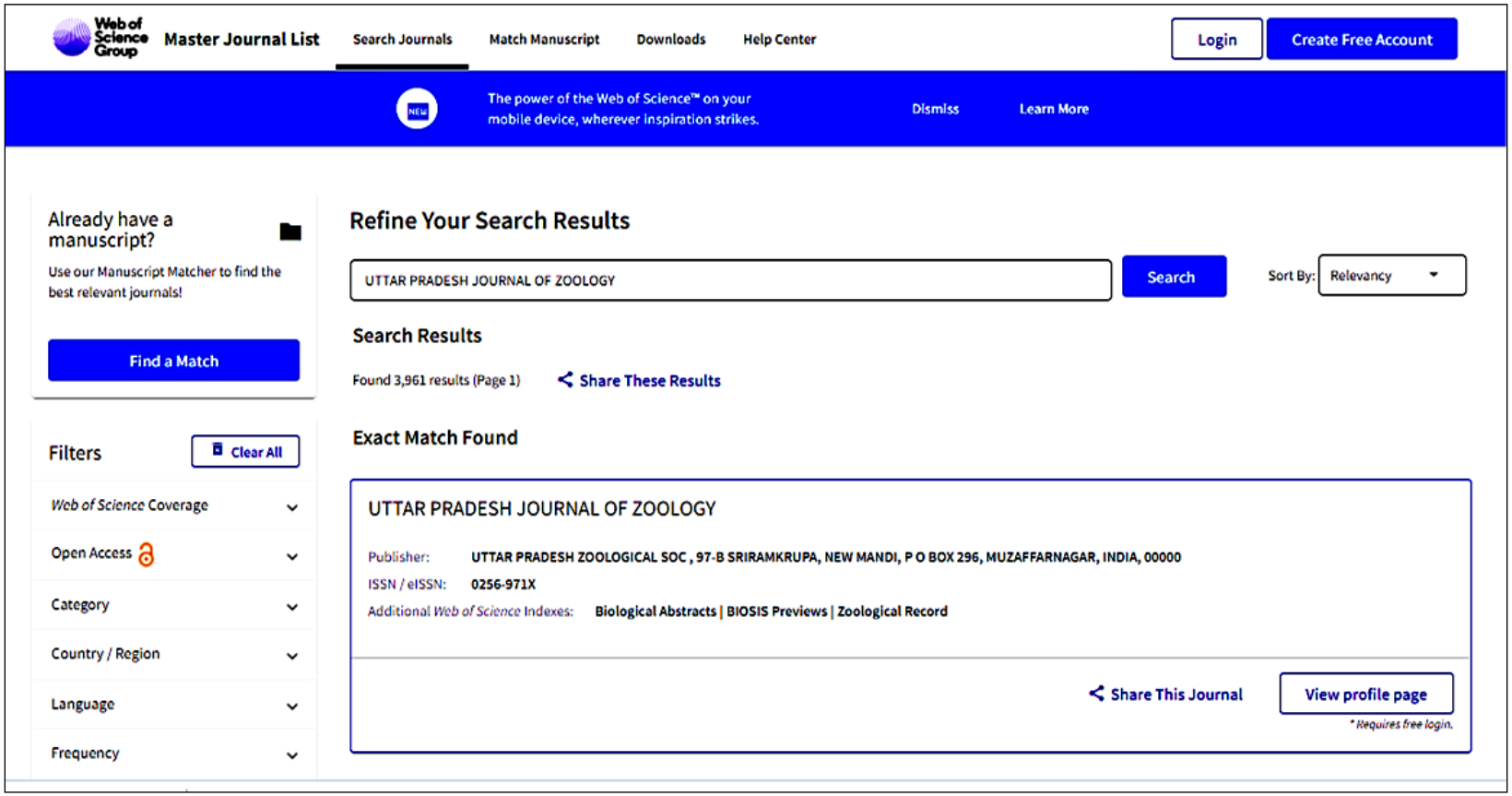
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PHYSICO-CHEMICAL ANALYSIS OF KUDUVAIYAR ESTUARINE WATER OF SOUTH EAST COAST OF INDIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

