STUDIES ON THE DISTRIBUTION PATTERNS OF TOXIC METALS IN CUTTLEFISH (*Sepia* Spp.) IN RELATION TO LEVELS IN FOOD FISHES ALONG THE WEST COAST OF INDIA AND SAFETY OF INDUSTRIAL PRODUCTS

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BY

LIJU FRANCIS. M.

CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY
COCHIN 682 029

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DECLARATION

I, Liju Francis.M, do hereby declare that the thesis entitled " **Studies on the distribution patterns of toxic metals in Cuttlefish** (*Sepia* spp.) **in relation to levels in food fishes along the west coast of India and safety of industrial products**" is an authentic record of research work carried out by me under the supervision and guidance of Dr. P. T. Lakshmanan, Principal Scientist, Central Institute of Fisheries Technology, Cochin and that no part of this work has previously formed the basis for the award of any degree, diploma, associate-ship, fellowship or other similar title of any University or Institution.

Liju Francis

Cochin-682 029
13-06-2003

LIJU FRANCIS. M

CERTIFICATE

This is to certify that this thesis bound herewith is an authentic record of the research carried out by Ms. Liju Francis. M, under my supervision and guidance in the Central Institute of Fisheries Technology, Cochin in partial fulfillment of the requirements for the degree of Doctor of Philosophy and that no part of this work thereof has been submitted for any other degree in any university.

Cochin-682 029
13-06-2003.

Dr. P. T. Lakshmanan
Principal Scientist & Supervising guide
Central Institute of Fisheries Technology
Cochin- 682 029

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Chapter 1

INTRODUCTION

Pollution of marine ecosystems by heavy metals is of environmental concern worldwide. Domestic sewage, industrial effluents, combustion emissions, mining operations and metallurgical activities are among the sources of anthropogenic metal inputs. Although heavy metals in trace concentrations are normal constituents of marine organisms, at high levels they are potentially toxic and may disrupt the biological activities of aquatic ecosystems. The ability of heavy metals to be concentrated in the organs of marine organisms accounts for their toxicity and also poses a direct threat to both aquatic biota and man (Watling, 1983).

The term 'trace metal' is used to designate the elements, which occur in small concentrations (<100 ppm) in natural biological systems. There are both essential and non-essential trace metals. It is well known that Cu, Ni, Zn, Cr, Co, Fe and Mn are essential to life (Mertz, 1981) and known as essential trace metals. The elements Al, Sb, Hg, Cd, Ge, V, Si, Rb, Ag, Au, Pb, Br, Ti etc are believed to be acquired by animal body from environmental contaminants, due to interaction of organism with the environment. These elements are usually unevenly concentrated in different organs and are called non – essential elements. Essential metals function either as an electron donor system or as ligands in complex enzymatic compounds in animals. Since essential elements are used by the organisms only in trace amounts, their enrichment in the organisms does not exceed the level which allows the enzyme system to function without

interference (Presley, 1997). However, if the heavy metal concentration at the source of supply is too high, the homeostatic mechanism ceases to function and the essential heavy metals act in either acutely or chronically toxic manner. Thus, in the event of extended bioaccumulation of heavy metals, the organism may be affected.

Bivalve molluscs are notorious for concentrating trace metals both from the environmental water as well through food web. These species reflect the concentration of heavy metals in the surrounding medium. Based on this property Goldberg (1978) had adopted 'mussel watch' programme to monitor the levels of heavy metals in the environment as reflected in the animal body. Of late metallothionines have been monitored in fish and shellfish to gather an early information about metal pollution.

1.1. Trace metals in the aquatic environment and its effect on aquatic life.

Trace metals are introduced in the environment from both natural sources and as a result of human activity (Penrose *et al.*, 1975; Phillips *et al.*, 1982; Martin and Scanes, 1996; Presley, 1997). A large number of heavy metals may be contributed by corrosion of metal pipes, smelting, refining, etc. Weathering is a natural source of dissolved and particulate trace metals. Geological weathering of rocks produces the clays and other minerals that make up the bulk of detrital sediments as well as the bulk of dissolved metals in seawater. Volcanic activity, either on land or in the sea,

is another natural source of metals. Data from several experiments during the 1990's (Radach and Heyer, 1997) showed that large pools of cadmium were contained in the sediments and were ingested by the benthos (Hall *et al.*, 1996). Phytoplankton may absorb metals and as a result, significant concentration of cadmium and mercury are found in kidney and livers of top predators. Pollution from metals, which are persistent and are bioaccumulated by marine organisms with serious public health implications (Phillips and Rainbow, 1993).

The environmental impact of a metal depends less on its source than on its behaviour. Its behaviour, including mobility, transport, transfer and biological uptake, depends strongly on the chemical and physical form of the metal. The toxicity of a metal is mainly determined by its ionic size, electron affinity, electro negativity, stability, solubility and its inherent capacity to adversely affect any biological activity (Wittmann, 1974). The heavy metals have high affinities for ligands containing sulphur and nitrogen, and hence are bound easily to organic molecules such as proteins, enzymes etc (Richardson, 1980).

Toxic metals change the biological structures and systems into inflexible and irreversible conformation leading to deformity in the body and finally death (Kudesia, 1980). Cd, Hg, Ni, Pb, Zn and As are known to produce a broad spectrum of lethal effects which includes histomorphological changes, deformities and biochemical alterations in the

cells. Diarrhea, nervous disorder, loss of memory, tissue damage, respiratory failure, liver necrosis, anaemia and hypertension are some of the symptoms of heavy metal poisoning.

Contamination of marine organisms with toxic metals such as mercury is of ecological and health concern worldwide (Goldberg, 1995). The presence and behaviour of mercury in aquatic systems is of great interest and importance since it is the only heavy metal which bioaccumulates and biomagnifies through all levels of the aquatic food chain (Lindqvist *et al.*, 1991). Mercury has many industrial uses such as in the manufacture of plastics, caustic soda, paints, certain fungicides and pesticides. The effluents coming from such factories pollute the aquatic environment. The first reported human poisoning by Hg in seafoods occurred in Jaipan, between 1953 and 1964, which is known as 'Minamata disease' (Nitta, 1972). Investigations revealed that the victims had eaten shellfish contaminated with mercury containing effluents from a nearby plastic industry. The methyl mercury compounds present in the effluent wastes discharged into the Minamata bay were gradually bioconcentrated by fish and shellfish in the bay. A similar incident of Minamata disease was reported from Niigata, where seafish and shellfish were eaten regularly from the inflowing Agano River carrying effluents from the electrical industrial plant. The discharge of wastes containing mercury from chlor – alkali plants, Rayon factory and paper industry causes mercury poisoning, which is evident in Chaliyar River in Kerala, Rushikulya River in Orissa and Thane

creek near Bombay. Mercury poisoning results in chromosomal damages resulting from the combination of mercurials with -SH groups of enzymes and $-NH_2$ groups of amino acids.

Cadmium is regarded as one of the most toxic metals. The poisoning implicated by cadmium containing food is known as 'itai-itai' disease in accordance with the patient's shrieks resulting from painful skeletal deformities. 'Itai-itai' disease was first reported in Jintsu River, Toyama Prefecturor, Japan (Friberg *et al.*,1974). The disease was characterized by kidney malfunction, drop in the phosphate level of the blood serum and loss of minerals from the bones. Anthropogenic sources of Cd include the mining and minerals processing industries, Zn smelting, paint and plastic industry, effluent from Ni/Cd batteries, urban runoff due to the elevated Cd concentrations in phosphate fertilizers etc.

Copper is an essential trace element for the fixation of Fe in haemoglobin and is not a potent liver toxin except in certain cases of genetic defects resulting in the inability to excrete copper, the primary homeostatic mechanism, for instance Wilson's disease. Copper in ionic form is found to be toxic and inhibits photosynthesis and affect the growth of unicellular algae (Nielson and Anderson, 1970). Cu in excessive amounts causes haemolysis, hepatotoxic and nephrotoxic effects. The phenomenon of green – sick oysters is caused by high content of copper in the environmental water. The effluents from copper refineries, pesticide

and fungicide manufacturing industries bring copper to the aquatic systems. In Taiwan, copper pollution due to the discharges from the local copper recycling operation has been reported (Hung, 1988) and this has caused serious toxicity in green oysters (Hung *et al.*, 1989). The highest level of Cu in the oysters collected from the polluted area was 4400 ppm (Hung and Han, 1991). Thus, higher level of Cu in the environment or marine organisms adversely affects quality and fishery and can cause great economic loss.

Zinc is another metal that is also toxic to fish and other aquatic organisms at higher concentrations (Pringle *et al.*, 1968). It has adverse effects on fish growth rate and cause mortality at higher concentrations. The main sources of Zn in the aquatic systems are the effluents from factories manufacturing zinc compounds, zinc plating wastes, galvanizing wastes, storage battery, rayon wastes, etc.

Lead is considered as a protoplasmic poison, which is a cumulative, slow - acting and subtle. The Greek poet-physician Nicander described the disease known as plumbism, which is caused by acute lead poisoning. The widespread and general use of lead due especially to its exceptional properties such as high degree of ductility and low corrosiveness has resulted in lead being concentrated in the environment. Lead is introduced into the environment by various industries such as storage batteries, production of chemicals including paints, gasoline additives and various

metal products (eg sheet, pipes). Lead is emitted in large amounts from municipalities, by incineration of waste products. High levels of Pb have been found in urban runoff. Because Pb is used as an antiknocking agent in gasoline in many countries, elevated levels of Pb was found in urban air and by precipitation it will be carried to the near by water bodies.

Manganese is another element, which is quite significant in the marine environment because of its reactivity. It is present in appreciable amounts in marine sediments. Mn affects trace metal distribution in the marine environment as a result of adsorption on manganese nodules (Morgan and Stumm, 1964; Murray, 1975; Cronan, 1980; Manheim, 1986). The geochemical distribution of Mn in seawater is quite irratic and is influenced mostly by its redox potential. Mn toxicity in humans is characterized by a severe psychiatric disorder resembling Schizophrenia, followed by a permanently crippling neurological disorder clinically similar to Parkinson's disease (Hurley and Keen, 1987).

Arsenic is another cumulative poison. Large quantities of arsenical compounds are released into the environment through mining operations and from industries producing biocidal formulations like herbicides, pesticides, war chemicals etc. Arsenic is also emitted during the burning of fossil fuels (Lederer and Fensterheim, 1982). Arsenic intoxication in humans has also been reported (WHO, 1981; Nriagu, 1988). Regulatory agencies of many countries have introduced permissible limits for As in Fish

and Fish products (Bebbington *et al.*, 1977; Phillips *et al.*, 1982) and in drinking water (Zielhuis and Wibowo, 1984; Farmer and Johnson, 1985). Fish and shellfish have a tendency to accumulate many folds of Arsenic in their body. Bioaccumulation and toxicity of As has been well documented (Moore and Ramamoorthy, 1984; Phillips, 1990). Recently in Bangladesh, high level of Arsenic content has been observed in ground water and implicated Black foot diseases in people residing in around 41 districts (Biswas *et al.*, 1998). It was also found that water from 96% of tube wells of many districts of Bangladesh were not suitable for drinking. The symptoms of As poisoning were diffused melanosis, spotted melanosis and spotted keratoses. The 'black foot disease' is caused by the chronic ingestion of inorganic arsenic.

Many other diseases like Bush Sickness, Black foot diseases, Gena velgum diseases, Wilson's diseases, White muscle disease are reported as manifestations of heavy metal poisoning. It is in light of the above, monitoring of heavy metals in marine fishes and shellfishes assumes importance from the view point of consumer safety.

Safety of seafoods are of paramount importance in the present global scenario. Seafood should be free from all kinds of hazards affecting human health. When considering heavy metal content in organisms suitable for human consumption, the most important aspects are its toxicity

towards humans and affinity for other ligands in the enzyme or protein matrix.

Higher levels of Cd and other toxic metals were observed in cephalopods, especially in many squid products imported to Italy (Cantoni, 1986) and in the cuttlefish products exported from India. Some of the frozen cephalopod products (mainly squid and cuttlefish) were detained or rejected in the late 80's, owing to higher levels of Cd. Recently, in 2001 some cuttlefish products exported from Veraval, India, also had high Cd concentration. Presence of high levels of cadmium in the economically important class of cephalopods has caused serious concern in the processing and export industry. In the light of these observations, investigations were carried out by Lakshmanan (1988a, 1989) and Lakshmanan and Stephen (1993) in seafood products particularly in cephalopods from the west coast of India. The survey indicated that finfishes in general, had only lower levels of Cd and other trace metals compared to squid and cuttlefish. The cephalopods showed an unique phenomenon of bioaccumulation of Cd and other trace metals in different organs of the body. Cephalopods being voracious fish eaters depend on a wide range of marine animals particularly crustaceans, molluscs and fishes and hence there exist the possibility of bioaccumulation of pollutants through food chain. The presence of these toxic chemicals/metals in cephalopods has to be monitored and sources ascertained.

To meet the global challenges in seafood trade, we need to ensure that our fish products are both safe and comply with international quality requirements and standards. Safety of seafood and consumer health are of paramount importance. In order to ensure seafood safety, the European Union and the US have introduced regulations and standards for various fish and fishery products. EU directive of 91/493/EEC and the regulations of US-FDA of July 1997, have made HACCP based (Hazard Analysis Critical Control Point) seafood quality assurance system mandatory for the industry as well as for all countries that want to export to EU and US, in order to ensure safety.

Cephalopods being an important marine export item from India, a detailed study on trace metal levels and uptake by cuttlefish are warranted. The world experience calls for sound environmental knowledge and high standards of environmental ethics. Monitoring of marine environment for Cd and other toxic metals, their probable source and implications of toxicity on the consumers form part of the study. These informations are of great significance for producing safe products and boosting the export potential of this seafood delicacy in the global market. Wholesome and safe cephalopod products would certainly enhance the market potential in the overseas market.

1.2. Cephalopod fishery and resources of India.

Cephalopods are fished from the seas around India from very early times and constitute one of the important exploited marine fishery resources of our country at present. Cuttlefish, squid and octopus are the three major groups of cephalopods which belong to the highly evolved class of the phylum Mollusca, namely Cephalopoda, animals with feet around head. The squid and octopus species are not dealt with in this thesis.

Cuttlefish is characterized by a large calcified internal shell, the cuttlebone, and an ovoid body somewhat flattened dorsoventrally. Cuttlefish are strong swimmers and voracious fish eaters. They live mostly in water column and are either neretic (200m depth zone) or oceanic (beyond 200m depth zone). They are generally bottom dwellers.

There are about 80 species of cuttlefish of commercial and scientific interest distributed in the Indian seas (Silas, 1968; Sarvesan, 1974; Oommen, 1977a). Silas (1968) has given an exhaustive account of the cephalopod species distributed in the Indian Ocean. The important species of cuttlefish available in Indian Waters are *Sepia pharaonis*, *Sepia prashadi*, *Sepia aculeata* and *Sepia elliptica*. Of these, *Sepia pharaonis* and *S.aculeata* dominated along the west coast of India. *Sepia pharaonis* showed differential growth and the rate of growth of females was higher than that of males. Higher catch rates were observed from the west coast centers.

Hornell (1917,1951) has given an account of the fishing gear and fishery for cuttlefish. Sarvesan (1974) has briefly reviewed the fishing methods by which cephalopods are caught in India. These include fishing with shore seines, boat seines, hooks and lines, hand lines and trawl nets. The fishery is limited to 50m depth line in the traditional trawling grounds as it is the case in most of the marine fisheries of the country. Prawns and fishes form the chief item of food of this species (Guerra, 1985; Boucher-Rodoni *et al.*, 1987; Castro *et al.*, 1990). Other crustacean items like crabs, stomatopods and polycheates also form the diet to a smaller extent. Cannibalism was often noticed in cuttlefish.

As a major fish-producing nation, Fisheries play an important role in the national economy. With the advent of the Law of the sea and the declaration of Exclusive Economic Zone (EEZ) by many nations, the cephalopod resource is gaining considerable importance from mere subsistence fisheries to directed fisheries in many developing countries.

Overall export of cephalopods have registered a growth of 5% by volume and 9.92% by value during 1999-2000 compared to 1998-99. Cephalopods are the third largest product group with a share of 21.21% in volume and 11.91% in value in export basket. During 1999-2000, cuttlefish constituted 45.08% in volume and 46.97% in value followed by squid and octopuses. The average unit value realization of cuttlefish has also increased to Rs. 286.22 crores during 1999-2000 from Rs. 273.31 crores

(1998-99). European Union continued to be the largest market for Indian cephalopods.

1.3. Utilization of cuttlefish

Cuttlefish were of economic importance both as food and an item of export. The high protein and low fat content of cuttlefish make them suitable for human consumption (Roper *et al.*, 1984). Padmanabhan (1970) has discussed the prospects of developing cephalopods into fishery products for internal and export trade and has given the methods of processing and preservation. Cuttlefish exported are processed in several styles such as cuttlefish whole, whole cleaned, cuttlefish fillet, tentacles, rings, wings, cuttlefish roes, IQF tubes, tray packed, ink and cuttlebone.

Apart from being a good source of human food and an effective bait in long line fisheries, cephalopods have many other uses. Cuttlefish are used as manure. The cuttlebone are commercially used in preparing fine abrasives and dentifrices (Dees, 1961). Certain medicinal properties are also attributed to the bones and ink of cuttlefish (*via* Boycott, 1957). The powdered cuttlebone is a good source of food for poultry and cage birds. Pulverized cuttlebone are used for cleaning the surface of woodwork and motor vehicles before they are painted (Sarvesen, 1974). They are also used in jewellery, making for moulding purposes.

1.4. Nutritional significance of cuttlefish

The nutritive value of cuttlefish and cuttlefish products are widely recognized. Cuttlefish meat has a very high nutritive value. The edible part of cuttlefish contains all essential minerals and the content of Zn, Mn and Cu were higher in cephalopods than fish meat. Consumption of marine invertebrates is of particular value in every stage of malnutrition, especially when there is a lack of animal protein. The cuttlefish contain 20% protein and very little fat and ash. Edible muscle of cuttlefish had around twenty essential amino acids. Cuttlefish has three times as much as collagen as fish and was found to contain 77-85% Myofibrillar protein, 12-20% Sarcoplasmic protein and 2-3% Connective tissue (Sugiyama, 1989).

Though the fat content is negligible, the muscle of head and arms of cuttlefish are known to contain high level of cholesterol. The lipid is found mainly in liver and also in the skin.

Cuttlefish are highly perishable at ambient temperature and the rate of quality degradation and spoilage are much faster compared to finfish. So, handling of cuttlefish requires special attention, the earliest, keeping the temperature very low. Hard rubbery texture, flabby and soft meat, high drip loss during thawing and pink or yellow discolouration of meat are some of the quality problems encountered in cuttlefish products. Furthermore, the presence of high levels of toxic metals adds to some of the important safety problems met in the cuttlefish export industry. Therefore, to increase

consumer acceptability, the quality of cephalopod raw materials and safety of finished products should be ensured.

1.5. Objectives of the study.

The main objectives of the present investigation are the following:

i. To monitor the levels of heavy metals, viz., Hg, Cd, Pb, Cu, Zn, Fe, Mn, Cr, and Ni in whole soft tissues as well as the edible (muscle) and the non – edible body components of the cuttlefish (*Sepia pharaonis*), found along the west coast of India and to provide base line data of trace metals in cephalopods.

ii. To determine the levels of toxic metals in cuttlefish caught from different geographic locations, viz., Cochin, Quilon, Mangalore and Mumbai region, so as to understand regional variation, if any, in metal distribution.

iii. To study seasonal variations of Cd and other toxic metals in cuttlefish caught off west coast of India.

iv. To study the levels of metals in food fishes, habitat water and their relation to metal levels in cuttlefish.

v. To evaluate Cd toxicity in experimental albino rats by feed trials using liver incorporated diets and observing the haematological and histopathological changes in the cells, and there by its toxicity to cephalopod consumer.

1.6. Review of literature.

Trace metals play an important role in the aquatic ecosystem. In marine organisms, uptake of heavy metals was reported by many researchers (Saiki *et al.*, 1955; Mullin and Riley, 1956; Martin and Goldberg, 1962). Brooks and Rumsby (1965) studied the biogeochemistry of trace metal uptake of Ag, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, V and Zn in three species of New Zealand bivalves. All the elements analysed showed more enrichment in molluscs than in the environment.

Since molluscs in general, concentrate in their bodies certain trace metals from the hydrosphere it becomes necessary to monitor the levels of these metals in seafoods. Trace metals in molluscs have been reported from various parts of the world (Decleir *et al.*, 1970; Bryan, 1973; Topping, 1973; Eustace, 1974; Ratkowsky *et al.*, 1974; Martin *et al.*, 1975; Bryan *et al* 1977; Nambisan *et al.*, 1977; Ishii *et al.*, 1978; Schipp *et al.*, 1978; Lakshmanan and Nambisan, 1983; Eisenburg and Topping, 1984; Ray, 1986; Sadiq and Alam, 1989; Skulsky *et al.*, 1989; Chen, 1998; Frias *et al.*, 1999; Lakshmanan *et al.*, 2001).

The pioneer work on the levels of trace metals in cephalopods was carried out by Martin and Flegal (1975) and stated that cuttlefish are very good cadmium integrators from the environment. These authors had reported the levels of a range of metals. Decleir *et al.* (1978) determined the protein bound copper and zinc in some organs of the cuttlefish *Sepia*

officinalis. Schipp *et al* (1978) reported on the distribution of copper and iron in central organs of the cuttlefish S*epia officinalis*. Cantoni *et al* (1986) determined the Zn/Cd ratio in cuttlefish imported into Italy from four different countries and reported that about 50% of the samples had Cd content in excess of the tolerance limit. Falandysz (1988, 1989, 1990, 1991) had made an exhaustive investigation on the levels of metals in fresh and processed squid, *Loligo patagonica* and *Loligo opalescens*. Lozano Soldevilla (1989) found concentration of Cu, Cd, and Fe in whole bodies of *Todarodes Sagittatus* to be high when compared with those in mantles and tentacles. Furthermore, the maximum values for Cu and Cd permitted by Spanish law exceeded in a few individual whole body samples. A survey conducted by Sapunar (1989) in cephalopods from industrially polluted Kastela and Kijelka bays and a control area in the Adriatic sea revealed significant difference in Cd and Hg levels between polluted and non polluted areas. Heavy metal content in the mantle (edible part) and the intestines of cuttlefish (*Todarodes sagittatus*) from North east Atlantic was reported by Oehlenschlaeger (1990) and found cadmium and lead content were low in the mantle but very high in the intestines. Ikebe *et al.* (1991) determined the content of 16 metals in fish and shellfish of Japan. Miramand and Bentley (1992) measured the concentration of eleven heavy metals in the tissues of cephalopods collected from the French coast of the English Channel and found that the digestive gland contained greater than 80% of the total body burden of Ag, Cd and Co. Trace metal concentration in

cuttlefish from processing factories of Thailand were extensively studied by Attaya *et al.* (1993) and concluded that Cd content in cuttlefish meat are generally at safe level. Martoja and Marcaillou (1993) reported in liver of cuttlefish, S*epia officinalis* L., the greater part of accumulated Cu is concentrated in spherulae, which are elaborated by the basal cells. Cisneros *et al.*, (1995) reported the Cd contents of 77 fresh and frozen samples of cuttlefish, and the cadmium concentration ranged from 0.98 to 3.30 mg/kg (wet wt) in the muscle of cuttlefish from Argentina. Lu chavhua (1995) determined the levels of Cu, Pb, Zn, Cd, Cr and Ni in cephalopods collected from the Northern area of South China Sea and reported that there were no significant levels in the edible muscle. In another study by Galarini *et al.*, (1996) the highest concentration of Cd was observed in cephalopods when 724 samples of marine and fresh water fish and shellfish from Umbria and Marche regions of Italy were analysed. Chen *et al.* (1998) assessed the contents of heavy metals in cephalopods from Zanijiang harbour waters and found the edible parts to be effected by Pb, Cd, Ni and Zn. Bustamante *et al.* (1998 a) analysed 350 individuals of 12 species of cephalopods from the French Atlantic coast to the sub-Arctic region and found high cadmium level in the cephalopods from the Sub-Arctic area than those from the lower latitudes. Jones *et al.* (2000) observed Zn concentration in the muscle of two species of cuttlefish from Cleveland Bay, in the range of 13-16 μg/g wet wt.

However, similar study in cephalopods from Indian waters is very scanty (Dious and Kasinathan, 1992). Ramamurthy (1979) and Patel and Chandy (1988) studied the base line levels of Hg in cephalopods. A comprehensive account of trace metals in cephalopods (squid and cuttlefish) was provided by Lakshmanan (1988a,b; 1989) and Lakshmanan and Stephen (1993) for the first time in India. This study has generated the idea of unique phenomenon of selective bioaccumulation of Cd in cephalopods.

Concentration of a metal in an organism mainly depends on the bioavailability of the metal, environmental conditions like salinity, dissolved oxygen, temperature and concentration of metals in their locality and the quality of food (Presley, 1997). Seasons do play a major role in the accumulation of trace metals from the habitat water. Seasonal distribution of heavy metals in molluscs have been reported worldwide. Brooks and Rumsby (1965) made a quantitative study of some trace metals in some bivalve molluscs of Newzealand. Segar *et al* (1971) gave the distribution of six major and thirteen minor elements in the shells and entire soft parts of 11 species of molluscs from Irish Sea. Another important piece of work is by Bryan (1973) on the occurrence and seasonal variations of Cu, Fe, Zn, Mn, Pb, Ni, Co, Cr, Cd and Al in the scallops *Pectin maximus* (L) and *Chalmys opercularis* (L). Hall *et al* (1974) had estimated the Hg content in certain clams and oysters. Eustace (1974) estimated the concentration of Cd, Cu, Zn, and Mn in certain species of finfish and shellfish caught from

Derwent estuary in Tasmania. Zingde *et al.*, 1975 have reported the concentration of some metals in *Saccostrea cucullata* from Goa region. Sankaranarayan *et al.*, 1978 studied seasonal variation in metal concentration in *Crassostrea madrasensis* from Cochin region. Other important investigations are that of Shiber (1980) in 12 species of molluscs, Boyden and Phillips (1981) in *Crassostrea gigas*, Gabbot (1983) in certain marine molluscs, Lakshmanan and Nambisan (1983) in bivalve molluscs, *Villorita cyprinoids*, *Meretrix casta* and *Perna viridis*, Cain and Luoma (1986,1990) in *Macoma balthica*, Talbot (1986) in *Saccostrea cucullata*, Vazquez *et al.* (1990) in *Crassostrea virginica*, Rajan *et al.*, (1991) in *Meretrix casta*, Mitra *et al.* (1995) in gastropod *Thais lacera*, Muralidharan & Raja (1997) in Pelecypod *Marcia recens*, Rivonker & Parulekar (1998) in *Perna viridis*, Senthilnathan & Balasubramanian (1998) in Oyster *Crassostrea madrasensis*, Frias (1999) in *Crassostrea irrdescens* and Olivier *et al* (2002) in oysters *Saccostrea commercialis*.

However, not much attention is given to study the distribution of metals in cephalopods during different seasons. Dious and Kasinathan (1992) studied the concentration of Fe, Mn, Zn and Cu in different body tissues of *Sepiella inermis* during different seasons.

Studies on the trace metal levels in coastal and marine waters of India have been studied in detail by Sengupta *et al.*, 1978 and Qasim and Sengupta, 1981. Other important works included are those of Braganca

and Sanzgiri, 1980; Fowler *et al.*, 1984; Kureishy *et al.*, 1993; Krishnakumar *et al.*, 1998; Senthilnathan *et al.*, 1998.

Marine organisms take up heavy metals to varying degrees with concentration factor in the order of 10^2-10^3. Trace metals can cause deleterious effects on density, diversity and productivity of aquatic organisms (Eisler, 1993; Moore and Ramamoorthy, 1984). Trace metals like Cd, Cu, Zn, Fe, Mn, As, Ba, Co, Mo, Ni, Sb, Sr, V and Zn can cause toxic effects to human beings with far reaching consequences (Mathew, 1991 a, b; Sharma, 1995).

Histomorphological alteration in the liver and kidney of albino rats have been reported by several workers (Cherian *et al.*, 1976; Weigal *et al.*, 1984; Dudley *et al.*, 1985; Elinder 1986; Anderson *et al.*, 1988; Sendelbach and Klaasen 1988 and Lin and Ho., 1992). Anderson *et al.*, 1988 found histopathological alterations in the livers of mice ten days after oral exposure to a single dose of $CdCl_2$ (30 mg/kg body wt), and Elinder (1986) reported that for detecting the long term effects of Cd on the liver, liver morphology is a more sensitive parameter than the liver-enzyme activities in the blood. Groten *et al.*, (1990) studied the toxicity of inorganic and liver incorporated cadmium in rats by feeding a test diet containing 30 mg Cd/kg. Chaterjee *et al.*, (1996) reported degenerative changes of hepatocytes, widening of the bowman's space in the cortical region of kidney, necrosis

and degeneration of tubular epithelium in rats treated with $CdCl_2$ (1 mg/kg/day) for four weeks.

Cuttlefish have been identified as voracious fish eaters by many authors (Bidder, 1966 and Boyle, 1990). Cannibalism was also noticed in some species of cuttlefish (Oommen, 1977b). Feeding habits of cuttlefish play an important role in the accumulation of Cd and other metals (Bryan *et al.*, 1983). The common food items are fish and prawns (Boucaud – Camou and Boucher – Rodoni, 1983; Guerra, 1985; Boucher – Rodoni *et al.*, 1987, Blanc *et al.*, 1998; Castro *et al.*, 1990; Pinczon du sel and Daguzan, 1992). Further more cuttlefish are a prey to a great variety of sea birds, marine mammals, fishes and cephalopods themselves (Silas, 1963; Clarke, 1986; Rodhouse *et al.*, 1992; Clarke, 1996; Croxall and Pierce, 1996; Smale, 1996). Oommen (1977) studied the structure of alimentary canal, digestive enzymes and food and feeding habits of this species from the South West coast of India.

Cephalopods, particularly cuttlefish are a significant source of Cd to their predators. This hypothesis was first proposed by Honda and Tatsukawa (1983) for striped dolphin from Japan. Muirhead and Furness (1988) supported this hypothesis, by explaining very high Cd concentrations in the tissues of cuttlefish eating seabirds from Gough islands. Works has been carried out on the cuttlefish predator relationship by Paludan – Muller *et al.*, 1993 and Caurant *et al.*, 1994. Cadmium is transferred from water

and food to the cuttlefish, and from cuttlefish to their predators through the food chain. Therefore, cuttlefish play an important role in the bioaccumulation of Cd by their predators (Law *et al.*, 1997; Sepulveda *et al.*, 1997). Bustamante *et al.*, 1998a observed that the bioaccumulation effect was found to be most evident at high latitudes. Food can be considered as the main source of metals in cephalopods (Bustamante *et al.*, 2000). Jiro Koyoma *et al.*, (2000) studied the relative contribution of Cd in water and food to bioaccumulation in oval squid and suggested that the main source of Cd in squid appear to be dietary.

The above literature review indicated a derth of information on the above topic from the Indian subcontinent. India, being a major exporter of seafood item including cephalopods, the safety of the marine products has to be ensured. Considering the importance in global trade, environmental contaminants particularly trace metals has to be monitored and safety has to be evaluated. The present study attempts to monitor Cd and other toxic metals in cephalopods, food fishes and habitat water from the west coast of India and to provide a base line information on the problem, which would greatly help the seafood industry.

Chapter 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Sample collection.

Collection of cuttlefish, food fishes and water samples were made from different pre-fixed stations namely Cochin, Quilon, Mangalore and Mumbai all along the West Coast of India during 1998-2000. Cuttlefish, *Sepia pharaonis* were collected at monthly intervals from Cochin Fisheries harbour, bimonthly intervals from Quilon and Mangalore Fisheries harbour and quarterly from Mumbai Fisheries harbour. A two tier sampling procedure was adopted; viz., onboard the Fishing vessel, MV *Sagarika* of Integrated Fishery Project, Cochin and the Research vessel, FORV *Sagar Sampada* of Department of Ocean Development and from the major fish landing centres on the west coast of India, as stated above. Location map is shown in Fig 2.1. During 1998, samples were collected onboard the vessel MV *Sagarika* from May 1998 to October 1998 using trawl operated at 30 – 70 m depth. The operational area confined to the region – Latitude 9^0 36' to 12^0 08' N, Longitude 74^0 50' to 76^0 02'E. Samples were also collected from FORV *Sagar Sampada* (depth range: 40-68 m) of Latitude 20^0 34' to 21^0 36'N and Longitude 68^0 58' to 70^0 13'E. Details of onboard sampling sites are presented in Table 2.1. Fish/shellfish and water samples were also collected from these stations. Cuttlefish samples were collected using trawl net and water samples were collected using the Conductivity Temperature Depth instrument ($SBE3^+$ model).

2.1.2. Sample handling and preparation.

Raw cuttlefish appeared very fresh as revealed by the sheen, general appearance and odour. Samples from landing centers (i.e. from various harbour), were collected, washed with potable water and properly iced and transported to the laboratory at minimum time. Cuttlefish collected from Cochin, Quilon and Mangalore fisheries harbours were iced and brought to the laboratory in insulated boxes and analysed immediately or kept frozen (-20^0 C) until further analysis. Samples from Mumbai Fisheries harbour were frozen and kept in the cold storage of a processing plant at −18^0 C and transported to CIFT, Cochin in an A/C compartment of a passenger train with minimum melting of the ice. After reaching CIFT, the samples were deiced, washed and reiced properly. These samples were graded and subjected to various analysis. Fishes separated from the mantle cavity of cuttlefish were identified and treated as food fishes, owing to their proximity. These samples were kept frozen until taken for heavy metal analysis. Water samples were preserved by adding con.HNO_3 (1 to 3 ml per litre of seawater) and kept in the refrigerated storage until analysis.

To study the regional variation of heavy metals, cuttlefish of commercial size of dorsal mantle length 21.2 ± 3.02 cm and weight 146.5 ± 26.5 g collected from Cochin, Quilon Mangalore and Mumbai fisheries harbours were used.

The seasonal variation of heavy metals were studied in commercial sized cuttlefish collected during premonsoon (January – May), monsoon (June – August) and postmonsoon (September – December) periods from 1998 to 2000.

2.1.3. Sample preparation.

Samples were prepared as whole animals as well as whole cleaned or fillets. The length and body weight of the cuttlefish were recorded whenever they are taken for analysis.

2.1.3.1. Whole cuttlefish

Whole cuttlefish (fresh/thawed) were washed with potable water; the cuttlebone was removed, and peeled. The peeled cuttlefish was finely chopped and homogenized in a blender. An aliquot of the homogenate was pressed within filter folds so as to remove the adhering water. This was used for trace metal and other biochemical analysis.

2.1.3.2. Body components

Distribution of metals in the various body components were studied by dissecting the animal and separating for body components like muscle, liver, gills, skin, ink and tentacles.

2.1.3.3. Food fishes/Environmental fishes

Fishes separated from the mantle cavity of cuttlefish and also those collected from the same habitat area of cuttlefish were washed with potable water and identified. The complete list of food fishes separated from the mantle cavity are given in Table 5.1. Their body length, weight were recorded and digested whole, using con. HNO_3 and $HClO_4$ as it is consumed whole by cuttlefish.

2.2. Reagents, chemicals and glasswares

The reagents and chemicals used were all of analytical reagent grade (AnalaR) from BDH – Glaxo Co. or of Sigma chemicals from M/s Sigma Chemical Co. (St. Luis, USA). Rectified spirit of 95% grade and its various dilutions were used in histopathological studies.

2.2.1. Reagent water

Deionised water from Milli-Q Reagent water system, which gave water of Conductivity 18.2 µs was used for the preparation of all reagents, standards and dilution water in all aspects of trace metal analysis of the digested samples.

