INVESTIGATIONS ON THE DISTRIBUTION CHARACTERISTICS OF HEAVY METALS IN SQUID (*LOLIGO* SPP.) IN RELATION TO LEVELS IN FOOD FISHES FROM THE WEST COAST OF INDIA WITH A PERSPECTIVE ON SEAFOOD SAFETY

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IN

MARINE SCIENCES

BY

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DECLARATION

I, Prafulla.V, do hereby declare that the thesis entitled **"Investigations on the Distribution Characteristics of Heavy Metals in Squid (*Loligo* spp.) in Relation to Levels in Food Fishes from the West Coast of India with a Perspective on Seafood Safety"** is a genuine record of research work done by me under the 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.

Prafulla. V.

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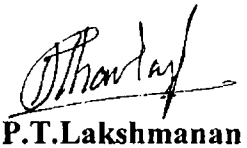
30th December, 2002.

CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried by Ms. Prafulla.V, 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.



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CHAPTER 1

Introduction

Environmental deterioration of the aquatic world due to heavy metals contamination commenced hand in hand with modernization, but it is only recently that man became aware of how trace metals in the aquatic environment could create deleterious effects on the aquatic flora and fauna.

The potential hazards of heavy metal pollution was triggered off by the Minamata disaster caused by the consumption of mercury contaminated shellfish and finfish from Minamata Bay (Nitta, 1972) and with the affliction of *Itai itai* disease caused by the consumption of foodstuffs contaminated with cadmium in Japan. Since then, there has been a spectacular rise in interest in the evaluation of trace metal concentrations in marine products.

The term 'trace metal' is used in current literature to designate the elements which occur in small concentrations (>100 ppm) in natural biological systems. For practical purposes, terms such as 'trace metals', 'trace inorganics', 'micronutrients' and 'trace elements' will be treated as synonymous with the term 'heavy metals' (Wittmann,1979).

At natural concentrations, trace elements either constitute the prosthetic group of enzymes or function as enzyme activators, but at elevated concentrations they act as inactivators of enzyme systems and as protein precipitants (Nair, 1984). The trace metals of serious concern include metals like Mercury (Hg), Cadmium (Cd), Lead (Pb),

Chromium (Cr), Nickel (Ni), Arsenic (As) and Selenium (Se). These cause injury to health through progressive and irreversible accumulation in the body as a result of ingestion in repeated small amounts. Accordingly, Pringle *et al.*, 1968 classified the metals into having (i) very toxic-effects (concentration below 1 ppm), (ii) moderately toxic-effects (concentrations between 1 and 100 ppm) and (iii) scarcely toxic-effects (rarely appear). The priority list of pollutants compiled by the Environmental Protection Agency of United States contains the eight widespread heavy metals – arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc (Moore & Ramamoorthy, 1984). It has been observed that the environmental levels of many of these metals are constantly being raised by an increased influx from different sources.

In general, it is possible to distinguish five different sources from which metal pollution of the environment originates: (i) weathering of rocks (ii) industrial processing of ores and metals (iii) effluents from metallurgical industries (iv) leaching of metals from garbage, solid waste dumps, sewages, dumping of wastes from tankers and cargo ships, and (v) other anthropogenic sources. In natural waters, metals may be present as dischargeable species, as soluble complexes, inorganic or organic particles and as adsorbed particulate matter.

In molluscs, particularly filter feeding bivalve molluscs are notorious for concentrating trace metals both from the environmental

water as well as through food web. These species reflect the concentration of heavy metals in the surrounding medium.

1.1. Trace metals in biota and their environment

In marine organisms, trace metal uptake occurs directly from the surrounding sea water across the permeable body surface, from food and imbibed sea water entering the gut (Depledge *et al.*, 1994). Essential metals such as Cu, Zn, Fe and Co form either an electron donor system or function as ligands in complex enzymatic compounds in animal body. 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. 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.

In contrast to the essential metals, non-essential metals bind to the metabolically active groups such as amino, sulphydryl-, carboxyl-, phenolic- or phosphoryl- groups. Such high affinities for biological molecules provide heavy metals with the potential to play toxic roles by substituting for an essential metal of lower affinity and distorting the geometry of the molecules. The toxicity of a metal is mainly determined by its ionic size, electron affinity, electron negativity,

stability, solubility and biological reactivity. The impact of heavy metals is caused mostly by the ionic forms which directly interact with or indirectly attack the structural and genetic make up of cells. Under normal circumstances, these metal ions are detoxified intracellularly by various processes which may involve the induction of antioxidant enzymes, induction of certain proteins such as metallothionein, ferritin and heat shock proteins. Although these detoxification processes are effective against toxicity of metals, they may become saturated and do not provide a permanent protection for chronic or excess exposure to determinal metals and hence metals tend to bioaccumulate in organisms. Bioaccumulation of toxic metals over a period of time, along the food chain at very high proportions from very low concentrations in water and sediment initiates harmful toxic effects in man and animals. Cd, Cr, Hg, Ni, Pb, Zn and As are known to produce a broad spectrum of lethal effects which includes histopathological changes, deformities and biochemical alterations. Symptoms range from diarrhoea, nervous disorder, gastrointestinal ulceration, loss of memory, tissue damage, respiratory failure, osteomalacea kidney dysfunction, liver necrosis, anaemia to hypertension. Thus, toxic metals exert their nefarious web to all the systems and functions of the body of marine organisms and man. In the aquatic environment, poisons especially salts of heavy metals exert a depressive action on fish. Most of them produce toxic effects through physical accumulation. Cr at 10 ppm levels in water is

considered to be toxic to several species of algae. Pb and Se destroy the erythrocytes. In the case of Cu, Hg and Ag the toxic effect is observed even at concentrations of 0.02 to 0.04 mg/L (Metelev *et al.*, 1983). In Ni and Zn poisoning the gills of the fish are covered with mucous and turn dark in colour. Pb acts haemolytically and As paralytically. Cu in excessive amounts causes haemolysis, hepatotoxic and nephrotoxic effects. In humans also these toxic metals have similar deleterious effect. It is in this light; monitoring of heavy metals in marine food fishes, safety of seafoods for consumption and consumer health attains greater importance.

Heavy metal levels in a few fishes, crustaceans and molluscs (gastropods, pelecypods) and environmental waters have been amply reported upon. However, concentrations of cadmium and other trace metals in cephalopods, one of the essential links in the marine trophic chains (Amaratunga, 1983; Rodhouse, 1989; Rodhouse & White, 1995) have rarely been documented. Studies addressing trace metal content in cephalopods from India received an added impetus in the late 80's when many of our consignments exported to Italy, France, Germany and Spain were rejected/detained. Later in 1994 -1995 three shipments of frozen whole cleaned squids valued at 30,750 US$ were detained by United States Food and Drugs Administration (USFDA). A shipment of whole cleaned squids (23 t) sent to Italy was again returned in 1996 due to the presence of high contents of Cd and *Salmonella*. As a result, presence of high levels of Cd in the

economically important class of cephalopods has caused serious concern in the export industry. In the light of this, investigations were carried out by Lakshmanan (1988a, b; 1989) and Lakshmanan & Stephen (1993) in seafood products particularly in cephalopods. The survey indicated that finfishes in general had only lower levels of trace metals when compared to squids and cuttlefishes. The cephalopods showed an unique phenomenon of bioaccumulation of Cd and other trace metals in different organs of the body. Cephalopods being predators themselves feed on a wide range of other marine animals particularly crustaceans, molluscs and fishes and hence there exists the possibility of bioaccumulation of pollutants through food chain. With the introduction of the European Union (EU) directives on seafoods, EU/91/EEC and the US regulation 1997, monitoring of chemical hazards, toxic metals, pesticides, *etc.,* have become mandatory. The global challenges in the seafood export trade have also brought to the fore the need to ensure that our fishery products are both safe and comply with the International quality requirements and standards. Cephalopods being an important component of the marine exports from India, a detailed study on trace metal uptake by squids are warranted. Monitoring of their marine environment, their probable source and nature of toxicity on the consumers form part of the study. These information are of great significance for boosting the export potential of this seafood delicacy in the global market.