A study has been undertaken to enlighten the water quality parameters of coastal water of Kuduvaiyar estuary and Kuduvaiyar east coast was selected as the reference site. The concentration of water quality parameters like pH, DO, Ammonium,Nitrate, BOD, Sodium, Chloride,Sulphate and Fluoride were done in the laboratory as per the standard methods compared with Fisheries and aquatic life standard value. The reason for choosing the coastal water level due to the continuous discharge of agricultural, domestic sewage and industrial effluent in the estuary. The present baseline information of the physicochemical properties of water would form a useful tool for further ecological assessment and monitoring of Kuduvaiyar estuarine water of South East coast of India. The conclusion of the present study is that physico-chemical parameters influence the Ichthyofaunal diversity and Kuduvaiyar estuary are not extremely pollutant but at the same time there is a possibility of gradual addition of pollutants in due course. It reveals that the estuarine environment is largely influenced by the annual cycle of monsoon.

Keywords: Physicochemical parameters; fisheries and aquatic life standard value; kuduvaiyar estuary.

1. INTRODUCTION

Estuarine and coastal areas are complex and dynamic aquatic environments. India has a long coastline of 8,129 km and of this 6,000 km is rich in estuaries, creeks, brackish water, lagoons and lakes. The southeast coast of India is an important stretch of coastline, where many major rivers drain into the Bay of Bengal and they are also richer in marine fauna and flora [1]. Agricultural, industrial and urban activities are considered to be major sources of addition of nutrients to aquatic ecosystems posing a big threat to

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fish stocks [2]. Eutrophication is of great environmental distress, leading to diverse problems such as toxic algal blooms, loss of oxygen, fish mortalities and eventually loss of biodiversity [3,4]. The impacts vary from relatively minor to major disruptions due to bioaccumulation and biomagnification processes [5,6].

Estuaries, the main contributors of fisheries in India, suffer from severe damage which receive large amounts of contaminants due to increased industrialization and urbanization along the coastal areas by continuous discharge of domestic sewage and industrial effluents. Overloading of the estuaries with contaminants for a longer period of time has resulted in the significant buildup of pollutants with a resulting impact on water properties [7,8]. Paramisivam and Kannan [9] and Rajalakshmi and Sukumaran [10] reported that factors related to water quality such as temperature, pH, salinity, dissolved oxygen, total organic carbon and nutrients are particularly important for determining the biota and ecosystem functions in coastal waters. Hence, the present study was therefore undertaken with a view to provide much needed information on the water quality parameters in the coastal water of Kuduvaiyar estuary and Kuduvaiyar east coast was selected as the reference site.

2. MATERIALS AND METHODS

2.1 Location of Sample Collection

The water sample was collected from Kuduvaiyarestuary, Nagapattinam, Tamil Nadu, India. The present study was carried out for the Kuduvaiyarestuary. During the study period (2019-2020), samples of water were collected fortnightly; the data were pooled seasonally to understand the seasonal effect. The Kuduvaiyar estuary is formed by the tributaries of Cauvery river and opens into an estuary on the South East coast of India. The three distinct seasons were pre-monsoon, monsoon and post-monsoon periods. The samples were collected from five different points of each site and were mixed together to prepare an integrated sample. The water pH were analyzed immediately on the spot after collection, whereas the analyses of DO, Ammonium,Nitrate, BOD,Sodium, Chloride,Sulphate and Fluoride were done in the laboratory as per the standard methods compare with Fisheries and aquatic life standard value (1996).

2.2 Physiochemical Parameter

The methods used for the analysis of various physico-chemical parameters were the same as given in Standard Methods for the Examination of water [11-13]; Golterman et al., [14] and National Environmental Engineering Research Institute [15].

3. RESULTS AND DISCUSSION

Pollution of the aquatic environment and its effects on the living resources, especially the fishery resources, has assumed considerable interest as well as importance in recent times. Most of the rivers which discharge large quantities of water into the coastal marine environment are polluted and these pollutants obviously end up in the inshore coastal waters. The vast marine environment has long been used as a site for the disposal of wastes. In some cases the polluted material is discharged directly into the sea and in other cases the pollutant reaches the rivers and Estuaries and finally ends up in the sea. Estuaries, the important contributors of fisheries in India, suffer from severe loss of fish production due to increased industrialization and urbanization along the coastal zone by continuous discharge of industrial effluents [7]. Rainfall is the most important cyclic phenomenon in tropical countries as it brings important changes in the hydrographical characteristics of the marine and estuarine environments. Under the influence of a variety of interrelated biotic and abiotic structural compounds and intensive chemical, physical and biological processes, estuaries are highly variable systems [16]. Data on the range of atmospheric and surface water pH, DO, Ammonium,Nitrate, BOD,Sodium, Chloride,Sulphate and Fluoride are given in Table 1.