2.2.2. Metal standard solutions

Metal standards were prepared using either pure metals or AnalaR BDH salts. In a few cases metal standards were procured from Sigma Chemicals Co. (St. Luis, USA)

A series of standard metal solutions in the optimum concentration range were prepared by appropriate dilutions of stock metal solutions with water containing 1.5 ml HNO_3/L. Stock standard solutions for Cu, Zn, Fe, Mn, Pb, Ni and Hg were prepared from analaR metals or metal salts as follows:

Lead - Dissolved 0.1598g AnalaR grade lead nitrate, $Pb(NO_3)_2$ in minimum amount of 1+1 HNO_3. Added 10 ml con. HNO_3 and diluted to 1000 ml with water. 1 ml of the solution = 100 μg Pb

Copper - Dissolved 0.1g pure copper metal in 2 ml con. HNO_3. Added 10 ml con. HNO_3 and diluted to 1000 ml with water. 1.00 ml = 100 μg Cu.

Zinc - Dissolved 0.1g Zn powder in 20 ml HCl (1+1) in 1L volumetric flask and diluted to volume with deionised water. 1.00 ml = 100 μg Zn.

Iron - Dissolved 0.1g iron wire in a mixture of 10 ml 1+1 HCl and 3 ml con. HNO_3. Added 5 ml con. HNO_3 and diluted to 1000 ml with water. 1.0 ml = 100 μg Fe.

Manganese - Dissolved 0.1g Mn metal in 10 ml con. HCl mixed with 1 ml con. HNO_3. Diluted to 1000 ml with water. 1.00 ml = 100 μg Mn.

Nickel - Dissolved 0.10g Ni metal in 10 ml hot con. HNO_3, cooled and diluted to 1000 ml with water. 1.0 ml = 100 μg Ni.

Mercury - Standard solution of mercury was prepared using AnalaR grade mercuric chloride (Glaxo, BDH). Weighed 0.1354 g of $HgCl_2$ and dissolved in 25 ml 5% HNO_3. About 1 ml of 1% $K_2Cr_2O_7$ solution was added and made up to 100 ml with 5% nitric acid. Secondary standards were prepared by diluting the required volume of primary standard using 5% HNO_3 and maintaining 0.01% $K_2Cr_2O_7$ in the solution.

Working standards for Cd and Cr were prepared from commercially available AAS grade stock standard obtained from Sigma Chemicals Co. and diluted suitably to the required level using pure water, acidified with nitric acid.

2.2.3. Stannous chloride solution (20% v/v)

20 g of high purity stannous chloride was dissolved in 10 ml distilled conc. HCl in a beaker and boiled for a minute. The solution was then cooled and diluted to 100 ml with distilled water. 1 – 2 g of tin metal was added to the solution after the preparation of the solution to maintain the concentration.

2.2.4. Citrate buffer.

Prepared an aqueous solution of 10% Diammonium hydrogen citrate and purified the buffer by repeated carbamate/freon extraction.

2.2.5. Ammonium Pyrrolidine Dithiocarbamate (APDC) and Diethyl ammonium Diethyl Dithiocarbamate (DDDC) (2% w/v)

Prepared an aqueous solution, 2% of each, purified the reagents by repeated extraction with freon. Stored the APDC/DDDC solution at 4^0 C. This solution was freshly prepared every day.

2.2.6. Neutral buffered formaldehyde solution (10%)

Mixed 100 ml 37 – 40% formaldehyde in 900 ml of water, 4g of sodium phosphate monobasic and 6.5g of sodium phosphate dibasic was added.

2.2.7. Stock Eosin solution (1%)

Dissolved 1g Eosin in 20 ml distilled water and made upto 100 ml with 95% alcohol.

Working Eosin solution: Dilute 1 part of the stock solution with 3 parts of 80% alcohol. Add 0.5 ml glacial acetic acid for every 100 ml stain.

2.2.8. Glasswares and plasticwares

Sample containers were washed first with water and detergent and further cleaned by soaking in 5% nitric acid for 24 h and finally rinsed 4 – 5 times with deionised water (Milli Q Water) to prevent metal contamination.

2.3. Methods

2.3.1. Trace metal analysis

2.3.1.1. Predigestion of samples

The homogenized meat after removing the adhering water by pressing within filter paper folds, were weighed into a Bethge's flask and subjected to pre digestion by adding 15 – 20 ml of A.R concentrated nitric acid and kept overnight. Triplicate samples were taken in all kinds of analysis.

2.3.1.2. Digestion procedure for determination of Mercury.

Digestion was carried out by wet oxidation under reflux with con. nitric acid and con. sulphuric acids in the ratio 4:1 (v/v) using the Bethge's apparatus. Digestion was carried out under closed condition to prevent the escape of volatile mercury.

2.3.1.3. Digestion procedure for determination of trace metals.

Metals such as Cd, Pb, Cu, Zn, Fe, Mn, Cr and Ni were determined in tissue samples by wet oxidation method (AOAC, 1990) using con. nitric

acid and perchloric acid in the ratio 5:1 (v/v). The samples were heated gently and cautiously at first, until the first vigorous reaction subsides, continued heating, until the organic matter was completely destroyed, indicated by a clear solution. If any traces of organic matter remained, as indicated by slight yellow colour, added little 10% hydrogen peroxide and boiled to make clear. This clear solution was cooled and made up to a known volume after filtration and kept in polythene bottles for AAS analysis.

A blank is also prepared using water and nitric acid as used for sample digestion. The above samples were directly fed to a Flame Atomic Absorption Spectrophotometer for metal determination.

2.3.1.4. Determination of trace metals by Flame Atomic Absorption Spectrophotometer (AAS)

Trace metal concentrations in the digested samples were determined directly by Flame Atomic Absorption Spectrophotometer (Model GBC 902).

The element to be analysed is introduced into a flame where it becomes dissociated from its chemical bonds into an unexcited, unionized ground state as individual atoms. The element at this state is capable of absorbing radiation at discrete lines of narrow wavelength. When a radiation at one of these wavelength is directed through the flame, the amount of this light absorbed as it passes through the flame is proportional to the concentration of element being analysed. The source of radiation is the respective Hollow metal cathode lamp of the metal under determination.

The concentration range, wavelength, slit width, lamp current, sensitivity and working range of standard metal solution used in the estimation of various metals by AAS are given in Table 2.2.

2.3.1.5. Determination of Mercury using Mercury Analyzer

Determination of ΣHg was carried out in a Mercury Analyser, MA-5840 manufactured by M/s. Electronic Corporation of India Ltd, Hyderabad.

Principle of the method: The digested sample solutions containing Hg^{2+} ions is reduced to metallic mercury, using stannous chloride and HCl. The liberated mercury is drawn into the absorption cell of the Mercury Analyzer which is irradiated by low pressure mercury vapour lamp. The mercury vapour lamp absorbs the radiation at 253.7 nm and causes a change in the transmittance which is proportional to the mercury present in sample solutions.

A calibration graph is prepared from the standard Hg^{2+} solutions (in the range of 50-200 ng/10 ml solutions). From the graph the concentration of different sample solutions were taken and results were expressed in ng/g or ppb wet weight.

A suitable aliquot of the sample was pipetted out in to the reaction vessel of the Mercury Analyser and the required amount of 10% nitric acid solution was added in order to maintain total volume at 10 ml. Two ml. of 20% stannous chloride solution was added and allowed to react for 5 mts.

The absorbance reading was noted on the meter as early as possible. The same procedure was adopted with a reagent blank and a series of standards. The reaction vessel was thoroughly cleaned before each measurement. Standard curve was prepared using absorbance attained for standard mercuric chloride solutions.

2.3.2. Extraction procedure adopted for seawater analysis.

Seawater analysis was carried out by preconcentration of the dissolved trace metals by chelation with a mixture of equal amounts of Ammonium Pyrolydine Dithiocarbamate (APDC) and Diethylammonium Diethyl Dithiocarbamate (DDDC). The pH was adjusted between 4 and 5 by a citrate buffer and extracted into Methyl Isobutyl Ketone (MIBK) followed by back extraction into nitric acid (Grasshoff *et al.*, 1976). Extracts were analysed for Cd, Pb, Cu and Zn by Flame Atomic Absorption Spectrophotometer.

Transferred 100 ml of seawater to a 250 ml Teflon separatory funnel. Adjust the pH to 4.5 by addition of 1 ml of citrate buffer. Added 1 ml chelating reagent solution followed by 20 ml Methyl Iso Butyl Ketone (MIBK). Continued the extraction by adding 10 ml MIBK to the funnel. The two extracts were combined and added 0.5 ml HNO_3 by a micropipette. The tubes were allowed to stand for 15 mts, to decompose the metal carbamate. 3.5 ml of pure water were added and shaken for two minute to ensure complete back extraction.

2.3.3. Histopathology

To study the toxic effects of cuttlefish liver bound cadmium and inorganic Cadmium as ($CdCl_2$), the liver and kidney tissues of experimental and control albino rats were subjected to histopathological studies following the method of Pearse (1968). The procedure consisted of the following steps.

(i) Fixation of tissues

Tissue samples (<5 mm thick) of liver and kidney of experimental and control albino rats were fixed in 10% neutral buffered formaldehyde solution for 6 – 18 h. They are then washed in running water overnight and stored in 70% alcohol.

(ii) Tissue processing

The tissue blocks are conveyed through a series of solvents as per the following schedule for dehydration, clearing and paraffin infiltration:

Alcohol 80%	:	1 h
Alcohol 90%		1 h
Absolute Alcohol (2 changes)		1 h each
Absolute Alcohol and Xylene (1:1)		½ h
Xylene (2 changes)		15 min each
Paraffin wax and Xylene		½ h each

Paraffin wax (3 changes)	1 h each

The tissues were then embedded in paraffin wax of melting point of 60 – 62^0 C. Sections are cut at 6 – 10 μm thickness in a rotary microtome and sections floated in a water bath between 38 – 49^0 C. The sections from the water are mounted on clean glass slides smeared with egg albumin. They are then dried and ready for staining.

(iii) Staining procedure

The slide containing the section is processed serially as follows:

Xylene I (2 changes)	3 minutes
Xylene II	3 minutes
100% Alcohol I	3 minutes
100% Alcohol	3 minutes
80% Alcohol	3 minutes
70% Alcohol	3 minutes
Distilled water	3 minutes
Hematoxylin stain	10 – 15 minutes
Tap water	3 minutes
Distilled water	3 minutes
Acid alcohol	1 dip
Distilled water	3 minutes
Distilled water	1 dip
Distilled water	3 minutes

Tap water and sodium bicarbonate	1 dip
Tap water	1 or 2 dips
Eosin working solution	1 minute
70% Alcohol	1 dip
95% Alcohol I	4 dips
95% Alcohol II	4 dips
100% Alcohol	4 dips
Xylene (2 changes)	10 minutes each

The processed tissue sections were mounted in DPX and the slides were examined by light microscopy and photographed using a binocular microscope and Nikon camera combination.

Table 2.1: Sampling stations for cuttlefish collected onboard M.V. *Sagarika* and FORV *Sagar Sampada.*

Latitude	Longitude	Depth	Research vessel, period of collection.
$10^0$39'N	$75^0$45'E	35m	MV *Sagarika* May 1998
$11^0$05'N	$75^0$37'E	30m	
$11^0$51'N	$75^0$02'E	48m	
$12^0$08'N	$74^0$50'E	52m	
$10^0$26'N	$75^0$52'E	30m	
$10^0$06'N	$75^0$51'E	44m	MV *Sagarika* June 1998
$9^0$47'N	$75^0$50'E	58m	
$9^0$36'N	$76^0$01'E	29m	MV *Sagarika* August 1998
$9^0$34'N	$75^0$57'E	59m	
$9^0$45'N	$75^0$51'E	59m	
$9^0$41'N	$76^0$01'E	45m	
$9^0$39'N	$76^0$02'E	40m	
$9^0$48'N	$75^0$56'E	45m	MV *Sagarika* October1998
$9^0$38'N	$76^0$02'E	38m	
$9^0$51'N	$75^0$58'E	36m	
$9^0$44'N	$75^0$56'E	46m	
$9^0$47'N	$75^0$52'E	43m	
$20^0$34'N	$70^0$13'E	64.0m	FORV *Sagar Sampada* March 1999.
$21^0$11'N	$69^0$40'E	43.0m	
$21^0$33'N	$68^0$56'E	61.8m	
$21^0$31'N	$69^0$11'E	68.5m	
$21^0$10'N	$69^0$24'E	62.5m	
$21^0$17'N	$69^0$11'E	40.0m	
$21^0$36'N	$68^0$58'E	61.0m	
$21^0$26'N	$69^0$06'E	61.0m	

Table 2.2: Instrumental parameters and concentration range of metals used in the determination of various trace metals using Flame Atomic Absorption Spectrophotometer.

Element	Wave length (nm)	Slit width (nm)	Lamp current-range (amperes)	Sensitivity (mg/L)	Working range
Cd	228.8	0.5	3 - 15	0.099	0.2 - 1.8
Pb	217.0	1.0	5 - 8	0.06	2.5 - 20
Cu	324.7	0.5	3 - 15	0.025	1.0 - 5.0
Zn	213.5	0.5	5 - 15	0.008	0.4 - 1.5
Fe	248.3	0.2	7 - 20	0.04	2.0 -9.0
Mn	279.5	0.2	5 - 15	0.02	1.3 - 6.0
Cr	357.9	0.2	6 - 20	0.05	2.0 - 15
Ni	232.0	0.2	3.5 - 20	0.04	1.8 - 8.0

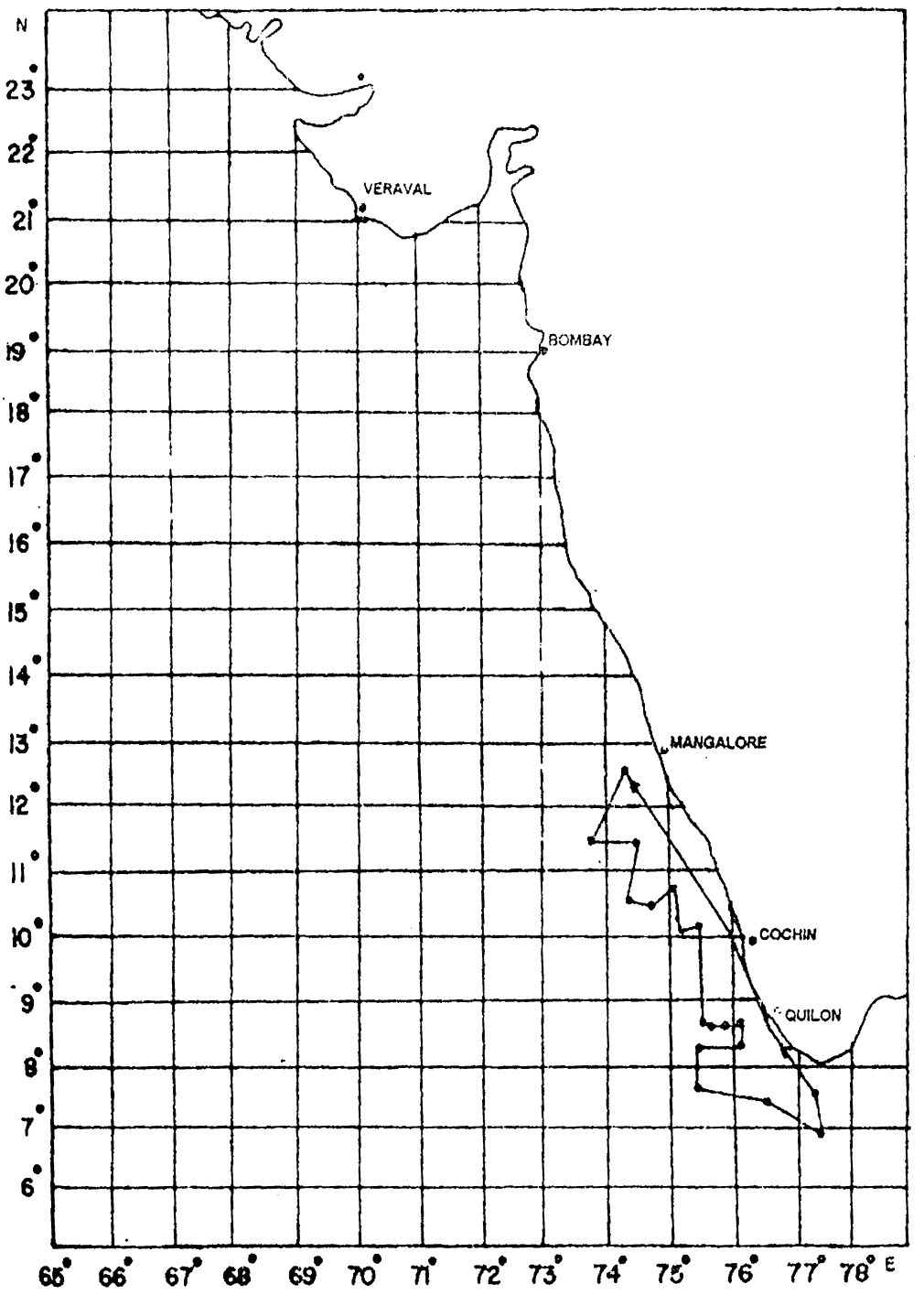


Fig 2.1. Sampling stations for cuttlefish collected onboard FORV *Sagar Sampada* (Cruise No. 191)

Chapter 3

GEOGRAPHICAL TRENDS IN THE DISTRIBUTION OF TRACE METALS IN CUTTLEFISH

3.1. Introduction

Cephalopods particularly cuttlefish and squid form an important component in the marine products export of India. Degradation of the quality of the marine environment with chemical contaminants especially heavy metals are of global concern. Considering the importance in global trade, the safety of products is of great concern. Environmental contaminants in these products are to be monitored regularly for their levels. However, variations were observed in the levels of metals at different geographic locations. Impact of industrial and human activities have increased the levels of environmental contaminants in the world over. In the present study, the geographical variation of various toxic metals in cuttlefish from the important landing centers of the west coast of India, is thought of monitoring with the objective of understanding spatial variations in metals at these different centres, viz., Cochin, Quilon, Mangalore and Mumbai. The data on heavy metals so generated may find a place in creating a GIS, for heavy metals levels in cuttlefish.

Not much information is available on the trace metal distribution in cuttlefish at different geographic locations in India. Dious and Kasinathan (1992) had reported the levels of some of the heavy metals in the body components of cuttlefish from Portnovo Sea. The levels of a range of trace metals in the cephalopods (both squid and cuttlefish) were studied in detail by Lakshmanan and Stephen (1993). They have reported that

cephalopods, in general are characterised by selective bioaccumulation of Cd and similar metals. The effect of environmental hazards on the seafood export of India was reported by Lakshmanan *et al.*, 2001. Elevated levels of Cd, Zn and Cu have been observed in the whole soft parts of squid and cuttlefish.

There is an increasing pressure on the quality and safety of seafood products from the importing countries and hence monitoring of the levels at various points is quite warranted. The present bio-monitoring program is taken up with a view to address the geochemical variation in heavy metal levels in cephalopods.

The need for constituting a GIS for trace metals is emphasized which would greatly help the seafood industry and boost export. In the present study, the levels of nine trace metals, viz., Hg, Cd, Pb, Cu, Zn, Fe, Mn, Cr and Ni in the cuttlefish, *Sepia* sp. collected from the four major fisheries harbour (Cochin, Quilon, Mangalore and Mumbai) along the west coast of India, their distribution in the body components and geographical trends are presented; which may form a data base for trace metals in cuttlefish. The study also identifies specific organs that may be particularly selective and sensitive to heavy metal accumulation.

3.2. Materials and Methods

The cuttlefish samples were collected at monthly intervals from Cochin and bimonthly intervals from Quilon and Mangalore region.

Samples could not be collected on a regular basis from Mumbai region. The samples were brought to CIFT laboratory and immediately analysed following wet digestion or kept frozen until analysis. Samples were digested whole and also after separating into various body components like muscle, liver, gills, skin, ink and tentacles. Following wet digestion, metal levels were determined by flame AAS or Cold vapour Atomic Absorption technique and procedures adopted are described in Chapter 2. The results of the study were subjected to statistical analysis by Two way ANOVA to find out geographical variation in metal levels, if any, in the distribution of heavy metals (Cd, Cu, Zn and Cr) in cuttlefish collected from the west coast of India.

3.3. Results

Concentration of heavy metals like Hg, Cd, Pb, Cu, Zn, Fe, Mn, Cr and Ni found in the whole soft parts, edible muscle and important body components like muscle, liver, gills, skin, ink and tentacles from the four regions are presented in Tables 3.1 to 3.4. The regional trends are illustrated in Figures 3.1 to 3.4. Results of Analysis of Variance (ANOVA) of some of the toxic and essential metals (eg. Cd, Cu, Zn, and Cr) in muscle and liver components are presented in Tables 3.6 to 3.13.

3.3.1. Regional variation in Mercury, Cadmium and Lead.

Concentrations of ΣHg, Cd and Pb from the four regions and their regional trends are presented in Tables 3.1 to 3.4 and Figs. 3.1 to 3.4. Hg, Cd and Pb are considered to be highly toxic metals. Mercury was the least abundant toxic metal found in cuttlefish from all four regions. In whole cuttlefish, ΣHg levels were in the range of 0.03 to 0.09 ppm (Cochin region), 0.02 to 0.08 ppm (Quilon region), 0.01 to 0.06 ppm (Mangalore region) and 0.05 to 0.17 ppm (Mumbai region). The highest value observed for ΣHg in whole cuttlefish was 0.17 ppm from Mumbai region. The edible muscle had, by and large a mean total mercury (ΣHg) concentration of <0.05 ppm; very low levels were invariably observed in samples from Quilon region. The highest mean value of Hg in edible muscle were observed in Cochin samples (0.04 ppm). In general, liver exhibited the highest level of the metal at all the four stations; the increasing order being Cochin > Mumbai > Mangalore > Quilon. The values of Hg in the liver ranged from 0.16 to 0.29 (Cochin region), 0.04 to 0.09 (Quilon region), 0.03 to 0.10 (Mangalore region) and 0.07 to 0.15 ppm (Mumbai region). Gills in cuttlefish along the west coast had ΣHg in the range of 0.01 to 0.07 ppm. ΣHg content in the skin were in the range 0.02 to 0.04, 0.01 to 0.05, 0.04 to 0.06 and 0.02 to 0.06 ppm in cuttlefish from Cochin, Quilon, Mangalore and Mumbai regions, respectively. The highest body burden of Hg in ink was noted from Mangalore region (Figs. 3.1 to 3.4). Tentacles of cuttlefish along the west

coast had ΣHg in the range of 0.03 to 0.08 ppm. The concentrations of Hg in the different body components obtained in the present study were low, and the distribution pattern being in the order liver > tentacles > ink > gills ≈ skin > muscle in Quilon, Mangalore and Mumbai regions. The geographic trends of ΣHg in muscle of cuttlefish were Cochin > Mumbai > Mangalore > Quilon, respectively.

Cadmium is a toxic metal from the viewpoint of public health. The cadmium content of whole cuttlefish was very high in all regions (Table 3.1 to 3.4); the highest value observed being 14.6 ppm from Mumbai region (Fig 3.4). Concentration of cadmium in whole soft tissues of cuttlefish at the four regions were 6.84 ± 2.21 ppm (Cochin region), 2.31 ± 0.61 ppm (Quilon region), 4.21 ± 0.23 ppm (Mangalore region) and 11.21 ± 3.69 ppm (Mumbai region). More than 90% of whole cuttlefish had cadmium content above 3ppm from all the regions. Average levels of Cd in edible muscle of cuttlefish was <3 ppm at all the four major regions (Fig 3.1 to 3.4). The edible muscle had mean Cd content 0.37 ppm, 0.39 ppm, 0.84 ppm and 0.83 ppm from Cochin, Quilon, Mangalore and Mumbai regions, respectively. Liver formed the major site of Cd accumulation. Mean Cd content in the liver of cuttlefish from Cochin, Quilon, Mangalore and Mumbai regions were 54.86 ppm, 56.29 ppm, 52.52 ppm and 63.67 ppm; samples from Mumbai region had the highest concentration of cadmium. Around 30% (Cochin), 33% (Quilon), 20% (Mangalore) and 40% (Mumbai)

samples had Cd content in liver above 100 ppm. Cadmium content in gills of cuttlefish showed concentrations of 2.00 ± 1.10, 3.09 ± 1.16, 3.53 ± 3.20, 10.80 ± 2.18 ppm at Cochin, Quilon, Mangalore and Mumbai regions, respectively. The highest levels were noted in Mumbai region and the lowest in Cochin region. Another major site of accumulation was in ink. Cd content in ink of cuttlefish varied from 0.05 to 92.2 ppm along the west coast of India. The highest value was reported from Quilon region (92.2 ppm). The increasing order of magnitude of Cd per unit weight of the body components of cuttlefish were liver > ink > gills > skin > tentacles > muscle for Cochin, Quilon and Mumbai regions. The regional trend of cadmium in muscle of cuttlefish from the different regions were: Mangalore > Mumbai > Quilon > Cochin.

The concentration of lead in the whole soft parts of cuttlefish varied from 0.46 to 1.50 ppm at Cochin; 0.25 to 0.71 ppm at Quilon, 0.15 to 0.67 ppm at Mangalore and 0.11 to 1.04 ppm at Mumbai region. The mean Pb content in the edible muscle was below 1 ppm in samples from the four regions; the range of values being 0 to 0.75 ppm at Cochin, 0 to 0.73 ppm at Quilon, 0 to 0.85 ppm at Mangalore and 0.14 to 0.71 ppm at Mumbai regions. The lower range of Pb, in general was zero, indicating more or less a cleaner environment with respect to lead. Liver in cuttlefish along the west coast had lead in the range of 0 to 1.14 ppm. The mean Pb content was the highest in gill samples of cuttlefish from Cochin region (0.38 ppm)

and the lowest level were noted in Mumbai region (Fig 3.4). The highest level of Pb was found in ink at all regions (Figs 3.1 to 3.4) and the range of values were 0 to 1.70 ppm, 0 to 1.15 ppm, 0.47 to 1.65 ppm and 0 to 1.18 ppm in samples from Cochin, Quilon, Mangalore and Mumbai regions, respectively. In the skin, Pb content ranged from 0 to 1.66 ppm, considering all the stations together.

The distribution pattern, in general, being

Cochin: ink > liver > muscle > gills > skin > tentacles

Quilon: ink > muscle > gills > tentacles > skin > liver

Mangalore: ink > muscle > skin > gills > tentacles ≈ liver

Mumbai: ink > liver > skin > tentacles > muscle > gills.

The highest value of Pb content was observed in the whole soft parts of cuttlefish collected from Cochin region (1.5 ppm) followed by Mumbai, Mangalore and Quilon.

3.3.2. Regional variation in Copper, Zinc, Iron and Manganese.

The trace metals Cu, Zn, Fe and Mn are essential to life (Mertz, 1981) and play a major role in biological processes. Concentration of Cu, Zn, Fe and Mn from the four regions and their regional trends are presented in Tables 3.1 to 3.4 and Figs 3.1 to 3.4.

Copper is regarded as an essential metal in mammalian nutrition due to its key role in many enzyme reactions. The mean copper concentration in whole of cuttlefish was the highest from Mumbai region (35.47 ppm)

(Table 3.4) and the lowest was in samples from Cochin region (10.88 ppm) (Table 3.1). Higher levels of copper were found in the liver, gills, skin and ink of cuttlefish collected from all the four regions (Figs 3.1 to 3.4). Muscle had the lowest Cu content and ranged from 0.17 to 6.95 ppm in cuttlefish, taking into account all the four regions. The level of copper in the liver of cuttlefish from Cochin, Quilon, Mangalore and Mumbai regions ranged from 12.24 to 133, 11.31 to 142, 11.44 to 135 and 10.44 to 123.7 ppm, respectively. More than 60% of liver samples from Cochin, Quilon and Mangalore region had Cu content above 50 ppm. Mean value of copper was the highest in gills of cuttlefish from Cochin region (33.83 ± 9.36 ppm), while in Mumbai region, samples exhibited the lowest level (13.32 ± 2.87 ppm). Significant levels of copper were observed in skin, ink and tentacles from different regions. The range and mean values of copper in skin of cuttlefish from Cochin, Quilon, Mangalore and Mumbai regions were 9.35 to 35.05 (17.90 ± 8.77),; 6.50 to 26.5 (17.51 ± 10.64),; 6.42 to 40.09 (21.58 ± 12.23),; 6.56 to 14.84 (11.72 ± 3.10) ppm, respectively. Level of Cu in the ink of cuttlefish varied between regions and the highest level was found in samples from Quilon region; the mean value being 29.01 ppm and the lowest level being in samples from Mangalore region (9.78 ppm). Tentacles of cuttlefish along the west coast had copper in the range 1.42 to 16.89 ppm. Among the body components, liver was again found to be the major site of accumulation followed by gills, skin, ink and tentacles. The

pattern of distribution of copper in the muscle in the four regions followed the order Quilon > Cochin > Mumbai > Mangalore.

Zinc is an integral component of numerous functional proteins where in it exerts specific properties over a wide range of physiological systems (Vallee and Falchuk, 1993). The mean value of zinc found in the whole soft parts of cuttlefish from Cochin, Quilon, Mangalore and Mumbai region were 19.12, 16.45, 17.38 and 20.81 ppm, respectively (Tables 3.1 to 3.4). However, much lower levels were observed in the muscle with values ranging from 6.03 to 19.53 ppm (Cochin), 4.37 to 19.62 ppm (Quilon), 4.48 to 10.40 ppm (Mangalore) and 9.06 to 13.46 ppm (Mumbai), respectively. Gills samples from Mumbai region exhibited the highest mean Zn content of 15.58 ppm (Fig 3.4) and the lowest from Quilon region (8.97 ppm). The other preferential site of accumulation were skin and tentacles. The concentration of Zn in skin of cuttlefish from Cochin, Quilon, Mangalore and Mumbai regions were 17.37 ± 11.61,; 12.93 ± 9.37,; 14.34 ± 0.84,; 21.91 ± 12.83 ppm, respectively. Mean value of Zn in skin was the highest in Mumbai region and the lowest in Quilon region. In the tentacles, the lowest levels of Zn were noted in Cochin samples (2.09 ppm) (Fig 3.1). The concentration of Zn in different organs of cuttlefish varied between regions and followed the order:

Cochin: liver > skin > ink > gills > tentacles > muscle

Quilon: liver > ink > skin > tentacles > muscle > gills

Mangalore: ink > liver > skin > gills > muscle > tentacles

Mumbai: ink > liver> skin > gills > muscle > tentacles.

In the muscle tissue, Quilon region showed higher mean value followed by Cochin, Mumbai and Mangalore. However, the variation was not very significant (Tables 3.1 to 3.4). The highest mean value of Zn in whole soft part was exhibited by samples from Mumbai region followed by Cochin, Mangalore and Quilon region.

Generally, low levels of iron were found in the muscle of cuttlefish (Figs 3.1 to 3.4). The range of values for Fe in cuttlefish muscle from Cochin, Quilon, Mangalore and Mumbai regions were 0.05 to 5.30, 0.18 to 2.10, 0.05 to 3.58, 2.63 to 6.02 ppm, respectively. The liver of cuttlefish is rich in iron content, the highest level was observed from Cochin region (220.4 ppm) (Fig. 3.1). The iron content was comparatively lower in liver samples from Quilon region, the over all mean and range being 38.68 ppm; (2.14 to 74.6 ppm), respectively. The highest mean value of Fe were noted in whole of cuttlefish from Mumbai region (12.16 ppm) and the lowest levels were noted in Cochin samples (4.04 ppm). Fe content in gills of cuttlefish along the west coast were in the range 0.92 to 36.18 ppm: the lowest level was at Quilon region (0.92 ppm) and the highest at Mangalore region (36.18 ppm). Iron content in skin were in the range of 3.6 to 11.69, 1.55 to 5.44, 1.78 to 14.08, 6.62 to 7.18 ppm in cuttlefish from Cochin, Quilon, Mangalore and Mumbai region, respectively. Ink and tentacles did not show much variation between the regions. Quilon region showed the highest mean value of Fe in ink and tentacles compared to other regions

(Fig 3.2). The geographic trend observed in whole cuttlefish were of the order: Mumbai > Mangalore > Quilon > Cochin.

Manganese is essential in animal body and its deficiency is impaired in the glucose utilization. The mean value of Mn in whole of cuttlefish from Cochin, Quilon, Mangalore and Mumbai regions were 0.39, 0.92, 0.78 and 0.51 ppm, respectively (Figs 3.1 to 3.4). The highest mean value of muscle were noted in samples from Quilon region and the lowest from Cochin region. Liver and ink, in general, contained higher level of Mn compared to other organs. The mean value of Mn found in the liver of cuttlefish from Cochin, Quilon, Mangalore and Mumbai regions were 2.47, 4.41, 7.42 and 3.32 ppm, respectively. The highest level of Mn in liver were found in samples from Mangalore region (13.46 ppm). Concentration of Mn in ink of cuttlefish were 2.29 ± 1.20, 3.81 ± 0.05, 4.20 ± 1.02, 2.71 ± 0.11 ppm from Cochin, Quilon, Mangalore and Mumbai regions, respectively. Further, the gills and skin were the other major site of accumulation. The order of abundance being:

Cochin: liver > ink > gills > skin ≈ tentacles > muscle

Quilon: liver > ink > skin > tentacles > muscle > gills

Mangalore: liver > ink > skin > gills > tentacles > muscle

Mumbai: liver > ink > gills > skin > muscle > tentacles.

In the whole soft parts and muscle tissue, Quilon region exhibited the highest manganese content when compared to other regions.

3.3.3. Regional variation in Chromium and Nickel.

Chromium is considered as one of the least toxic of trace elements. Chromium (iii) as a part of the human tolerance factor was identified as an essential micronutrient for mammals. The distribution pattern and regional trends of Cr and Ni in cuttlefish are presented in Tables 3.1 to 3.4 and Figs 3.1 to 3.4. In whole cuttlefish, Cr content varied from region to region and were in the range 0.01 to 7.31 ppm at Cochin, 0.52 to 3.79 ppm at Quilon, 0.08 to 8.12 ppm at Mangalore and 0.38 to 1.86 ppm at Mumbai region. However, Cr content in the muscle of cuttlefish was below the tolerance limit at all the regions. The mean values being 0.64, 0.55, 0.83 and 0.70 ppm from Cochin, Quilon, Mangalore and Mumbai regions, respectively. The lower range of Cr in the edible muscle was zero in Cochin, Quilon and Mangalore region (Table 3.1 to 3.3). Cr was the highest in liver from Mangalore region when compared to other regions the mean value being; 8.21 ± 4.84 (1.78 to 14.42) ppm, (Table 3.3). Concentration of Cr in gills of cuttlefish were 0.29 ± 0.33, 1.90 ± 1.47, 0.65 ± 0.43, 1.20 ± 0.95 ppm from Cochin, Quilon, Mangalore and Mumbai regions, respectively. Mean values of Cr in skin of cuttlefish varied between regions and were in the range 0 to 4.85 ppm along the west coast of India. The level of Cr was the highest in ink when compared to other body components at Cochin, Quilon and Mumbai regions, the mean values being 11.22, 3.39 and 2.56 ppm (Figs 3.1, 3.2 and 3.4). Chromium content in the tentacles did not show variation with region. The mean values being 0.87 ± 1.24 ppm at Cochin, 1.07 ±

0.93 ppm at Quilon, 0.30 ± 0.31 ppm at Mangalore and 0.69 ± 0.58 ppm at Mumbai region. The concentration of Cr in the different organs of cuttlefish varied among regions and followed the order:

Cochin: ink > liver > skin > tentacles > muscle > gills

Quilon: ink > liver > gills > skin > tentacles > muscle

Mangalore: liver > ink > muscle > gills > skin > tentacles

Mumbai: ink > liver > gills > skin > muscle > tentacles.

The geographic trend in the distribution of Cr in the whole soft tissues were in the order: Mangalore > Cochin > Quilon > Mumbai.