Wholesome and safe cephalopod products would certainly enhance the market potential especially in the overseas market.

1.2. Cephalopod fishery of India

Cephalopods are exclusively marine predators and voracious carnivores with very high metabolic and conversion rates. They feed on live prey throughout their life cycle. There are about 650 species of squids in the world oceans, which are diverse in form, size, and nature (Voss, 1973; Worms, 1983). Of these, 80 species are of commercial and scientific interest in the Indian seas (Silas, 1968; Sarvesan, 1974; Varghese, 1976). This rich cephalopod fauna constitute one of the important exploited marine fishery resources of our country. The average annual cephalopod landings for the 1999 -2000 was estimated at 92,292 t and it constituted the fourth largest item in our export basket. The E.U. is the major importer of cephalopods from India accounting for about 53 % of the total export in terms of value.

The major groups of cephalopods of commercial importance belonging to the Phylum Mollusca are the cuttlefishes, nautilus, octopods and squids. Squids are elongated, torpedo shaped cephalopods with posterior-lateral fins and ten circum-oral appendages bearing chitinous rings or hooks at the distal end. Squids in India are represented by several genera (Silas, *et al.* 1985). The two major types of squids are the neretic squids – "Myopsidae" squids with eyes completely covered with corneal membrane or near shore squids

and the Oceanic squids – "Oegopsidae" eyes not covered with corneal membrane, those occurring in the oceans and seas of the world.

1.3 Importance of squids in Indian fisheries

Neretic squids constitute less than 40 % of the total cephalopod catch. Six species of neretic squids, which are commercially exploited along the Indian coasts, are *Loligo duvauceli, Doryteuthis sibogae, Doryteuthis singhalensis, Sepioteuthis lessoniana, Loligo uyii, and Loliolus investigatoris.* Of these, *Loligo duvauceli* is the most abundant and forms an exclusive fishery for this genus along the west coast of India. Therefore the present study is confined to the various manifestations of the impact of heavy metals on this particular species. Around 200 species of oceanic squids are known to occur in the world oceans (Silas, 1985). Exploratory works and planktological studies carried out indicate several species occur in the Indian seas. The cruises conducted on board the research vessel FORV *Sagar Sampada* yielded only *Ancistrocheirus* spp. the oceanic squid found at depths of 200-350 m.

Silas *et al.* (1985) reported on the biology of *Loligo duvauceli.* The species is heterosexual and males grow to a larger size than females. Along the west coast the males attain maturity in the size range 108-170 mm and females in the range 90-170 mm. Maturation is prolonged and takes place throughout the year. They are mainly caught using the trawls, hooks and line, shore seines and boat seines

and are found in the water column throughout the year. Prawns and fishes form the chief item of food of this species. Other crustacean items like crabs euphasids and stomatopods also formed part of the diet. Cannibalism was often noticed. Squids, in turn, form forage to a wide variety of fishes, marine mammals and sea birds.

Squids dominated the cephalopod landings at Mangalore, Tadri, Malpe and Kozhikode. The analysis of species-wise composition in major landing centers indicated that *Loligo duvauceli* dominated the squid landings and at times formed the only species in some centres. Overall export of squid from India increased by 8.26 % in terms of volume and 7.2 % in terms of value during 2001 as compared to 2000 . Squids exported are processed in several styles such as squid whole, whole cleaned, tubes, rings, tentacles, peeled whole, stuffed squids, squid fillet, squid roes and IQF tubes, rings and tentacles. The export of new product forms like squid wings and tray packed squids continued during this year also.

Squids were considered to be a poor man's food for a long time. Later on studies showed that the squid meat has a very high nutritive value. Furthermore, the edible portions consisting of the mantle, arms, tentacles and fins formed 60 – 80 % of the body weight which is much higher as compared in finfishes. Squid meat had three times as much as collagen as fish and was found to contain 12-20 % sarcoplasmic protein, 72-85 % myosin and 2-5 % stroma proteins (Sugiyama, 1989).

The high protein and low fat content of the meat make them most suitable for human consumption (Roper *et al.*, 1984). Now squids are prized seafood export item. In addition, liver extracts of squids are used for human consumption and in the dehydrated form, as food for livestock (Takahashi, 1965). Squids also are in demand in the fishing industry as bait in the tuna long line fishing (Silas & Pillai, 1982). The viscera of squids are a good poultry feed, good source of manure and serve as fishmeal.

The nutritive value of squids and squid products are widely recognized. However, poor post-handling results in hard, rubbery texture, flabby and soft meat, high drip loss during thawing and pink or yellow discolouration of the meat. Furthermore, the presence of high levels of toxic metals add to some of the important quality problems met in the squid export industry. Therefore to increase consumer acceptability, the quality of cephalopod raw materials and safety of finished products should be ensured.

1.4 Objectives of the study

The main objectives of the present investigation are the following:

i. To provide base line on the concentration of heavy metals, viz., Hg, Cd, Cu, Zn, Fe, Mn, Cr and Ni in whole soft tissues as well as the edible (muscle) and the non-edible body components (liver and gills) of the most abundant squid species, *L. duvauceli*, found along the west coast of India.

ii. To study regional trends in the distribution characteristics of heavy metals in *L. duvauceli* caught off Cochin, Quilon, Mangalore and Mumbai regions.

iii. To study seasonal variations of heavy metals in *L. duvauceli* caught off Cochin, Quilon and Mangalore regions.

iv. To study the comparative distribution of heavy metals in neretic and oceanic squids

v. To study the comparative levels of heavy metals in squids, associated fish fauna and environment.

vi. To evaluate Cd toxicity in experimental albino rats following haematological and histopathological investigations.

1.5. Review of literature

The concentration of heavy metals is known to be significantly higher in the marine biosphere than in the hydrosphere since the early 19th century. Much of this early work has been summarized by Vinogradov (1953). A series of reviews by Goldberg (1957, 1961) have highlighted an increasing interest in the biosphere, particularly in relation to trace element uptake by marine organisms. Uptake of heavy metals by marine organisms was reported in the early years by (Fox & Rampage, 1931; Korringa, 1952; Saiki *et al.,* 1955; Mullin & Riley 1956; Martin & Goldberg 1962). Brooks & Rumsby (1965)

studied the biogeochemistry of trace metal uptake of Ag, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, V and Zn in three species of New Zealand bivalves. Interestingly. all the elements analysed showed more enrichment in the molluscs than in the environment. Following this a large number of studies have been carried out by several workers on heavy metals in molluscs.