pH of water is an important environmental factor, the fluctuation of pH is linked with chemical changes, species composition and life processes. It is generally considered as an index for suitability of the environment [17]. Ellis [18] has observed that a pH range of 6.7 to 8.4 is suitable for the growth of Aquatic biota. In Kuduvaiyar estuary pH ranged from (7.9 to 8.7) indicating slight alkaline nature of water. pH varied with the seasons as lowest in monsoon months (7.9) and highest during pre monsoon (8.7) while post monsoon pH was 8.3 (Fig. 1). Our findings are in concordance with Mohan Raj et al. [19] and Ravichelvan et al. [20]. From the present study the pH result indicating slight alkaline nature which could be due to solutes, shows buffering action, i.e. H^+ ions are compensated with OH^- ions. It has been mentioned that the increasing pH appears to be associated with increasing use of alkaline detergents in residential areas and alkaline material from wastewater in industrial areas [21].

Table 1. Physico-chemical analysis of Kuduvaiyar estuary water of east coast of Tamil Nadu (2019-2020)

Parameters	Season			*Fisheries and aquatic life (1996) Std.
	Pre-monsoon (March to May)	Monsoon (June to Sept.)	Post-monsoon (Oct. to Dec.)	
pH	8.7	7.9	8.3	6.0-9.0
DO (mg/L)	5.9	5.2	5.3	4.0-6.0
Ammonium (mg/L)	0.62	0.45	0.58	0.5
Nitrate (mg/L)	38.57	32.67	35.92	40
BOD (mg/L O_2)	4.57	2.34	3.72	3
Sodium (mg/L)	132.49	97.51	125.74	120
Chloride (mg/L)	302.28	213.47	286.51	300
Sulphate (mg/L)	86.92	73.21	80.65	100
Fluoride (mg/L)	0.65	0.54	0.59	0.75

** Maximum allowable concentrations of selected water quality variables for Fisheries and aquatic life uses. DO: Dissolved oxygen; BOD: Biological oxygen demand*

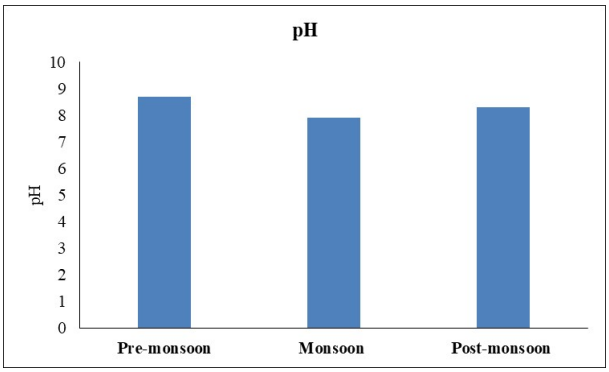

Fig. 1. pH of Kuduvaiyar estuary water of east coast of Tamil Nadu

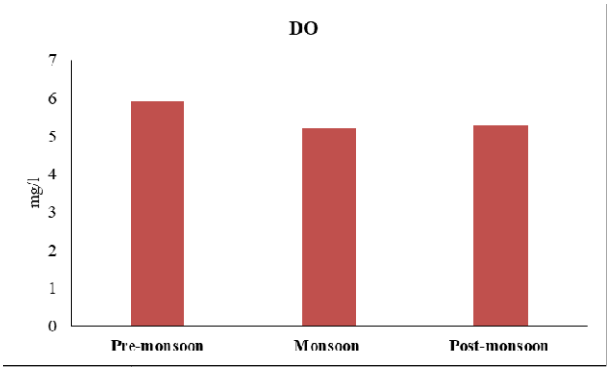

Fig. 2. DO of Kuduvaiyar estuary water of east coast of Tamil Nadu

The value of dissolved oxygen is remarkable in determining the water quality criteria of an aquatic ecosystem. The Dissolved oxygen is the regulator of metabolic activities of organisms and thus governs metabolisms of the biological community as a whole and also acts as an indicator of trophic status of the