Nickel in excess are toxic to aquatic organisms and persistent in the aquatic environment. The distribution of Ni is shown in Fig 3.1 to 3.4. Nickel content in whole cuttlefish varied from region to region and were in the range 0.16 to 8.87 ppm at Cochin, 0.03 to 2.06 ppm at Quilon, 0.56 to 0.73 ppm at Mangalore and 2.08 to 3.01 ppm at Mumbai region. However, Ni content in the muscle of cuttlefish was below the tolerance limit at all the regions. The mean values being 0.30, 0.50, 0.23 and 0.78 ppm, respectively. Slightly elevated levels of Ni were found in the liver of cuttlefish from Mangalore region (3.99 ppm) and Mumbai region (3.66 ppm) (Table 3.3 and 3.4). Ni levels ranged from 0 to 7.37 ppm in gills along the west coast of India and the highest value observed was in Mangalore region (7.37 ppm) (Fig 3.3). In skin samples, the lower range, in general, was zero and the highest mean value exhibited was in Mumbai region (3.05 ppm). The concentration of Ni in ink of cuttlefish from Cochin, Quilon,

Mangalore and Mumbai regions were 3.77 ± 1.25, 4.35 ± 3.04, 4.69 ± 3.34, 6.92 ± 4.11 ppm, respectively. Ink samples of cuttlefish contained the highest level of Ni for all the stations. The levels of Ni in tentacles were relatively low in samples from all the regions and were in the range 0 to 2.96 ppm. The distribution pattern of Ni in the body components from the four regions were of the order:

Cochin: ink > skin > liver > tentacles > gills > muscle

Quilon: ink > tentacles > liver > skin > gills > muscle

Mangalore: ink > liver > gills > skin > tentacles > muscle

Mumbai: ink > liver > skin > gills > muscle > tentacles.

Two way Analysis of Variance (ANOVA) of the data was employed to compare the group means between the four regions, viz., Cochin, Quilon, Mangalore and Mumbai region in respect of Cd, Cu, Zn and Cr. The results are presented in Table 3.6 to 3.13.

Results showed that there is no significant difference in Cd content in the muscle and liver of cuttlefish between the four regions (Table 3.6 and 3.7). Cuttlefish from Quilon region exhibited significantly higher copper in muscle ($p<0.01$), when compared to other regions (Table 3.8). Similarly, in liver also Quilon region shows significantly higher copper ($p<0.05$), when compared to Cochin, Mangalore and Mumbai regions (Table 3.9). Zinc content in the muscle and liver of cuttlefish from Quilon region were significantly higher ($p<0.05$ and $p<0.01$) when compared to other regions

(Table 3.10 and 3.11). There was no significant difference in Cr levels in muscle samples between the four regions (Table 3.12). Mangalore region is having significantly higher value ($p<0.05$) of Cr in liver than Cochin and Quilon region (Table 3.13).

3.4. Discussion

It can be seen that, whole soft parts of the cuttlefish, invariably exhibited higher levels of all the metals than found in the muscle. In the edible muscle, levels of all metals were below the tolerance limit. The concentration of mercury are far below the limit of $1 mgkg^{-1}$ permitted for sea foods (FAO, 1983). Barska *et al* (1988a, b) reported low levels of Hg in *Loligo patagonica* and *Illex argentinus*. The values for Hg obtained in the present study are quite comparable with the levels reported by Ramamurthy (1979), Patel and Chandy (1988), Jasmine *et al.*, (1989), Lakshmanan and Stephen (1993) and Lakshmanan *et al.*, (2001). Low levels of Hg in *Loligo* sp. were reported by Falandysz (1989, 1990) and Oehlenschlaeger (1991). The concentration of Hg in the different body components obtained in the present study were also low, and the distribution pattern being in the order liver > tentacles > ink > gills > skin > muscle in Quilon, Mangalore and Mumbai region. The results indicated that cephalopod molluscs did not exhibit unusual tendency for Hg accumulation.

The cadmium content of whole cuttlefish was very high in all regions and exceeded the tolerance limit. Around 90% of whole cuttlefish had Cd

content above 3ppm. Martin and Flegal (1975) reported very high levels of Cd and similar metals in the intestines of squid, *Loligo patagonica* and found first detailed study in the distribution of metals in squid. High levels of Cd in cephalopods have been reported from various parts of the world by other researchers (Tanaka *et al.*, 1974; Horowitz and Presley, 1977; Sugiyama, 1981; Smith *et al.*, 1981; Falandysz, 1989; Bustamante, 1998; Bustamante *et al.*, 1998a). Raw whole squid contained an average 4.0 mg Cd/kg as reported by Falandysz (1991) and the highest level was found in the liver, as in the present study. Higher levels of Cd (2.9 to 10 mg/kg wet wt) in the edible parts of canned squid, *Loligo patagonica* were reported by Falandysz (1989). Elevated levels of Cd (>2 ppm) have been observed in the whole soft parts and liver of cuttlefish (Lakshmanan *et al.*, 2001). Cantoni *et al* (1986) reported that about 50% of the cuttlefish samples imported into Italy had Cd content in excess of the tolerance limit. Tonsavic *et al* (1988) found that in Yugoslavia 50% of squid samples exceeded the tolerance limit of (1 mg/kg), the highest Cd concentration being 3.0 mg/kg. Cozzani *et al.*, (1990) found that 40 of 422 samples of cephalopods had higher levels of Cd and was found to be >3ppm. Oehlenschlaeger (1990) found Cd content were low in the mantle but very high in intestines of cuttlefish *Todarodes sagittatus*, caught from Northeast Atlantic. Bustamante *et al* (1998a) found that the concentrations of Cd in different Loliginidae species from French and Irish waters was significantly low (0.10 µg/g wet wt.). The liver of cuttlefish, *Sepia officinalis*, caught on the French

coast also exhibited higher Cd content (Miramand and Bentley, 1992). In the present study, liver formed the major site of Cd accumulation in cuttlefish from all the regions. However, all the mantle and tentacles samples had relatively low values for cadmium and was far below the tolerance limit.

Cephalopods have been considered as a significant source of Cd to their predators (Muirhead and Furness, 1988; Law *et al.*, 1997; and Bustamante *et al.*, 1998 a). High levels of Cd have been found in striped dolphins (Honda and Tatsukawa, 1983), Arctic marine mammals (Dietz *et al.*, 1998), Juan Fernandez fur seals (Ochoa- Acuna and Francis, 1995) and it has been attributed to high cephalopod consumption in the diet. The environmental factors and feeding habits of cuttlefish, wherein a variety of fish, shellfish and crustaceans are consumed, probably contribute to high levels of the metals in cuttlefish (Bryan *et al.*, 1983). Being a voracious fish eater, and carnivorous in nature, cuttlefish receive and accumulate heavy metals from their food fishes and also from cephalopods. Cannibalism that has been noticed in cuttlefish (Okutani, 1990) might have also contributed to high Cd levels.

The distribution pattern of lead showed that the highest level of the metal was found in the ink, at all regions. Concentration of lead in muscle of cuttlefish were less than the permissible limit (1.5 mg kg^{-1}) and were comparable to the values reported in *L. opalescens* (Falandysz, 1991). The levels of Pb observed in the liver of cuttlefish were similar to the values

reported in *Sepia pharaonis* (Lakshmanan and Stephen, 1993). The lower range of Pb, in general was zero, indicating more or less a cleaner environment. Another reason may be due to the sparingly soluble property of lead compounds and consequently non-availability to bio-mass in the marine environment. Almost all the samples from all the four regions had lead content below 1 ppm, except few samples from Cochin and Mumbai had values above 1 ppm.

Zinc content in the edible muscle of cuttlefish are quite comparable to the values observed in *L. patagonica* (12 mg/kg wet wt) reported by Falandysz (1989). Jones *et al.* (2000) observed Zn concentration in the muscle of two species of cuttlefish from Cleveland Bay in the range of 13-16 µg/g wet wt. The level of zinc found in whole soft tissues of cuttlefish from the four regions were in the range 7.15 to 27.55 ppm and are comparable to the values reported in *Sepia pharaonis* by Lakshmanan and Stephen, 1993. Smith *et al* (1984) observed wide ranges in Zn content in the digestive gland of squid, *Nototodarus gouldi*. In general, the level of Zn reported for various molluscan products were quite high (Lakshmanan and Nambisan, 1983; Talbot, 1986; Rivonker and Parulekar, 1998). However, the level of zinc in *S. pharaonis* in the present study was found to be quite normal and did not vary greatly.

The level of copper observed in the muscle of cuttlefish are similar to the values reported in the mantle of *Sepiella inermis* in Portnovo samples

by Dious and Kasinathan (1992). The copper levels in muscle are also quite comparable to the levels in *L. duvauceli* reported by Tariq *et al.* (1991). Very high levels of Cu in the liver of *L. opalescens* (200 to 300 mg/kg wet wt.) was reported by Falandysz (1991). Martin and Flegal (1975) and Oehlenschlaeger (1991) also reported high copper content in the liver of *L. opalescens*. Copper content in the liver of cuttlefish are quite comparable to the values reported by Lakshmanan and Stephen (1993) (56.16 ± 45.67 ppm). Since cuttlefish requires Cu for the synthesis of the respiratory pigment, haemocyanin, a high level in the body may be attributed to the functional necessity (Smith, 1984).

The level of Fe found in the mantle and tentacles in the present study are comparable to the values observed in *L. patagonica* (1.8-3.4 mg kg^{-1}) (Falandysz 1989). Liver of *Todarodes pacificus* from Japan showed higher level of Fe in visceral organs than in the edible parts (Ueda *et al.*, 1979). Lozano Soldevilla (1989) found Fe content to be high in whole bodies of *Todarodes sagittatus* when compared to those in mantles and tentacles. Wide ranges in the concentrations of the elements Fe, Mn, Pb and Zn were observed in the digestive gland of squid, *Nototadarus gouldi* by Smith *et al* (1984). The level of Mn in the whole of cuttlefish observed in the present study compares well with the values for whole *L. opalescens* (0.31 ± 0.25 mg kg^{-1}) reported by Hall *et al* (1978).

Whole soft parts of cuttlefish, invariably exhibited higher levels of all metals than found in the muscle, which must be owing to the contribution from other body components like liver, gills, ink and tentacles that contained higher levels of the metals. Barska *et al.* (1988a, b) conducted a base line data of heavy metals like Cd, Zn, Pb, Cu, Hg in *L. patagonica* and *Illex argentinus* caught by the polish fishery and reported high levels of heavy metals especially Cd in whole canned squid (*L. patagonica*). They also observed migration of metals from the viscera to the mantle. Martin and Flegal (1975) noted that trace metals migrate out of the liver into the muscle and thus results in higher levels in the edible muscle. Cisneros *et al.* (1995) reported that Cd was accumulated mainly in the viscera of cuttlefish and careful washing reduced Cd content to a high degree. Schulz – Schering (1995) suggested that as Cd concentrations of the cephalopod products resulted from high concentrations in intestine, the visceral parts must be removed carefully before it is processed for human consumption.

Present study shows that there is variation in heavy metal concentration in cuttlefish from different regions along the west coast of India. Environmental difference in conjunction with age, nature and availability of food are the probable factors responsible for the wide range of metal concentration found in cuttlefish from the four different geographical regions.

Table 3.1: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components and whole soft parts of cuttlefish from Cochin Region.

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	65	0.04±0.01 (0-0.04)	0.37±0.34 (0-0.67)	0.47±0.25 (0-0.75)	2.10±0.98 (0.49-6.73)	10.30±3.78 (6.03-19.53)	2.97±1.86 (0.05-5.30)	0.29±0.16 (0-0.48)	0.64±0.59 (0-1.49)	0.30±0.24 (0-0.82)
Liver	62	0.19±0.10 (0.16-0.29)	54.86±44.8 (4.63-114)	0.53±0.47 (0-1.14)	67.96±48.57 (12.24-133)	46.65±37.69 (2.14-146.56)	75.27±56.36 (2.47-220.4)	2.47±2.77 (0-10.13)	2.23±1.50 (0.71-4.58)	1.31±0.81 (0-2.53)
Gills	51	0.05±0.02 (0.03-0.07)	2.00±1.10 (0.98-3.10)	0.38±0.35 (0-0.87)	33.83±9.36 (9.36-46.83)	11.15±6.62 (6.62-11.05)	8.28±5.22 (5.22-8.28)	1.30±0.80 (0.80-1.31)	0.29±0.33 (0.29-0.63)	1.05±0.88 (0.88-1.06)
Skin	55	0.02±0.02 (0.02-0.04)	1.79±1.74 (0-5.50)	0.27±0.68 (0-1.66)	17.90±8.77 (9.35-35.05)	17.37±11.61 (3.49-38.48)	7.32±2.97 (3.6-11.69)	1.20±0.45 (0-3.14)	1.85±1.20 (0-4.85)	2.64±2.48 (0-5.67)
Ink	56	0.04±0.02 (0.04-0.06)	3.10±5.71 (0.06-19.44)	0.72±0.59 (0-1.70)	15.91±9.25 (2.94-27.22)	16.27±5.16 (5.27-22.4)	5.86±1.89 (0-6.96)	2.29±1.20 (0-4.25)	11.22±8.40 (0-19.37)	3.77±1.25 (0-4.22)
Tentacles	48	0.07±0.02 (0.04-0.08)	0.59±0.49 (0-1.49)	0.24±0.18 (0-0.92)	5.84±2.72 (1.42-12.06)	10.47±4.69 (2.09-19.38)	6.63±4.10 (0.69-15.19)	1.20±0.54 (0-2.09)	0.87±1.24 (0-3.98)	1.06±0.84 (0-2.26)
Whole	58	0.08±0.02 (0.03-0.09)	6.84±2.21 (6.1-8.81)	0.88±0.39 (0.46-1.50)	10.88±2.48 (5.15-14.06)	19.12±3.44 (7.15-22.15)	4.04±0.45 (1.72-2.36)	0.39±0.24 (0-0.62)	4.34±3.36 (0.01-7.31)	3.35±3.09 (0.16-8.87)

*number of samples analysed

Table 3.2: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components and whole soft parts of cuttlefish from Quilon Region.

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	48	0.01±0.04 (0.01-0.03)	0.39±0.34 (0-0.95)	0.41±0.25 (0-0.73)	2.18±2.12 (0.17-6.95)	10.73±4.51 (4.37-19.62)	1.35±0.56 (0.18-2.10)	1.05±1.14 (0.42-3.10)	0.55±0.35 (0-0.81)	0.50±0.48 (0-0.98)
Liver	43	0.06±0.02 (0.04-0.09)	56.29±55.38 (14-123)	0.26±0.16 (0-0.39)	78.51±64.28 (11.31-142)	52.15±52.71 (1.66-126)	38.68±29.15 (2.14-74.6)	4.41±2.73 (2.07-10.09)	2.28±1.36 (0.45-3.77)	1.44±0.85 (0.24-2.64)
Gills	47	0.03±0.01 (0.01-0.05)	3.09±1.16 (1.98-4.33)	0.28±0.18 (0-0.79)	31.07±10.62 (21.94-42.9)	8.97±3.36 (5.56-19.06)	3.60±2.44 (0.92-7.09)	0.53±0.31 (0-0.96)	1.90±1.47 (0.34-4.89)	1.06±0.77 (0-2.12)
Skin	45	0.03±0.01 (0.01-0.05)	0.44±0.27 (0-0.93)	0.23±0.11 (0-0.36)	17.51±10.64 (6.50-26.5)	12.93±9.37 (6.47-24.4)	3.68±1.30 (1.55-5.44)	1.76±2.19 (0-4.96)	1.08±0.74 (0.35-2.53)	1.23±0.77 (0-2.31)
Ink	41	0.04±0.04 (0-0.08)	16.80±34.9 (0.05-92.2)	0.81±0.55 (0-1.15)	29.01±24.69 (11.58-73.5)	24.90±19.07 (4.99-39.4)	6.73±3.13 (4.00-11.23)	3.81±0.05 (1.58-3.84)	3.39±2.15 (1.25-7.12)	4.35±3.04 (2.77-9.05)
Tentacles	42	0.06±0.01 (0.04-0.07)	0.43±0.41 (0.04-1.38)	0.24±0.23 (0-0.80)	6.84±3.44 (3.85-14.13)	10.98±6.49 (5.58-24.88)	7.14±6.40 (1.00-19.33)	1.22±0.88 (0-0.76)	1.07±0.93 (0.08-3.03)	1.48±1.14 (0-2.96)
Whole	33	0.04±0.04 (0.02-0.08)	2.31±0.61 (1.43-3.29)	0.49±0.22 (0.25-0.71)	21.61±8.54 (9.36-30.34)	16.45±7.75 (14.75-27.55)	5.09±0.12 (4.98-5.18)	0.92±2.12 (0.05-2.08)	1.67±1.54 (0.52-3.79)	1.20±0.83 (0.03-2.06)

*number of samples analysed

Table 3.3: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components and whole soft parts of cuttlefish from Mangalore Region.

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	39	0.02±0.01 (0.01-0.04)	0.84±0.07 (0.01-0.88)	0.73±0.18 (0-0.85)	1.97±1.75 (0.43-5.64)	8.35±1.54 (4.48-10.40)	2.19±0.93 (0.05-3.58)	0.56±0.84 (0-1.29)	0.83±0.82 (0-2.47)	0.23±0.17 (0-0.45)
Liver	36	0.08±0.03 (0.03-0.10)	52.52±47.9 (4.66-107)	0.25±0.06 (0-0.38)	67.96±40.64 (11.44-135)	35.15±25.98 (3.08-76.05)	67.05±12.65 (38.77-76.01)	7.42±5.45 (0-13.46)	8.21±4.84 (1.78-14.42)	3.99±3.52 (0-7.38)
Gills	35	0.04±0.03 (0.01-0.07)	3.53±3.20 (0.57-9.97)	0.35±0.29 (0-0.84)	29.93±8.37 (12.95-36.1)	10.07±7.35 (0.99-24.16)	12.44±10.94 (0.99-36.18)	2.25±1.93 (0-5.69)	0.65±0.43 (0-1.37)	1.48±2.64 (0-7.37)
Skin	31	0.05±0.01 (0.04-0.06)	0.65±0.44 (0.13-1.28)	0.42±0.22 (0.16-0.85)	21.58±12.23 (6.42-40.09)	14.34±0.84 (12.9-15.2)	5.91±4.05 (1.78-14.08)	4.05±3.46 (1-10.47)	0.60±0.45 (0.24-1.58)	0.38±0.18 (0-0.66)
Ink	33	0.06±0.03 (0.02-0.09)	0.79±0.32 (0.62-1.02)	0.86±0.28 (0.47-1.65)	9.78±3.24 (5.55-12.68)	48.51±0.39 (32.10-48.62)	6.72±1.93 (5.18-8.98)	4.20±1.02 (1.92-5.68)	6.07±4.46 (1.09-9.72)	4.69±3.34 (1.20-9.53)
Tentacles	30	0.07±0.02 (0.03-0.09)	0.88±0.78 (0-1.88)	0.15±0.02 (0-0.15)	8.06±6.88 (1.58-16.89)	7.46±1.15 (4.79-8.34)	5.10±2.04 (2.28-7.61)	1.67±1.41 (0.06-3.59)	0.30±0.31 (0.20-0.61)	0.27±0.12 (0.02-0.42)
Whole	29	0.05±0.01 (0.01-0.06)	4.21±0.23 (1.48-5.32)	0.66±0.01 (0.15-0.67)	12.24±3.12 (7.81-16.48)	17.38±1.30 (7.0-18.91)	5.12±1.31 (2.05-8.02)	0.78±0.20 (0.09-1.18)	5.34±0.12 (0.08-8.12)	0.63±0.03 (0.56-0.73)

*number of samples analysed

Table 3.4: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components and whole soft parts of cuttlefish from Mumbai Region.

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	20	0.03±0.02 (0.02-0.04)	0.83±0.46 (0.47-1.36)	0.42±0.32 (0.14-0.71)	1.98±1.11 (0.44-4.07)	10.50±1.58 (9.06-13.46)	3.20±1.48 (2.63-6.02)	0.30±0.19 (0.17-0.50)	0.70±0.27 (0.33-1.06)	0.78±0.12 (0.68-0.94)
Liver	19	0.09±0.03 (0.07-0.15)	63.67±34.7 (30.95-140)	0.47±0.02 (0-0.68)	57.19±15.09 (10.44-123.7)	24.09±15.54 (9.00-52.8)	56.12±5.46 (3.71-79.8)	3.32±0.47 (0.69-3.77)	2.31±1.18 (0.76-3.12)	3.66±0.83 (1.28-4.52)
Gills	18	0.04±0.02 (0.02-0.06)	10.80±2.18 (8.37-14.4)	0.20±0.02 (0.10-0.25)	13.32±2.87 (9.31-15.91)	15.58±4.81 (9.77-20.33)	11.04±0.30 (1.28-11.82)	0.90±0.12 (0.28-1.02)	1.20±0.95 (0.38-2.25)	2.11±1.29 (0.19-2.97)
Skin	15	0.03±0.02 (0.02-0.06)	0.87±0.73 (0.45-1.47)	0.58±0.12 (0.42-0.71)	11.72±3.10 (6.56-14.84)	21.91±12.83 (3.04-39.48)	6.95±0.29 (6.62-7.18)	0.49±0.34 (0.16-0.86)	0.90±0.59 (0.09-1.64)	3.05±1.08 (0-4.03)
Ink	13	0.04±0.02 (0.02-0.06)	11.77±25.2 (0.74-68.8)	0.91±0.44 (0-1.18)	12.12±7.38 (1.70-19.52)	34.10±24.10 (3.26-75.11)	6.14±0.59 (4.41-6.51)	2.71±0.11 (0-2.79)	2.56±0.62 (0-2.59)	6.92±4.11 (2.18-9.48)
Tentacles	14	0.06±0.02 (0.04-0.08)	0.90±0.70 (0.23-2.05)	0.45±0.16 (0-0.72)	4.32±0.89 (3.42-5.66)	10.14±0.42 (9.80-10.89)	2.55±0.04 (2.0-2.59)	0.08±0.01 (0-0.09)	0.69±0.58 (0.26-1.72)	0.72±0.02 (0.50-0.75)
Whole	09	0.07±0.02 (0.05-0.17)	11.21±3.69 (8.09-14.6)	0.81±0.91 (0.11-1.04)	35.47±3.95 (31.28-42.6)	20.81±1.42 (19.19-22.58)	12.16±3.14 (8.55-15.11)	0.51±0.08 (0.04-0.68)	1.31±0.82 (0.38-1.86)	2.07±0.08 (2.08-3.01)

*number of samples analysed

Table 3.5: Environmental contaminants, tolerances, action levels and guidance levels as per FDA and EU regulations

Toxic Elements	Limits as per FDA	Food commodity	Limits as per EU norms (for all marine products) mg/Kg
Arsenic	76 86	Crustacea Molluscan bivalves	-
Cadmium	3 4	Crustacea Molluscan bivalves	0.5-3.0*
Chromium	12 13	Crustacea Molluscan bivalves	-
Lead	1.5 1.6	Crustacea Molluscan bivalves	0.5-10*
Nickel	70 80	Crustacea Molluscan bivalves	-
Mercury	1.0	All fish	1.0
Zinc	-	-	50

* varying between EU countries

Table 3.6: Analysis of variance (ANOVA) of Cadmium in muscle of cuttlefish. (Regional variation)

Source	SS	dF	mS	F
Total	915.896	117	-	-
Between Regions	21.4926	3	7.16	1.017 (NS)
Error	788.846	112	7.04	

Table 3.7: Analysis of variance (ANOVA) of Cadmium in liver of cuttlefish. (Regional variation)

Source	SS	dF	mS	F
Total	187939.7	72	-	-
Between Regions	665.509	3	221.836	0.1179(NS)
Error	126027.4	67	1881	

Table 3.8: Analysis of variance (ANOVA) of Copper in muscle of cuttlefish. (Regional variation)

Source	SS	dF	mS	F
Total	20289.82	118	-	-
Between Regions	1730.109	3	576.70	5.022 ($p<0.01$)
Error	12976.46	113	114.83	

Table 3.9: Analysis of variance (ANOVA) of Copper in liver of cuttlefish. (Regional variation)

Source	SS	dF	mS	F
Total	146825.27	76	-	-
Between Regions	15038.89	3	5012.96	3.0978($p<0.05$)
Error	135026.96	71	1901.78	

Table 3.10: Analysis of variance (ANOVA) of Zinc in muscle of cuttlefish. (Regional variation)

Source	SS	dF	mS	F
Total	1727.565	117	-	-
Between Regions	111.3209	3	37.106	2.683($p<0.05$)
Error	1566.205	112	13.983	

Table 3.11: Analysis of variance (ANOVA) of Zinc in liver of cuttlefish. (Regional variation)

Source	SS	dF	mS	F
Total	86882.52	74	-	-
Between Regions	13196.30	3	4398.76	4.319($p<0.01$)
Error	70272.6	69	1018.44	

Table 3.12:Analysis of variance (ANOVA) of Chromium in muscle of cuttlefish (Regional variation)

Source	SS	dF	mS	F
Total	14.2046	79	-	-
Between Regions	0.89206	3	0.297	1.767 (NS)
Error	12.499	74		

Table 3.13: Analysis of variance (ANOVA) of Chromium in liver of cuttlefish. (Regional variation)

Source	SS	dF	mS	F
Total	187.974	72	-	
Between Regions	21.048	3	7.016	3.143 ($p<0.05$)
Error	149.609	67	2.232	

Fig 3.1. Heavy metal concentrations (mean, ppm,wet wt.) in the body components and whole soft parts of cuttlefish from Cochin region.

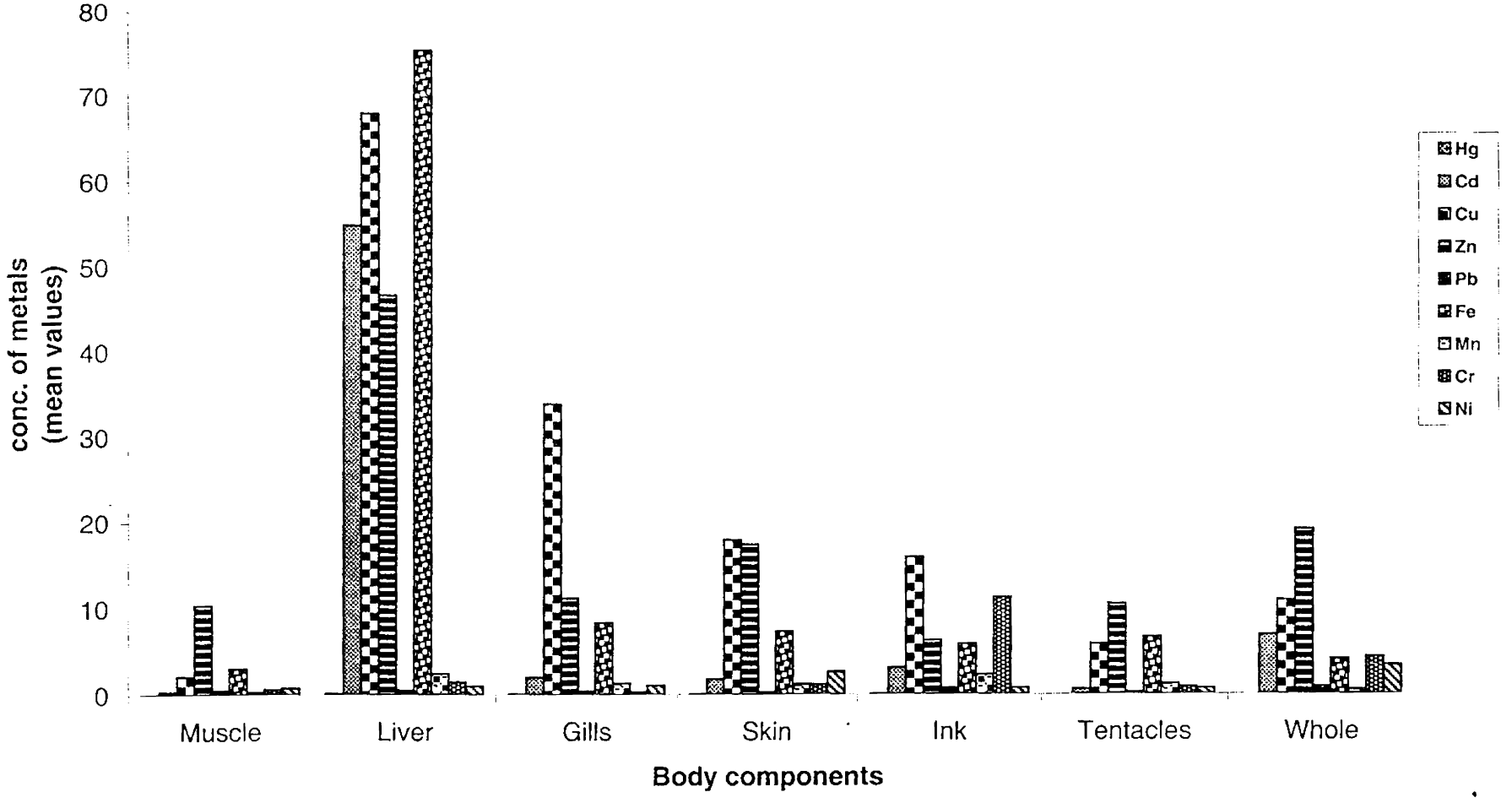


Fig 3.2. Heavy metal concentrations (mean, ppm,wet wt) in the body components and whole soft parts of cuttlefish from Quilon Region

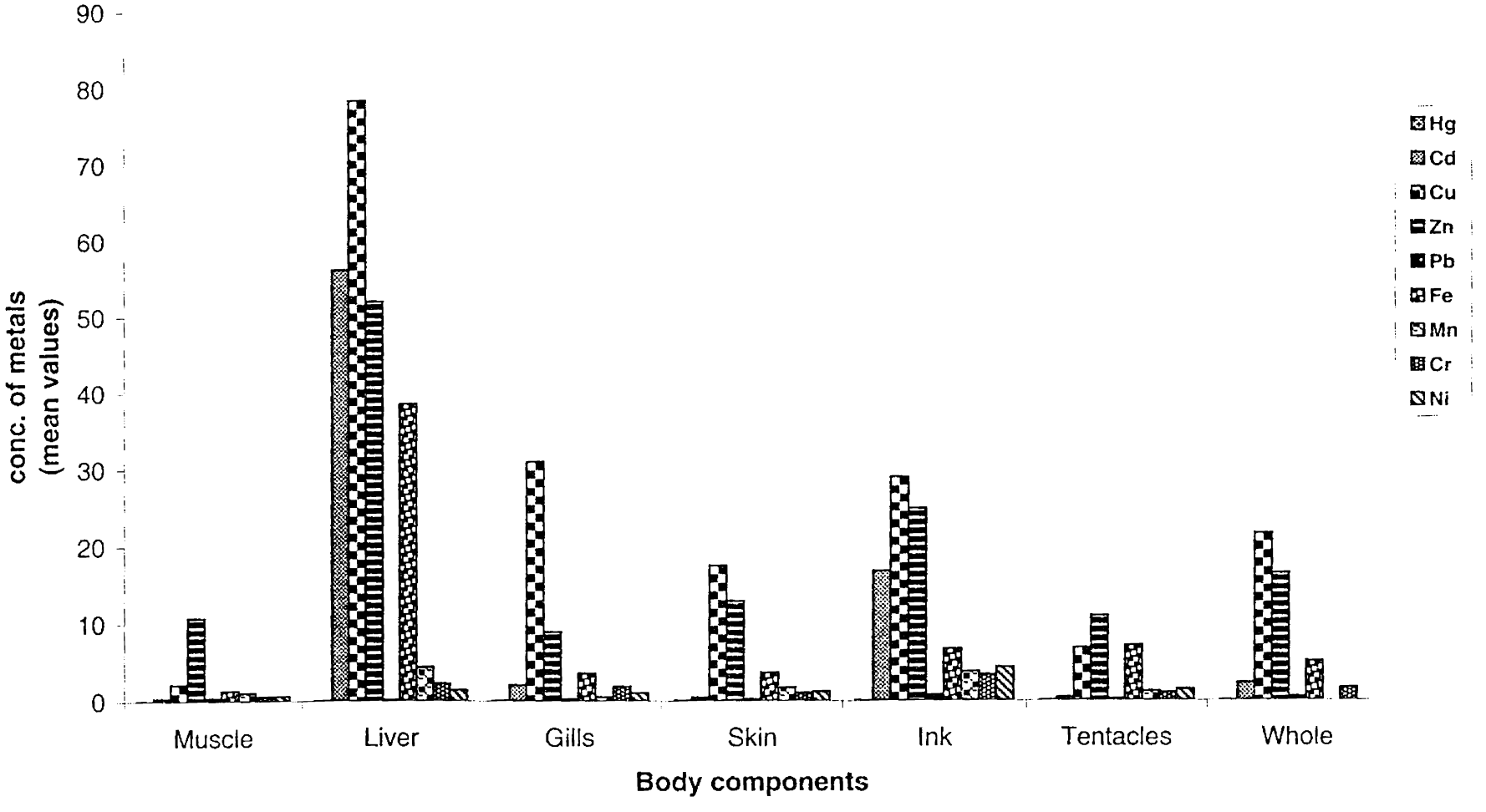


Fig 3.3. Heavy metal concentrations (mean,ppm,wet wt) in the body components and whole soft parts of cuttlefish from Mangalore Region.