The heavy metal levels in gastropods and in lamellibranch molluscs have been reported from the various parts of the world. (Pringle *et al.,* 1968; Nickless *et al.*, 1972; Windom, 1972; Bryan, 1973; Thrower & Eustace, 1973a, b; Topping, 1973; Ratkowsky *et al.* 1974; Phillips, 1976; Bryan & Hummerstone, 1977; Bryan *et al.* 1977; Nambisan *et al.*, 1977; Bolach *et al.* 1981; Eisenberg & Topping, 1983; Lakshmanan & Nambisan, 1983; Phillips & Muttarsin, 1985; Sadig & Alam,1989; Krishnakumar *et al.,* 1998; Schumacher *et al.,* 1994; Lu-Chavhua, 1995; Muralidharan & Raja, 1997; Chen, 1998; Rivonker & Paruleker,1998; Frias *et al.*, 1999; Lakshmanan, *et al.*, 2001).

Comparably very little information is available on the trace metal concentrations in the most highly developed molluscs, the members of class Cephalopoda (Bryan, 1984).

Studies concerning trace elements in cephalopod cuttlefishes and octopuses are limited to species targeted by commercial fisheries such as in *Nautilus macromphalus* (Bustamante *et al.*,2000); *Sepia*

officinalis (Leatherland & Burton, 1974; Decleir *et al.*, 1978; Schipp & Hevert, 1978; Miramand & Bentley, 1992; Martoja & Marcaillou, 1993; and Bustamante *et al.* 1998a) and in *Eldone cirrhosa, Octopus vulgaris* and other Octopods (Ghiretti – Magaldi *et al.,* 1958; Rocca, 1969; Renzoni *et al.*, 1973; Ueda *et al.*, 1979; Miramand & Guary, 1980; Miramand & Bentley, 1992; Barghigiani *et al.*, 1993; Bustamante *et al.,* 1998b; Storelli & Marcotrigiano, 1999). The notable indigenous works on trace metal uptake by cephalopods are those of Ramamurthy (1979), Lakshmanan (1988a,b; 1989), Patel & Chandy (1988), Jasmine *et al.* (1989), Krishnakumar *et al.* (1990), Dious & Kasinathan (1992), Lakshmanan & Stephen (1993), Prafulla *et al.* (2000) and Lakshmanan *et al.* (2001).

A few studies have been carried out on squids. Studies have been carried out on the essential metals like Cu, Fe, Mn, Zn and potential pollutants like Ag, Cd, Hg, Cr, Ni, Pb and V in both neretic and oceanic squids. Eustace (1974) studied a wide range of species from the Derwent Estuary in Tasmania and found < 0.05 μg Cd/g wet wt in the oceanic squid *Notodarus gouldi.* National Oceanic and Atmospheric Administration (1975) report quoted Cd concentrations (by wet wt for whole animals) of 0.18 to 0.34 μg/g for the short finned oceanic squid *Illex illecebrosus* from the North Atlantic, < 0.05 to 0.054 μg/g for the neretic squid *Loligo opalescens* from Californian coast. Analysis of Ag, Cd, Cu, Zn and Fe concentration in the livers of squid,

Loligo opalescens of Central California by Martin and Flegal (1975) revealed significant correlations between Cu and Ag, Cd and Zn. Copper concentrations were three orders of magnitude higher than the Cu concentration in the visceral masses of pelecypods. Livers from *Loligo opalescens* contained 22.6 to 26.5 μg Cd/g by dry wt, whereas those of oceanic squid, *Ommastrephes bartami* contained 71 to 694 μg/g. Livers of *Symplectoteuthis oulaniensis* contained 427 to 1106 μg/g was found in. *Doryteuthis bleekeri, Stenoteuthis bartrami* and *Todarodes pacificus* of Japan showed higher levels of Fe, Cu, Zn, Co in visceral organs than in the edible parts, although Cs and Mn were almost the same (Ueda *et al.*, 1979). Tanaka *et al.* (1983) studied the sub cellular distribution of heavy metals in the untreated liver of squid and compared it with data from the livers of cadmium and silver exposed rats. Wide ranges in concentrations of the elements Ag, Al, Ca, Cd, Cu, Fe, Mn, Mg, Pb and Zn were observed in the digestive gland of *Nototodarus gouldi* a squid by Smith *et al.* (1984). Cantoni *et al.* (1986) determined the Zn/Cd ratio in squids imported into Italy from four different countries and reported that about 50 % of the sample had Cd content in excess of the tolerance limit. Clark (1986) reported high level of Cd (1,900 mg/kg dry wt) in the oceanic squid, *Symplectoteuthis oualaniensis*. Falandysz *et al.* (1987) reported on the concentrations of Cd, Pb, Cu and Zn in tinned squid. The molecular association of Cu, Zn, Cd and ^{210}Po in the digestive gland of the squid *Nototodarus gouldi* was studied extensively by Finger & Smith (1987).

Barska *et al.* (1988a, b) conducted a base line study of heavy metals like Pb, Cd, Zn, Cu, Hg in *Loligo patogonica* and *Illex argentinus* caught by the polish fishery and noted high levels of heavy metals especialiy Cd in whole canned squid (*Loligo patagonica*). They also observed diffusion of metals from the viscera to the mantle. Higher trace metal levels were found in inedible tissues of *Illex argentinus* caught from the continental shelf of Argentina (Falandysz, 1988). Tomasevic *et al.* (1988) determined the toxic and essential elements levels in samples of Yugoslav produced and imported marine products. They found that 50% of squid samples have exceeded the tolerance limit for Cd (1mg/kg). Lozano Soldevilla (1989) found concentration of Cu, Cd, Fe in whole bodies of *Todarodes sagittatus* to be high when compared with those in mantles and the 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 Kijeka 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 contents in squids (*Loligo* spp.) and squid products from Southeast Asia was reported by Oehlenschlaeger (1989). The effect of Hg, Cd, Cu on the development and viability of *Loligo vulgaris* embryos was extensively studied by D'Aniello *et al.* (1990), while Castillo *et al.* (1990) investigated the presence of heavy metal binding proteins in squid *Onychoteuthis borealijaponica* and found that liver

contained a significantly higher Cd concentration (154 ± 37mug/g.d.w) than any of the other organs examined. Forty of 422 samples of squids (*Loligo* spp.) imported to Italy exceeded Italian tolerances for Cd content (Cozzani *et al.,* 1990). Concentrations of Hg and other trace metals were reported in various tissues of the squid, *Loligo opalescens* (Falandysz 1990; 1991 and Oehlenschlaeger 1991). Ikebe *et al.* (1991) determined the content of 16 metals in fish and shellfish (including squid) of Japan. Concentrations of 11 heavy metals (Ag, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn and Hg) in *L.duvauceli* off the Arabian Sea coast of Pakistan showed considerable site and species differences (Tariq *et al.,* 1991). Miramand & Bentley (1992) measured the concentrations of 11 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. Kurihara *et al.* (1993) studied the concentrations of Cd in livers of several kinds of squids and suggested ways to its elimination. Trace metal concentrations in squids from the Adriatic Sea and coastal area of Tarragona Province, Spain respectively were extensively studied by Antonetti *et al.* (1994) and Schumacher *et al.* (1994). A review study of the contamination of molluscs with Cd and other heavy metals was presented by (Lopez, 1994). Lu Chavhua (1995) evaluated the concentrations of Cu, Pb, Zn, Cd, Cr and Ni in cephalopods collected from the Northern area of South China Sea and stated there were no significant effects on the