water body [22]. Maximum dissolved oxygen was recorded during pre-monsoon months (5.9mg/l) which might be due to the cumulative effect of higher wind velocity coupled with heavy rainfall. This could also be because of freshwater mixing from Kuduvaiyar estuary and low metabolic rate of organisms. Minimum dissolved oxygen was recorded (Fig. 2) during post monsoon and monsoon months (5.3 and 5.2 mg/l respectively) due to high temperature, availability of huge quantities of untreated domestic sewage with enriched inorganic reductant and high metabolic rate of organisms. Similar observations were drawn by Ravichelvan et al. [20] and Sahu et al., [23]. Dissolved oxygen is the regulator of metabolic activities of organisms and thus governs metabolisms of the biological community as a whole and also acts as indicator of trophic status of the water body [22].

Tarzwell [24] has suggested that a minimum of 3 mgl^{-1} dissolved oxygen is necessary for healthy fish and other aquatic life. The present study showed minimum values of dissolved oxygen during monsoon and post monsoon which are not sufficient for most of the aquatic organisms. Interestingly even when DO was much below the optimum level, the rotifers were present in abundance indicating their wide range of tolerance. One of the reasons for the fatality of many teleosts observed during monsoon in Kuduvaiyar estuary might be the low level of dissolved oxygen. Dissolved oxygen is the most important indicator of the health of a water body and its capacity to support a balanced aquatic ecosystem of plants and animals. Waste water containing organic pollutants depletes the dissolved oxygen and may lead to impact benthic communities by producing acute changes in their distribution, abundance, and diversity of species [25]. The lower dissolved oxygen also implies that the estuaries were more polluted downstream.

Biological Oxygen Demand (BOD) is the amount of oxygen required by the living organisms engaged in the utilization and ultimate destruction or stabilization of organic water. It is a very important indicator of the pollution status of a water body. In the conducted experiments, BOD was high in Domestic and Industrial Effluent due to high organic load and excessive growth of total microorganisms [26]. This may be as a result of escape of organic matter (organic) into the river mostly from faecal waste deposition by the surrounding urban area and human settlements. Control Sample revealed BOD value below detection limit, because it was distilled water and had no organic load. In the present study BOD was lowest in monsoon months (2.34 mg/l) and highest during pre-monsoon (4.57mg/l) while post monsoon BOD was 3.72 mg/l (Fig. 3).

In the present study ammonium, nitrate, sodium, chloride, sulphate and fluoride were lowest in monsoon months (0.45, 32.67, 97.51, 213.47, 73.21 and 0.54mg/l) and highest during pre-monsoon (0.62, 38.57, 132.49, 302.28, 86.92 and 0.65mg/l) while post monsoon months were 0.58, 35.92, 125.74, 286.51, 80.65 and 0.59mg/l (Fig. 4 to 9). The observed parameters were compared with Fisheries and aquatic life (1996) Standard value for aquatic organisms.

Sulphate (SO_4^{2-}) concentrations in water are the results of natural weathering of minerals, atmospheric deposition, and industrial discharges along with agricultural run-off and sewage [27]. The burning of fossil fuels, particularly high-sulphur coal and diesel, is also a major source of sulphur to the atmosphere which and precipitation could serve as a source of sulphate content of aquatic ecosystem. Sulphates are of concern because the unpleasant odour it gives to the waste water and corrosion problem due to the reduction of sulphates to hydrogen sulphideunder anaerobic conditions.