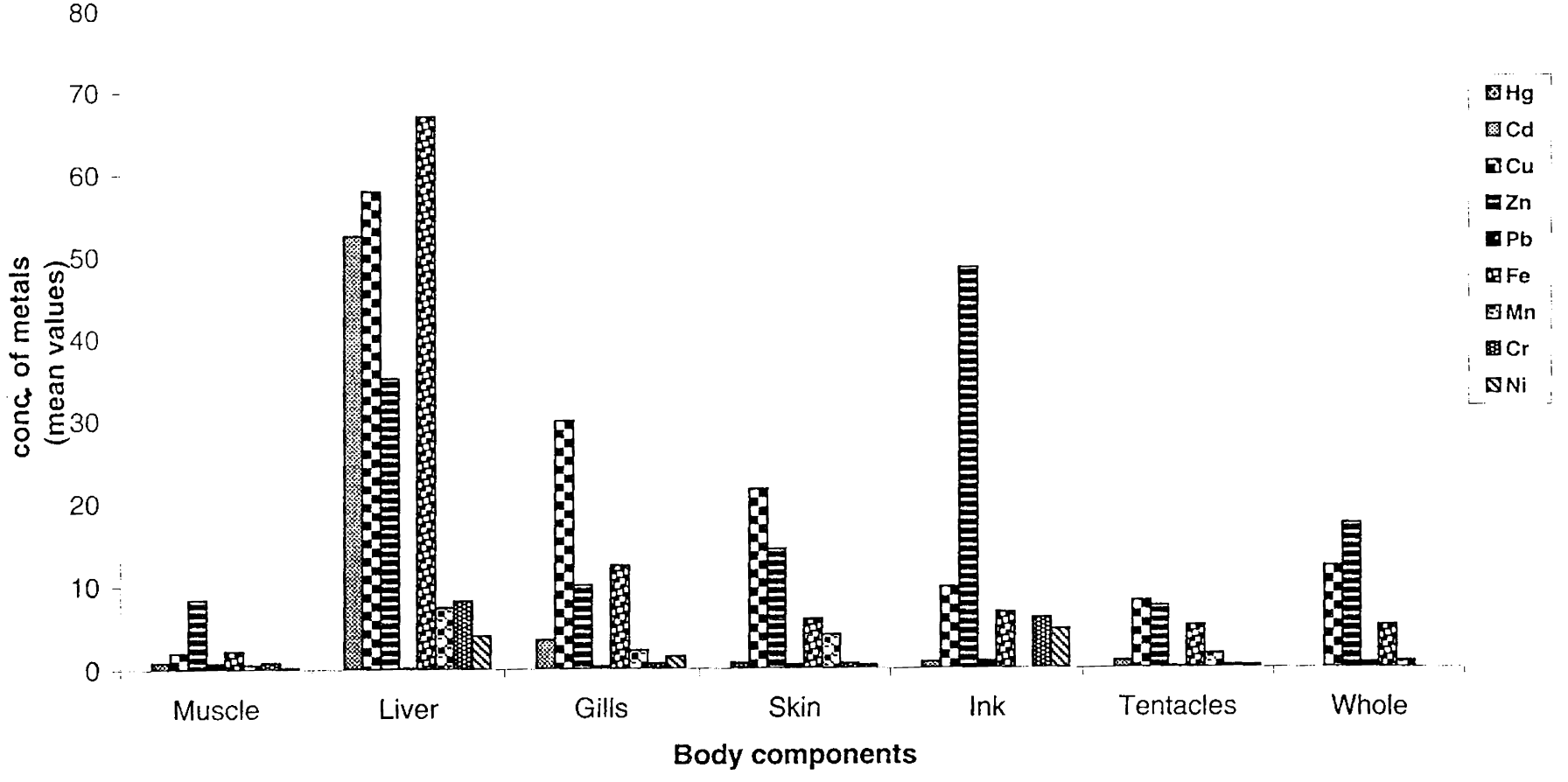
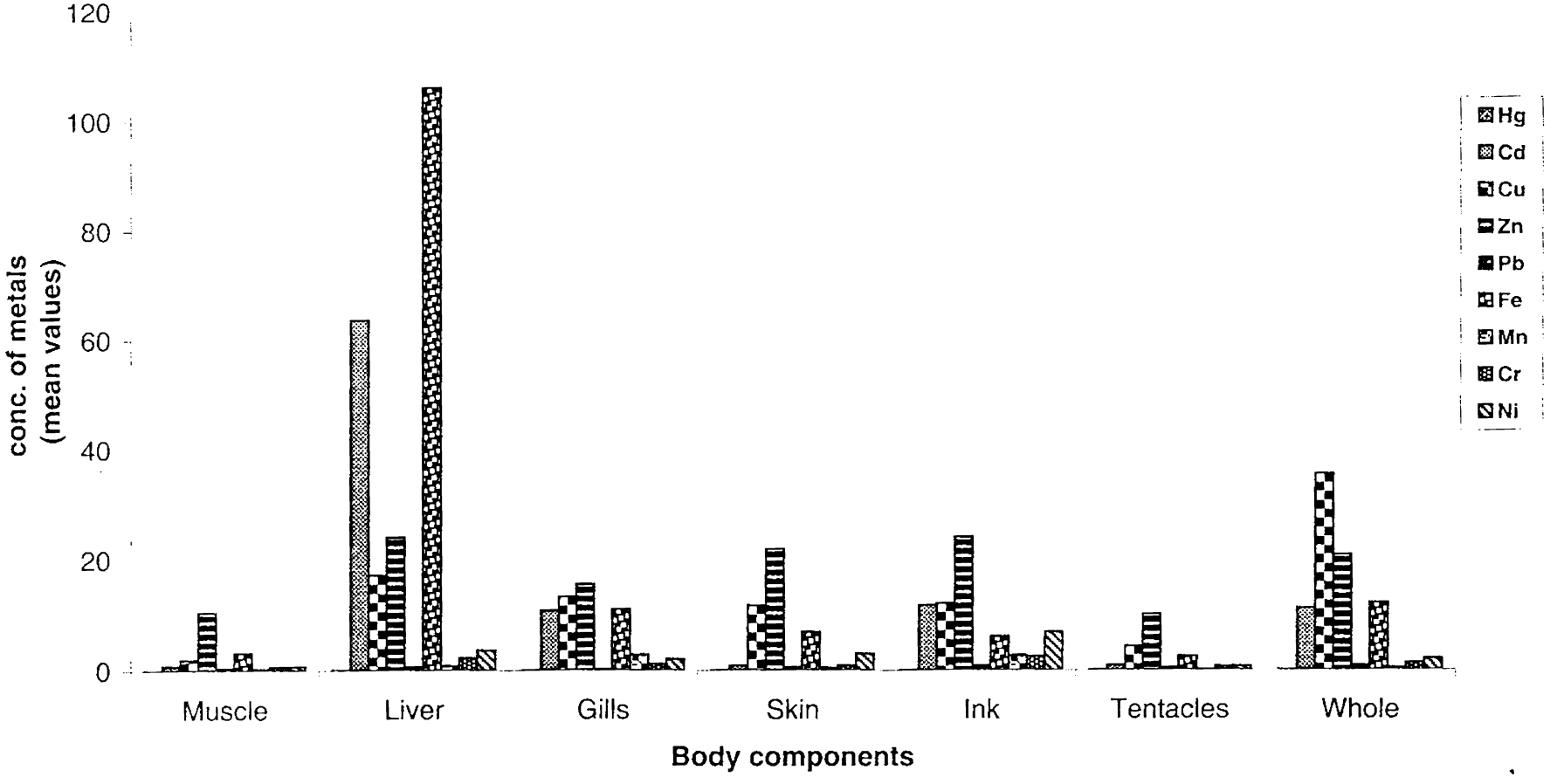


Fig 3.4. Heavy metal concentrations (mean,ppm,wet wt) in the body components and whole soft parts of cuttlefish from Mumbai Region



Chapter 4

SEASONAL VARIATION OF HEAVY METALS IN CUTTLEFISH

4.1. Introduction

Heavy metals are present in all phases of environment air, water and land. When chemicals such as heavy metals are released in to the environment, they are subjected to various physicochemical and biochemical changes as a result of adsorption, absorption, chelation or precipitation as hydroxide or carbonate. Environmental factors such as water salinity, the level of dissolved oxygen (redox condition of the environment), the pH of water, dissolved and particulate organic matter etc are the factors, which determine the level of trace metals in the aquatic environment (Wittmann, 1974; Waiwood and Beamish, 1978; Everall, 1987). The environmental water may be contaminated by effluents coming out from mining and metallurgical industries, anthropogenic sources and above all the seasonal variations in physiochemical parameters as a result of underwater currents, rainwater, and also due to different kinds of transportation in the ocean water. The biological cycle of the animals such as physiological condition, spawning and maturation etc also play a major role in metal levels in aquatic organisms, most of which are dependent on seasonal changes in the environmental factors and consequently the bio – availability of metals. In the light of the above, the study of the seasonal variation of metal assumes importance, so that, the period when there is higher levels of metals in the organisms can be provided.

In recent years, monitoring the concentration of heavy metals in marine and fresh water organisms has received much attention. As a mandatory requirement of many fish producing as well as importing countries, regular monitoring of the seafood products for various kinds of hazards including heavy metals are required. Seasonal variations of heavy metals in molluscs have been reported by Bryan (1973); Fowler and Oregioni (1976), Kumagai and Saeki (1980), Shiber (1980), Gabbot (1983), Lakshmanan and Nambisan (1983), Cain and Luoma (1986, 1990), De-Gregori *et al.*, (1996), Muralidharan and Raja (1997), Rivonker and Parulekar (1998) and Senthilnathan and Balasubramanian (1998). However, not much attention is given to study the distribution of metals in cephalopods during different seasons. Dious and Kasinathan (1992) studied the concentration of Fe, Mn, Zn and Cu in different body tissues of *Sepiella inermis*, during different seasons. In view of this, seasonal variation in metals in cuttlefish were carried out inorder to find out any hazardous level of trace metals in cuttlefish or in its body components during different seasons of the year.

In the present study, the levels of toxic as well as essential metals in the edible muscle as well as in various body components were monitored in samples collected from Cochin, Quilon and Mangalore region during premonsoon (Jan-May), monsoon (June-Aug) and postmonsoon season (Sept-Dec). Samples were collected regularly, at monthly intervals from Cochin and Quilon, and bimonthly intervals from Mangalore region.

4.2. Materials and Methods

Cuttlefish samples were collected from different fish landing centers of the west coast of India and determination of metal levels in the edible muscle and body components were carried out as described in Chapter 2. Samples of the same size grade were taken for analysis so as to eliminate variation in metal levels owing to size difference. Commercial size grade cuttlefish of length 21.2 ± 3.02 cm and weight 146.5 ± 26.5 g were taken for metal analysis. Triplicate determinations were carried out in each case. Statistical analysis were carried out using Two way ANOVA to compare group means and to determine significant difference, if any, in the distribution of heavy metals (eg. Cd, Cu, Zn and Cr) in cuttlefish collected during different seasons along the west coast of India.

4.3. Results

Concentration of nine heavy metals viz, Hg, Cd, Pb, Cu, Zn, Fe, Mn, Cr and Ni in cuttlefish, *Sepia pharaonis* has been determined during the whole cycle of the year, namely premonsoon, monsoon and postmonsoon seasons for the different regions, viz., Cochin, Quilon and Mangalore. The results are presented in Tables 4.1a to 4.3c and Figs. 4.1a to 4.3c. The results indicated that there is significant seasonal variation in the distribution of heavy metals in the species collected from all the three regions. Results of ANOVA (Analysis of Variance) in the edible muscle and liver of cuttlefish are presented in Tables 4.2 to 4.9.

4.3.1. Seasonal variation in Mercury, Cadmium and Lead.

Concentration of ΣHg, Cd and Pb in cuttlefish varied with seasons (Tables 4.1a to 4.3c and Figs. 4.1a to 4.3c). Hg was the least abundant trace metal found in cuttlefish during all the seasons from the regions. The edible muscle had mercury content (ΣHg) varying from 0 to 0.06 ppm during premonsoon season, 0.01 to 0.05 ppm during monsoon period and 0 to 0.04 ppm during postmonsoon period from Cochin region. At Quilon, the corresponding values for total Hg ranged from 0.01 to 0.03 (0.02 ppm); 0.04 to 0.16 (0.10 ppm) and 0.01 to 0.05 (0.04ppm); the mean values are given in parenthesis. Cuttlefish samples from Mangalore region also exhibited appreciable variation with season. In the edible muscle, the values being 0 to 0.05 ppm (0.04 ppm); 0.01 to 0.09 (0.06 ppm) and 0.02 to 0.08 (0.05 ppm) during premonsoon, monsoon and postmonsoon period. Of the three regions, the highest value recorded was 0.16 ppm from Quilon region during monsoon period. All other values are below these range and none of the edible muscle of cuttlefish from these regions exceeded the tolerance limit during any of the season. In general, higher levels were obtained during monsoon period at all stations. The distribution of ΣHg followed the order.

Cochin : monsoon > premonsoon > postmonsoon

Quilon : monsoon > postmonsoon > premonsoon

Mangalore : monsoon > postmonsoon > premonsoon

Among the body components, liver had the highest value for ΣHg at all regions. The mean and range of ΣHg are presented in the tables (4.1a to 4.3c). The highest mean value of Hg in the liver of cuttlefish was 0.17 ppm during monsoon season from Cochin region. The corresponding values for total Hg in the liver of cuttlefish from the other two regions representing three seasons are 0.04 to 0.06; 0.03 to 0.10 and 0.08 to 0.22 ppm during premonsoon, monsoon and postmonsoon seasons from Quilon region and from Mangalore region, total mercury content in the liver of cuttlefish ranged from 0.03 to 0.10; 0.03 to 0.16 and 0.03 to 0.12 ppm during premonsoon, monsoon and postmonsoon season, respectively. Gills formed another major site of Hg accumulation. Gills was found to be the highest during postmonsoon season in cuttlefish from Cochin region (0.13 ppm). The ink like fluid from cuttlefish had appreciable level of ΣHg and the highest value (0.17 ppm) was recorded during premonsoon period and followed the order: premonsoon > monsoon > postmonsoon, from all the regions. The levels for other body components were relatively low and are presented in Tables 4.1a to 4.3c. The results indicated that with respect to total Hg, the level is far below the tolerance limit and hence need not be concerned as a significant hazard, in cephalopod products in the near future.

The presence of Cd in seafoods is of serious concern in recent years, due to its cumulative effect and toxicity, from the viewpoint of public health. The poisoning implicated by Cd containing food is known as 'itai-

itai' disease, which causes renal dysfunction and osteomalacia. The modern consumers demand seafood or any food item free from all kinds of hazards. The Cd content in the muscle and body components of cuttlefish from the three regions covering all the seasons: premonsoon, monsoon and postmonsoon are presented in Tables 4.1a to 4.3c and Figs. 4.1a to 4.3c. The Cd content in the muscle of cuttlefish had an average value < 1.0 ppm in samples covering the entire season. However, very few samples from Mangalore region had Cd content more than 2 ppm. The edible muscle value ranged from 0.04 to 0.67, 0.13 to 0.82 and 0.16 to 0.45 ppm during premonsoon, monsoon and postmonsoon period from Cochin region. In samples from Quilon and Mangalore region, the corresponding values were respectively, 0.32 to 0.50, 0.28 to 0.60, 0.16 to 0.94 ppm and 0 to 2.36, 0.28 to 0.59 and 0.01 to 0.89 ppm during premonsoon, monsoon and postmonsoon period. The distribution pattern is as follows:

Cochin : monsoon > postmonsoon > premonsoon

Quilon : postmonsoon > monsoon > premonsoon

Mangalore : premonsoon > monsoon > postmonsoon

Liver formed the major site of Cd accumulation in samples from all the regions; irrespective of seasons. Cuttlefish liver had Cd content in the range of (7.2 to 76.51) ppm during premonsoon period from Cochin region (Table 4.1a); while the levels in samples from Quilon and Mangalore regions were in the range of 10.96 to 41.8 ppm (Table 4.2a) and 6.29 to

54.05 ppm (Table 4.3a) respectively. During monsoon seasons, liver exhibited Cd in the range of 24 to 138.5 ppm from Cochin region (Table 4.1b) while in other regions, Cd content were in the range of 22.66 to 123 ppm (Quilon) and 39.52 to 140 ppm (Mangalore) (Table 4.2b and 4.3b). Gills component of cuttlefish from Quilon and Mangalore region exhibited the highest Cd concentration during premonsoon period (Fig 4.2a and 4.3a) and the postmonsoon samples from Cochin had the highest value (Fig. 4.1c). Ink had comparatively higher levels of Cd during premonsoon season from Cochin and Quilon region and the mean value was >3 ppm. Further, the skin and tentacles, which are also important sites of Cd accumulation, contributed significantly to total body burden of Cd in cuttlefish.

The results of the study indicated that the level of Pb in the edible muscle of cuttlefish was quite low and ranged from 0 to 1.18 ppm; 0 to 1.45 ppm; 0 to 0.68 ppm during premonsoon, monsoon and postmonsoon season in samples from Cochin region. Around 50% of the edible muscle of cuttlefish samples did not exhibit any Pb content. The highest mean Pb content in the muscle was noted during premonsoon period in Quilon and Mangalore region; the values being 0.86 ppm and 0.76 ppm. The lowest mean Pb content was found in Cochin region during postmonsoon season and followed the order:

Cochin : monsoon > premonsoon > postmonsoon and

Quilon / Mangalore : premonsoon > postmonsoon > monsoon.

Liver was found to be the major storage organ for Pb also. Liver samples of cuttlefish caught during the premonsoon season off Quilon and Mangalore region showed comparatively higher levels of Pb (mean values being 0.89 and 0.91 ppm) than monsoon and postmonsoon periods. At Cochin region, the seasonal distribution pattern of Pb in liver followed the order:

monsoon > premonsoon > postmonsoon

Even though, the liver exhibited higher value for Pb; the lower range was invariably zero. Among the various body components in cuttlefish, ink like fluid showed the highest level of Pb during all the seasons from Cochin region. The Pb content ranged from 0 to 4.65 ppm in all the ink samples from the west coast of India during all the seasons. Gills component of cuttlefish from Cochin and Quilon region exhibited higher mean Pb content during postmonsoon period than monsoon and premonsoon period (Fig. 4.1c and 4.2c). Skin recorded the highest mean value of Pb (1.94 ppm) from Quilon region during premonsoon season; while Pb content in the skin was the lowest (0.48 ppm) in Mangalore samples during the season. Pb accumulation in the tentacles were comparatively higher during monsoon period than premonsoon and postmonsoon period at both Cochin and Quilon regions; the range being 0.33 to 1.53 ppm (Table 4.1 b) and 0.81 to 1.81 ppm (Table 4.2b).

4.3.2. Seasonal variation in Copper, Zinc, Iron and Manganese

Significant seasonal variation in Cu, Zn, Fe and Mn could be observed in cuttlefish samples from the three regions and results are presented in Tables 4.1a to 4.3c and Figs. 4.1a to 4.3c. Comparatively higher levels of copper were observed in the muscle and other body components of cuttlefish. Copper content in the muscle of cuttlefish ranged from 1.1 to 6.02 ppm; 2.0 to 10.53 and 0.44 to 2.02 ppm from Cochin region during premonsoon, monsoon and postmonsoon season, respectively (Table 4.1a to 4.1c). Monsoon period exhibited the highest level of Cu in muscle followed by premonsoon and postmonsoon from Cochin region. A similar trend of distribution of Cu was seen in samples from other regions as well. Seasonality was also observed in Cu content in other body components of cuttlefish. The mean copper concentration in liver is the highest for cuttlefish during monsoon season from Quilon region (146.9 ppm) (Fig. 4.2b). Gills of cuttlefish showed comparatively higher mean values for samples collected from Quilon (32.09 ppm) and Mangalore (38.24 ppm) region during premonsoon season. Higher levels (44.3 ppm) of Cu was observed in gills of samples collected during postmonsoon season from Cochin region (Fig 4.1a to 4.1c). The level of copper in ink was the highest in Cochin region during premonsoon period (Table 4.1 a); while in Quilon region higher values were observed during monsoon period (Table 4.2 b). The levels in other body components were relatively low in all seasons.

Zinc is an essential element for which the individual variations are known to be limited as a consequence of homeostasis process (Caurant, 1999). There was no significant variation in the level of zinc in cuttlefish muscle with season. Zn content varied between 6.0 to 11.6; 8.09 to18.80 ppm and 8.49 to 15.52 ppm during premonsoon, monsoon and postmonsoon period respectively, in samples from Cochin area. In samples from Quilon region, the mean Zn content was 15.42 ± 4.43 ppm during premonsoon followed by 7.82 ± 1.21 ppm during monsoon and 10.24 ± 5.69 ppm during postmonsoon period, the highest mean value of Zn in muscle tissue was found during premonsoon period and the lowest during monsoon period. (Fig 4.2a and 4.2b). Mean Zn content in the muscle of cuttlefish from Mangalore region was in the order: postmonsoon > premonsoon > monsoon. The distribution of Zinc in liver of cuttlefish was the highest during monsoon period at all regions; the mean values being 91.62 ppm (Cochin), 94.41 ppm (Quilon), 77.72 ppm (Mangalore region). Concentration of Zn in gills of cuttlefish also varied with season at different regions and followed the order:

Cochin : postmonsoon > monsoon > premonsoon

Quilon : postmonsoon > premonsoon > monsoon

Mangalore : premonsoon > postmonsoon > monsoon

The mean Zn content in ink was highest for cuttlefish during premonsoon period from Quilon region (52.80 ± 28.09 ppm). The distribution pattern of Zn in different organs of cuttlefish from Cochin region followed the order: liver > skin > tentacles > muscle > gills > ink and in samples from Quilon, the order being liver > ink > skin > tentacles > muscle >gills during premonsoon period (Table 4.1a and 4.2a). Cuttlefish from Mangalore region, the order of distribution of Zn being liver > ink > gills > skin > muscle > tentacles.

Generally, low levels of iron were found in the muscle of cuttlefish during all the three seasons. In the edible muscle, Fe content varied in the order: monsoon > postmonsoon > premonsoon at all the regions (Figs. 4.1a to 4.3c). The liver of cuttlefish seemed to be a rich source of iron content, followed by gills. The mean value of Fe content in the liver showed higher values during premonsoon period at all stations and were in the range of 8.69 to 146.0, 8.32 to 63.12 and 8.87 to 86.0 at Cochin, Quilon and Mangalore region, respectively. Seasonality was also observed in Fe content in other body components. At Cochin region, average Fe content in ink samples were 9.65 ± 10.16, 3.70 ± 0.27 and 3.90 ± 0.27 ppm during premonsoon, monsoon and postmonsoon period respectively. At Quilon region, levels varied in the order postmonsoon > premonsoon > monsoon. The highest mean value of Fe in ink was recorded at Quilon region during postmonsoon period (46.89 ppm). The average value of Fe in tentacles of cuttlefish from Cochin region varied with season and were in the order:

monsoon > postmonsoon > premonsoon. At Quilon region, mean Fe content in tentacles were respectively, 6.45, 6.91 and 13.28 ppm during premonsoon, monsoon and post monsoon seasons.

Mn content varied markedly with season in both edible and non-edible components of cuttlefish. The mean value of Mn in the muscle of cuttlefish was generally high in postmonsoon period at Quilon (1.39 ppm) and Mangalore region (1.07 ppm), while at Cochin region, Mn was high during monsoon period (1.32 ppm). The distribution of Mn in the body components of cuttlefish are presented in Tables (4.1a to 4.3c). Higher level of Mn were found in liver and ink of cuttlefish, followed by gills or skin during the three seasons from the three regions. Mn accumulation in the liver was comparatively higher during postmonsoon period than during other seasons at Quilon and Mangalore region. While, at Cochin region, Mn content in liver varied from 0 to 1.95, 1.24 to 10.13 and 0.19 to 0.65 ppm, during premonsoon, monsoon and post monsoon periods. Samples from Quilon and Mangalore recorded the highest mean value of Mn in gills during postmonsoon season. At Cochin region, Mn content in gills were in the order: monsoon > postmonsoon > premonsoon. Level of Mn in the skin of cuttlefish was quite low and ranged from 0 to 2.89, 0 to 1.25 and 0.21 to 1.08 ppm during premonsoon, monsoon and postmonsoon seasons from Cochin region. At Quilon region Mn content varied in the order: postmonsoon > premonsoon > monsoon. The highest value of Mn in skin were observed during postmonsoon season from Mangalore region (10.47

ppm). Concentration of Mn in ink were in the range of 0 to 1.87, 3.80 to 5.25 and 3.23 to 5.25 ppm during premonsoon, monsoon and postmonsoon in samples collected from Cochin region. Mn level in the ink were comparatively higher during premonsoon period than during other seasons at Quilon and Mangalore region. In tentacles, Mn content varied in the order monsoon > postmonsoon > premonsoon at Cochin and postmonsoon > premonsoon > monsoon at Quilon region.

4.3.3. Seasonal variation in Chromium and Nickel

Mean concentration, range and standard deviations of Cr and Ni from the three regions covering the entire seasons are presented in Tables 4.1a to 4.3c and illustrated in Figs. 4.1a to 4.3c.

Chromium is one of the least toxic of the trace elements and the mammalian body can tolerate 100 – 200 times its total body content of Cr without harmful effects. In cuttlefish muscle from Cochin region, mean Cr content was 0.60 ± 0.57 ppm during premonsoon followed by 0.30 ± 0.24 ppm during monsoon and 0.65 ± 0.07 ppm during postmonsoon period. Here, mean Cr content was more or less similar during premonsoon and postmonsoon periods. Cuttlefish samples from Quilon and Mangalore region had the highest level of muscle Cr during monsoon, the mean values being 0.71 and 0.36 ppm, respectively. Cr content in the liver showed a marked seasonal variation, the highest mean values were found during premonsoon period in Cochin and Mangalore regions. While, at Quilon

region, Cr content in liver varied from 0.52 to 0.70, 0.25 to 0.72 and 0 to 3.77 ppm during premonsoon, monsoon and post monsoon periods. At Cochin and Quilon regions, mean Cr content in gills was comparatively high during postmonsoon period (4.56 and 1.99 ppm respectively) than during monsoon and premonsoon period. At Mangalore region, Cr content in gills varied in the order: premonsoon > postmonsoon > monsoon. Among the various body components examined in cuttlefish, ink like fluid showed the highest level of Cr during all the seasons at all stations (Fig. 4.1a to 4.3c). The highest value observed was 17.95 ppm from Quilon region during premonsoon season. Mean concentrations of Cr in skin samples from Cochin region were 1.34 ± 0.50, 0.25 ± 0.17 and 0.47 ± 0.32 ppm during premonsoon, monsoon and postmonsoon periods, respectively. The distribution pattern of Cr in skin from Quilon region were postmonsoon > premonsoon > monsoon. In skin, the least Cr content was found in monsoon period in cuttlefish from Cochin region (0.25 ± 0.17 ppm) and at Quilon region (0.57 ± 0.12 ppm) than in other seasons. In the tentacles, mean Cr content varied with seasons and were in the order: monsoon > postmonsoon > premonsoon at Cochin region. At Quilon region, concentration of Cr varied from 0.66 to 1.26, 0.50 to 0.74 and 0.32 to 3.03 ppm during premonsoon, monsoon and postmonsoon period.

Nickel is included in the category of highly toxic and non-essential element to biological system. In the edible muscle, Ni content varied from 0 to 1.42, 0 to 1.54 and 0.67 to 2.20 ppm during premonsoon, monsoon and

postmonsoon seasons, respectively at Cochin regions. At Quilon and Mangalore regions, Ni levels in muscle followed the increasing order: monsoon > postmonsoon > premonsoon. Liver samples exhibited higher level of Ni during monsoon seasons from Quilon and Mangalore regions (Table 4.2b and 4.3b) while in Cochin region, the distribution pattern being postmonsoon > monsoon > premonsoon. In gills, concentration of Ni was the highest during postmonsoon period from Cochin region (1.90 ± 0.04 ppm) and at Quilon region (1.63 ± 1.50 ppm) than in other seasons; the order of distribution being postmonsoon > monsoon > premonsoon. Postmonsoon season recorded higher mean value of Ni in skin, ink and tentacles of cuttlefish from Cochin region. At Quilon region, Ni content in ink and tentacles exhibited the highest value during monsoon months and the order being: monsoon > postmonsoon > premonsoon.

Two way Analysis of Variance (ANOVA) was employed to compare the group mean between the three different seasons viz., premonsoon, monsoon and postmonsoon seasons of Cd, Cu, Zn and Cr in the edible muscle and liver of cuttlefish. Statistical analysis showed that Cd content in muscle is significantly higher ($p<0.01$) during monsoon season than the other two seasons (Table 4.2). Cu content in the muscle of cuttlefish during monsoon season was significantly higher ($p<0.01$) than premonsoon and postmonsoon season (Table 4.4). Statistically, no significant difference was noted in Zn content in the muscle of cuttlefish during different seasons

(Table 4.6). There is no significant variation in Cr content in the muscle of cuttlefish during different seasons (Table 4.8)

Monsoon samples showed significantly higher Cd ($p<0.01$) and Cu ($p<0.05$) content in liver of cuttlefish when compared to other seasons (Table 4.3 & 4.5). There is no significant difference in Zn content in the liver of cuttlefish during different seasons (Table 4.7). Chromium content in the liver of cuttlefish during premonsoon seasons were significantly higher ($p<0.05$) than monsoon and postmonsoon period (Table 4.9).

4.4. Discussion

Concentration of heavy metals, viz; Hg, Cd, Cu, Zn, Fe, Mn, Cr and Ni showed marked difference in metal levels in cuttlefish muscle with seasons collected form Cochin, Quilon and Mangalore region. Levels of various heavy metals observed in cuttlefish in the present study are comparable to the levels reported by other researchers from different parts of the globe (Brooks and Rumsby, 1965; Falandysz, 1988 & 1990; Lakshmanan 1988 a, b; 1989; Dious and Kasinathan, 1992 and Lakshmanan and Stephen, 1993).

Mercury content in cuttlefish were quite low during all seasons, the values are far below the limit of 1mg kg^{-1} permitted for seafood by many fish importing nations and USFDA. A similar mean value of Hg were observed during August (Falandysz, 1990) in the edible and non-edible tissues of *Illex argentinus* caught in autumn at the continental shelf of Argentina

(Falandysz, 1988). The variation in ΣHg levels were within the ranges found by other researchers in Indian waters (Patel and Chandy, 1988; Jasmine *et al.*, 1989; Lakshmanan and Stephen, 1993). Mercury is known to be accumulative in higher tropic level organism of the food chain (Honda *et al.*, 1987). Since main food items of cephalopods are lower tropic organisms such as fishes and crustaceans, accumulation of ΣHg is low during all seasons in cuttlefish. In the present study, cuttlefish exhibited weak affinity for mercury accumulation and did not seem to cause any health hazard.

Average levels of Cd in the muscle of cuttlefish from the three seasons were well below the tolerance limit. Liver formed the major site of Cd accumulation from all the regions during different seasons. In the present study, monsoon season showed significantly higher ($p<0.01$) cadmium content in muscle and liver of cuttlefish. A similar higher concentration of Cd was noted in gills, viscera and mantle of *Mytilus galloprovincialis* during monsoon season by Serra *et al.*, 1999. Higher level of Cd concentration was observed during monsoon season in oyster, *Crassostrea madrasensis* (Senthilnathan and Balasubramanian, 1998). The monsoon floods causes large changes in salinity and pH (Bryan *et al*, 1978) and this might have definite effect on the distribution, species variation etc of heavy metals present in the water and sediment. The low pH values (a consequence of low salinity) may facilitate the dissolution of precipitated form of the metal (hydroxide, carbonate or chloro complexes)

and increase the amount of ionic species in solution (Sundararaj *et al*, 1972; Zirino, 1972; Van der weijden *et al*, 1977; Subramanian *et al.*, 1979). This coupled with the increased rate of filtration would naturally increase the level of metals in these organisms (James, 1966). High variability in feeding habits of cephalopods were noted by Bustamante *et al.* (1998). Blanc *et al.* (1998) reported that feeding intensity in cuttlefish was significantly higher during June-August. Results of the present study showed high concentration of Cd during monsoon season, suggesting a crucial role for feeding habits of cuttlefish in seasonal metal accumulation.

Mean lead content in the edible muscle of cuttlefish was all below 1.00 ppm in samples from the three regions and the values ranged from 0 to 1.45 ppm (Cochin region), 0 to 1.38 ppm (Quilon region) and 0 to 1.16 ppm (Mangalore region), taking into account all the three seasons together. The values of Pb content in the mantle of cuttlefish were of the same magnitude as observed in *Illex argentinus* during autumn as reported by Falandysz, 1988. Among the various body components examined, ink like fluid in cuttlefish exhibited the highest level of Pb in samples from all the three regions and during all the seasons. The next major site of accumulation of Pb was either skin or liver. Gills also contributed significantly to Pb content in cuttlefish. The lower range of lead, in general was zero, indicating a cleaner environment. A greater concentration of Pb in ink as observed in the present study was reported in cuttlefish, *Sepia pharaonis* by Lakshmanan and Stephen (1993) and also in *Illex argentinus*

by Falandysz, 1988. Oysters and clams of Taiwan also showed higher Pb content from May to September (Hsu and Wang, 1979) and it was speculated that seasonal Pb changes arise due to food and more land drainage in summer along the west coast.

Lower levels of Cu were found in the edible muscle of cuttlefish. Copper content in the muscle of cuttlefish *Sepia pharaonis* during postmonsoon season are comparable to Cu level in autumn concentration found in *Illex argentinus* (Falandysz, 1988). In the present study, concentration of Cu was the highest in liver, followed by gills, ink, skin and tentacles. Martin and Flegal (1975) also had reported very high levels of Cu in the visceral mass of squid. Statistical analysis of the results (ANOVA) showed that the Cu content in muscle ($p<0.01$) and liver ($p<0.05$) is significantly higher during monsoon season. Dious and Kasinathan (1992) also observed high concentration during monsoon period in the different tissues of *Sepiella inermis*, and low during summer months. It is confirmed that the low saline media has higher capacity to maintain metals either in solution or in suspension (Fowler, 1976; Phillips 1976; Phillips, 1977) facilitating more bioavailability. Another reason for increasing concentration during monsoon period may be attributed to the industrial effluents coming out from various chemical industries from the region.

Zn content in cuttlefish also varied with seasons, however; the amplitude of variation in the muscle was small. A common pattern of distribution of Zn with seasons was seen at all the three stations. The

distribution pattern of Zn in cuttlefish muscle with seasons followed the order

Cochin: monsoon > postmonsoon > premonsoon

Quilon: premonsoon > postmonsoon > monsoon

Mangalore: postmonsoon > premonsoon > monsoon.

Zn content in the edible muscle of cuttlefish are quite comparable to the values observed in *L. patagonica* as reported by Falandysz, 1989. Phillips (1976) observed Zn concentration in bivalve *Mytilus,* four times higher in winter than in summer, an effect that was especially pronounced in upper water layer.

The Fe content was high during monsoon period and low during premonsoon period in the muscle of cuttlefish, and are quite comparable to that of *Sepiella inermis* as reported by Dious and Kasinathan (1992). This higher level of Fe in cuttlefish muscle may again attributed to the influx of monsoon waters, which dissolve most of the insoluble iron complexes, owing to low pH conditions (Fowler, 1976; Phillips, 1976). A definite seasonal trend was noted in the liver of cuttlefish and are quite comparable to the elevated Fe concentrations noted in digestive gland of scallop *Chlamys* sp. (January-February) and *Pecten* sp. (February) as reported by Bryan (1976). Mn content varied markedly with seasons in different body components of cuttlefish. The gills and digestive gland varied appreciably in Mn concentrations at different seasons in scallops (Bryan, 1976). The

Mn in the muscle of *Sepiella inermis* showed higher level during monsoon period (Dious and Kasinathan, 1992). A similar trend of Mn distribution was observed in cuttlefish at Cochin region during monsoon period. Monsoon floods cause changes in salinity and pH and might have brought metals into solutions and increased the bioavailability of metals (Dious and Kasinathan, 1992)

Seasonal variation of Cr in cuttlefish are comparable to Cr content in mussels of Richard Bay Harbour (Vermeulen and Wepener, 1999). Among the various body components, ink showed the highest Cr content during all the seasons and are comparable to Cr content reported by Falandysz (1988) in squid, *Loligo patagonica.* Distribution pattern of Ni varied with seasons and in muscle, ink and tentacles higher level were noted during monsoon season in all the regions. A quite comparable maximum in monsoon was found in molluscs from Beirut (Shiber, 1980).

There are several factors responsible for the variation of heavy metals in molluscs. Seasonality changes, environmental conditions like salinity, pH, temperature, concentration of metals in their locality and the feeding habits or food available (Phillips, 1980; Presley, 1997). Another reason may be due to anthropogenic local inputs or from local sources and river runoff areas. Seasonal changes of heavy metals in the bivalves *P. maximus* and *Chlamys opercularis* was studied by Bryan (1973) and observed that metal concentration were inversely related to phytoplankton productivity and the food chain play a major role in the bioaccumulation of

trace metals. Olivier *et al.*, 2002 also reported that the phytoplankton productivity may be more important in bioaccumulation of metals. The highest concentrations of trace metals in *Mytilus galloprovincialis* during spring were observed by Fowler and Oregioni (1976). They attributed this to the reproductive state of the organisms and to the high winter run-off. Other studies have suggested that temperature and salinity as causative agents for seasonality in metal concentrations. Seasonal variation in metal concentration in the oyster *Crassostrea gigas* was found to be mainly due to changes in the weight of the whole soft parts, which in turn were due to gonadial development and spawning (Boyden and Phillips, 1981). Lakshmanan and Nambisan (1983) studied the seasonality of trace metals Fe, Cu, Zn and Pb in bivalve molluscs, *Villorita cyprinoides*, *Meretrix casta* and *Perna virdis*. They found the highest concentration of these metals during monsoon period and metal concentration decreased in these species during summer months. Dious and Kasinathan (1992) studied the concentration of Fe, Mn, Zn and Cu in different body tissues of *Sepiella inermis* during different seasons, and found the highest concentration in monsoon period.

Feeding habits of cuttlefish play an important role in the accumulation of metals (Bryan *et al*, 1983). Cuttlefish have been identified a voracious fish eaters (Bidder, 1966; Castro and Guerra, 1990). Variability in metal concentrations in cuttlefish could be as a function of their sexual maturity (Oommen, 1977), their geographical origin, ecological behaviour

(Bustamante *et al.*, 1998), reproductive cycle (Gabbot, 1983) causing changes in the affinity of these compounds to metals (Oesterberg, 1974) and perhaps significantly their diverse feeding habits. Blanc *et al.* (1998) reported that feeding intensity in cuttlefish was significantly higher during June-August. In addition cannibalistic behaviour has been noted in several species of cuttlefish (Oommen, 1977; Okutani, 1990) and were found to increase with age of cuttlefish indicating that the dietary habits of cuttlefish play a major role in metal accumulation.

The present study shows that concentrations of metals in cuttlefish during different seasons are relatively lower in the edible parts than the other non-edible parts. In the edible part all the metals accumulated relatively lower concentration than in other parts, because it receives only traces of metals through their food. The data generated in the present study may form baseline values for trace metals in cuttlefish during different seasons for the three regions.