edible tissue. Schulz & Schering (1995) observed Cd concentration in the range of 0.08 – 10.87 mg/kg in 34 out of 102 squid samples analysed. Shakir *et al.* (1995) measured the levels of essential metals like Cu, Zn, Fe in *Dosidicus gigas* and *Loligo duvauceli.* In another study by Galarini *et al.* (1996) highest concentration of Cd was observed in cephalopods when 724 samples of marine and freshwater fish and shellfish from Umbria & Marche regions of Italy, were analysed. Yamada *et al.* (1997) studied metal concentration in liver of squids collected from coastal waters and open oceans. Chen *et al.* (1998) assessed the contents of heavy metals in cephalopods from Zanjiang Harbour waters and found the edible parts to be effected by Pb, Cd, Ni and Zn. Bustamante *et al.* (1998a) 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. Cd and total Hg concentration determined in flesh and hepatopancreas of *Illex condetii* caught in the South Adraitic Sea showed higher concentration in the former than in the latter (Storelli & Marcotrigiano, 1999). Jiro Koyama *et al.* (2000) studied the bioaccumulation of water borne and dietary Cd by neretic squid *Sepioteuthis lessoniana* and its distribution among organs and suggested that the main source of Cd for squid would appear to be dietary.

Studies on heavy metal content in squids by Indian authors are quite sparse (Lakshmanan, 1988a; 1989; Jasmine *et al.,* 1989;

Krishnakumar *et al.*, 1990; Lakshmanan & Stephen 1993; Prafulla *et al.*, 2000; Lakshmanan *et al.*, 2001;).

Studies on the seasonal distribution of heavy metals in molluscs have been reported worldwide. Important works included are those of Bryan (1973) in Scallops *Pecten maximus* (L) and *Chalmys opercularis,* Fowler & Oregioni (1976) in *Mytilus galloprovincialis,* Ishii *et al.* (1978) in marine molluscs, Frazier (1976) in Oyster *C. virginica,* Hsu & Wang (1979) in Taiwan Oysters, Walker & Foster (1979) in *Balanus balanoides,* Kumagai & Saeki (1980) in Clam *Tapes japonica*, Orren *et al.* (1980) in mussel *Chloromytilus meridionalis,* Shiber (1980) in 12 species of molluscs, Boyden & Phillips (1981) in *Crassostrea gigas*, Gabbot (1983) in marine molluscs, Lakshmanan & Nambisan (1983) in bivalve molluscs *V.cyprinoides, M.casta and P.viridis,* Bouquengneau *et al.* (1984) in marine animals, Cain & 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,* Poorzada & Kozli (1992) in Australian oysters, Swaileh (1996) in bivalve *Arctica islandica*, Mitra *et al.* (1995) in gastropod *Thais lacera*, Carrascal *et al.* (1996) in *Ostrea edulis*, De-Gregori *et al.* (1996) in mussels *Chorito maico, Almeyas* sp. *and Navajuelas chilenas,* Luk-Yanova & Martem Yanova (1996) in Scallop *Mizuhopectel yessoensis,* Muralidharan & Raja (1997) in Pelecypod *Marcia recens,* Rivonker & Parulekar (1998) in *Perna viridis,*

Senthilnathan & Balasubramanian (1998) in Oyster *Crassostrea madrasensis* and Frias Espericueta (1999) in *Crassostrea irrdescens*.

The references in squid are scanty, Falandysz (1988) studied distribution characteristics of heavy metals in *Illex argentinus* and *Loligo* spp. during autumn. Studies related to seasonal variation of essential metals (Cu and Mn) have been reported by Motoe *et al.* (1997) in firefly squid (*Watesenia scintillans)*

However, trace metal concentrations in coastal and marine waters of India have been studied. (Topping, 1969; Mckim & Benoit, 1971; Goldberg, 1972; Sengupta *et al.,* 1978; Danielson, 1979; Sanzgiry & Caroline, 1979; Braganca & Sanzgiri, 1980; Matkar *et al.,* 1981; Qasim & Sengupta, 1981; Sanzgiry *et al.,* 1983; Bryan, 1984; Fowler *et al.*, 1984; Sathyanarayana, *et al.*, 1985; Mohapatra,1993; Gouda & Panigrahi, 1992; Sanzgiry *et al.,* 1992; Kureishy *et al.,* 1993; Krishnakumar *et al.,* 1998; Palanichamy & Rajendran 2000). Most of these authors have noted that the mean values of Cu, Zn and Pb in water of almost all the above centres were below the Environmental Protection Agency limits except for some localized pollution (Mohapatra, 1993).

It has been established that aquatic ecosystems are not usually able to eliminate heavy metals from waste discharges by their own natural processes. Hg, Cd, As, Cu and other basic metals tend to accumulate and move up through biological food chains causing

deleterious toxic effects. The toxic effects of some of the metals like Cu, Zn, Fe, Mn, As, Ba, Co, Mo, Ni, Sb, Sc, Sr, V and Zn to human beings have been dealt by few authors (Sittig, 1973; Sharma, 1995; Mathew, 1991a, b).

Studies addressing acute toxicity of Cd to human beings was described as early as 1858 (Savot, 1858), although Cd poisoning as such was not definitively recognized for another 62 years. produced in Sweden the first of a series of studies relating specific symptoms and a high incidence of illness and death among industrial workers to cadmium oxide exposure. Prominent among the reactions produced were emphysema of the lungs and renal damage, Anosmia, yellowing of the teeth and mild liver damage were also observed. Acute Cd poisoning can result from inhalation of Cd fumes or dust or from ingestion of heavily contaminated food or water (Yasumura *et al.*1980) and severe gastrointestinal symptoms and several deaths following Cd ingestion have been reported (Gleason *et al.*, 1969). After gaining entrance into the body, cadmium is sequestered in the liver and kidneys. More than half the total body burden of Cd is found in these two organs, with the larger fraction in the kidney (Friberg *et al.*, 1974; Elinder *et al.*, 1976). Tipton and Cook (1963) reported that the liver has five times more Cd per unit weight than other parts of the body.