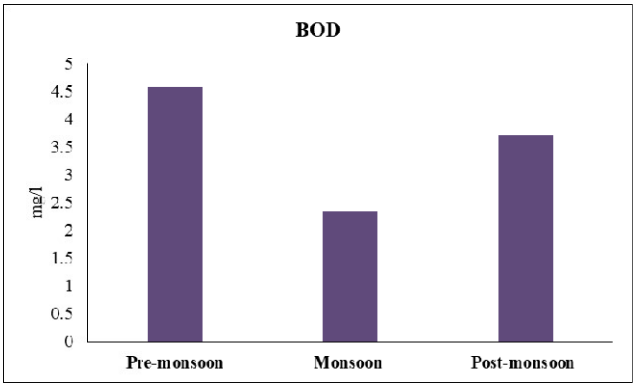

Fig. 3. BOD of Kuduvaiyar estuary water of east coast of Tamil Nadu

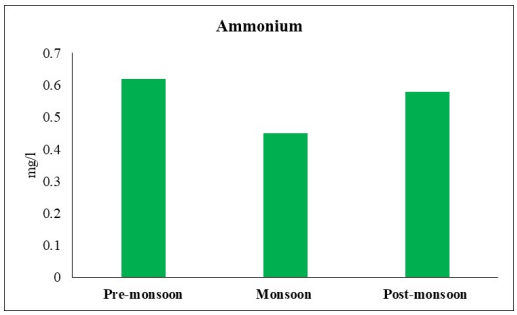

Fig. 4. Ammonium of Kuduvaiyar estuary water of east coast of Tamil Nadu

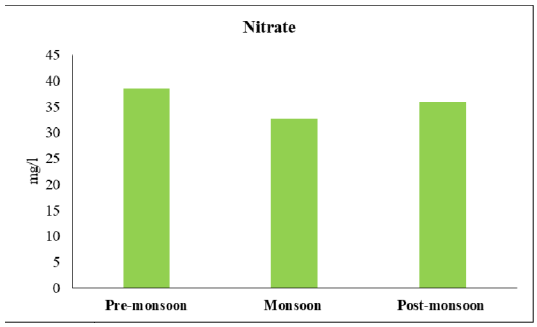

Fig. 5. Nitrate of Kuduvaiyar estuary water of east coast of Tamil Nadu

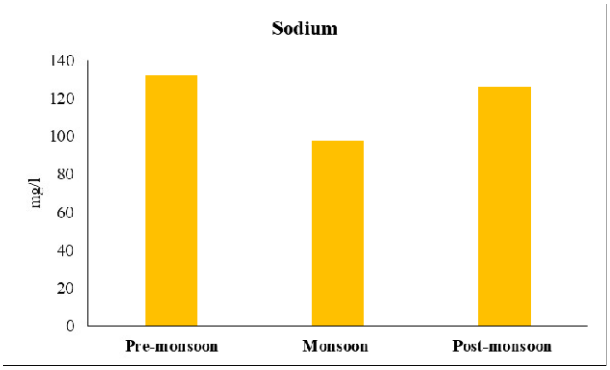

Fig. 6. Sodium of Kuduvaiyar estuary water of east coast of Tamil Nadu

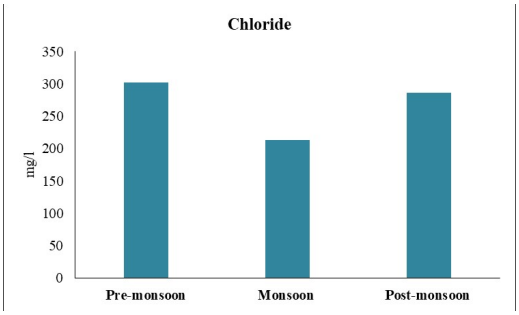

Fig. 7. Chloride of Kuduvaiyar estuary water of east coast of Tamil Nadu

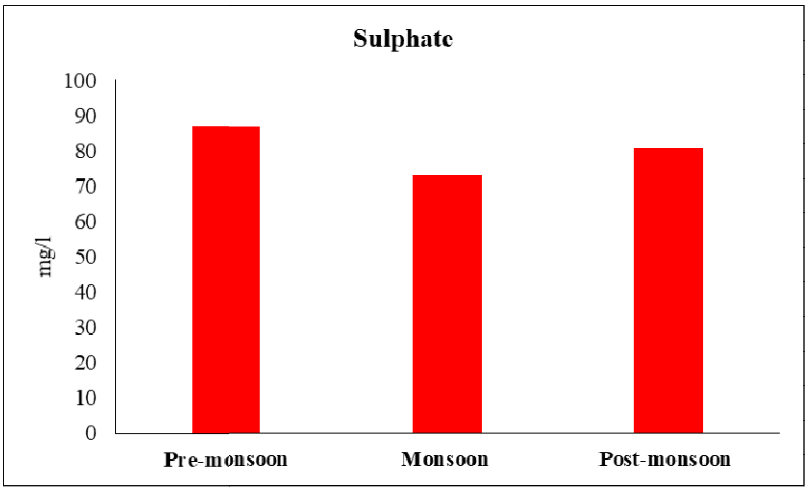

Fig. 8. Sulphate of Kuduvaiyar estuary water of east coast of Tamil Nadu

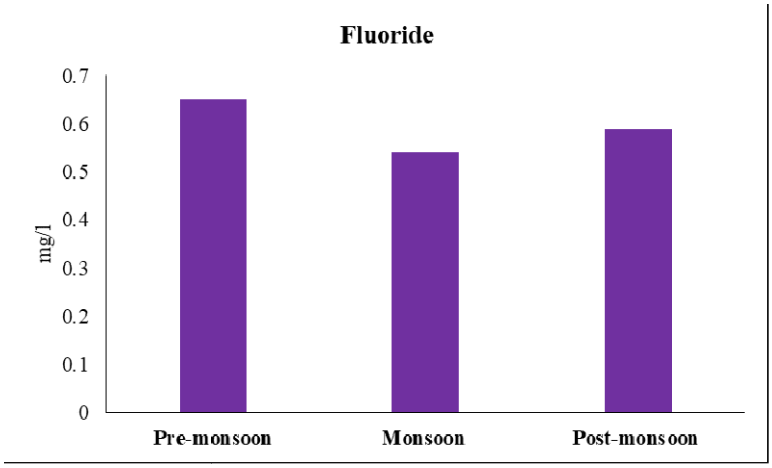

Fig. 9. Fluoride of Kuduvaiyar estuary water of east coast of Tamil Nadu

Because of the great reactivity, it is difficult to find fluorine in its elemental state and exists either as inorganic fluorides or as organic fluoride compounds (e.g., freons). In the global environment, inorganic fluorides are much more abundant than organic fluoride compounds. The major natural sources of inorganic fluorides are weathering of fluoride minerals and volcanic eruptions [28]. However, the increased presence of Fl^- in the Kuduvaiyar estuary than the reference site might be the result of anthropogenic activities including the industrial and urban runoff especially due to its proximity to the industrial complex. Rajalakshmi and Sukumaran [10] workers have reported that activities involving aluminium smeltering, discharge of fluoridated municipal waters, manufacturing of brick, ceramics, glass and fluoride chemicals, may cause significant increases in the fluoride concentration of surface waters.

4. CONCLUSION

Among the entire parameters investigated, exhibited significant variations in Kuduvaiyar estuarine water The present baseline information of the physicochemical properties of water would form a useful tool for further ecological assessment and monitoring of Kuduvaiyar estuarine water of South East coast of India. The conclusion of the present study is that physico-chemical parameters influence the Ichthyofaunal diversity and Kuduvaiyar estuary are not extremely pollutant but at the same time there is a possibility of gradual addition of pollutants in due course. It reveals that the estuarine environment is largely influenced by the annual cycle of monsoon.