Table 4.1a: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components of cuttlefish during premonsoon Season (Cochin Region)

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	26	0.04 ±0.02 (0-0.06)	0.22±0.19 (0.04-0.67)	0.54±0.34 (0-1.18)	2.46±2.09 (1.1-6.02)	8.09±2.06 (6.0-11.6)	2.22±1.90 (0.2-2.98)	0.73±0.25 (0-1.56)	0.60±0.57 (0-1.5)	0.48±0.40 (0-1.42)
Liver	20	0.10±0.05 (0.05-0.19)	30.89±22.69 (7.2-76.51)	0.56±0.38 (0-1.19)	111.0±93.6 (12.2-221)	33.55±30.65 (4.0-101)	74.53±55.93 (8.69-146)	1.07±0.86 (0-1.95)	1.71±1.49 (0-3.14)	0.64±0.62 (0-1.35)
Gills	24	0.05±0.03 (0.02-0.08)	0.93±0.85 (0-1.02)	1.13±0.90 (0-2.8)	29.0±5.40 (23.6-36.9)	7.87±4.27 (1.8-13.3)	33.40±11.24 (4.5-50.1)	0.72±0.59 (0-1.5)	2.00±1.05 (0-3.15)	0.41±0.41 (0-1.13)
Skin	21	0.06±0.01 (0.04-0.07)	1.64±0.99 (0-2.97)	0.55±0.28 (0-1.03)	26.51±17.6 (4.51-59.9)	17.44±10.74 (5.82-24.18)	10.32±10.68 (6.02-35.77)	1.73±0.87 (0-2.89)	1.34±0.50 (0-1.98)	0.66±1.27 (0-3.06)
Ink	20	0.09±0.08 (0.05-0.17)	4.18±1.81 (0-6.48)	2.92±1.84 (0-4.65)	35.12±32.0 (3.72-70)	3.42±2.79 (4.20-6.97)	9.65±10.16 (6.70-29.58)	0.84±0.49 (0-1.87)	7.57±2.02 (0-10.34)	0.18±0.29 (0-0.73)
Tentacles	19	0.10±0.01 (0-0.11)	0.24±0.17 (0-0.38)	0.71±0.68 (0-1.27)	5.75±3.27 (1.45-12.0)	9.33±5.66 (2.16-14.47)	5.74±3.84 (0-8.76)	0.19±0.10 (0-0.48)	0.52±0.36 (0-0.98)	0.52±0.78 (0-1.68)

*number of samples analysed

Table 4.1b: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components of cuttlefish during monsoon Season (Cochin Region)

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	25	0.05±0.02 (0.01-0.05)	0.37±0.19 (0.13-0.82)	0.85±0.67 (0-1.45)	4.95±5.43 (2-10.53)	11.58±3.19 (8.09-18.80)	5.06±4.21 (0.37-9.21)	1.32±0.89 (0-2.43)	0.30±0.24 (0-0.53)	0.58±0.46 (0-1.54)
Liver	28	0.17±0.10 (0.03-0.30)	92.09±70.72 (24-138.5)	0.88±0.45 (0-1.39)	84.66±62.7 (0.49-174)	91.62±55.75 (20.8-148)	44.94±22.47 (2.47-82.91)	4.74±3.11 (1.24-10.13)	0.68±0.57 (0-1.93)	2.39±3.14 (0-5.64)
Gills	22	0.05±0.01 (0.02-0.07)	1.41±1.23 (0.37-1.68)	1.05±0.87 (0-2.05)	24.38±8.10 (18.39-34)	10.10±1.58 (7.78-13.52)	11.64±4.69 (4.59-16.69)	4.31±2.95 (0.84-9.08)	0.49±0.47 (0.08-0.48)	1.40±1.34 (0-2.34)
Skin	26	0.04±0.03 (0.01-0.09)	4.48±1.28 (3.37-5.65)	1.28±1.03 (0.22-3.23)	11.63±2.47 (8.69-13.5)	8.79±1.48 (7.04-10.08)	11.02±1.62 (7.05-15.20)	0.92±0.50 (0-1.25)	0.25±0.17 (0-0.42)	0.14±0.13 (0-0.24)
Ink	20	0.04±0.03 (0.02-0.09)	1.34±1.30 (0.21-2.68)	2.96±0.17 (0-3.56)	9.33±7.35 (2.94-15.7)	10.41±8.82 (1.02-20.14)	3.70±0.27 (3.39-3.89)	5.18±0.08 (3.80-5.25)	3.25±0.85 (1.10-5.08)	0.46±0.07 (4.0-5.14)
Tentacles	24	0.05±0.20 (0.08-0.25)	1.21±1.09 (0.17-3.05)	0.92±0.55 (0.33-1.53)	4.96±1.89 (2.02-6.55)	9.21±3.19 (4.27-13.26)	8.80±7.36 (2.43-15.19)	2.87±1.30 (1.14-4.85)	3.44±3.35 (0.26-7.32)	0.63±0.70 (0-1.86)

*number of samples analysed

Table 4.1c: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components of cuttlefish during postmonsoon Season (Cochin Region)

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	30	0.03±0.02 (0-0.04)	0.31±0.13 (0.16-0.45)	0.30±0.25 (0-0.68)	1.37±0.70 (0.44-2.02)	11.19±2.38 (8.49-15.52)	5.04±3.92 (1.39-8.80)	0.32±0.19 (0.15-0.67)	0.65±0.07 (0.59-6.73)	1.19±0.87 (0.67-2.20)
Liver	26	0.10±0.04 (0.02-0.15)	55.2±22.6 (19.31-78)	0.53±0.08 (0.15-0.59)	77.2±52.17 (12.53-130)	48.21±24.80 (26.60-79)	25.16±1.03 (15.08-25.89)	0.62±0.04 (0.19-0.65)	0.99±0.16 (0.20-1.11)	7.23±5.95 (0.66-11.72)
Gills	28	0.10±0.03 (0.03-0.13)	9.96±4.91 (2.67-15.4)	1.24±0.29 (1.03-3.49)	31.62±12.56 (12.64-44.3)	16.16±1.21 (15.20-17.7)	5.04±0.12 (4.2-5.02)	4.05±2.31 (0.73-7.85)	4.56±3.05 (0-6.36)	1.90±0.04 (0.20-2.93)
Skin	25	0.01±0.12 (0-0.13)	2.64±1.69 (0.77-4.91)	1.69±0.40 (0.56-1.70)	13.11±7.89 (6.96-30.29)	12.80±7.75 (4.87-26.53)	11.44±0.23 (9.52-11.69)	0.60±0.37 (0.21-1.08)	0.47±0.32 (0.15-0.59)	0.93±0.40 (0.54-1.42)
Ink	23	0.03±0.01 (0.01-0.05)	1.88±0.48 (1.25-2.41)	2.85±0.99 (1.02-3.56)	19.39±6.37 (9.03-24.09)	4.8±0.01 (1.24-4.81)	3.90±0.27 (2.20-3.83)	5.18±0.08 (3.23-5.25)	5.02±0.32 (3.12-6.05)	4.63±0.71 (2.25-5.13)
Tentacles	26	0.03±0.13 (0.03-0.15)	0.87±0.46 (0.33-0.89)	0.45±0.67 (0-1.41)	3.91±2.26 (0.94-5.63)	9.21±0.34 (5.02-9.29)	5.97±3.15 (3.56-9.45)	0.24±0.11 (0.13-0.36)	0.56±0.33 (0-1.82)	1.15±1.41 (0.22-3.20)

*number of samples analysed

Table 4.2a: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components of cuttlefish during premonsoon Season (Quilon Region)

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	31	0.02±0.01 (0.01-0.03)	0.40±0.14 (0.32-0.50)	0.86±0.58 (0-1.38)	2.01±1.48 (0.98-4.28)	15.42±4.43 (10.57-19.7)	0.66±0.58 (0.18-1.15)	0.64±0.08 (0.58-0.74)	0.59±0.41 (0.32-1.13)	0.32±0.13 (0.26-0.47)
Liver	25	0.05±0.01 (0.04-0.06)	21.91±9.83 (10.96-41.8)	0.89±1.29 (0-2.39)	98.01±47.9 (29.73-142)	84.36±7.06 (61.0-92.4)	59.00±14.16 (8.32-63.12)	4.24±0.14 (3.06-4.42)	0.55±0.10 (0.52-0.70)	0.90±0.05 (0.32-0.91)
Gills	21	0.05±0.02 (0.04-0.07)	0.84±0.81 (0.33-1.77)	0.74±0.90 (0-1.49)	32.09±15.12 (21.93-57.9)	15.19±9.25 (6.63-28.72)	1.89±0.03 (0.98-1.92)	0.77±0.10 (0.70-0.89)	1.04±0.67 (0.32-1.84)	0.56±0.68 (0-2.08)
Skin	26	0.02±0.01 (0.02-0.04)	0.57±0.25 (0.24-0.93)	1.94±1.70 (0-3.20)	29.91±11.50 (16.93-38.8)	25.59±10.14 (17.49-43.1)	1.55±0.01 (0.85-1.56)	0.98±0.20 (0.68-1.02)	1.56±0.64 (0.17-2.30)	0.21±0.02 (0.19-0.23)
Ink	22	0.08±0.05 (0.03-0.13)	4.02±2.05 (0.53-8.20)	1.20±0.20 (0-2.48)	19.81±11.22 (6.86-26.61)	52.80±28.09 (20.36-69.4)	11.52±3.21 (2.89-15.20)	22.71±2.12 (18.77-22.71)	9.48±7.56 (3.39-17.95)	0.93±0.36 (0.67-1.19)
Tentacles	20	0.46±0.01 (0.05-0.20)	0.88±0.25 (0.58-3.56)	0.20±0.18 (0-0.50)	15.13±0.28 (6.86-17.12)	16.24±2.12 (9.63-18.20)	6.45±1.12 (0.84-7.12)	0.40±0.32 (0.28-0.81)	1.40±0.96 (0.66-1.26)	0.26±0.10 (0.12-0.38)

*number of samples analysed

Table 4.2b: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components of cuttlefish during monsoon Season (Quilon Region)

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	15	0.10±0.04 (0.04-0.16)	0.43±0.05 (0.28-0.60)	0.67±0.13 (0-1.33)	3.95±0.39 (1.85-6.52)	7.82±1.21 (4.85-8.99)	1.58±0.16 (1.47-1.77)	0.42±0.21 (0-0.72)	0.71±0.11 (0.58-0.81)	1.38±0.11 (1.26-1.42)
Liver	12	0.08±0.02 (0.03-0.10)	119.58±6.21 (22.66-123)	0.41±0.32 (0-1.02)	146.9±109.7 (20.25-210)	94.41±12.11 (26.68-95.0)	30.24±10.02 (2.15-42.50)	1.58±0.23 (0.87-1.85)	0.45±0.12 (0.25-0.72)	1.81±0.14 (0.52-4.01)
Gills	11	0.03±0.02 (0.02-0.05)	0.77±0.08 (0.48-0.91)	0.46±0.30 (0.30-0.82)	22.27±0.07 (18.69-22.3)	6.67±0.29 (4.52-8.20)	6.77±0.57 (3.57-7.72)	0.46±0.06 (0.28-0.59)	1.12±0.15 (0.85-1.25)	0.79±0.09 (0.57-0.80)
Skin	13	0.04±0.03 (0.02-0.07)	0.06±0.01 (0.05-0.07)	0.94±1.64 (0-2.84)	27.49±0.41 (20.05-27.4)	5.97±0.01 (4.05-5.98)	3.67±0.01 (2.85-3.68)	n.d	0.57±0.12 (0.21-0.66)	1.41±0.35 (0.69-1.66)
Ink	11	0.06±0.01 (0.02-0.07)	0.19±0.02 (0.13-0.38)	1.02±0.88 (0.45-1.56)	45.90±10.41 (5.01-60.0)	2.07±0.14 (1.32-2.17)	9.51±0.40 (3.89-9.80)	7.89±0.06 (1.34-10.28)	2.7±0.49 (0.95-3.20)	5.19±0.03 (2.51-5.22)
Tentacles	12	0.04±0.01 (0.01-0.06)	0.33±0.03 (0.15-0.39)	1.01±0.44 (0.81-1.81)	6.29±0.08 (5.41-6.34)	8.48±1.35 (6.85-9.89)	6.91±0.26 (5.89-8.05)	n.d	0.72±0.02 (0.50-0.74)	1.82±0.37 (0.74-1.96)

*number of samples analysed
n.d = not detected

Table 4.2c: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components of cuttlefish during postmonsoon Season (Quilon Region)

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	18	0.04±0.01 (0.01-0.05)	0.55±0.49 (0.16-0.94)	0.76±0.57 (0-1.28)	1.49±0.52 (0.99-2.29)	10.24±5.69 (6.57-24.08)	1.08±0.52 (0.90-2.10)	1.39±1.30 (0.11-3.52)	0.26±0.32 (0-0.84)	1.11±0.95 (0-2.91)
Liver	15	0.13±0.07 (0.08-0.22)	94.54±72.42 (0.15-213.5)	0.61±0.67 (0-1.23)	88.80±50.03 (21.28-174.)	57.34±10.34 (4.05-70.5)	42.24±28.05 (2.14-56.29)	5.50±3.26 (2.23-10.13)	1.49±1.36 (0-3.77)	1.50±1.18 (0-3.06)
Gills	12	0.05±0.02 (0.04-0.06)	0.76±0.65 (0-1.82)	0.92±1.70 (0-4.90)	25.9±8.30 (21.32-37.5)	16.84±3.48 (9.23-32.10)	31.73±18.1 (2.27-56.7)	2.17±1.44 (1.02-4.88)	1.99±1.81 (0-4.05)	1.63±1.50 (0-5.33)
Skin	13	0.05±0.01 (0.03-0.05)	0.59±0.36 (0-1.13)	1.11±1.05 (0-2.12)	14.75±5.64 (5.36-26.59)	8.95±5.22 (7.00-116.5)	5.14±2.40 (2.73-11.09)	1.91±1.36 (0-4.96)	4.64±3.48 (0-8.07)	1.74±1.06 (0-3.76)
Ink	11	0.04±0.06 (0.03-0.09)	3.00±3.31 (0.49-1.27)	2.39±0.17 (0-3.62)	33.50±29.75 (11.58-73.5)	25.51±18.57 (1.88-52.5)	46.89±49.48 (4.00-90)	3.81±0.05 (1.78-3.84)	6.89±0.50 (3.85-7.25)	1.45±1.68 (0-2.77)
Tentacles	11	0.05±0.02 (0.03-0.07)	0.39±0.47 (0-1.29)	0.63±0.66 (0-1.53)	7.63±4.41 (2.04-12.01)	8.77±4.05 (0.47-13.27)	13.28±16.45 (1.03-40.16)	2.71±1.65 (0-3.19)	1.34±1.05 (0.32-3.03)	1.46±0.90 (0-2.06)

*number of samples analysed

Table 4.3a: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components of cuttlefish during premonsoon Season (Mangalore Region)

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	17	0.04±0.01 (0-0.05)	1.75±1.16 (0-2.36)	0.76±0.66 (0-1.16)	1.37±0.63 (0.43-1.79)	8.41±0.49 (7.75-8.84)	1.77±0.92 (0.59-2.68)	n.d	0.11±0.01 (0-0.11)	n.d
Liver	12	0.09±0.02 (0.03-0.10)	29.75±17.20 (6.29-54.05)	0.91±0.58 (0-2.73)	99.04±65.76 (19.97-201)	55.36±16.66 (35.63-76.0)	74.32±11.44 (8.87-86.0)	n.d	6.77±0.18 (5.25-6.89)	n.d
Gills	13	0.06±0.02 (0.02-0.09)	4.57±4.43 (0.44-10)	0.85±0.57 (0.59-1.76)	38.24±23.71 (11.98-63.4)	17.45±7.43 (7.94-24.16)	12.15±8.29 (4.87-19.51)	0.20±0.10 (0.15-0.21)	1.02±0.16 (0.80-1.14)	0.23±0.02 (0.18-0.25)
Skin	12	0.06±0.02 (0.05-0.09)	0.67±0.37 (0.25-0.99)	0.48±0.22 (0.31-0.55)	18.38±9.14 (8.24-25.4)	11.02±7.34 (0-14.87)	9.51±5.24 (4.88-14.08)	0.20±0.12 (0.15-0.21)	1.02±0.16 (0.91-1.14)	0.22±0.03 (0.17-0.25)
Ink	11	0.07±0.02 (0.05-0.09)	0.80±0.17 (0.62-1.02)	1.09±0.95 (0-1.65)	9.08±3.94 (5.55-12.68)	32.34±28.01 (0-48.79)	6.72±1.93 (5.18-8.98)	n.d	8.56±1.64 (6.81-9.72)	1.57±0.52 (1.20-1.94)
Tentacles	11	0.05±0.02 (0.02-0.08)	0.96±1.02 (0.07-1.89)	0.90±0.79 (0-1.49)	10.64±8.29 (1.56-16.89)	6.73±1.26 (4.79-7.55)	5.88±2.28 (3.17-7.61)	0.07±0.02 (0.03-0.09)	0.73±0.04 (0.65-0.77)	n.d

*number of samples analysed
n.d = not detected

Table 4.3b: Distribution pattern of trace metals (Mean ± S.D and Range, ppm, wet weight) in the body components of cuttlefish during monsoon Season (Mangalore Region)

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	13	0.06±0.02 (0.01-0.09)	0.44±0.15 (0.28-0.59)	n.d	4.56±2.49 (0.63-6.64)	6.57±2.39 (4.48-8.90)	2.90±1.28 (0-4.81)	0.82±0.31 (0-1.22)	0.36±0.19 (0.23-0.49)	0.24±0.34 (0-0.48)
Liver	12	0.11±0.03 (0.03-0.16)	86.20±53.48 (39.52-140)	0.49±0.69 (0-1.82)	87.75±60.73 (32.26-144)	77.72±4.48 (13.33-61.8)	35.94±8.90 (2.10-45.31)	0.70±0.57 (0.30-1.54)	0.82±0.62 (0.10-3.24)	5.80±4.95 (0.15-9.40)
Gills	10	0.03±0.01 (0.03-0.08)	3.40±2.48 (1.18-5.64)	0.57±0.81 (0-1.15)	28.77±16.92 (12.94-46.1)	5.79±5.03 (0-9.09)	31.73±30.81 (9.95-53.52)	0.54±0.77 (0-1.09)	0.38±0.18 (0.23-0.58)	2.30±3.26 (0-4.61)

*number of samples analysed
n.d = not detected

Table 4.3c: Distribution pattern of trace metals (Mean ± S.D and Range; ppm, wet weight) in the body components of cuttlefish during postmonsoon Season (Mangalore Region)

BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
Muscle	18	0.05±0.04 (0.02-0.08)	0.31±0.39 (0.01-0.89)	0.67±0.21 (0.37-0.85)	1.06±0.09 (0.96-1.15)	8.50±1.78 (8.17-10.4)	2.76±0.52 (2.33-3.58)	1.07±0.81 (0.10-2.29)	0.23±0.13 (0.08-0.41)	0.18±0.17 (0.03-0.45)
Liver	11	0.10±0.06 (0.03-0.12)	56.68±54.59 (9.39-104.1)	0.80±0.71 (0-1.06)	1.46±0.03 (1.46-8.0)	28.08±24.15 (3.08-66)	40.80±2.40 (38.77-43.5)	10.45±0.59 (9.80-11.20)	n.d	4.75±3.33 (1.08-7.37)
Gills	10	0.04±0.03 (0.02-0.08)	2.57±1.83 (0-4.27)	0.72±0.73 (0.84-1.64)	24.50±15.77 (0.98-41.8)	6.25±4.01 (0.96-12.36)	15.59±13.67 (0.95-36.18)	2.72±1.91 (1.18-5.69)	0.55±0.35 (0.39-0.79)	0.15±0.12 (0-0.33)
Skin	13	0.05±0.03 (0.03-0.08)	0.64±0.50 (0.13-1.28)	1.65±0.59 (0.68-2.29)	24.47±14.35 (6.37-40.09)	14.14±0.99 (12.9-15.2)	4.40±1.50 (0.68-2.85)	4.43±3.39 (1.0-10.47)	1.17±2.06 (0.14-6.55)	0.37±0.23 (0-0.40)
Ink	11	0.05±0.04 (0.01-0.06)	0.78±0.66 (0.31-1.25)	1.95±1.09 (0.47-3.09)	11.17±0.11 (9.21-11.25)	20.52±10.10 (3.24-42.5)	19.20±1.32 (4.82-25.50)	0.45±0.31 (0.13-0.95)	1.95±2.09 (1.09-3.25)	6.77±2.45 (4.84-9.53)
Tentacles	09	0.09±0.01 (0.02-0.10)	0.43±0.46 (0-0.93)	0.93±0.73 (0-1.70)	5.58±5.01 (2.22-14.37)	8.08±0.47 (7.38-8.81)	3.99±1.95 (2.12-6.21)	1.93±1.10 (0.27-3.59)	0.16±0.18 (0-0.49)	0.12±0.16 (0-0.42)

*number of samples analysed
n.d = not detected

Table 4.2: Analysis of variance (ANOVA) of Cadmium in muscle of cuttlefish. (Seasonal variation)

Source	SS	dF	mS	F
Total	915.896	117	-	-
Between Seasons	105.569	3	52.78	7.497(p<0.01)
Error	788.846	112	7.04	

Table 4.3: Analysis of variance (ANOVA) of Cadmium in liver of cuttlefish. (Seasonal variation)

Source	SS	dF	mS	F
Total	187939.7	72	-	-
Between Seasons	61246.80	3	30623.40	16.088(p<0.01)
Error	126027.4	67	1881	

Table 4.4: Analysis of variance (ANOVA) of Copper in muscle of cuttlefish. (Seasonal variation)

Source	SS	dF	mS	F
Total	20289.82	118	-	-
Between Seasons	5583.26	3	2791.63	24.309($p<0.01$)
Error	12976.46	113	114.83	

Table 4.5: Analysis of variance (ANOVA) of Copper in liver of cuttlefish. (Seasonal variation)

Source	SS	dF	mS	F
Total	146825.27	76	-	-
Between Seasons	11782.92	3	5891.46	3.986($p<0.05$)
Error	135026.96	71	1901.78	

Table 4.6: Analysis of variance (ANOVA) of Zinc in muscle of cuttlefish. (Seasonal variation)

Source	SS	dF	mS	F
Total	1727.565	117	-	-
Between Seasons	50.042	3	25.02	1.789(NS)
Error	1566.205	112	13.983	

Table 4.7: Analysis of variance (ANOVA) of Zinc in liver of cuttlefish. (Seasonal variation)

Source	SS	dF	mS	F
Total	86882.52	74	-	-
Between Seasons	3413.622	3	1706.81	1.675(NS)
Error	70272.6	69	1018.44	

Table 4.8: Analysis of variance (ANOVA) of Chromium in muscle of cuttlefish (Seasonal variation)

Source	SS	dF	mS	F
Total	14.2046	79	-	-
Between Seasons	0.8133	3	0.4065	2.419(NS)
Error	12.499	74		

Table 4.9: Analysis of variance (ANOVA) of Chromium in liver of cuttlefish. (Seasonal variation)

Source	SS	dF	mS	F
Total	187.974	72	-	
Between Seasons	17.325	3	8.662	3.880($p<0.05$)
Error	149.609	67	2.232	

Fig 4.1a. Seasonal variation of heavy metals(mean,ppm,wet wt) in the body components of cuttlefish from Cochin Region during premonsoon season.

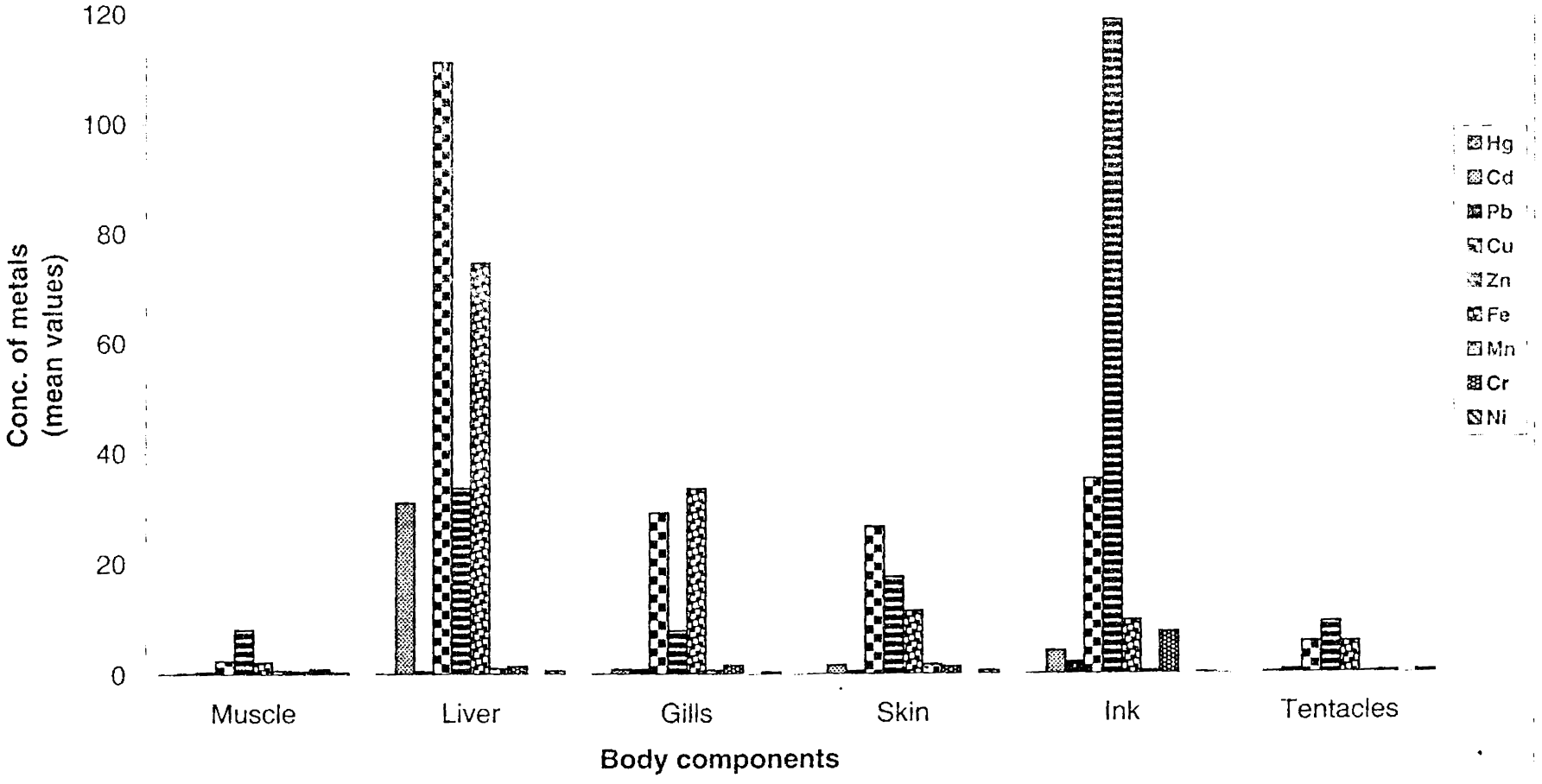


Fig 4.1b. Seasonal variation of heavy metals (mean,ppm,wet weight) in the body components of cuttlefish from Cochin Region during monsoon season.

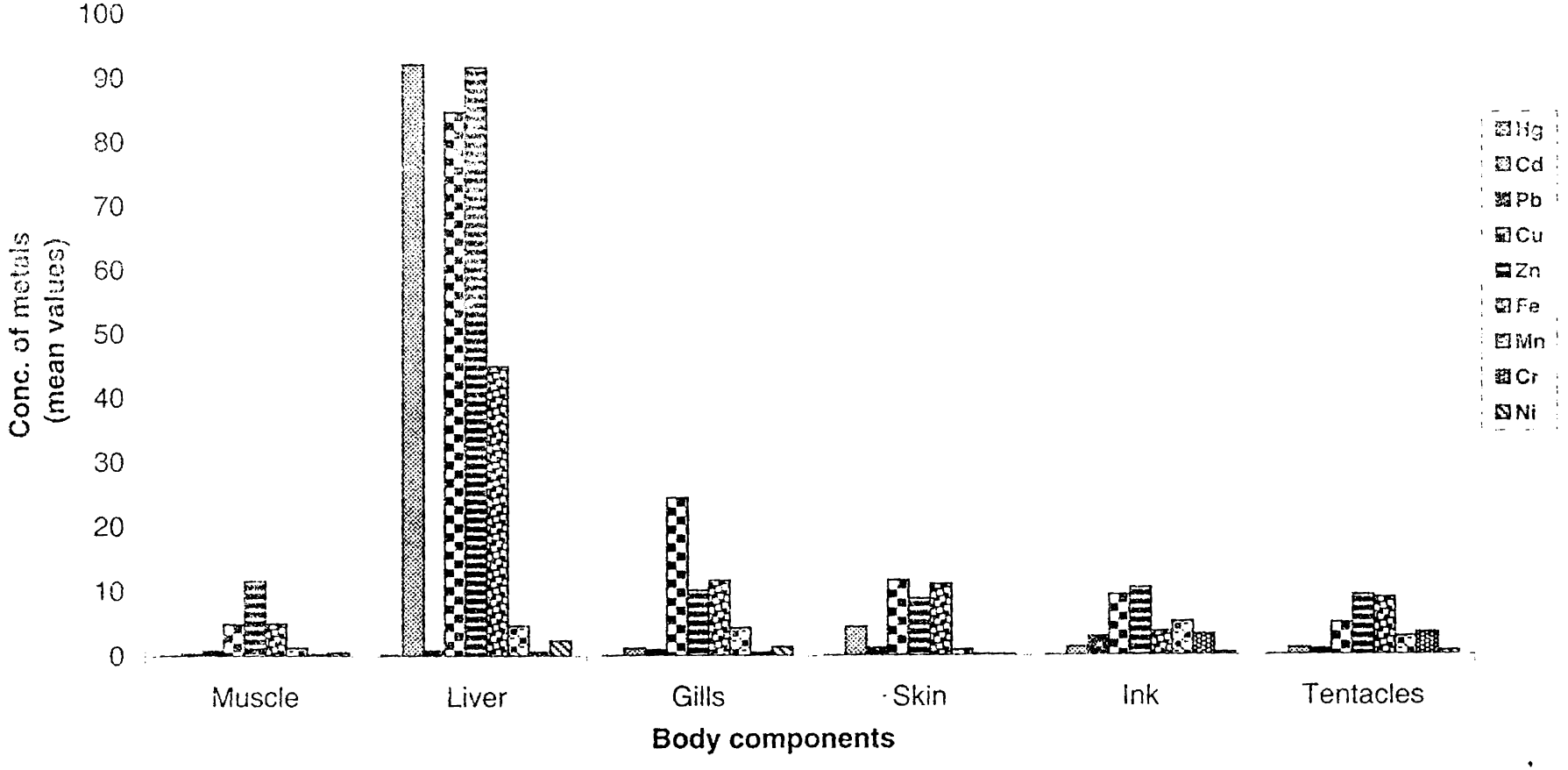


Fig 4.1c. Seasonal variation of heavy metals(mean,ppm,wet weight) in the body components of cuttlefish from Cochin Region during postmonsoon season.