As there occurs a selective accumulation of Cd in organs like kidney and liver, the dietary intake of this element is not likely to

increase with the rise of this metal in the environment, unless these organs are consumed (Sharma, 1979). Several studies have been performed in mammals to relate the dietary exposure of Cd to the residues of this metal in various edible tissues and food products from animals. Baker *et al.* (1975) fed different fed different levels of Cd to laying hen and broiler chickens for twelve weeks. Considerable increase in Cd residues was observed only when the dietary Cd was 48 ppm. Willams *et al.* (1976) fed sorghum and corn on soil with application of cadmium containing sludges to meadow vole (dietary cadmium 2.76 and 1.09 ppm, dry matter respectively) and reported that no accumulation of Cd was observed in their muscle. The liver Cd after a 40 day feeding trial was 1.86 and 0.43 ppm (dry matter) on sorghum and corn diets, respectively. The corresponding kidney cadmium levels were 2.84 and 0.42 ppm. Doyle *et al.* (1974) added different levels of Cd, upto 60 ppm, to the diet of lambs for 191 days and noted increase in Cd levels only at the highest dietary level for this metal. Vogt *et al.* (1977) fed broiler chickens with various levels of dietary Cd (1 to 80 ppm) for 4 and 7 weeks and found that kidney and liver accumulated Cd upto 65 and 50 ppm respectively. Sharma & Shupe (1977) reported the relationship between dietary Cd to the residues of this metal in different tissues of three food- producing species; cattle, swine and chicken.

Histopathalogical effects of Cd and other heavy metals in aquatic organisms have been reported worldwide. Carmichael (1981)

and Friberg *et al.* (1985) exposed bay scallops to 0.7 ppm Cd in flowing sea water for 5 days and noted massive extrusions of the calcified concentrations of most of the kidney epithelial cells. An attempt was made by Dalwani *et al.* (1985) to correlate alterations in heme metabolism due to Cd with the histopathalogical manifestations in liver of fish exposed to sub lethal Cd levels. Usharani & Ramamurthi (1989) observed histopathalogical alterations in the liver of *Tilapia mossambica* ranging from degeneration of hepatocytes to fatty acid changes in the peripancreatic hepatocytes.

Clinical toxicity in rats due to Cd accumulation in liver and kidney leading to pathological lesions in organs (Stowe *et al.*, 1972; Hoffman *et al.*, 1975; Nordberg *et al.,* 1975; Sendelbach & Klaassen (1988); Cherian *et al.*, 1976; Itokawa *et al.,* 1978; Squibb *et al.,* 1979; Maitani *et al.,* 1984; Dudley *et al.*,1985; Elinder, 1986; Anderson *et al.*, 1988; Chatterjee *et al.*, 1996). The only work that contained any reference to toxicity of inorganic and liver incorporated Cd in rats is that of Groten *et al.* (1990) and study on the hepato-toxicity and hepato carcinogenity in rat fed squid by Lin & Ho (1992) who investigated whether the incidence of cancer in rats could be increased by consumption of squid diet.

The diet of squids largely consists of fishes and pelagic crustaceans (Soeda, 1950; Squires, 1957, 1966; Aldrich, 1964; Fields, 1965; Oommen 1977; Kore & Joshi, 1975; Voss, 1973; Nixon, 1987;

and Boyle, 1990). Cannibalism by large squids on smaller ones is also known in some species (Worms, 1983; Baddyr, 1988; Dawe, 1988; Lipenski & Linkowski, 1988; Sauer & Lipenski, 1991). Neretic and pelagic squids prey mainly on fish and other cephalopods (Rocha *et al.*, 1993; Pierce *et al.*, 1994 and Collins & Pierce, 1996). Many published accounts exists dealing with determination of diet of squid by analysis of stomach content. Bidder (1950), Pierce *et al.* (1994) and Collins *et al.* (1994) on *Loligo forbesi*; Coelho *et al.* (1997), Santos *et al.* (1998) on *Illex argentinus*, on *Ommastrephes sloani pacificus;* Verrill (1882), Squires (1966) on *Illex illecebrosus*; Okiyama (1965) for *Todaradus pacificus*; Kore & Joshi (1975), Varghese (1976) on *Loligo duvauceli.* During their life cycle, cephalopods prey on a variety of species and a large range of size groups (Boucher-Rodoni *et al.*,1987), widening their range of prey species as they grow (Nixon, 1987; Lipenski, 1987 & 1992). Further more squids are a prey to a great variety of sea birds, cetaceans, marine mammals, fishes and cephalopods themselves (Silas, 1963; Clarke, 1977, 1986; Rodhouse *et al.* 1992. Clarke, 1996; Croxall & Pierce,1996; Smale, 1996).

Squids are a significant source of Cd to their predators. This hypothesis was first proposed by Honda & Tatsukawa (1983) for striped dolphin from Japan. Muirhead & Furness (1988) supported this hypothesis to explain very high cadmium concentrations in the tissues of squid eating seabirds from Gough islands. A lot of work has been carried out on the squid predator relationship (Paludan-Muller *et*

CHAPTER 2

Materials and Methods

2.1. Materials

2.1.1. Animals and water samples

A two tier sampling procedure was adopted - Samples were collected in west coast of India and onboard the fishing vessel, MV *Sagarika* (IFP) and research vessel, FORV *Sagar Sampada* (DOD) during her cruises along the west coast of India.

Monthly collections of the squid, *Loligo duvauceli* were made between 1998-1999 and 1999-2000 from Cochin, Quilon and Mangalore Fisheries Harbours and quarterly from Mumbai Fisheries Harbour. Neretic squids, (*Loligo* spp.) were collected onboard the fishing vessel M.V. *Sagarika* from May 1998 to October 1998 using trawl operated at 30 - 70 m depth. The operational area covered lat. 9° 34' to12° 08' N and long. 74° 50' to 76° 02' E for onboard collections from M.V. *Sagarika* and 20°34' to 21°36' N lat. to 68°58' to 70°13' E long. for onboard collections from FORV *Sagar Sampada*. Details of onboard sampling sites are shown in Table 2.1. Oceanic squids (*Ancistrocheirus* spp.) were collected onboard the research vessel FORV *Sagar Sampada*, (Cruise No. 191) during 2001 at 200-350 m depth. The operational area as confined to the region 6°58' to 13°30' N lat. and 74°15' to 77°50' E long. The sampling sites of oceanic squids are illustrated in Fig. (2.1). Fishes and water samples from the same habitat area as that of neretic and oceanic squids were also collected

onboard the vessels. Squid samples were collected using trawl net and water samples were collected using the Conductivity Temperature Depth instrument (SBE3^{+} model).

To study the regional distribution of heavy metals, *Loligo duvauceli* of commercial size range of dorsal mantle length 10-25 cm and weight 80 to 200 g collected from Cochin, Quilon, Mangalore and Mumbai Fisheries Harbours were used.

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

Squids collected from Cochin, Quilon, Mangalore and Mumbai fisheries harbours were iced and brought to the laboratory in insulated boxes and analysed immediately or deep frozen (-20° C) until further analysis. Preservation of the seawater samples were done by adding 1.5 ml con. nitric acid for every 1L of the sample and were stored in chill (4°C) until further analysis. Squids captured onboard were deep frozen (-20°C) for later analysis.

2.2. Reagents, Chemicals and Glasswares

AnalaR grade acids, stains and reagents were used. The acids used for estimation of trace metals were redistilled. Rectified spirit of

95% grade and its various dilutions were used in histopathological studies.

2.2.1. Glasswares and Plasticwares

All glasswares/plastic bottles 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 copious amounts of deionised water (Milli Q water system) to prevent metal contamination.