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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ACUTE TOXICITY STUDIES OF CADMIUM ON CRAB *Sesarma quadratum* (Decapoda)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

An estuary is a partially enclosed coastal body of brackish water with one or more rivers or streams flowing into it, and with a free connection to the open sea. Estuaries form a transition zone between river environments and maritime environments and are an example of an ecotone.. Since many species of crustaceans inhabit estuaries, numerous studies have aimed at examining the bioaccumulation and effects of various toxicants in these animals. Heavy metals can accumulate in the tissues of aquatic organisms and cause a range of hazardous effects to all organisms through biomagnifications. The aimed of this study was to investigate the acute toxicity effects of cadmium on the crab (*Sesarma quadratum*). Crab samples were exposed to different concentrations (5, 10, 15, 20 and 25µg/L) of cadmium for 96h and their cumulative mortality was calculated in 24 hours intervals. Results were analyzed by SPSS 20 to obtain a number of cumulative mortality and lethal

Keywords: *Sesarma quadratum;* LC_{50}; cadmium; acute toxicity; heavy metal pollution.

1. INTRODUCTION

Crabs are generally covered with a thick exoskeleton, composed primarily of highly mineralized chitin and armed with a pair of chelae (claws). Crabs vary in size from the pea crab, a few millimeters wide. Crab is packed with protein, which is important for building and maintaining muscle. Crab also contains high levels of omega-3 fatty acids, vitamin B12, and selenium. These nutrients play vital roles in improving general health while helping prevent a variety of chronic conditions. The omega-3 fatty acids in crab provide many benefits related to heart health. These important nutrients may help lower triglycerides, reduce blood clotting, and make it less likely that you'll develop an irregular heartbeat. Many of the nutrients found in crab, including vitamin B12 and folate, help reduce the risk of vitamin deficiency