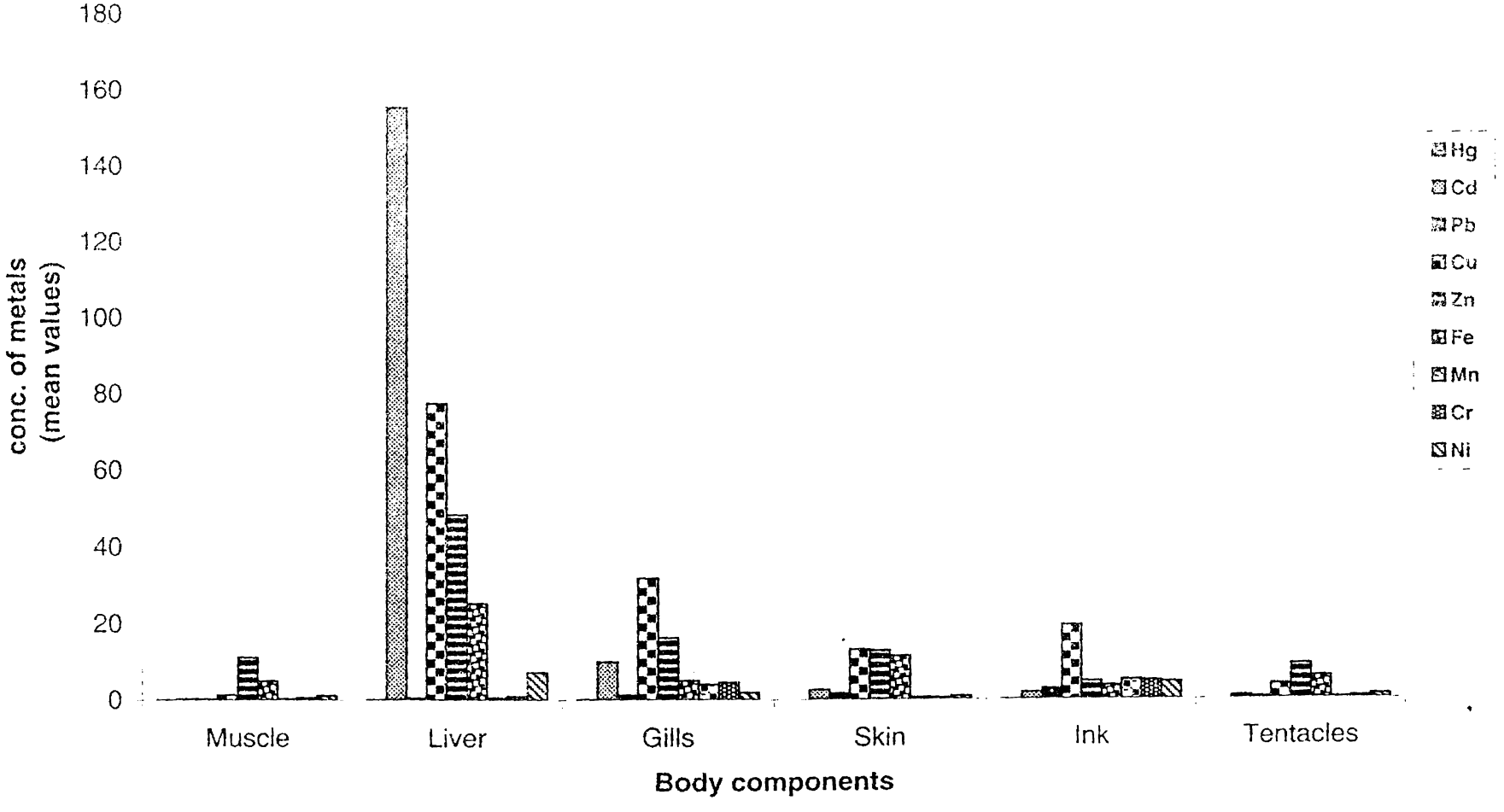


Fig 4.2a. Seasonal variation of heavy metals (mean,ppm,wet weight) in the body components of cuttlefish from Quilon region during premonsoon season

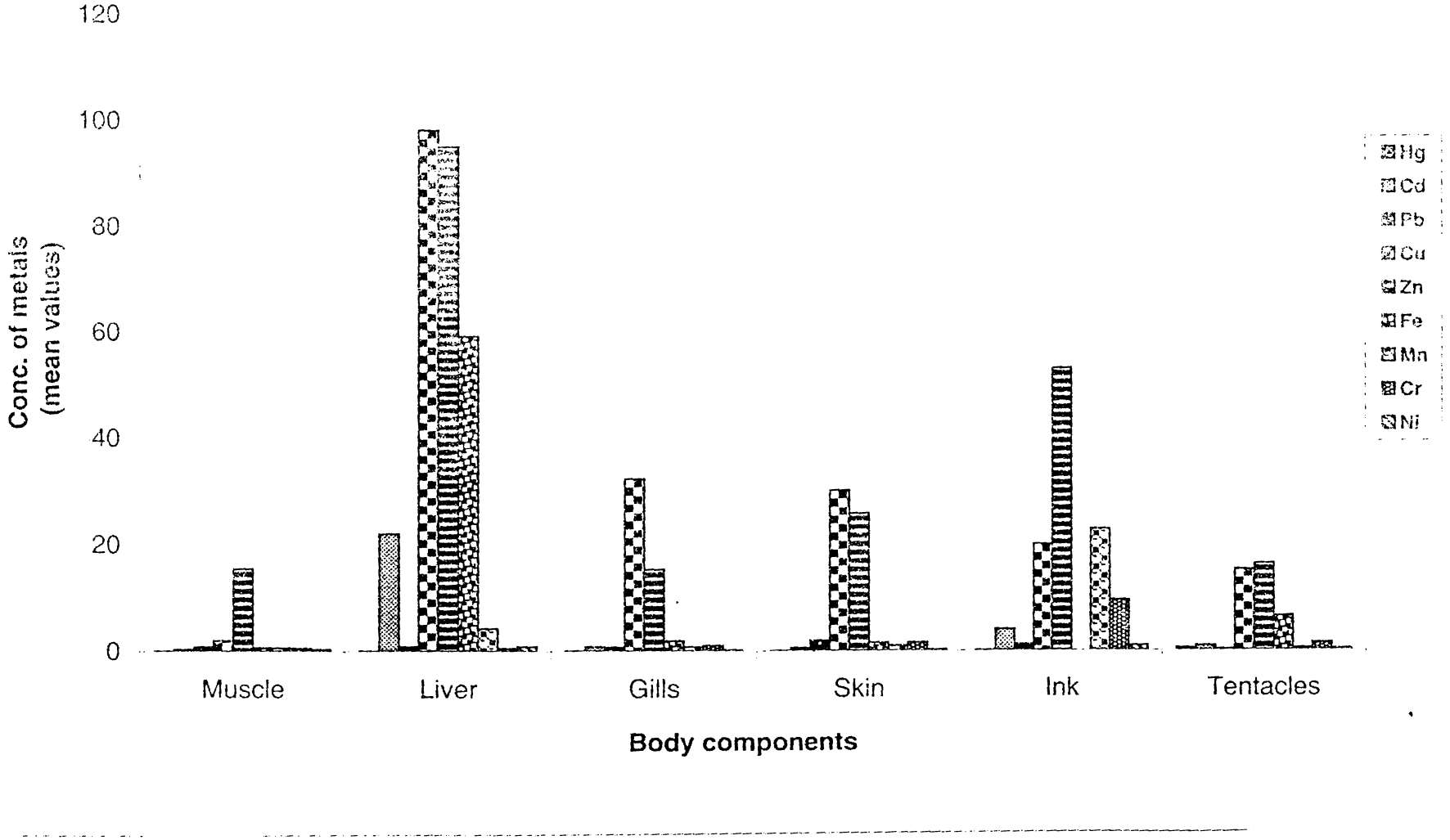


Fig 4.2b. Seasonal variation of heavy metals (mean,ppm,wet weight) in the body components of cuttlefish from Quilon Region during monsoon season

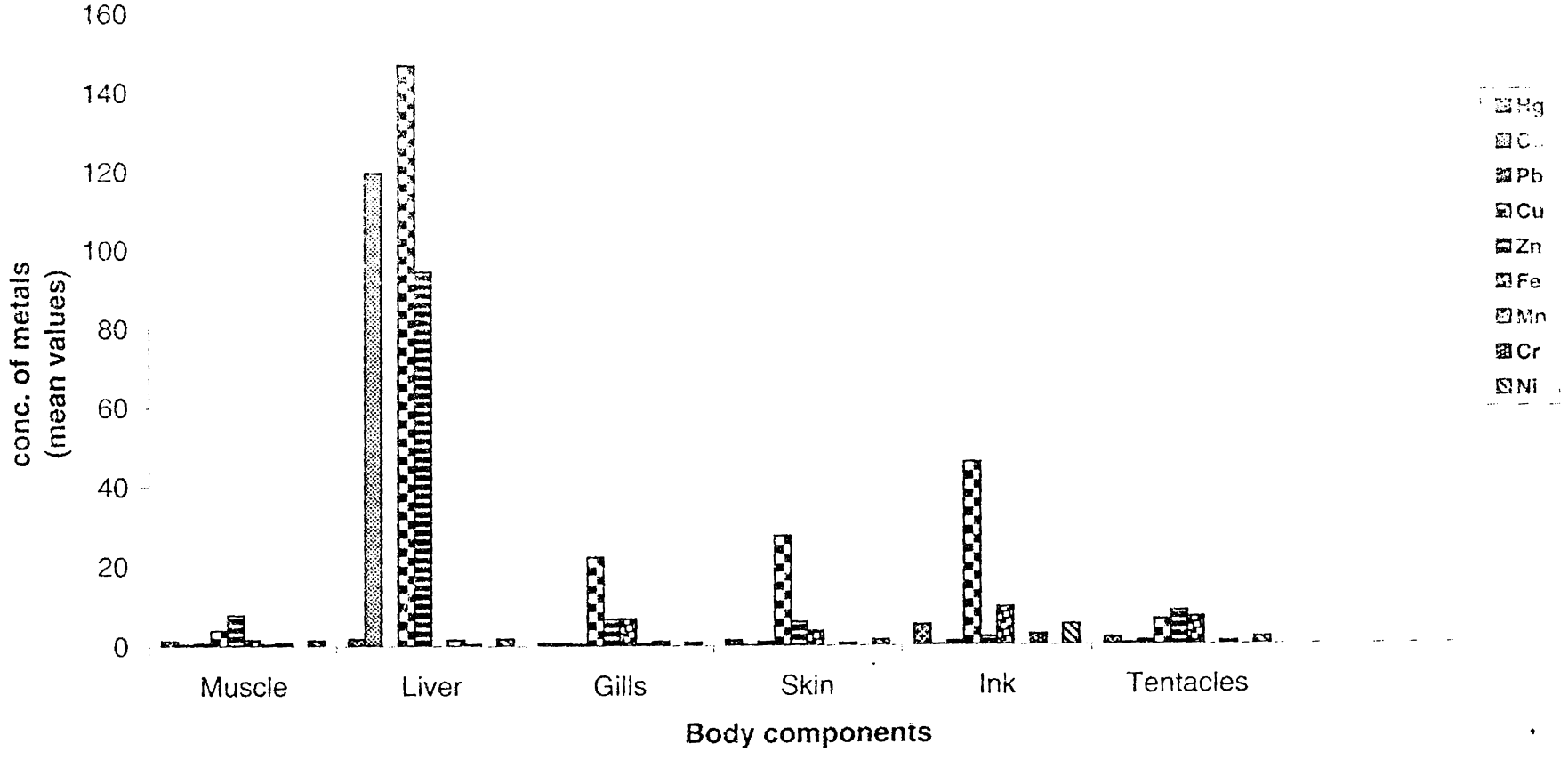


Fig 4.2c. Seasonal variation of heavy metals (mean,ppm,wet weight) in the body components of cuttlefish from Quilon Region during postmonsoon season

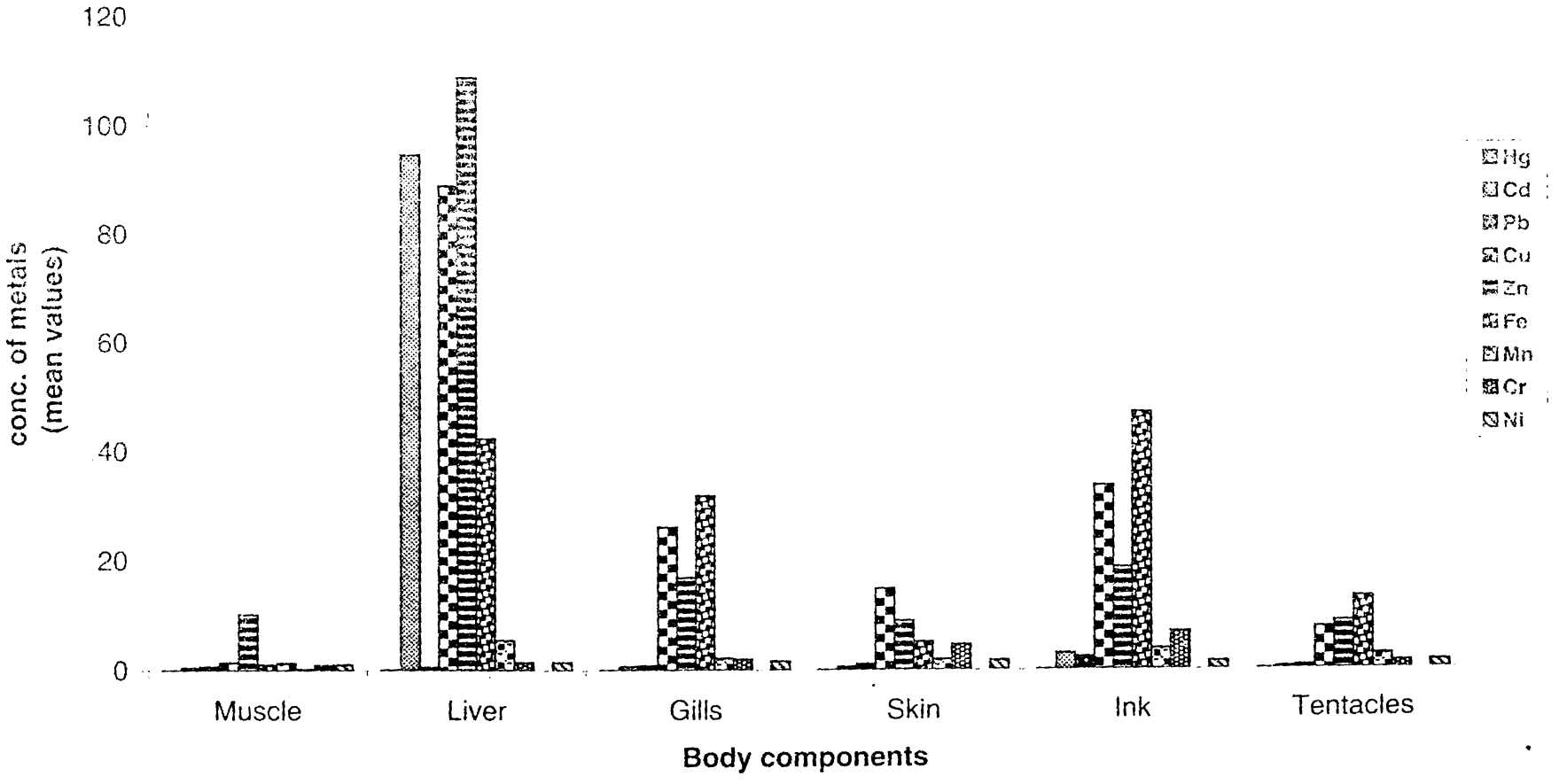


Fig 4.3a. Seasonal variation of heavy metals (mean,ppm,wet weight) in the body components of cuttlefish from Mangalore Region during premonsoon season

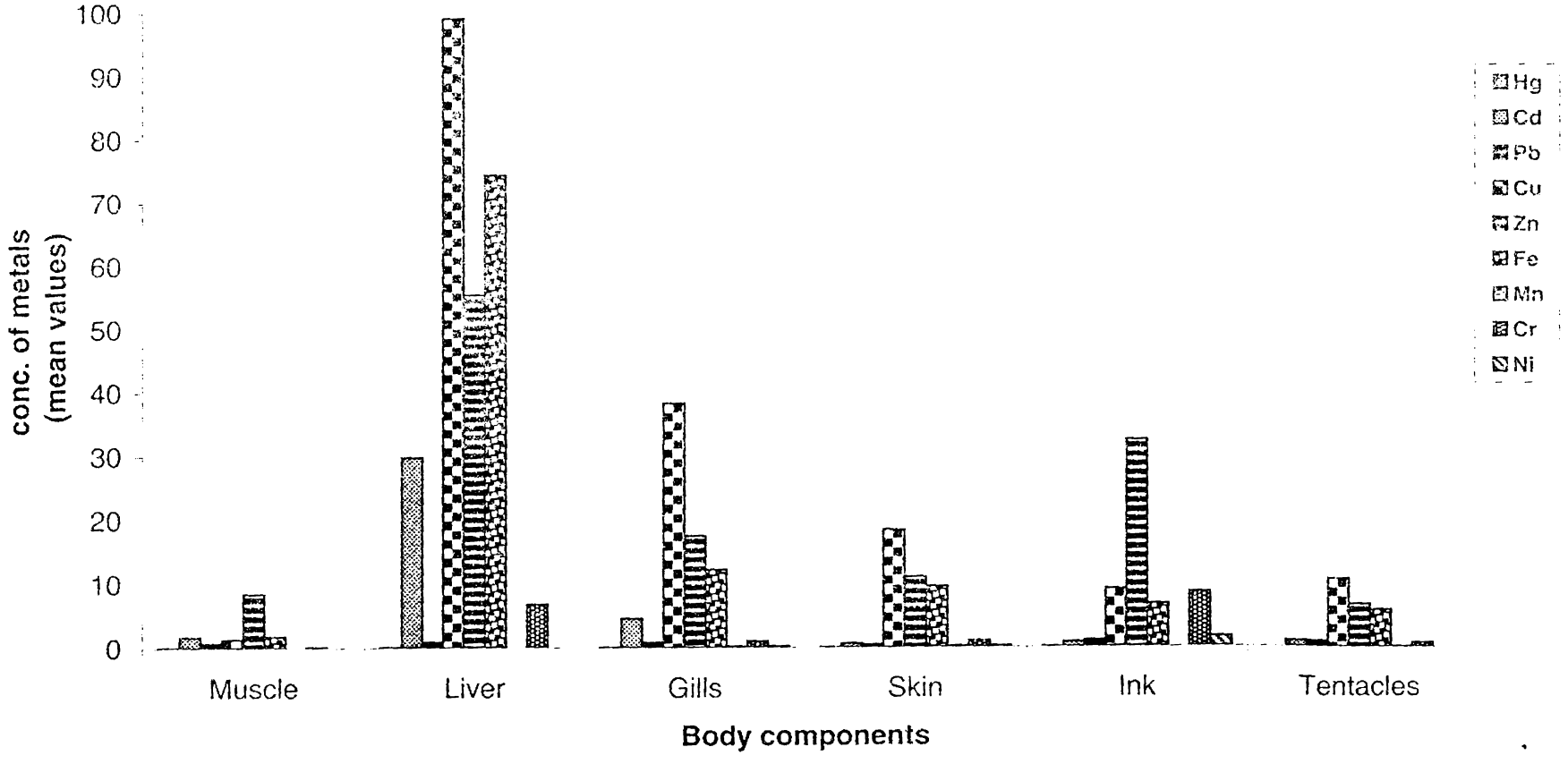


Fig 4.3b. Seasonal variation of heavy metals (mean,ppm,wet weight) in the body components of cuttlefish from Mangalore Region during monsoon season.

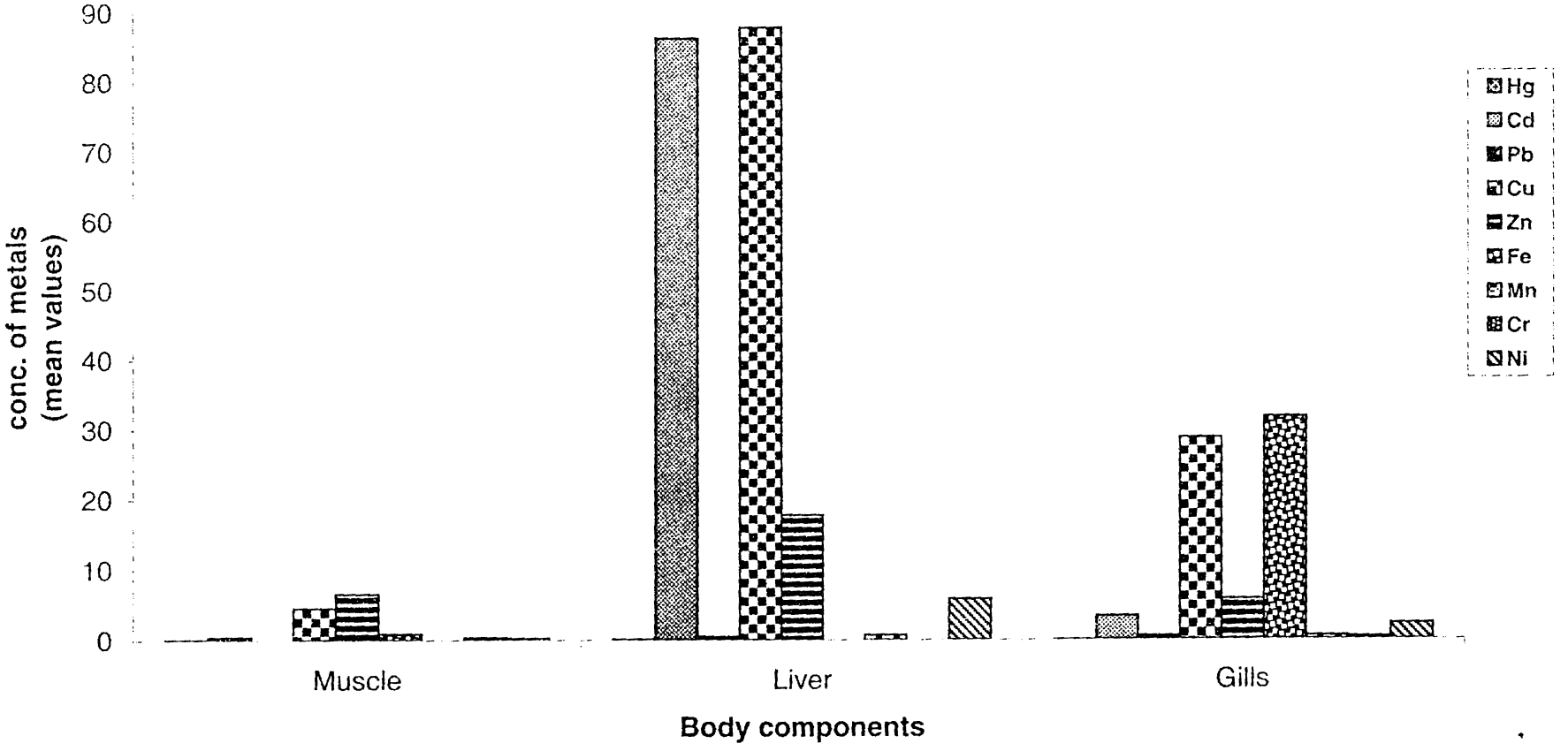
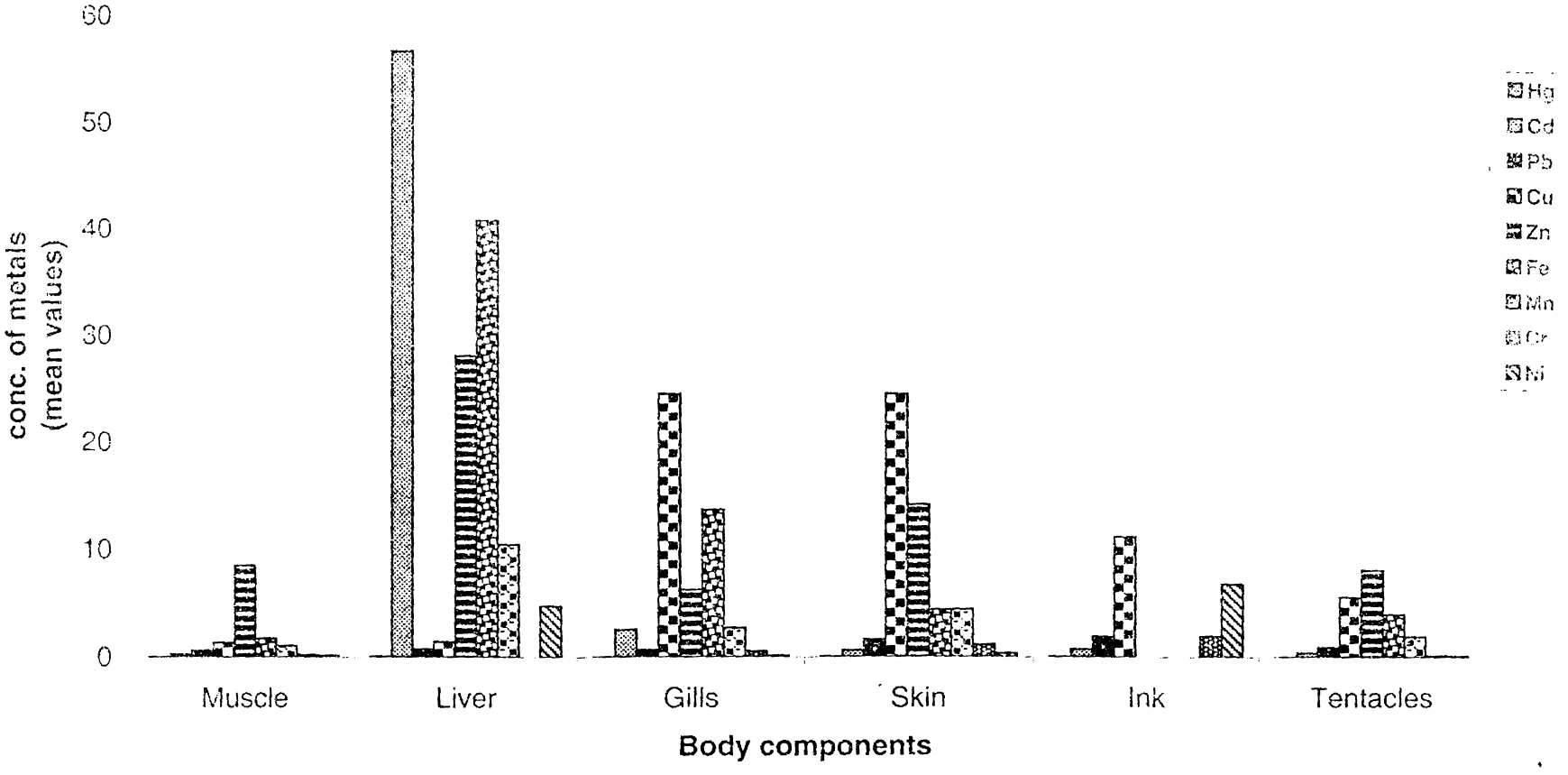


Fig 4.3c. Seasonal variation of heavy metals (mean,ppm,wet weight) in the body components of cuttlefish from Mangalore Region during postmonsoon season.



Chapter 5

HEAVY METAL ACCUMULATION IN CUTTLEFISH IN RELATION TO LEVELS IN FOOD FISHES

5.1. Introduction.

Aquatic animals including food fishes are capable of absorbing and concentrating heavy metals from the environment. Benthic organisms such as filter feeding molluscs concentrate Cd and other toxic metals in their tissue to a very high level. The metal uptake usually take place from direct consumption of food, namely small fishes, crustaceans, crabs etc, dissolved metal ions from water, sediment and detritis, in the case of benthic feeders. In the aquatic animals they are strongly bonded to the organic matrix such as enzymes, proteins or such ligands and may be stored as metallothionines. The accumulated metals may be strongly bonded to sulphydril (-SH) or amino ($-NH_2$) groups or proteins. The metal uptake process is influenced by speciation of the metals in solution or water ie. the bio-availability of the metal, salinity, pH, oxygen saturation, temperature and other dissolved organic compounds in water (Bryan, 1976; Luoma, 1990).

Cephalopods, particularly cuttlefish are also noted for its ability to concentrate heavy metals to very high levels than observed in fishes. Being a voracious fish eater, this organism integrate certain metals to a very high levels (Bidder, 1966; Boyle, 1990). During the growth process they enter into cannibalism at some stage and prey upon their young ones (Verrill, 1982; Okutani, 1990). Feeding habits of cuttlefish play an important role in the accumulation of Cd and other metals (Oommen, 1977; Bryan *et al.*, 1983). Boucaud and Rodoni (1983) identified certain fishes and prawns

as the food of cuttlefish. Guerra (1985) and Castro *et al.* (1990) also reported the same type of food habits in cuttlefish.

Being a voracious fish eater and carnivorous in nature, cuttlefish receive and accumulate heavy metals from their food fishes and also from cephalopods. In an attempt to find out the source of cadmium and other metals in cephalopods, the fishes separated from the mantle cavity (attributing them to be their food fishes) and also caught from the same trawl net along with cuttlefish were analysed for trace metals and tried to find out their interrelationship. Oommen (1977) reported that fishes caught in the same trawl net along with cuttlefish are treated as food fishes, owing to its proximity of the food fishes to cephalopods. The author identified a number of fishes such as *Nemipterus japonicus*, *Platycephalus scaber*, *Saurida tumbil*, *Cynoglossus* sp., and *Rastrelliger kanagurta*, and the crustaceans, *Metapenaeus* sp., *Penaeus* sp., from the stomach of cuttlefish. This approximation was justified from the findings of Graham (1994) where it is observed that larger prey are cut into chunks by the beak before ingestion, coupled with rapid digestion, make identification of prey and quantification of diet from gut content particularly difficult. In the present study, fishes were separated from the mantle cavity of cuttlefish and were identified and metal levels determined. An attempt was made to correlate metal levels in food fishes with the levels found in cuttlefish. The complete list of food fishes separated from the mantle cavity are given in

Table 5.1. Similarly, fishes caught from the same trawl net were also identified and attributed to the food fishes of cuttlefish.

5.2. Materials and Methods.

Cuttlefish samples for the study were mostly collected Onboard the fishing vessel MV *Sagarika* or FORV *Sagar Sampada*. The samples were chilled and brought to the laboratory and animals of the same size grade were selected. Fishes and crustaceans separated from the mantle cavity of cuttlefish and fishes caught along with cuttlefish in the same trawl net of MV *Sagarika* in two different cruises from May to June 1998 at depths 30-58 m lat 9^0 47' N to 12^0 08' N and long 75^0 02' N to 75^0 50' E were subjected to metal analysis. The length and weight range of the fishes were of 10 – 16 cm and 35 – 100 g. These fishes were wet digested, whole (as it is consumed by cuttlefish) for metal determination by Flame AAS, as described in Chapter 2. The edible muscle of cuttlefish were also processed and digested for metal analysis. Statistical analysis involved regression analysis of heavy metal levels in cuttlefish with that of metal levels in fishes. Water samples collected from the same trawl area were also analysed for metals so as to find out the environmental levels of metals in habitat water. The levels of metals in habitat water are presented in Tables 5.5 and 5.6.

5.3. Results.

The levels of trace metals viz., Cd, Cu, Zn and Cr in the body components (eg. muscle, liver and gills) of cuttlefish collected onboard the fishing vessel FORV *Sagar Sampada* and MV *Sagarika* are presented in Table 5.2 and the levels found in whole fishes (food fishes) are given in Table 5.3. The concentration of heavy metals in fishes caught in the same trawl net are presented in Table 5.4. Trace metal concentration in water samples collected on board FORV *Sagar Sampada* at 30 – 65m depth and 200 – 350m depth are presented in Tables 5.5 & 5.6.

5.3.1. Metal levels in cuttlefish caught onboard the vessel FORV *Sagar Sampada* and MV *Sagarika.*

The results of the analysis and distribution pattern of Cd, Cu, Zn and Cr in cuttlefish are shown in Table 5.2, which indicate both mean and range of values. The mean Cd content in the muscle of cuttlefish were <1 ppm and ranged between 0.19 to 1.54 ppm. Liver samples of cuttlefish had mean Cd concentration of 50.23 ± 24.1 ppm with range being 4.04 to 113.7 ppm. Gills samples of cuttlefish also recorded high Cd content with mean value being 18.57 ± 7.67 ppm. The order of abundance of Cd in the body components of cuttlefish were liver > gills > muscle.

Comparatively higher levels of Cu were detected in muscle of cuttlefish, caught onboard the fishing vessels; the highest value being 14.55 ppm. The range of values of Cu in the liver of cuttlefish varied from 16.70

to 119 ppm. In the edible muscle, Zn content varied from 6.05 to 18 ppm. Concentration of Cu and Zn in different body components of cuttlefish followed the order liver > gills > muscle. The prevalence of Cr in the muscle of cuttlefish were lower, the concentration being 0.77 ± 0.49 ppm. Among the body components analysed, liver of cuttlefish again exhibited comparatively higher values (Table 5.2).

5.3.2. Metal levels in fishes separated from the mantle cavity of cuttlefish.

The frequently occurring fishes separated from the mantle cavity of cuttlefish were *Platycephalus tuberculatus*, *Priacanthus hamrur*, *Nemipterus japonicus*, *Saurida tumbil*, *Cynoglossus* sp, *Lactarius lactarius* and the prawn, *Penaeus indicus* and the complete list of fishes separated from the mantle cavity are presented in Table 5.1. Metals were determined in whole fish as it was consumed by cephalopods, so that a realistic picture of the metal uptake could be obtained. The concentration of metals viz., Cd, Pb, Cu, Zn, Cr and Ni in the whole fishes separated from the mantle cavity are presented in Table 5.3. Comparatively higher levels of Cd content was observed in these fishes, *P. tuberculatus* (0 to 3.29 ppm), *P. hamrur* (1.04 to 1.18 ppm), *N. japonicus* (0.62 to 3.38 ppm), *S. tumbil* (0.03 to 2.57 ppm), *P. indicus* (1.31 to 2.72 ppm) and *Cynoglossus* sp. (0.22 to 4.53 ppm). The highly toxic metal Cd exceeded 1 ppm in most cases in whole fishes.

The level of Pb and Zn were highest in *P. tuberculatus* compared to other fishes (Table 5.3). Concentration of Pb and Zn were 9.77 ± 6.62 and

9.17 ± 2.52 ppm, respectively. Lower level of copper were found in *P. tuberculatus* and exhibited in the range of 0.48 to 5.91 ppm. The mean concentration of Cr and Ni were 2.21 ± 1.07 ppm and 1.19 ± 0.13 ppm, respectively. In *P. hamrur*, mean concentration of cadmium was 1.12 ± 0.05 ppm and elevated level of Pb was found and was in the range of 2.43 to 4.18 ppm. Cr level varied from 0.39 to 1.22 ppm in *P. hamrur*. In *N. japonicus*, mean Cd content was 1.78 ± 1.09 ppm; with the highest level of 3.38 ppm. Concentration of lead varied from 0 to 0.96 ppm. Copper and chromium content in *N. japonicus* were 2.99 ± 1.74 and 1.92 ± 0.91 ppm, respectively. Zinc content in *N. japonicus* sample was 9.13 ± 4.15; with the highest level of 13.91 ppm. The average concentration of cadmium in *S. tumbil* were 1.19 ± 0.92 ppm. The mean concentration of lead and chromium in *S. tumbil* were 3.83 ± 1.96 ppm and 2.39 ± 1.53 ppm, respectively. Lower value of lead, in general, was zero in *P. tuberculatus*, *N. japonicus*, *S. tumbil*. Zinc level in *S. tumbil* varied from 0.15 to 8.45 ppm. The mean value of Ni in *S. tumbil* was 4.39 with the highest value being 14 ppm. Higher values of cadmium have been recorded in *Cynoglossus* sp with a mean value of 3.28 ppm. Mean concentration of copper and zinc in *Cynoglossus* sp were 7.12 ± 1.69 ppm and 7.55 ± 3.84 ppm. *Cynoglossus* sp. showed high levels of Cd and Cu when compared to other species. In *P. indicus*, mean Cd content was 1.91 ± 0.69 ppm; with the highest value being 2.72 ppm. The toxic metals, viz., Pb and Ni were

also high and the highest values being 6.65 and 10 ppm, respectively. Concentration of Pb and Zn in *L. lactarius* showed marked difference. The values being 4.29 ± 0.99 and 9.07 ± 0.11 ppm.

5.3.3. Metal levels in fishes caught in the same trawl net along with cuttlefish.

Fishes caught in the same trawl net from MV *Sagarika* along with cuttlefish were identified and metal levels were determined and are presented in Table 5.4. *Priacanthus hamrur, Dactyloptera orientalis, Lutjanus lutjanus, Saurida tumbil, Upeneus* sp, and *Alectus indica* were the fishes identified and analysed. Invariably higher levels of Cd were found in the whole soft parts of *S. tumbil* and *D. orientalis*. The mean Cd content in the two species being 3.78 ± 3.49 ppm and 0.95 ± 0.84 ppm, respectively. Cd content was comparatively lower in all other species and was below 1 ppm. The copper concentration was the highest in *S. tumbil* (3.79 ± 3.51 ppm); while in the case of other fishes concentration of Cu ranged from 0.06 to 3.06 ppm. The mean Zinc content in *D. orientalis* and *S. tumbil* were 6.79 and 6.41 ppm, respectively. Chromium content in the muscle of *Upeneus* sp. showed the highest value of 5.74 ppm and in other fishes the level of Cr were in the range of 0 to 2.51 ppm. Nickel content was comparatively higher in all fishes and ranged between 0 to 34.44 ppm. The mean Ni level in *D. orientalis, S. tumbil, Upeneus* sp. were 15.89 ± 13.76, 11.02 ± 3.86 and 14.37 ± 17.71 ppm, respectively, which are comparatively higher.

5.3.4. Metal levels in water samples collected from the same habitat area of cuttlefish.

As a part of monitoring of environmental levels of various metals, seawater samples were collected onboard the vessel '*Sagarika*' of Integrated Fisheries Project and FORV *Sagar Sampada*, Cochin from the same region from where cuttlefish were caught. The metal levels in the water samples were determined as described in Chapter 2. The results are summarized in Table 5.5 and 5.6.

The levels of Cd, Pb, Cu and Zn in seawater samples at 30 – 58m depth were in the range of 0.0005 to 0.013, 0.010 to 0.020, 0.008 to 0.021 and 0.013 to 0.078 µg/L, respectively. The metal levels in habitat water were found to be low and were at normal levels. The habitat water collected at 200 – 350m depth, also contained trace metals at low levels. The levels of metals are presented in the Table (Table 5.6). In deep water, concentration of Cd, Cu, Zn, Pb and Cr were 0.0003 to 0.006; 0.003 to 0.007; 0.028 to 0.042; 0.022 to 0.046 and 0.006 to 0.008 µg/L, respectively. There is not much variation in Cd level between surface and deep water, while Cu, Zn and Pb showed slightly higher levels in deep water.

5.3.5. Correlation of metal levels in food fishes Vs metal levels in cuttlefish.

With a view to identify the probable source of Cd and other toxic metals in cuttlefish, the experimental data were subjected to statistical analysis using correlation and regression. Wherever significant correlation were detected, regression equation were worked out using the method of

least squares. The goodness of fit of regression were shown in the Figs 5.1a to 5.1c and 5.2a to 5.2b. The regression equation for metal levels in various species of fish versus metal levels in cuttlefish are as follows:

Regression analysis of metal levels in cuttlefish Vs metal levels in food fishes.

	Nemipterus japonicus		
Cadmium	y = 1.98 + 1.10 x	r = 0.989	p<0.05
Copper	y = 7.34 + 1.85 x	r = 0.704	p<0.05
Zinc	y = -5.13 + 1.31 x	r = 0.715	p<0.05
Chromium	y = 0.58 + 0.41 x	r = 0.560	p<0.10
	Saurida tumbil		
Cadmium	y = -8.32 +1.85 x	r = 0.764	p<0.05
Copper	y = 6.85 + 1.72 x	r = 0.930	p<0.05
	Cynoglossus		
Cadmium	y = -0.31 + 1.76 x	r = 0.593	p<0.10
Copper	y = -40.12 + 2.98 x	r = 0.912	p<0.05
Zinc	y = 7.45 + 0.65 x	r = 0.720	p<0.05
	Fishes caught along with cuttlefish		
	Dactyloptera orientalis		
Cadmium	y = 0.31 + 0.08 x	r = 0.710	p<0.05
Zinc	y = -8.90 + 5.43 x	r = 0.586	p<0.10
	Saurida tumbil		
Cadmium	y = 0.08 + 0.81 x	r = 0.609	p<0.10
Zinc	y = -5.92 +1.57 x	r = 0.821	p<0.05

5.4. Discussion.

Regression analysis were carried out between levels of metals in cuttlefish and levels found in food fishes separated from the mantle cavity. Significant positive correlations existed between metal levels in food fishes and metal levels in cuttlefish. Cd content in *Nemipterus japonicus* ($p<0.05$), *Saurida tumbil* ($p<0.05$) and *Cynoglossus* sp ($p<0.10$) showed significant positive correlation with that of cuttlefish. Copper level in *N. japonicus* ($p<0.05$), and *S. tumbil* ($p<0.05$) also showed significant positive correlation with metal levels in cuttlefish. Significant positive correlation could be obtained for Cr in *N. japonicus* ($p<0.10$) and the corresponding regression equation are presented above.