2.2.2. Reagent water

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

2.2.3. 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 metals or metal salts as follows:

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

Copper – Dissolved 0.1g pure copper metal in 2 ml con. HNO_3. Added 10.0 ml con. HNO_3 and diluted to 1000 ml with water. 1.00 ml ≡ 100 µg Cu.

Zinc – Dissolved 1g Zn powder in 20 ml HCl (1+1) in 1L volumetric flask and diluted to volume with deionised water. Concentration of the solution is 1000 ppm.

Iron – Dissolved 0.1 g 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. 1.00 ml ≡100 µg Fe.

Manganese- Dissolved 0.1000 g manganese 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 0.10 g Nickel metal in 10 ml hot con. HNO_3, cooled and diluted to 1000 ml with water. 1.00 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 of 5% HNO_3. About 1 ml of 1% $K_2Cr_2O_7$ solution was

added to it and made up to 100 ml with con. HNO_3. 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 standard solutions for Cd and Cr were prepared from commercially available AAS grade stock standard obtained from Sigma Chemicals Co.

2.2.4. Stannous Chloride solution (20% v/v)

20 g of high purity stannous chloride was dissolved in 10 ml distilled con. 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.

2.2.5. Neutral buffered formaldehyde solution (10%)

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

2.2.6. Stock Eosin solution (1%)

Dissolve 1 g of 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 of glacial acetic acid for every 100 ml of stain.

2.3. Methods

2.3.1. Preparation of muscle homogenate

2.3.1.1. Whole Squids

Whole squids (fresh/thawed) were washed with potable water. The squid pen was removed. Squids were peeled, finely chopped and homogenized in a mixer. An aliquot of the homogenate was pressed within filter paper folds so as to remove the adhering water. This was then used for digestion and subsequent analysis.

2.3.1.2. Body Components

Another lot of squids were dissected for different body components such as liver, gills, muscle. using stainless steel scissors, knives and forceps. The tissues were homogenized and aliquots taken for metal analysis.

2.3.1.3. Whole fishes

Fishes separated from the mantle cavity of squids and also those collected from the same habitat area of squids were washed with

potable water. Their body length, weight were recorded and digested whole until it became clear and aliquots taken for metal digestion.

2.3.2. Trace metal analysis

2.3.2.1. Predigestion of samples

15 ml of con. nitric acid was added to 10 -15 g of squid/fish mince samples and kept overnight for predigestion at room temperature in a fume hood. Triplicate samples were taken in all kinds of analysis.

2.3.2.2. 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 modified Bethge apparatus. Digestion carried out under closed condition to prevent the escape of volatile metals.

2.3.2.3. Digestion procedure for determination of metals by Flame Atomic Absorption Spectrophotometer (AAS)

Metals such as Cd, Cu, Zn, 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, adding more con. HNO_3 in small portions as needed to prevent charring, 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 the digestion was completed. The solution was cooled, filtered through Whatman filter paper, diluted to a definite volume with deionised water in volumetric flasks and kept in polythene bottles for AAS analysis. Blanks were carried through the same procedure as the squid samples. A reagent blank was prepared by taking the same volume of acid mixture and other ingredients except the tissue samples.

2.3.2.4. Determination of Mercury using Mercury analyzer

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.

Mercury content in samples were determined by Cold Vapour AAS technique using Mercury Analyser (model MA5840) designed by Analytical Chemistry Division, BARC, Bombay with a sensitivity of 3 ng absolute for 1% absorption and of 0.1 µg/L detection limit.

A 10 ml suitable aliquot of the sample was pipetted out in to the reaction vessel of the apparatus and the required amount of 10% nitric acid solution was added in order to maintain total volume at 10 ml. 2 ml of 20% stannous chloride solution was then added and allowed to react for 5 min. 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.

2.3.2.5. Determination of trace metals by Flame Atomic Absorption Spectrophotometer

Trace metal concentrations in the digested samples were determined by Flame Atomic Absorption Spectrophotometer (Model GBC 902). The samples were directly aspirated over the flame (air acetylene flame) and using the concentration mode the corresponding readings were noted. Calibration of the instrument was carried out using corresponding solutions.

The concentration range, wavelength, slit width, lamp current, sensitivity and working range used in the estimation of various metals in AAS are given in Table 2.2. Standard addition technique was employed to validate the method.

2.3.3. Analysis of water samples for metals

Sea water 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 Diethyl Ammonium Dithiocarbamate (DADC). pH adjusted between 4 and 5 by a citrate buffer and extracted into Methyl Isobutyl Ketone (MIBK) followed by back extraction into nitric acid. The method followed is based on that of Grasshoff *et al.* (1976). Extracts were analysed for Cd, Cu and Zn by Flame Atomic Absorption Spectrophotometer.

2.3.4. Histopathology

To study the toxic effects of squid liver bound cadmium and inorganic Cd ($CdCl_2$) the liver and kidney tissues of experimental and control Albino rats were subjected to histopathological studies following the method of Pearse (1968). The procedure consist 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 though 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)	1/2 h
Xylene (2 changes)	15 min each
Paraffin wax and xylene	1/2 h each
Paraffin wax (3 changes) (melting pt. 58-60° C) :	1 h each

The tissues were then embedded in paraffin wax of melting point of 60-62°C. Sections are cut at 6-10μm thickness in a rotary microtome and sections floated in a water bath between 38-49°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 II		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 neretic squid collected onboard M.V. *Sagarika* and FORV *Sagar Sampada*

Latitude	Longitude	Depth	Research vessel, period of collection
10°39' N	75°45' E	35m	
11°05' N	75°37' E	30m	
11°51' N	75°02' E	48m	M.V. *Sagarika*
12°08' N	74°50' E	52m	May 1998
10°26' N	75°52' E	30m	
10°06' N	75°51' E	44m	M.V. *Sagarika*
09°47' N	75°50' E	58m	June 1998
09°36' N	76°01' E	29.0m	
09°34' N	75°57' E	59.0m	
09°45' N	75°51' E	59.0m	M.V. *Sagarika*
09°41' N	76°01' E	45.0m	August 1998
09°39' N	76°02' E	40.0m	
09°45' N	75°56' E	45.0m	
09°38' N	76°02' E	38.0m	
09°51' N	75°58' E	36.0m	M.V. *Sagarika*
09°44' N	75°56' E	46.0m	October 1998
09°47' N	75°52' E	43.0m	
20°34' N	70°13' E	64.0m	
21°11' N	69°40' E	43.0m	
21°33' N	68°56' E	61.8m	
21°31' N	69°11' E	68.5m	FORV *Sagar*
21°10' N	69°24' E	62.5m	*Sampada*
21°17' N	69°11' E	40.0m	March 1999
21°36' N	68°58' E	61.0m	
21°26' N	69°06' E	61.0m	

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

Element	Wave length (nm)	Slit width (nm)	Lamp current (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