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anemia. Estuaries and coastal zones receive pollutant inputs from both specific and nonspecific sources, especially such ecosystems as seaports, cities, or other industrialized coastal areas that receive chronic inputs of metals. Since many species of crustaceans inhabit estuaries, numerous studies have aimed at examining the bioaccumulation and effects of various toxicants in these animals [1,2]. Metals are substances with high electrical conductivity, malleability and luster that voluntarily lose their electrons to form cations. They are found naturally in the earth's crust and their compositions vary among different localities, resulting in spatial variations of metal concentrations. Metal distribution in the atmosphere is determined by the properties of the given metal and by various environmental factors [3]. Heavy metals enter the surroundings by natural means and through human activities. The contamination of natural aquatic resources with heavy metals released from industrial, domestic and other anthropogenic activities has become a matter of concern over the past few decades [4]. Harmful effects of these heavy metals on aquatic organisms can be detected by performing toxicity tests that allow establishing a dose-response relationship [5,6] which help society in predicting acute and chronic effects on aquatic fauna as well as in regulating toxic chemical discharges into the water bodies [7]. No toxicity work recording cadmium was carried out in this crab. This work was aimed to find out the acute toxicity of cadmium (Cd) on the crab *Sesarma quadratum*.

2. MATERIALS AND METHODS

2.1 Collection and Acclimation of Experimental Crab

Sesarma quadratum crabs (Fig. 1) were collected from Kuduvaiyar estuary situated in the Southeast coast, near Nagapattinam, Tamil Nadu, India (Station: Lat 10° 45' N, Long 79° 96' E. The Kuduvaiyarriver is a tributary of the major river, 'Cauvery' of South India. The Crab were acclimatized under laboratory conditions for 7 days prior to the start of this experiment. Glass aquaria of 10 liter marine water capacity were used in this experiment. Fresh air was supplied to each aquaria through an air pump fitted with a capillary system.

2.2 LC_{50} Determination

After acclimation, healthy crab of *Sesarma quadratum* were chosen for the LC_{50} determination of cadmium by static renewable bioassay. Crabs were not fed during the experimental period. Various concentrations (5, 10, 15, 20 and 25µg/L) of the test solutions were prepared from cadmium chloride stock solutions. A group of 10 laboratory acclimatized crabs of a particular species having the same weight, size and age were introduced into each test concentration of cadmium. Triplicates and appropriate controls were maintained for each concentration. LC_{50} values were calculated as per Spearman-Karber methods [8,9]. Toxicity tests were conducted in accordance with the method recommended by Sprague [10]. Median lethal concentrations of 96h were calculated by Finney [11] probit analysis using SPSS Ver.20 Log_{10} Base calculation.

Fig. 1. ***Sesarma quadratum***

3. RESULTS AND DISCUSSION

Acute lethal toxicity bioassays are useful for providing a measurement of the relative toxicity of substances, for assessing the sensitivity of the species' different stages of life to a particular substance, and for determining concentrations of chronic toxicity so as to assess water quality criteria. Moreover, mixed toxicity bioassays provide information on the global effects of mixtures present in environments and allow evaluation of the magnitude of the effects by determining additive, synergistic, and/ or antagonistic responses.

To determine the LC_{50} value graphs were plotted between % mortality and log concentrations of toxicant. The concentrations obtained by drawing a perpendicular line against 50% mortality and calculated their antilog value. In the present investigation, LC_{50} of *Sesarma quadratum*at 96 hrs was 12.30µg/L for cadmium. Results of present studies (Table 1. and Fig. 2.) clearly indicated that the rate of mortality for any fixed time increased with increase in concentration and for a particular concentration with increase in exposure time and a regular mode of toxicant due to accumulation up to dangerous levels leading to death. Table 1. showed the relation between the cadmium concentration and the mortality rate of *Sesarma quadratum*. The results obtained for 96 hours of toxicity experiments of

cadmium crabs were estimated by Finney's probit analysis method.

The observed percentage of mortality of *Sesarma quadratum*for cadmium for different concentrations was given in Table 1. Fig. 2 showed the probit line graph of the cadmium toxicity data and probit kill vs log concentrations. The 96h LC_{50} study observed that *Sesarma quadratum*was significantly more susceptible to cadmium toxicity. Present 96 hours of crabs mortality were concentration (µg/L) of cadmium-dependent $R^2 = 0.9592$.