Dactyloptera orientalis and *Saurida tumbil* from the same habitat area also exhibited significant correlation. The levels of cadmium and zinc in *D. orientalis* and *S. tumbil* showed significant positive correlation with the levels of these metals in cuttlefish muscle ($p<0.05$ and $p<0.10$ for Cd and Zn in *D. orientalis*; $p<0.10$ and $p<0.05$ for Cd and Zn in *S. tumbil*). The corresponding regression equation are presented overleaf. These results indicated that the metal levels in cephalopods is very much influenced by metal levels in food fishes and indicating one of the probable source of cadmium and other toxic metals in cephalopods. Thus, cuttlefish accumulates higher levels of Cd and other toxic metals and are considered to be an important vector of this element to top marine predators.

Present study showed that metal content in the food fishes separated from the mantle cavity of cuttlefish is quite significant (varied from 5% to 10%), and play a major role in metal accumulation in cuttlefish. Cannibalism has also been noted in several species of cuttlefish (Oommen, 1977; Verrill, 1982; Okutani, 1990) and were found to increase with the growth of cuttlefish, indicating that the feeding habits of cuttlefish might have played a pivotal role in metal accumulation. The levels of Cd, Pb, Cu, Zn and Cr determined in the habitat water were at normal level and there is no indication of pollution in the environment by heavy metals.

Table 5.1: Fishes/shellfish found in the mantle cavity of cuttlefish.

Sl. No.	Food fishes
1	*Platycephalus tuberculatus*
2	*Priacanthus hamrur*
3	*Nemipterus japonicus*
4	*Saurida tumbil*
5	*Cynoglossus*
6	*Lactarius lactarius*
7	*Paeneus indicus*
8	*Rosy snapper*
9	*Terapon jarbua*
10	*Apogonichthys*
11	*Sardinella longiceps*
12	*Anchiovies*

Table 5.2: Trace metal concentrations (Mean ± S.D and Range, ppm, wet weight) in the body components of cuttlefish caught onboard MV *Sagarika* and FORV *Sagar Sampada* at 30-70m depth, off west coast of India.

Body components	n*	CADMIUM	COPPER	ZINC	CHROMIUM
Muscle	15	0.65 ± 0.50 (0.19 - 1.54)	9.54 ± 2.89 (0.88 - 14.55)	9.82 ± 4.86 (6.05 - 18)	0.77 ± 0.49 (0.26 - 1.55)
Liver	18	50.23 ± 24.1 (4.04 - 113.7)	73.18 ± 50.32 (16.70 - 119)	57.40 ± 23.2 (36.99 - 82)	3.80 ± 0.32 (0.52 - 6.26)
Gills	20	18.57 ± 7.67 (12.95 - 27.31)	24.42 ± 7.7 (14.12 - 48.6)	40.07 ± 17.8 (23.45 - 58)	1.85 ± 2.68 (2.95 - 4.75)

*number of samples analysed

Table 5.3: Distribution pattern of heavy metals (Mean ± S.D and Range, ppm, wet weight) in fishes separated from the mantle cavity of cuttlefish collected off Cochin.

FISH/SHELLFISH	n*	CADMIUM	LEAD	COPPER	ZINC	CHROMIUM	NICKEL
Platycephalus tuberculatus	08	1.82 ± 0.99 (0 - 3.29)	9.77 ± 6.62 (0 - 14.67)	2.78 ± 2.46 (0.48 - 5.91)	9.17 ± 2.52 (5.97 - 11.10)	2.21 ± 1.07 (1.28 - 3.56)	1.19 ± 0.13 (1.06 - 1.3)
Priacanthus hamrur	10	1.12 ± 0.05 (1.04 - 1.18)	3.36 ± 0.84 (2.43 - 4.18)	1.48 ± 1.16 (0.21- 2.45)	6.80 ± 4.58 (1.41 - 11.76)	0.95 ± 0.29 (0.39 - 1.22)	1.87 ± 0.36 (1.57 - 2.2)
Nemipterus japonicus	09	1.78 ± 1.09 (0.62 - 3.38)	0.71 ± 0.48 (0 - 0.96)	2.99 ± 1.74 (0.62 - 6.20)	9.13 ± 4.15 (0 - 13.91)	1.92 ± 0.91 (0.89 - 3.03)	1.70 ± 0.62 (1.16 - 2.3)
Saurida tumbil	12	1.19 ± 0.92 (0.03 - 2.57)	3.83 ± 1.96 (0 - 6.97)	3.52 ± 2.77 (0 - 7.09)	6.83 ± 3.27 (0.15 - 8.45)	2.39 ± 1.53 (0.41 - 4.94)	4.39 ± 5.47 (0.44 - 14)
Cynoglossus	06	3.28 ± 2.19 (0.22 - 4.53)	n.d	7.12 ± 1.69 (1.08 - 4.26)	7.55 ± 3.84 (3.31 - 10.59)	0.54 ± 0.11 (0.40 - 0.62)	n.d
Lactarius lactarius	08	0.30 ± 0.42 (0.06 - 0.70)	4.29 ± 0.99 (2.01 – 5.10)	3.18 ± 0.07 (3.0 - 3.23)	9.07 ± 0.11 (8.04 - 9.78)	1.16 ± 0.04 (1.01 - 1.21)	3.58 ± 0.02 (1.24 - 3)
Penaeus indicus	07	1.91±0.69 (1.31 – 2.72)	5.35 ± 0.91 (4.39 – 6.65)	2.83 ± 1.81 (0 – 5.0)	8.13 ± 0.19 (7.96 – 8.31)	1.64 ± 0.45 (0.98 – 2.08)	6.31 ± 0.20 (6.02 – 10)

*number of samples analysed
n.d =not detected

Table 5.4: Distribution pattern of heavy metals (Mean ± S.D., Range, ppm wet wt) in fishes caught in the same trawl net during May – June 1998 onboard the fishing vessel MV *Sagarika* from the west coast of India

FISHES	n*	CADMIUM	LEAD	COPPER	ZINC	CHROMIUM	NICKEL
Priacanthus hamrur	12	0.32 ± 0.13 (0 - 0.50)	0.51 ± 0.41 (0 - 0.95)	0.80 ± 0.28 (0.45 - 1.12)	5.48 ± 0.89 (4.07 - 6.35)	0.84 ± 1.03 (0 - 2.38)	2.54 ± 1.65 (0.49 - 4.09)
Dactyloptera orientalis	10	0.95 ± 0.84 (0 - 1.58)	0.97 ± 0.92 (0 - 1.84)	1.81 ± 1.43 (0.55 - 3.06)	6.79 ± 2.30 (4.71 - 8.90)	1.18 ± 0.66 (0.37 - 1.95)	15.89 ± 13.76 (3.53 - 24.15)
Lutjanus lutjanus	08	0.31 ± 0.22 (0 - 0.50)	2.52 ± 2.91 (0 - 5.17)	0.57 ± 0.37 (0.06 - 0.94)	4.94 ± 1.57 (3.07 - 6.60)	0.64 ± 0.74 (0 - 1.66)	4.05 ± 4.53 (0 - 9.54)
Saurida tumbil	13	3.78 ± 3.49 (0.71 - 6.85)	3.32 ± 3.49 (2.71 - 3.97)	3.79 ± 3.51 (0.74 - 6.84)	6.41 ± 3.72 (3.63 - 10.50)	1.83 ± 0.88 (0.58 - 2.51)	11.02 ± 3.86 (7.52 - 14.49)
Upeneus sp	09	0.55 ± 0.34 (0 - 0.88)	0.23 ± 0.46 (0 -0.93)	0.51 ± 0.28 (0.17 - 0.79)	4.09 ± 1.16 (2.57 - 5.03)	3.31 ± 2.12 (0 - 5.74)	14.37 ± 17.71 (9.86 - 34.44)
Alectus Indica	11	0.30 ± 0.24 (0 - 0.59)	1.16 ± 0.45 (0 -3.26)	1.40 ± 1.09 (0.12 - 2.89)	5.79 ± 1.20 (4.43 - 7.20)	1.46 ± 0.61 (0.59 - 2.24)	3.72 ± 2.29 (0 - 6.03)

*number of samples analysed

Table 5.5: Trace metal concentrations in water samples collected on board MV *Sagarika* at 30-58 m depth. (Range expressed in µg/L)

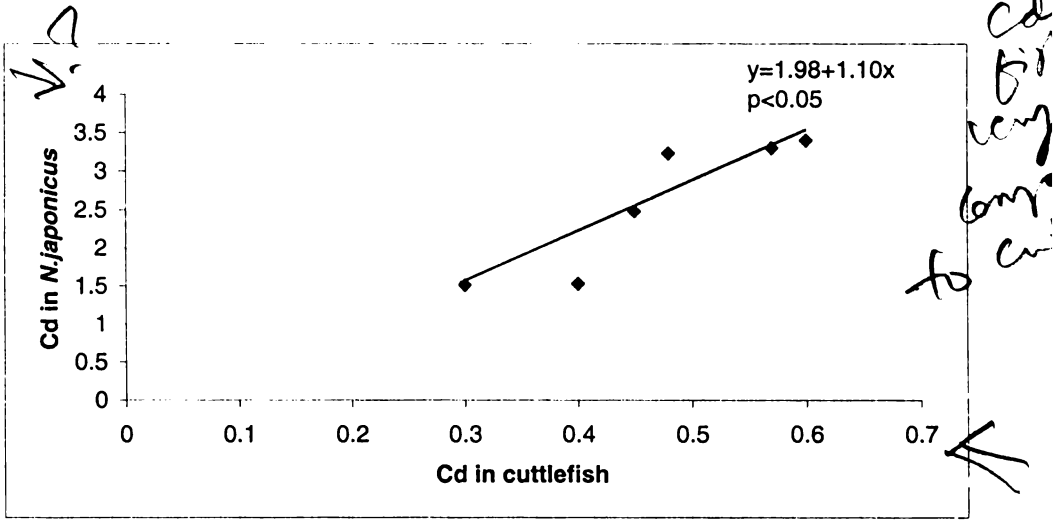
	CADMIUM	LEAD	COPPER	ZINC
Bottom water	0.0005-0.013	0.010-0.020	0.008-0.021	0.013-0.078

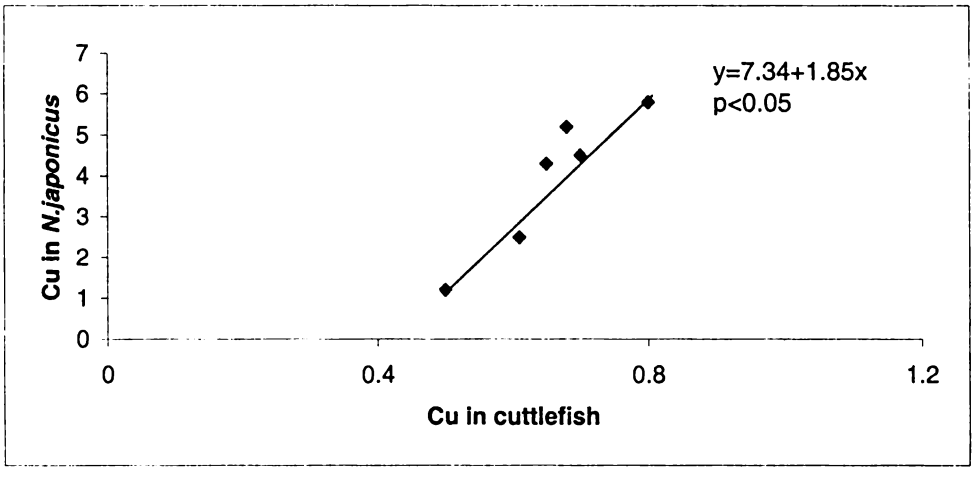
Table 5.6: Trace metal concentrations in water samples collected on board FORV *Sagar Sampada* at 200-350m depth. (Range expressed in µg/L)

	CADMIUM	LEAD	COPPER	ZINC	CHROMIUM
Surface water*	0.0003-0.005	0.019-0.031	0.002-0.005	0.020-0.032	0.006-0.009
Deep water*	0.0003-0.006	0.022-0.046	0.003-0.007	0.028-0.042	0.006-0.008

*number of samples analysed = 12

Fig 5.1a : Regression plot of metal levels in fishes separated from the mantle cavity of cuttlefish vs. metal levels in cuttlefish (expressed in ppm)





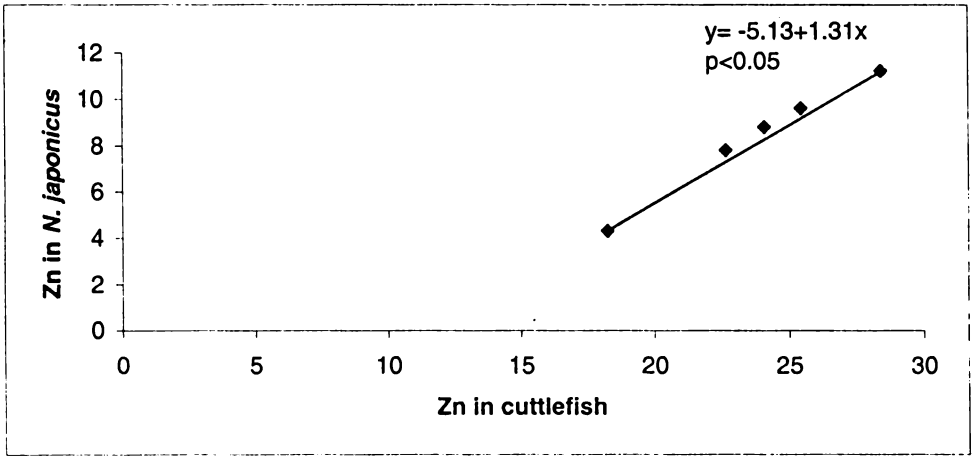
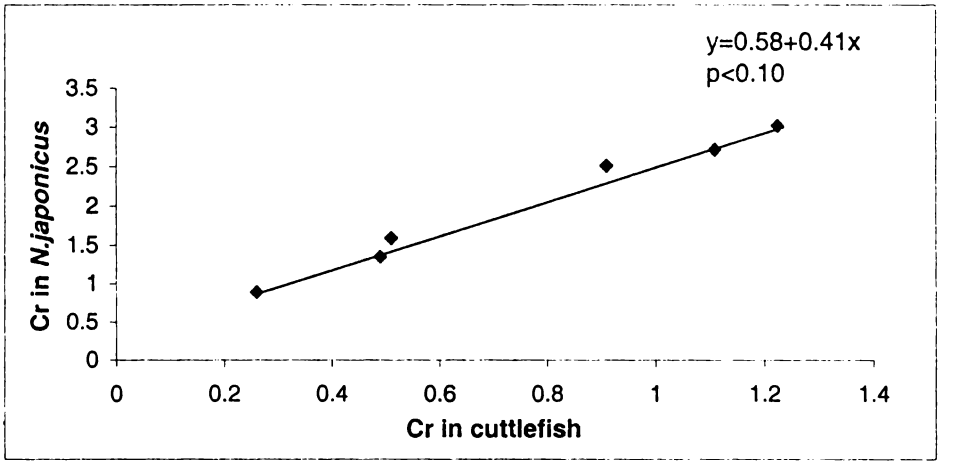
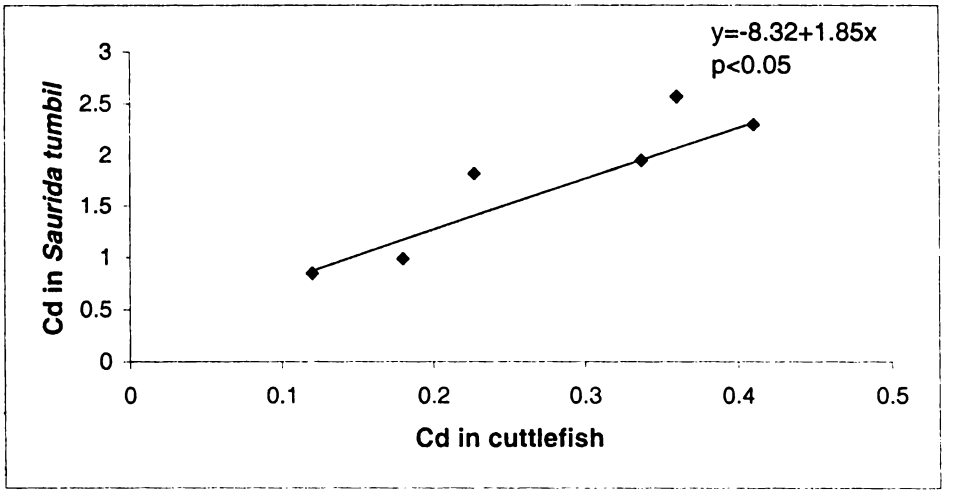


Fig 5.1b : Regression plot of metal levels in fishes separated from the mantle cavity of cuttlefish vs. metal levels in cuttlefish (expressed in ppm)





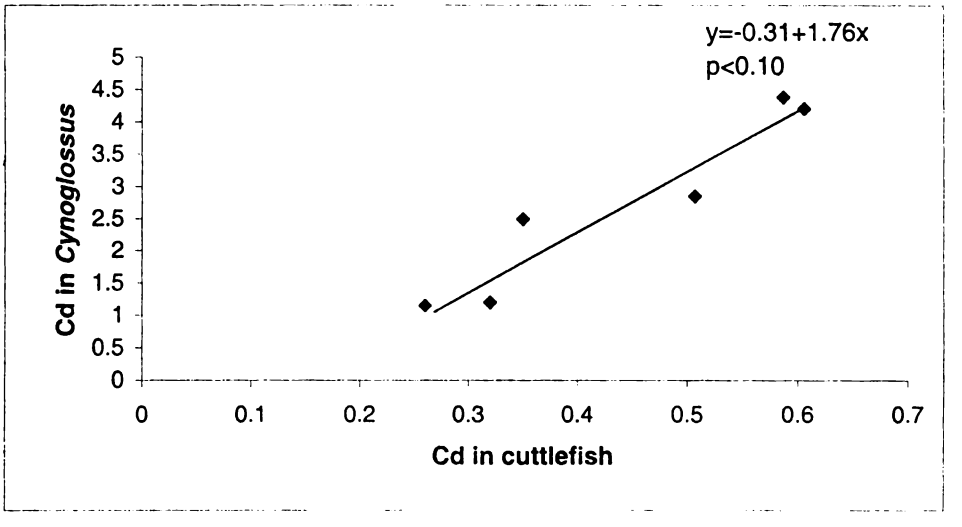
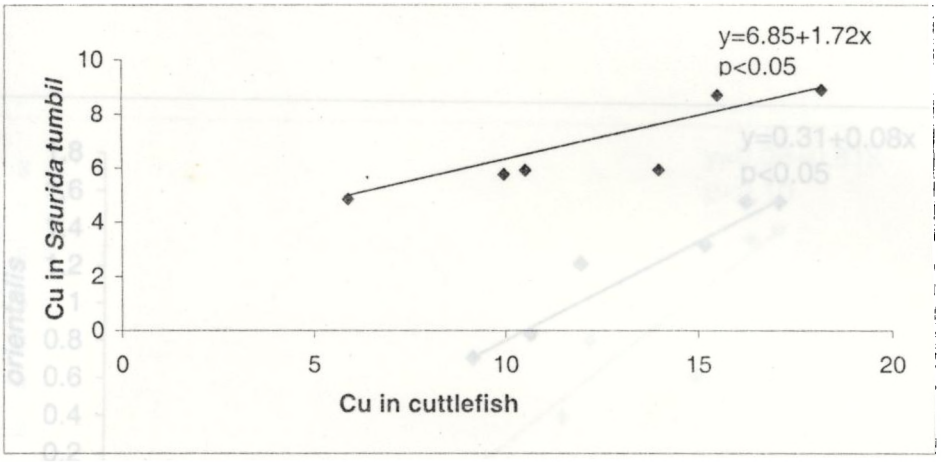
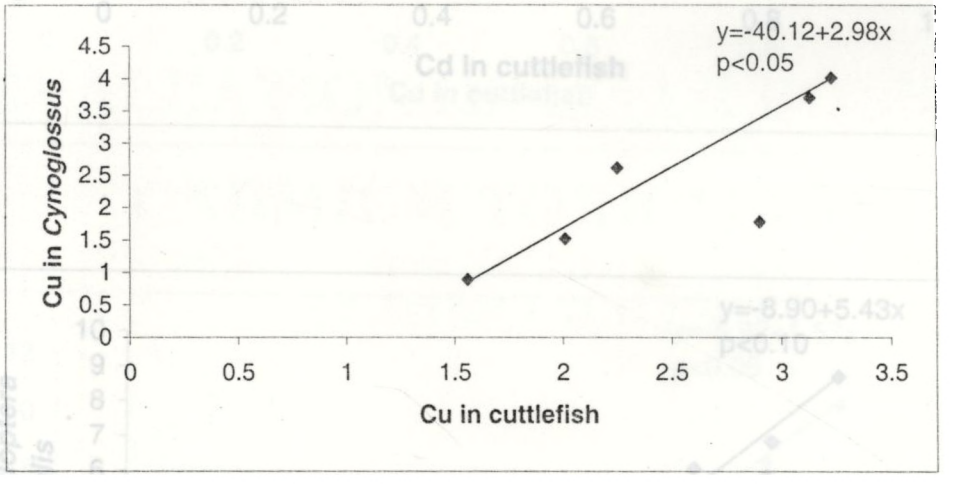


Fig 5.1c : Regression plot of metal levels in fishes separated from the mantle cavity of cuttlefish vs. metal levels in cuttlefish (expressed in ppm)





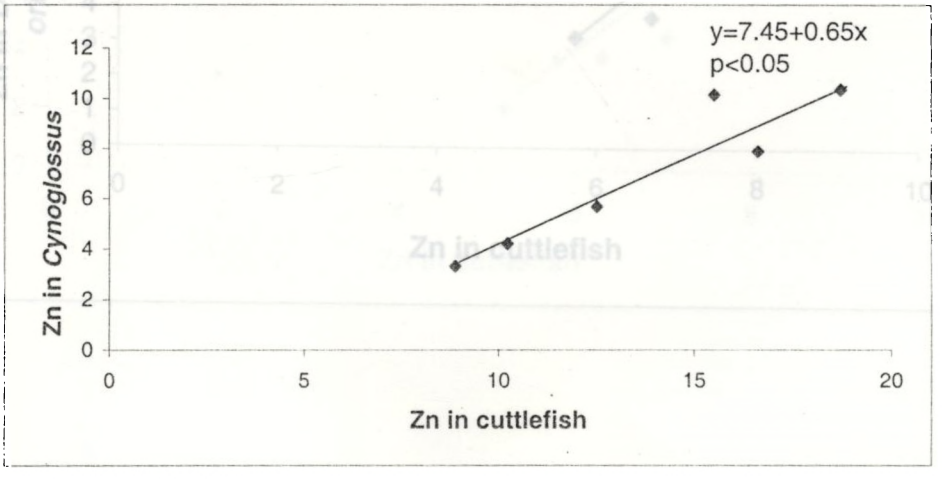
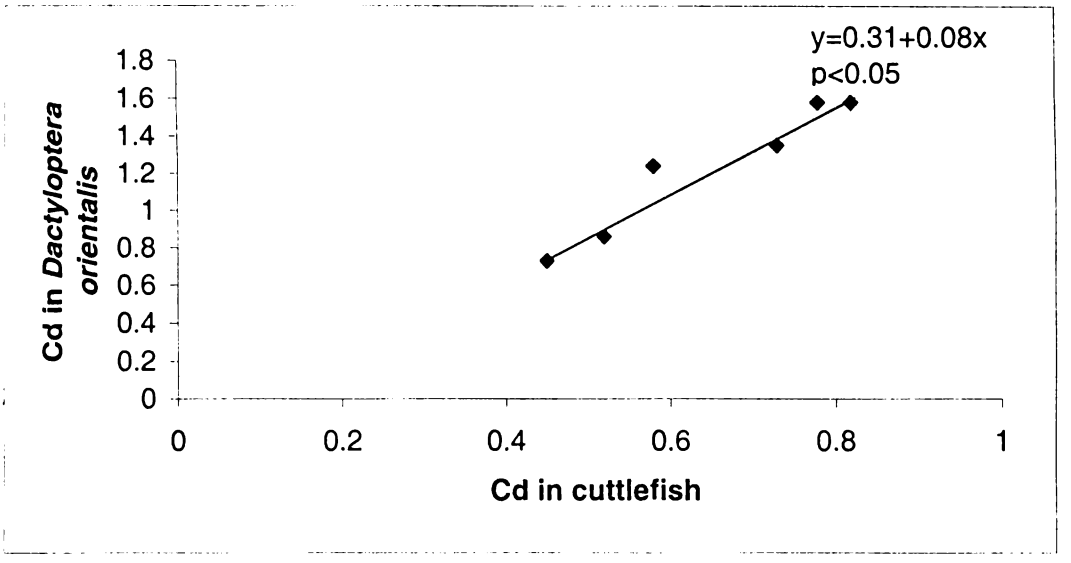


Fig 5.2a : Regression plot of metal levels in fishes caught in the same habitat area of cuttlefish vs. metal levels in cuttlefish (expressed in ppm)



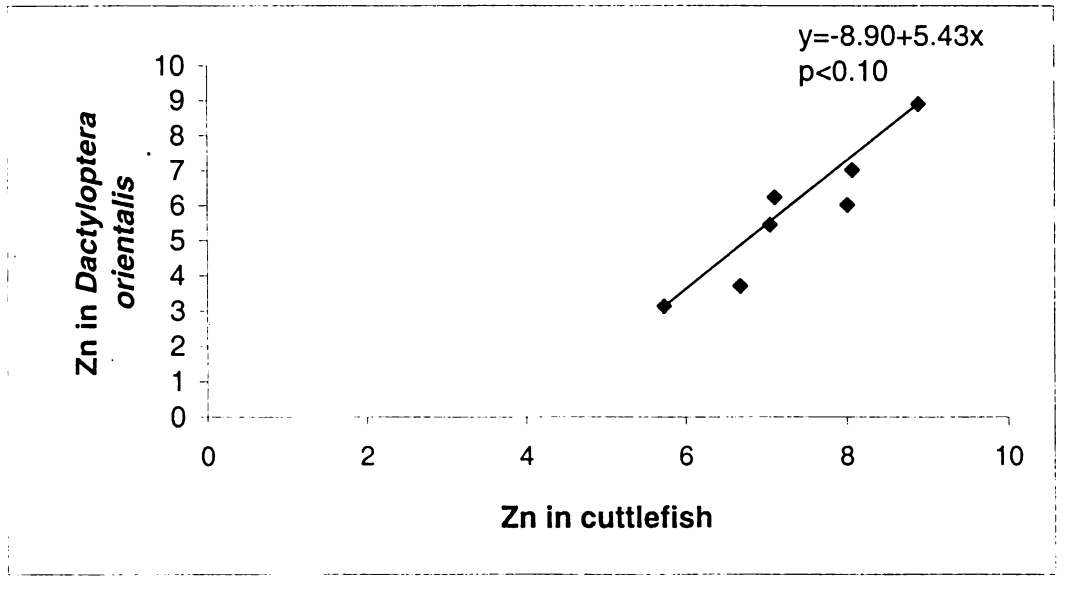
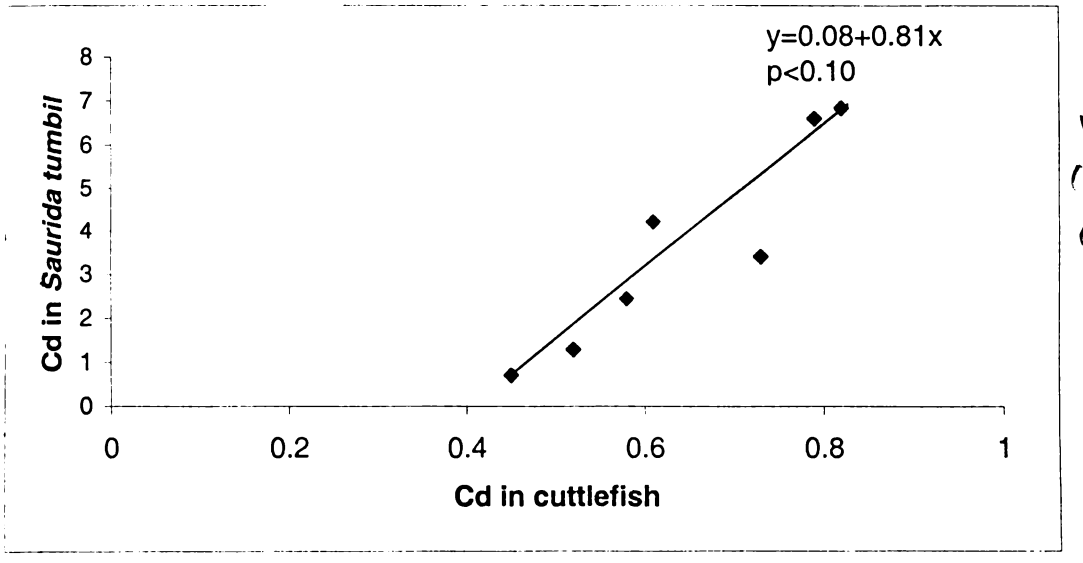
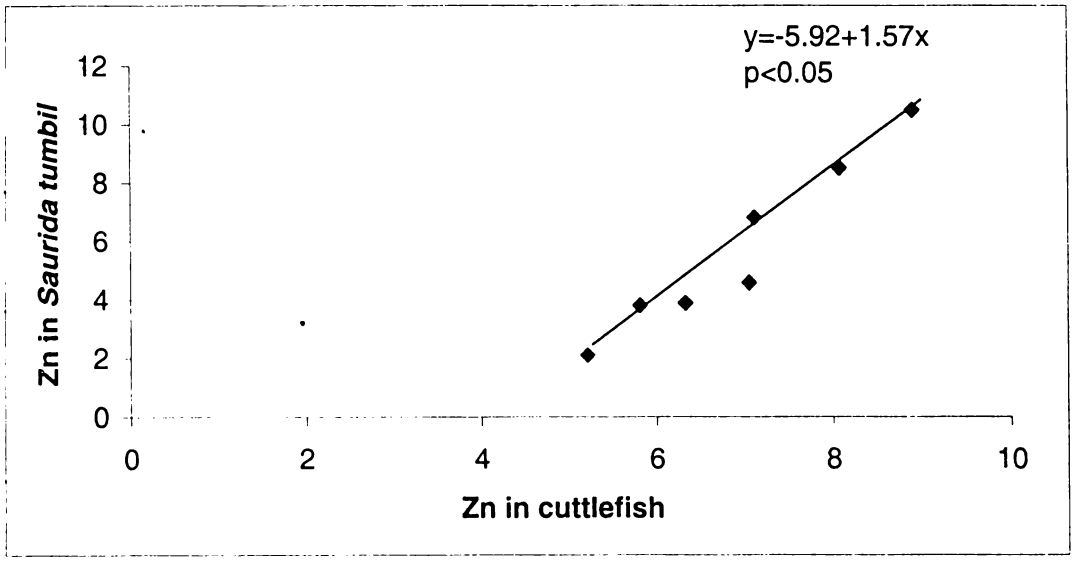


Fig 5.2b : Regression plot of metal levels in fishes caught in the same habitat area of cuttlefish vs. metal levels in **cuttlefish** (expressed in ppm)





Chapter 6

EVALUATION OF LIVER BOUND CADMIUM TOXICITY IN ALBINO RATS

6.1. Introduction

Cephalopods and molluscs, in general are notorious for metal accumulation. Martin and Flegal (1975) and Honda and Tatsukawa (1983) had reported that cuttlefish are very good cadmium integrators of metals from the environment. Cephalopods have been considered as a significant source of Cd to their predators (Muirhead and Furness, 1988). The major source of environmental exposure to cadmium for the general population is through food and specifically, seafood (Foulkes, 1986)

Cadmium is a highly toxic metal and Cd^{2+} implicate serious ailments among man and animals. Cd is a cumulative toxin and produces nephrotoxicity and interferes in calcium metabolism. As cadmium is found in very high level in cephalopods, particularly in liver (Falandysz, 1989, 1990, 1991; Lakshmanan and Stephen, 1993), a liver incorporated diet was fed to experimental albino rats in order to study the toxic effect of muscle bound cadmium and also to compare the toxicity of $CdCl_2$ in experimental albino rats. A number of studies on cadmium toxicity to experimental animals have been carried out as inorganic salt (Uthe *et al.*, 1980; Chapatawala *et al.*, 1982; Elinder, 1986; Anderson *et al.*, 1988; Groten *et al.*, 1990; Chatterjee *et al.*, 1996). The toxicity of organically bound Cd in laboratory animals is scant (Cherian, 1976; Maitani *et al.*, 1984) and also that none of these authors had compared the cadmium toxicity with inorganically bound cadmium. The toxicity evaluation becomes more

meaningful when it is fed to the experimental animals in a natural form, as it is generally present in their food. In a preliminary experiment, the toxicity of diet incorporated Cd to a level of 30 mg Cd/Kg in food revealed that the metal is not toxic to the animal up to 30 ppm. (Sinkeldam *et al.*, 1986). Other researchers (Friberg *et al.*, 1974; Kjellstrom, 1986) also reported that no renal toxicity was caused using 30 mg Cd/Kg feed. While studying the selective bio-accumulation of Cd and other metals in cephalopods, particularly in cuttlefish and squid, it was observed that liver of cephalopods accumulates cadmium and other toxic metals to very high levels (Lakshmanan and Stephen, 1993). So, these liver components if incorporated in the human diet may implicate toxic effects. The objective of the present study is to evaluate the toxic effects of cuttlefish liver incorporated cadmium to experimental albino rats by continuously feeding the rats and comparing the toxic effect of inorganic Cd as $CdCl_2$ along with the diet. Therefore, the effect of a continuous oral intake of cadmium containing diet in albino rats was conducted in order to find out the toxic effect to the consumer, if any. In the present study the toxicity of cadmium in albino rats would be evaluated by measuring the haematological, histopathological, nephrotoxicity and other oral toxicity as revealed by anaemia, general weakness, low food and water intake etc.

6.2. Materials and Methods

6.2.1. Animals

Four weeks old albino rats were selected for the experiment. They were housed in stainless steel metabolism cages for a period of five days for acclimatization. The animals were maintained in an A/C chamber at temperature 22-24^{0}C and at relative humidity 40-70%. Drinking water was supplied in glass bottles attached to the cages which were refilled daily. Weighed quantity of the diets were provided in stainless steel bowl in the enclosure area of the respective cages.

6.2.2. Preparation of vacum dried cuttlefish liver sample

Liver portions were separated from a number of cuttlefish (length 20-25 cm and weight 140 -145 g), pooled, cooked and homogenized in a warring blender. The homogenized mass were vacuum dried in a vacuum oven (50^0 C) until the moisture content was minimum. The homogenized, and dried liver were wet ashed and metal levels were determined.

6.2.3. Preparation of diet.

Rat feed obtained from M/s. Lipton India, Bangalore were pulvarised and mixed with calculated quantities of vacuum dried cuttlefish liver so as to contain 40 ppm cadmium in the mixed diet. A second diet containing the basal diet supplemented with $CdCl_2$ to a dietary level of 40 mg Cadmium/Kg was also prepared. A control diet without any Cd content was also

prepared. The lipid level was made equal by adding pig liver in control diet and in $CdCl_2$ diet. The proximate and nutrient composition of the three diets were determined by standard methods and presented in Table 6.1.

6.2.4. Experiment

Albino rats were separately caged for acclimatization for a period of five days and fed with control diet and sufficient quantity of drinking water. After acclimatization, the animals were weighed and caged separately. Healthy animals of the same weight range (72-78 g) were selected for the study in order to eliminate the effect of size difference in toxicity. The animals were fed with respective diets – diet without cadmium for control rats (Diet C), diet containing $CdCl_2$ (Diet A) and diet with cuttlefish liver (Diet B). These three diets would be designated as Diet C, Diet A and Diet B. The animals were also supplied with required quantity of water. The quantity of the diet was increased daily depending upon the requirement. Following the initial weight, the weight of the animals were recorded every week. The food intake, water consumption and the volume of urine in individual cages were recorded daily.