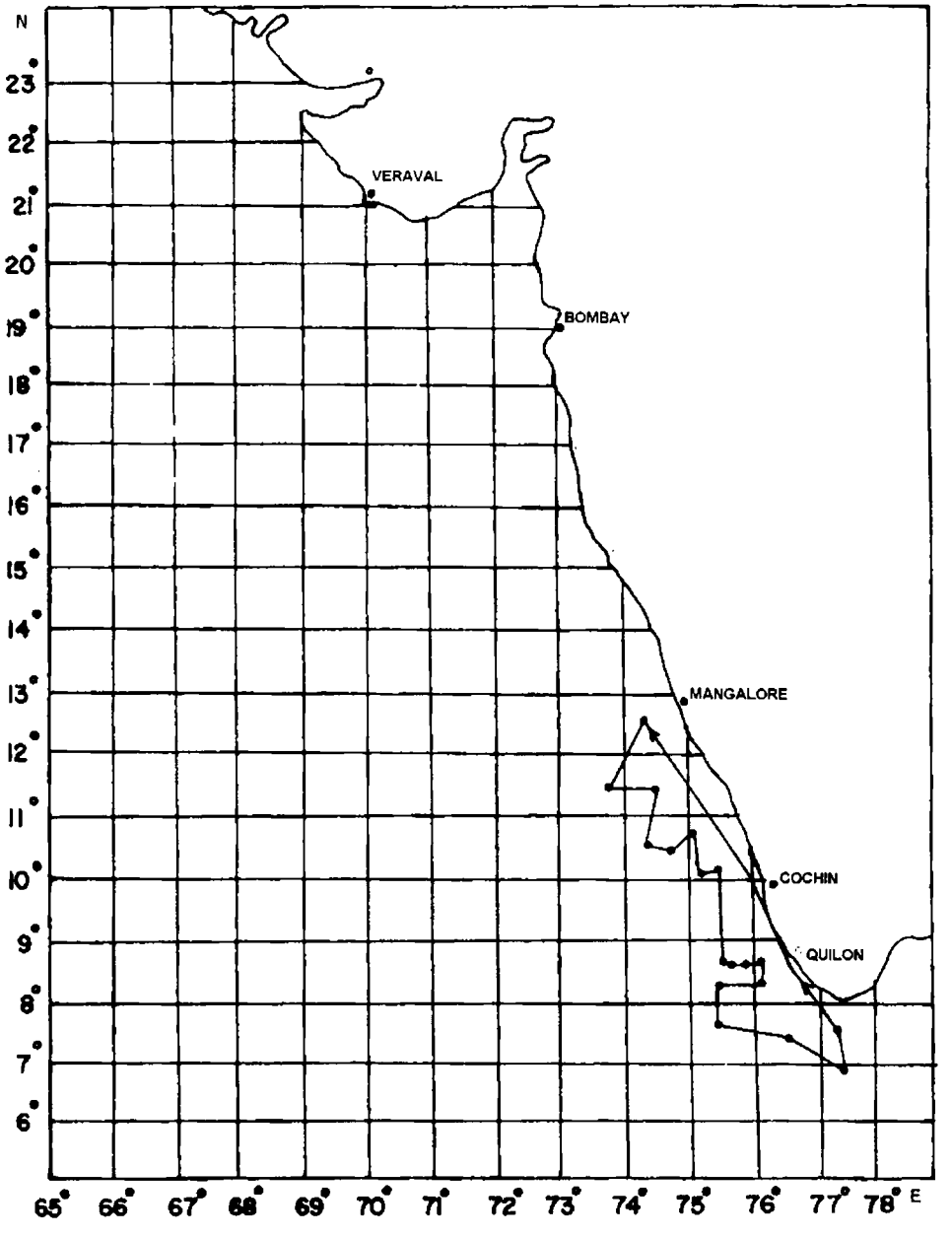


Fig.2.1. Sampling stations for oceanic squids collected onboard FORV *Sagar Sampada* (Cruise No. 191)

CHAPTER 3

Regional trends in the distribution of metals in *Loligo duvauceli*

3.1. Introduction

Heavy metals have been recognized as serious pollutants of the aquatic environment with deleterious effects on associated organisms. There is, therefore, great concern throughout the world on the impact of heavy metals on the quality and safety of the food that man gets from the aquatic sources.

Mercury, cadmium, lead, copper, zinc, iron manganese, chromium, nickel and other heavy metals are known to accumulate in a great number of marine invertebrates, especially bivalve and gastropod molluscs (Bryan, 1984). However, concentration of Cd and other metals in cephalopods, one of the essential links in marine trophic food chains have rarely been documented.

The studies concerning heavy metals in squids from different parts of the world are limited (Martin & Flegal, 1975; Ueda *et al. 1979;* Smith *et al.*, 1984; Finger & Smith, 1987; Tomasevic *et al.,* 1988; Falandysz 1990, 1991; Yamada *et al.,* 1997; Bustamante *et al.*, 1998a) and studies on squids from Indian waters are quite sparse (Lakshmanan,1988a, 1989; Jasmine *et al.,* 1989; Krishnakumar *et al.,* 1990; Lakshmanan & Stephen, 1993; Prafulla *et al.,* 2000 and Lakshmanan *et al.,* 2001)

Hence, in the present study, the levels of nine metals, *viz.*, Hg, Cd, Pb, Cu, Zn, Fe, Mn, Cr and Ni in the squid, *Loligo duvauceli* from the four major Fisheries Harbours along the west coast of India, namely Cochin, Quilon, Mangalore and Mumbai, their distribution in the body and geographic trends have been dealt with. This study also identifies specific organs that may be particularly selective and sensitive to heavy metal accumulation.

3.2. Materials and Methods

The details of sample collection, sample preparation and determination of metal levels in them by Flame AAS or Cold Vapour Atomic Absorption technique and procedures adopted are described in detail in Chapter 2. Statistical analyses were carried out using one way ANOVA followed by student t-test to compare the means and to determine significant differences if any, in the distribution of heavy metals in squids collected from different regions along 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 tissues of squid, *L. duvauceli* and in various body components like muscle, liver and gills collected off Cochin, Quilon, Mangalore and Mumbai regions are presented in

Tables 3.1 to 3.4. The regional trends are illustrated in Figs. 3.1 to 3.5. Table 3.5 shows heavy metal levels in whole squid and squid whole cleaned samples from the west coast of India. Results of Analysis of Variance (ANOVA) of some of the toxic and essential metals in whole soft parts and muscle component are presented in Tables 3.7 and 3.8. The distribution characteristics of metals in the whole soft tissues and body components of *L. duvauceli* are described below.