Data analyzed by SPSS ver. 20, Regression equation and r^2value was calculated by log. Concentration vs probit value, LCL: 95% of Lower Confidence Limits, UCL: 95% of Upper Confidence Limits. Since the significance level is *P*> 0.050, no heterogeneity factor is used in the calculation of confidence limits.

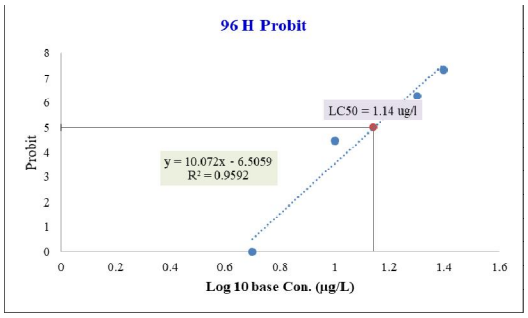

Fig. 2. 96 Hours of LC_{50}values of cadmiumon estuarine crab *Sesarma quadratum*

Acute toxicity caused by various toxicants on estuarine animals can be evaluated by quantitative parameters like survival or mortality of test animals and sensitivity of different crabs species to toxicants. Toxicity in crab is the culmination of a series of events involving various physical, chemical and biological processes. Toxicity studies measure a response of an organism to biologically active substances [12] and are useful in determining water quality. The wide variation in sensitivity of different species to different heavy metals depends on various factors like age, sex, weight, physical stage of the animal and presence or absence of enzyme system that can detoxify the pollutants [13].

Cd is a common inorganic contaminant of coastal sediments and waters due to anthropogenic pollution and natural sources [14,15]. It can be accumulated in aquatic animals (e.g. crabs, shrimps, oysters and mussels) after entering through different ways such as respiratory tract, digestive tract, surface penetration etc. [16]. Cd in water can be absorbed by aquatic organisms via respiratory system, digestive system and body surface without significant excretion [17].

Studies concerning the influence of Cd on the ultrastructure of crabs have appeared in the past few years. The published studies have focused on the destruction of membrane systems and morphologic changes of cells. Cd can accelerate cellar lipid peroxidation and cause the accumulation of lipid peroxides. These free radicals and their reaction products, peroxides, can often cause various biological macromolecules, including DNA, to change structures and properties through chemical reactions, such as hydrogen abstraction, oxidation sulfhydryl and carbon chain destruction. Cd can also decompose the unsaturated fatty acid into malondialdehyde (MAD) by peroxiding and cause biological macromolecules to crosslink into abnormal macromolecules which degrade membrane structure and alter the membrane permeability [18].

Table 1. Toxicity of cadmium on crab *Sesarma quadratum*

Concentration (µg/L)	**Log Con. (µg/L)**	**Number of exposed crab**	**96 hours**		
			Number of mortality	**% of mortality**	**Probit**
5	0.69	10	0	0	0
10	1.00	10	3	30	4.48
15	1.17	10	7	70	5.52
20	1.30	10	9	90	6.28
25	1.39	10	10	100	7.33
Control		10	Nil	Nil	Nil
		LC_{50}value			
	LC_{50}value (µg/L)			**Chi-Square (Df = 3)**	
96 Hours	**LC_{50}**	**LCL**	**UCL**	**X^2 value**	**Sig.**
	12.30	9.57	14.58	0.379	0.945

Studies regarding the effects of Cd on ovarian development in crabs and shrimps have been conducted since the 1990s. The majority of experiments showed that Cd inhibited ovarian growth, reduced hatch rates of the fertilized eggs and led to embryonic deformity. Reddy *et al.* [19] found Cd could inhibit 5-HT-induced ovarian maturation in the red swamp crayfish, *Procambarus clarkia*. Lee et al. [20] documented that Cd deformed eyespots, reduced hatching success, and inhibited growth of oocytes of *Callinectes sapidus*. Naqvi et al. [21] reported that *P. clarkia* treated with Cd hatched 48 eggs with a hatching rate of only 17%.

4. CONCLUSION

The present study was an attempt to find the effect of cadmium toxicity on crab *Sesarma quadratum*. The 96h LC50 study observed that Sesarma quadratumwas significantly more susceptible to cadmium toxicity. Present 96 hours of crabs mortality were concentration (µg/L) of cadmium-dependent. The toxicity study on crabs will be very useful to provide a future understanding of ecological impact.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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