6.2.5. Termination of feeding trials.

After six weeks of feeding, the animals were killed by placing in an ether atmosphere. Toxicity evaluation was carried out by haematological, histopathological and metal uptake studies. Muscle, liver and kidney were dissected and the metal levels of cadmium, copper and zinc were

determined using Flame Atomic Absorption Spectrophotometer. Blood samples were collected for Haematological analysis in heparinized bottles. The tissue samples were processed and embedded in paraffin wax, sectioned at 5μm, stained with Haematoxylin and Eosin, and then examined microscopically by standard procedure as described in Chapter 2.

6.3. Results

Average body weight of control and experimental albino rats are presented in Table 6.2. Determination of pH of the urine of control and experimental albino rats are presented in Table 6.3. Concentration of nutrient elements (Ca, Na and K) in the urine of albino rats fed with cadmium incorporated diets as well as control diets are shown in Table 6.4. The metal concentrations in the body components of control and Cd-fed albino rats are presented in Table 6.5. Results of the haematological analysis of experimental albino rats fed with diets A, B and C are presented in Table 6.6. Photographs of liver and kidney tissues of control and experimental albino rats are shown in Plates 6.1 to 6.4.

Observation of the experimental albino rats indicated that all rats appeared healthy and no sign of anaemia was revealed. The growth rate as manifested by overall weight of the animals were similar, without significant difference except in albino rats fed with diet A which had comparatively lower weight than rats fed with diet B and diet C (Table 6.2).

The pH of urine of control albino rats ranged from 9.15 to 9.31and in experimental rats, the pH ranged from 8.96 to 9.29. There is not much significant difference in pH in the urine of experimental and control albino rats. The colour of the urine at different experimental cages were also similar, but slightly intense dark brown colour was seen in rats fed with cadmium diet.

6.3.1. Levels of nutrient elements (Ca, Na, K) in the urine of albino rats fed with cadmium incorporated diet.

Concentration of nutrient elements viz., Ca, Na and K in the urine of albino rats fed with diet containing inorganic and muscle bound Cd at 40 ppm are presented in Table 6.4. Results of the study showed that excretion of Ca through urine of rats fed with Cd diets showed higher values than that present in control animal. At '0' day (beginning of the experiment), Ca level in the urine of control as well as experimental animals were 14.01, 13.98 and 14.09 μg. At the end of six weeks, the calcium excretion increased considerably in experimental rats from the initial level of 13.98 to 57.58 μg (diet A) and from 14.09 to 63.90 μg (diet B). While, in control rats (diet C) the level increased from the initial value of 14.01 to 35.65 μg, only.

It can be seen that there is significant increase in Ca excretion in both the experimental rats fed with diet containing either $CdCl_2$ and liver bound cadmium. Thus, inorganic Cd ($CdCl_2$) as well as organically bound Cd (liver bound Cd) are toxic to albino rats. The toxic effect of Cd in the diet (both organically bound and inorganic) to the albino rats may probably

be attributed to interference in Ca metabolism and affecting the liver function. Similar is the case with other two metals namely, sodium and potassium where the higher level of excretion may be due to alterations in the functioning of kidney. The higher level of excretion of all the nutrient elements was observed in the case of diet A animals also, suggesting that both organically bound Cd and inorganic Cd do play the same role. While, the excretion of Na and K in rats fed with diet B is similar to that of control group (diet C) may be explained as due to the excess level of Na and K in the cuttlefish liver (Table 6.4).

6.3.2. Metal accumulation in experimental albino rats.

At the termination of the experiment after six weeks, the animals were killed by ether anesthesia and muscle, liver and kidney were dissected and separated. Metal levels of cadmium, copper and zinc were determined using Flame Atomic Absorption Spectrophotometer following wet digestion of the body parts of albino rats. Results are presented in Table 6.5. Concentration of cadmium in the muscle of control albino rats (diet C) was 0.09 ± 0.08 ppm while, albino rats fed with $CdCl_2$ diet (diet A) and the rats fed with cuttlefish liver incorporated diet (diet B) had cadmium content of 0.13 ± 0.11 and 0.33 ± 0.29 ppm, respectively. Results showed that mean cadmium content in the muscle tissue of rats fed with cuttlefish liver bound cadmium had higher level than $CdCl_2$ fed groups. In liver tissue also, cadmium concentration was higher in rats fed with diet A and diet B (Table

6.5). The kidney of rats fed with diet A showed slightly higher concentration of Cd (5.88 ppm) than rats fed with cuttlefish liver bound cadmium (5.78 ppm). The distribution pattern of Cd in the body components of albino rats were in the order kidney > liver > muscle. Rats fed with cuttlefish liver bound cadmium was observed to have higher concentration of cadmium in muscle and liver than diet A fed animals.

Copper content in the muscle of three groups of albino rats viz., control, diet A and diet B were 0.24 ± 0.19, 0.76 ± 0.74 and 0.88 ± 0.77 ppm, respectively. Copper content in the liver of albino rats fed with diet A had exhibited higher level of Cu (3.53 ± 0.25 ppm) compared to animals in other experimental set up (diet C and B). The copper content in the kidney increased considerably in both cadmium fed groups. The values were 5.18 ± 3.18, 7.25 ± 2.67, 7.39 ± 2.67 ppm, respectively in control groups, diet A fed rats and diet B fed rats. The distribution pattern of Cu being kidney > liver > muscle.

The zinc content in the muscle of albino rats maintained in the three experimental cages, viz., diet C, diet A and diet B, respectively were 5.15 ± 0.12, 5.26 ± 2.02 and 11.79 ± 4.67 ppm at the end of six weeks. Mean zinc content in the liver of cadmium treated rats (diet A and diet B) had significantly higher levels (34.19 and 70.75 ppm) than control rats (18.74 ppm). Similarly, Zn content in the kidney tissues of Cd-treated rats

were more or less equal and higher than Zn content in the control rats (10.23 ppm).

Results of the present indicated that Cd, Cu and Zn levels in muscle, liver and kidney of experimental albino rats fed with cadmium incorporated diets increased considerably during the experimental period and implicated toxicity in albino rats.

6.3.3. Haematological Evaluation.

The results of the haematological analysis of control and experimental albino rats are presented in Table 6.6 along with standard values for these parameters. The blood samples were examined for Hb content, Packed Cell Volume (PCV), Red Blood Cells (RBC), Total leucocytes count (TC), Differential leucocytes count (DC) and Platelet count at the Doctors Diagnostic Centre. The haematological observation clearly indicated lower Hb content, Packed Cell Volume (PCV), total and differential leucocytes count compared to control animals (Table 6.6), indicating that liver bound Cd and inorganic Cd are toxic to albino rats. The Hb content decreased from control value of 14.2 gm/dl to 12.6 gm/dl in both cases of experimental rats during six weeks period. Haemoglobin content showed a significant reduction in experimental animals compared to control animals. Packed Cell Volume (PCV) also decreased considerably in experimental animals to the extent of 39 and 38% respectively, in albino rats fed with diet B and A. WBC count also declined and reduced by 40%

in diet B animals and 60% in diet A animals. These results clearly indicated cadmium toxicity in albino rats. There is no apparent reduction in the platelet count in the experimental animals (liver fed diet) compared to control animals. However, in the inorganic cadmium incorporated diet (Diet A), platelet count showed a decrease and the value declined to 1.4 lakh from control level of 1.5 lakh.

6.3.4. Histopathological study.

The liver and kidney tissues of experimental and control albino rats were prepared for light microscopic examination and histochemical studies. Observations of the slides of control group of kidney and liver section showed normal architecture of tissues (Plate 6.1 and 6.2). The liver and kidney section of experimental albino rats fed with cadmium incorporated diets showed histomorphological alterations. The kidney tissues of experimental rats (Cd - 40 ppm) showed congestion and shrinkage of glomeruli and adhesion to the bowman's capsule (Plate 6.3). Similarly, liver tissues of Cd-fed albino rats showed the presence of pyknotic nuclei and mild bilary epithelial proliferation (Plate 6.4). Accumulation of leucocytes around necrosed area and focal area of necrosis was also seen.

6.4. Discussion.

During the course of the experiment, the animals all gained weight. However, the net gain in animals fed with diet A had a lower weight

compared to control as well as diet B fed animals (Table 6.2). This decrease in weight may be attributed to the adverse effect of $CdCl_2$ diet to the albino rats. Another reason may be due to the utilization of fat deposits by the animal for the synthesis of glucose as $CdCl_2$ increased the activities of glucogenic enzymes (Chapatwala *et al.*, 1982). A slight lower value of pH of urine was noticed in the beginning of the experiment in the rats fed with diet A and later, the pH of urine in all the cages were found to be similar. There was no abnormal value of pH in any of the experimental rats. However, the colour of urine in experimental animals was more intense compared to control rats. The general activity, feed consumption, water intake etc did not indicate any significant difference in animal behaviour.

The levels of Ca, Na and K in the urine of experimental rats clearly indicated considerable difference between control and experimental rats. There was a significant increase in Ca excretion in both the experimental rats fed with diet containing cadmium (Table 6.4). In control and diet B fed animals, the excretion of Na and K was similar. However, the excretion of Na and K was more in diet A fed animals. The increased excretion of these metals in $CdCl_2$ diet animals may be induced by cadmium chloride. Groten *et al.*, 1990 also observed a similar toxic effect in albino rats fed with liver incorporated Cd. A comparative excretion of Na and K in control and cuttlefish liver bound Cd may be attributed to the excess level of Na and K in the cuttlefish liver.

Haematological analysis indicated a lower Hb content, Packed Cell Volume (PCV), Total count and Platelet count compared to control animals. These results indicated that both organically bound cadmium and inorganic cadmium had adverse effect on the health of albino rats. Although nephrotoxicity was regarded as the characteristic toxic effect, decreased haemoglobin concentration and decreased packed cell volume are considered as the early signs of toxicity (Elinder, 1986). A similar reduction in Hb, RBC and PCV was reported by Groten *et al.* (1990) in rats fed with diets containing tissue-incorporated cadmium and cadmium salt in four weeks old experiment. Mary *et al.* (1997) also reported a similar reduction in Hb, RBC, PCV and DC count in mice administered with lead nitrate for 21 days.

During the course of six weeks study in albino rats, it was observed that there was significant uptake of metals from the diet by albino rats. The analysis of body components of albino rats viz., muscle, liver and kidney for toxic metals (Cd, Cu and Zn) indicated considerable levels for these metals in experimental animals compared to control group (Table 6.5). There was significant increase in the level of Cd in the liver and kidney of experimental albino rats fed with both diet A and diet B; the uptake by kidney may be slightly higher than compared to liver (Table 6.5). Copper content also increased significantly in the liver of experimental rats. However, metal uptake by the muscle tissue was minimum. Rats fed with diet B (cuttlefish liver) had higher levels of these metals in all the body components

analysed. Again, Zn content in the rats fed with diet B were very high (68.8 to 91.10 ppm). The higher levels of Zn and other metals in cuttlefish liver fed albino rats may be attributed to the natural form of the metals in the liver and hence a more preferred diet of the albino rats. Extent of toxic effects of $CdCl_2$ and cuttlefish liver bound cadmium differs possibly because of the differences in the cadmium concentrations in the liver and kidney. Moreover, the sensitivity of the target organs may also be different to the toxic compounds (Groten *et al.*, 1990). Similar findings have been reported by Maitani *et al.* (1984) in mice after a single oral administration of $CdCl_2$ or liver-incorporated cadmium.

Liver and kidney tissues of albino rats fed with diet A and diet B showed similar histopathological changes. Histomorphological alterations in the liver and kidney of albino rats have been reported by several workers (Hoffman *et al.*, 1975; Itokawa *et al.*, 1978; Weigal *et al.*, 1984; Dudley *et al.*, 1985; Elinder, 1986 and Anderson *et al.*, 1988). Chaterjee *et al* (1996) reported degenerative changes of hepatocytes, widening of the bowman's space in the cortical region of kidney, necrosis and degeneration of tubular epithelium in rats treated with $CdCl_2$ (1 mg/Kg/day) for four weeks as observed in the present study. Anderson *et al.*, 1988 found histopathological changes in the livers of mice exposed for ten days to a single dose of $CdCl_2$ (30 mg/Kg body weight), and Elinder (1986) reported, that for detecting the long term effects of cadmium, liver morphology is a

more sensitive parameter. Dudley *et al.* (1985) noted morphological signs of toxicity only after four weeks of exposure to cadmium.

In the present study, cadmium treated groups showed histopathological changes in liver and kidney tissues. As liver is an important detoxifying organ, cell damage affects normal metabolic cycle of the liver (Metabolism of fat, synthesis of vitamin K, serum protein etc may be affected). These histomorphological alterations clearly indicate the deleterious effects of Cd to normal functioning of liver and kidney (plate 6.3 and 6.4) and there by its pathological impact on the cephalopod consumer.

Table 6.1: Proximate and nutrient composition of diet for experimental albino rats

Proximate composition			
Constituents	**Control (Diet C)**	**Rat feed+$CdCl_2$ (Diet A)**	**Rat feed+Cuttlefish liver (Diet B)**
Moisture (%)	11.42	13.38	12.68
Protein (%)	60.96	60.04	57.85
Fat (%)	4.72	5.01	3.38
Carbohydrate (%)	15.88	14.13	18.73
Ash (%)	7.02	7.44	7.36
Nutrient composition (µg/g)			
Calcium	2.21	2.36	2.54
Sodium	0.20	0.22	0.06
Potassium	0.14	0.13	0.19
Trace metal composition (ppm dry wt. basis)			
Cadmium	4.49	40.25	41.41
Copper	49.39	13.94	21.64
Zinc	42.97	33.42	50.88

Table 6.2: Body weight of control and experimental albino rats fed with Cd incorporated diet. (Expressed in g)

Duration of exposure (weeks)	Control (Diet C)	Rat feed+$CdCl_2$ (Diet A)	Ratfeed+Cuttlefish liver (Diet B)
1	72	76	77
2	82	82.75	89
3	122	117	126
4	153.1	145.35	155.2
5	180	167.2	179.5
6	200	185.4	198.3

Table 6.3: pH of the Urine of control and experimental albino rats fed with Cd incorporated diet

Duration of exposure (weeks)	Control (Diet C)	Rat feed+$CdCl_2$ (Diet A)	Rat feed+Cuttlefish liver (Diet B)
1	9.15	8.96	9.13
2	9.11	9.22	9.18
3	9.24	9.21	9.26
4	9.17	9.31	9.30
5	9.29	9.31	9.31
6	9.31	9.29	9.26

Table 6.4: Concentration of nutrient elements in the urine of albino rats fed with Cd incorporated diet. (Expressed in μg/day)

Duration of exposure (weeks)	Control (Diet C)	Rat feed+$CdCl_2$ (Diet A)	Rat feed+Cuttlefish liver (Diet B)
		Ca	
1	14.01	13.98	14.09
2	14.57	9.45	5.60
3	16.58	15.45	21.75
4	20.55	25.91	22.50
5	23.0	34.01	33.80
6	35.65	57.58	63.90
		Na	
1	19.09	18.98	19.09
2	19.80	12.0	8.40
3	13.0	12.86	28.50
4	15.0	18.87	15.63
5	23.0	18.70	22.20
6	39.53	79.9	33.30
		K	
1	70.09	68.89	69.10
2	71.50	58.12	28.0
3	56.87	72.25	42.72
4	59.50	78.19	57.80
5	68.10	61.87	69.0
6	75.55	106.76	74.70

Table 6.5: Concentration of trace metals in the body components of control and experimental albino rats fed with Cd incorporated diets (Mean ± S.D., Range, ppm wet wt)

Metal	Body components	Control (Diet C)	Rat feed+$CdCl_2$ (Diet A)	Rat feed+Cuttlefish liver (Diet B)
CADMIUM	Muscle	0.09 ± 0.08 (0.12 - 0.15)	0.13 ± 0.11 (0.13 - 0.25)	0.33 ± 0.29 (0.45 - 0.55)
	Liver	0.05 ± 0.04 (0.07-0.09)	3.24 ± 1.51 (0.76-4.33)	5.12 ± 0.06 (5.03-5.18)
	Kidney	0.12 ± 0.13 (0.13 - 0.25)	5.88 ± 1.05 (4.62 - 6.81)	5.78 ± 1.58 (3.01 - 6.82)
COPPER	Muscle	0.24 ± 0.19 (0.04 - 0.43)	0.76 ± 0.74 (0.04 - 2.15)	0.88 ± 0.77 (1.29 - 1.37)
	Liver	2.95 ± 1.85 (0.82 - 4.12)	3.53 ± 0.25 (3.26 - 3.84)	3.17 ± 1.18 (1.06 - 3.77)
	Kidney	5.18 ± 3.18 (1.49 - 7.08)	7.25 ± 2.67 (3.28 - 9.46)	7.39 ± 2.67 (5.0 - 11.97)
ZINC	Muscle	5.15 ± 0.12 (5.05 - 5.29)	5.26 ± 2.02 (1.85 - 7.33)	11.79 ± 4.67 (6.40 - 14.43)
	Liver	18.74 ± 3.96 (16.39 - 23.32)	34.19 ± 4.03 (30.12 - 40.80)	70.75 ± 19.20 (68.80 - 91.10)
	Kidney	10.23 ± 2.49 (7.36 - 11.77)	17.14 ± 3.30 (14.15 - 22.77)	16.76 ± 7.21 (5.87 - 23.4)

Table 6.6: **Haematological analysis of experimental albino rats fed with diets containing cuttlefish liver with bound cadmium at 40 ppm and inorganic cadmium as $CdCl_2$ (40 ppm)**

Parameter tested	Std. Value*	Control (Diet C)	Rat feed+Cuttlefish liver (Diet B)	Rat feed+$CdCl_2$ (Diet A)
Packed cell volume Average % Range	46 39-53	44	39	38
Haemoglobin gm/dl Average % Range	14.6 12-17.5	14.2	12.6	12.6
RBC Average % Range	$8.9x10^6$ mm^3 $7.2 - 9.6 x10^6$	$5.7 x10^6$	$5.0 x10^6$	$5.1 x10^6$
WBC Total count DC	$6\text{-}12x10^3$ mm^3	5000 P-17%, L-83%	2000 P-5%, L-95%	3000 P-12%, L-88%
Platelet count (in mm^3)	$340 x10^3$	1.5 lakh	1.6 lakh	1.4 lakh

* Source : Indian Council of Medical Research (1980)

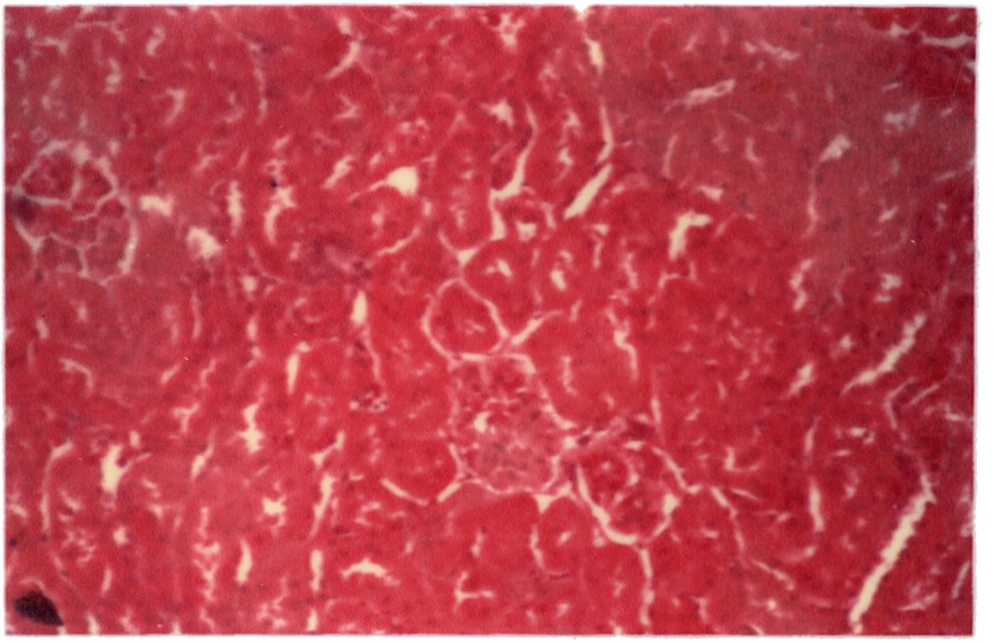


Plate 6.1. ***Microphotograph of kidney tissue of control albino rats*** **(H.E. x 20)**

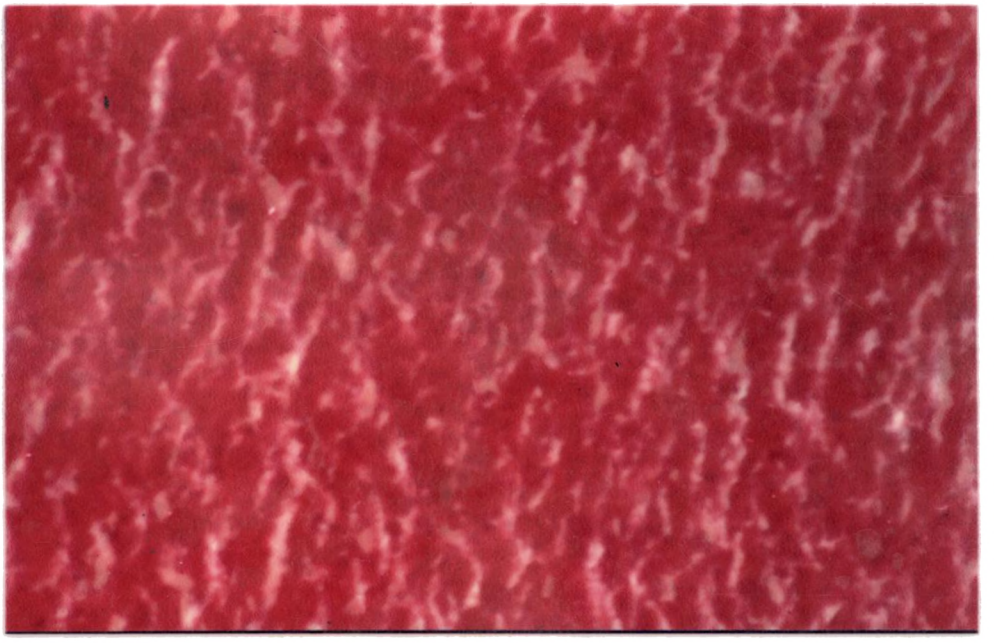


Plate 6.2. ***Microphotograph of liver hepatocytes of control albino rats*** **(H.E. x 20)**

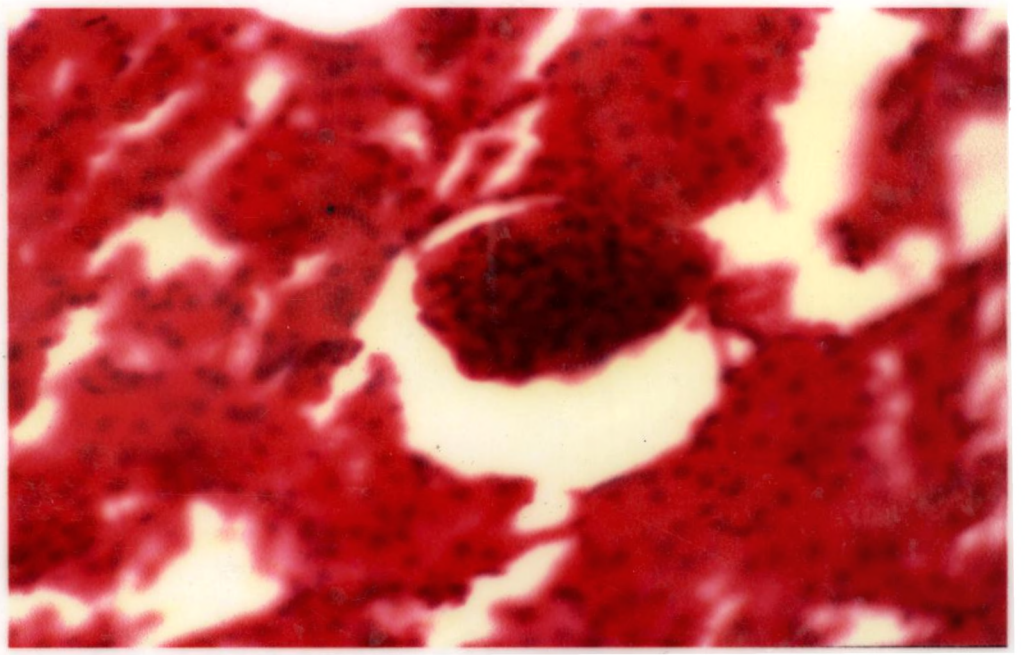


Plate 6.3. ***Microphotograph of kidney tissue of experimental albino rats fed with diet containing Cd at 40 ppm level showing shrinkage of glomeruli*** **(H.E. x 20)**

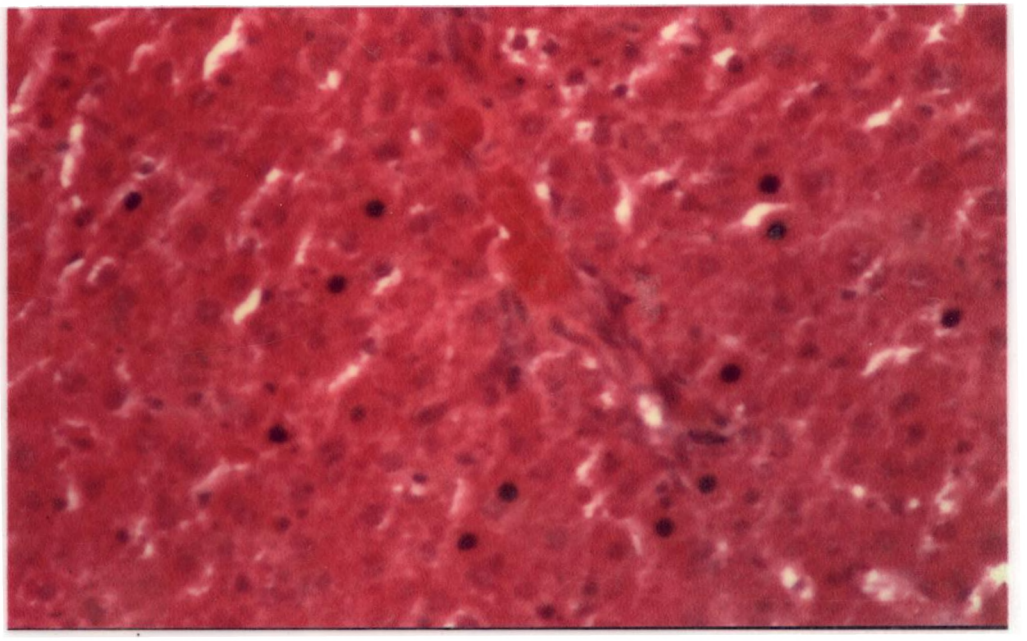


Plate 6.4 ***Microphotograph of liver tissue of experimental albino rats fed with diet containing Cd at 40 ppm level showing pyknotic nuclei and mild bilary epithelial proliferation*** **(H.E. x 20)**

SUMMARY OF RESULTS

Summary of Results.

The presence of higher levels of cadmium and other toxicants in the economically important class of cuttlefish caused concern during the past few decades. Environmental contaminants not only endanger fish resources but can also reach human body through seafood. There is, therefore a need to improve the quality and safety of seafood products in the international market as well as for domestic trade. Developed nations are very much concerned over the safety of food items which may contain pesticide residues, harmful chemicals, heavy metals, bio-toxins, pathogenic organisms etc causing health hazards. Many consignments of cuttlefish exported from India were rejected or detained by European Union on the ground that the samples had higher levels of cadmium or salmonella contamination. To meet the global requirements in seafood trade, India has to produce safe and high quality products. With the implementation of EU directive (EU/91/493/EEC) and the US regulations of 1997, it has become mandatory to monitor the levels of various hazards in seafoods.

This thesis presents a comprehensive account of the baseline data of important trace metals, viz., Hg, Cd, Pb, Cu, Zn, Fe, Mn, Cr and Ni in the edible and non-edible body components of cuttlefish (*Sepia pharaonis*) caught along the west coast of India viz., Cochin, Quilon, Mangalore and Mumbai region. The results of the study are presented in different chapters (Chapters 3 to 6). The thesis deals with regional and seasonal variations of

different trace metals, their distribution in the different body components of cuttlefish caught along the west coast of India. The study also deals with the levels of metals in food fishes, habitat water and their relation to metal levels in cuttlefish. Animal feeding study was also carried out in experimental albino rats using cuttlefish liver incorporated diets and observing the haematological and histopathological changes in the cells.

Chapter 3 deals with distribution of heavy metals in cuttlefish caught off Cochin, Quilon, Mangalore and Mumbai regions and their regional variation. The essential and non-essential metals in muscle of cuttlefish varied from region to region. The geographic trend of ΣHg in muscle of cuttlefish were Cochin > Mumbai > Mangalore > Quilon. Metal levels in cuttlefish (eg. Cd, Cu, Zn and Cr) exhibited significant regional variation and the level of significance ranged between 1 to 5%. There is no significant difference in Cd content in the muscle and liver of cuttlefish between the regions. Cuttlefish from Quilon region exhibited significantly higher copper in muscle ($p<0.01$) and liver ($p<0.05$) when compared to Cochin, Mangalore and Mumbai region. Zinc content in the muscle and liver of cuttlefish from Quilon region were significantly higher ($p<0.05$ and $p<0.01$) when compared to other regions. Mangalore region exhibited the highest mean values of Pb and Cr in the muscle followed by Cochin, Mumbai and Quilon region, respectively.

The distribution pattern of Cd and other metals indicated, that liver formed the major site of metal accumulation. Average levels of cadmium in the edible muscle of cuttlefish was <3ppm. In general, the pattern followed was liver > ink > gills > skin > tentacles > muscle. The distribution of lead showed that the highest level of the metal was found in ink at all stations; pattern of distribution were in the order ink > liver > muscle > gills > skin > tentacles. It can be seen that, the whole soft parts of the cuttlefish invariably exhibited higher levels of all the metals than found in the muscle. More than 90 and 65% of whole cuttlefish samples had Cd and Pb content above the tolerance limit, respectively. However, the mean content of all the metals analysed were significantly lower in the edible parts and far below the tolerance limits.

Chapter 4 deals with the seasonal variation of metals in cuttlefish (*Sepia pharaonis*) at the different regions. Results of the analysis of metal levels from the three regions indicated significant seasonal variation for almost all the metals in the edible muscle, liver and some of the body components. Edible muscle invariably exhibited lower levels of metals, during all the seasons. Results of Analysis of Variance (ANOVA) of some of the toxic and essential metals (eg. Cd, Cu, Zn and Cr) during different seasons in the edible muscle and liver of cuttlefish are presented in Tables 4.2 to 4.9. The level of significance ranged between 1 to 5%. Monsoon season showed significantly higher values for cadmium ($p<0.01$) in muscle and liver in comparison with premonsoon and postmonsoon season.

Copper content in muscle ($p<0.01$) and liver ($p<0.05$) is significantly higher during monsoon periods. There is no significant difference in zinc content in the muscle and liver of cuttlefish between the seasons. The mean value of Fe in muscle were high during monsoon period, at all stations and followed the order: monsoon > postmonsoon > premonsoon.

Premonsoon season is having significantly higher values of Cr ($p<0.05$) and Fe in liver than other seasons at three regions. Liver formed the major site of heavy metal accumulation in samples from all the three regions; irrespective of seasons. Pb content in the edible muscle of cuttlefish was below the tolerance limit (1.5 ppm) in samples from all stations during different seasons. Distribution pattern of Ni varied with seasons and in muscle, ink and tentacles higher levels were noted during monsoon season from Quilon region. In general, higher levels of most metals were noted during monsoon period.

Chapter 5 attempts to establish the source of cadmium and other toxic metals in cuttlefish by determining metal levels in fishes separated from the mantle cavity of cuttlefish and also fishes caught from the same trawl area. The exhaustive data on trace metals are presented in various tables. Regression analysis were carried out between levels of metals in cuttlefish and levels found in food fishes separated from the mantle cavity. Significant positive correlation (varied from 5% to 10%) existed between metal levels in food fishes and metal levels in cuttlefish. For instance,

Cadmium content in *Nemipterus japonicus* (r = 0.989, p<0.05), *Saurida tumbil* (r = 0.764, p<0.05) and *Cynoglossus* sp (r = 0.593, p<0.10) showed significant positive correlation with the levels of metals in cuttlefish. Similarly, Cu content in *N. japonicus* (r =0.704, p<0.05), *Saurida tumbil* (r = 0.930, p<0.05) and *Cynoglossus* sp (r = 0.912, p<0.05) showed significant positive correlation with metal levels in cuttlefish. The level of significance are indicated in brackets against each species.

Dactyloptera orientalis and *Saurida tumbil* from the same habitat area also showed significant positive correlation: the levels of cadmium and zinc in *D.orientalis* and *Saurida tumbil* were r = 0.710, p<0.05 for Cd; r = 0.586, p<0.10 for Zn in *D.orientalis* and r = 0.609, p<0.10 for Cd; r = 0.821, p<0.05 for Zn in *S. tumbil*. The regression equations for metal levels in various species of fish versus metal levels in cuttlefish are as follows:

Regression analysis of metal levels in cuttlefish Vs metal levels in food fishes.

Food fishes			
Nemipterus japonicus			
Cadmium	y = 1.98 + 1.10 x	r = 0.989	p<0.05
Copper	y = 7.34 + 1.85 x	r = 0.704	p<0.05
Zinc	y = -5.13 + 1.31 x	r = 0.715	p<0.05
Chromium	y = 0.58 + 0.41 x	r = 0.560	p<0.10

Saurida tumbil			
Cadmium	y = -8.32 +1.85 x	r = 0.764	p<0.05
Copper	y = 6.85 + 1.72 x	r = 0.930	p<0.05
Cynoglossus			
Cadmium	y = -0.31 + 1.76 x	r = 0.593	p<0.10
Copper	y = -40.12 + 2.98 x	r = 0.912	p<0.05
Zinc	y = 7.45 + 0.65 x	r = 0.720	p<0.05
Fishes from Habitat water			
Dactyloptera orientalis			
Cadmium	y = 0.31 + 0.08 x	r = 0.710	p<0.05
Zinc	y = -8.90 + 5.43 x	r = 0.586	p<0.10
Saurida tumbil			
Cadmium	y = 0.08 + 0.81 x	r = 0.609	p<0.10
Zinc	y = -5.92 +1.57 x	r = 0.821	p<0.05

The results of the regression analysis indicated that the metal levels in cephalopods is very much influenced by metal levels in food fishes and thus indicating one of the probable source of cadmium and other toxic metals in cuttlefish. The levels of Cd, Cu, Zn, Pb and Cr determined in the habitat water were at normal level and there is no indication of pollution in the environment by heavy metals.

Chapter 6 presents the results of evaluation of toxicity of liver bound cadmium in experimental albino rats and its histological and haematological

effects. The results showed that both organically bound and inorganic cadmium was toxic to the animal. Haematological analysis indicated a lower Hb content, Packed Cell Volume (PCV), Total count and Platelet count compared to control animals. The liver and kidney sections of experimental albino rats fed with cadmium incorporated diets showed histomorphological alterations.

The changes observed were:

Kidney

- Congestion and shrinkage of glomeruli
- Thickening of Bowman's capsule.

Liver

- Mild bilary epithelial proliferation
- Presence of pyknotic nuclei.

These histomorphological alterations clearly indicated the deleterious effects of Cd to normal functioning of liver and kidney and thereby its pathological impact on the cephalopod consumer.

There was accumulation of toxic metals (Cd, Cu and Zn) in the liver and kidney of experimental albino rats. The level of Cd, Cu and Zn in muscle, liver and kidney of experimental albino rats fed with cadmium incorporated diets increased considerably during the experimental period. Liver and kidney tissues of experimental albino rats was the major site of

metal accumulation, indicating that higher levels of toxic metals in cephalopods can cause hazard to the consumer.

The source of Cd and other toxicants has to be further investigated and the reason for the strange phenomenon of cadmium selective accumulation by cuttlefish has to be unravelled so as to get a better understanding of higher level of Cd in cephalopods.

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