3.3.1. Levels of Mercury, Cadmium and Lead.

The results of concentrations of ΣHg, Cd and Pb from the four regions and their geographic trends are presented in Tables 3.1 to 3.4 and Figs. 3.1 to 3.5. These three metals are included in the category of highly toxic metals. Mercury was the least abundant toxic metal found in *L. duvauceli* from all the four regions. In whole soft tissues, ΣHg levels were in the range of 0.035 to 0.098 ppm (Cochin region), 0.023 to 0.089 ppm (Quilon region), 0.027 to 0.056 ppm (Mangalore region) and 0.055 to 0.059 ppm (Mumbai region). The highest value observed for ΣHg in whole *L. duvauceli* was 0.098 ppm and a reduction of around 30% of ΣHg content was seen in the edible muscle. The edible muscle in *L. duvauceli* along the west coast had ΣHg in the range of 0.010 to 0.071 ppm. Highest mean values of ΣHg in edible muscle were noted in Cochin samples (0.042 ppm). Among the other body components analysed, only the liver exhibited a value of > 0.10 ppm for Hg and

concentrations were 0.156 ± 0.060, 0.075 ± 0.034, 0.086 ± 0.022 and 0.039 ± 0.011 ppm from Cochin, Quilon, Mangalore and Mumbai regions respectively. Liver of *Loligo duvauceli* caught off the Cochin region recorded the highest values, *viz.*, 0.231 ppm (Table 3.1). Only 18% of the liver samples showed ΣHg content above 0.2 ppm. ΣHg content in gills were in the range 0.032 to 0.073, 0.021 to 0.062, 0.017 to 0.065 and 0 to 0.062 ppm in *L. duvauceli* from Cochin, Quilon, Mangalore and Mumbai regions respectively. Highest body burden of ΣHg in gills was noted in Cochin region samples compared to levels in other regions (Figs. 3.1 to 3.3). The distribution pattern of ΣHg in the body components analysed were of the order: liver > gills > muscle at all the four regions. The geographic trends of ΣHg in the whole soft tissues and muscle of *L.duvauceli* were Cochin > Mumbai > Mangalore> Quilon and Cochin > Quilon > Mangalore > Mumbai respectively.

As in the case of ΣHg whole squid had a higher level of Cd than in the edible muscle; the highest value observed being 6.07 ppm from Cochin region. Concentrations of Cd in whole soft tissues of *L.duvauceli* at the four regions were 2.228 ± 1.378 ppm (Cochin region), 0.713 ± 0.577 ppm (Quilon region), 0.754 ± 0.077 ppm (Mangalore region) and 0.996 ± 0.207 ppm (Mumbai region). Around 20% of the whole squid had Cd content > 3 ppm, the tolerance limit

(Table 3.5). However, the Cd content in the muscle of *L.duvauceli* was < 3 ppm at all the four regions (Fig 3.1 to 3.4). Mean Cd content in the edible muscle at the four regions were 0.475 ppm (Cochin region), 0.613 ppm (Quilon region), 0.233 ppm (Mangalore region) and 0.583 ppm (Mumbai region). Liver was the major site of Cd accumulation in *L.duvauceli* from all the regions and it contributed significantly to the total body burden of Cd. Liver samples from Cochin region had Cd content in the range of 2.313 to 187.56 ppm, while the Cd content in liver samples from Mangalore, Quilon and Mumbai regions were in the range of 3.235 to 45.045 ppm, 0.742 to 24.670 ppm and 26.057 to 74.351 ppm respectively. Around 65% of the samples had Cd content above 3 ppm. Overall in all the regions together, 22% of samples had Cd content in liver >100 ppm. Cd content in gills of *L. duvauceli* exhibited concentrations of 2.086 ± 2.035, 5.273 ± 4.804, 0.854 ± 0.247 and 2.417±1.540 ppm at Cochin Quilon, Mangalore and Mumbai regions respectively. All the liver and gill samples from Quilon region had Cd content above 2 ppm. Lowest levels were noted in gill samples of Cochin region (0.144ppm). However, 35% of the gill samples from Cochin region showed Cd content above 2 ppm. The increasing order of abundance of Cd in the body components of squid were liver > gills >muscle. This was true for samples from all the four regions. A similar geographic trend as that of ΣHg was noted in the distribution of Cd in the whole soft tissues of *L.duvauceli*. However, in the muscle tissue

collected from the different regions the increasing order of Cd was as follows: Quilon > Mumbai > Cochin > Mangalore.

In the whole soft parts of *L.duvauceli* Pb levels varied from 0.564 to 1.985 ppm at Cochin, 0.321 to 2.011 ppm at Quilon, 0.721 to 1.969 ppm at Mangalore and 0.134 to 2.014 ppm at Mumbai. In the whole samples analysed 11% showed Pb content above the tolerance limit of 1.5 ppm. However, mean Pb content in the edible muscle was below 1 ppm in the samples from the four regions; the range of values being 0 to 1.414 ppm at Cochin, 0 to 1.185 at Quilon, 0 to 1.277 ppm at Mangalore and 0 to 1.133 ppm at Mumbai. The lower range of Pb, in the various body components analysed in general was zero. The liver concentration of Pb ranged from 0 to 12.316 ppm covering all the stations. The mean Pb content was highest in gill samples of *L. duvauceli* from Cochin (1.959 ppm) and lowest average levels were noted in Quilon region samples (Fig. 3.3). The distribution pattern in general being liver > gills> muscle. The mean Pb content in *L.duvauceli* in the whole soft parts varied from region to region and were of the order: Cochin > Quilon > Mumbai > Mangalore.

3.3.2. Levels of Copper, Zinc, Iron and Manganese.

Cu, Zn, Fe, and Mn are essential to marine organisms and play a major role in biological processes. Elevated levels of Cu were noted in squid samples from all the regions. The mean Cu content in whole

soft tissues of *L.duvauceli* was highest in the squids from Cochin region (37.640 ppm) and the lowest was in samples from Mangalore region (4.958 ppm). Among the various body components, muscle had the lowest Cu content and ranged from 0 to13.798 ppm in *L.duvauceli* covering all the regions. Comparatively higher levels of Cu were found in the liver and gills of *L.duvauceli* from the four regions (Fig. 3.1 to 3.3). The range of values of Cu in the liver of *L.duvauceli* from Cochin, Quilon, Mangalore and Mumbai regions were 8.585 to 182.70, 11.553 to 107.66, 0.479 to 44.52 and 21.132 to 136.67 ppm, respectively. 54% of Cu in liver samples from Cochin region and 11% of liver samples from Quilon region showed Cu levels above 50 ppm. However, Cu content in the muscle samples from all the four regions reflected low levels of Cu. Highest average levels of Cu in gills were noted in *L.duvauceli* samples from Mumbai region and concentrations were 53.918±69.293 ppm, while Mangalore region samples exhibited lowest concentrations of 14.832±10.280 ppm. At Cochin and Quilon regions Cu concentrations were more or less similar (Table 3.1 and 3.2). The distribution pattern of Cu in the four regions were Cochin > Quilon > Mumbai > Mangalore. Cu content in whole soft tissues of *L. duvauceli* from the west coast of India were of the order: muscle < gills < liver The mean Cu content in muscle tissue samples from Cochin region was slightly higher than the other three regions.

The range of values of Zn in the whole soft tissues of *L.duvauceli* from Cochin, Quilon, Mangalore and Mumbai regions were 5.314 to 20.371, 10.598 to 43.430, 6.048 to 12.816 and 2.040 to 14.716 ppm, respectively. In general, the level of zinc was highest in the liver of *L.duvauceli* (Figs. 3.1 to 3.3) except in samples from Quilon region that had a comparatively higher mean value of Zn (35.81 ppm) in gills. None of the samples from Quilon region exhibited Zn concentrations above 50 ppm. Around 45% of the liver samples from Cochin region and around 35% of liver samples from Mangalore region had Zn content above 50 ppm. However, much lower levels were observed in the muscle with values ranging from 2.995 to 24.440 ppm (Cochin), 2.139 to 32.41 ppm (Quilon), 3.339 to 14.631 ppm (Mangalore) and 5.571 to 13.285 ppm (Mumbai). Liver samples of *L.duvauceli* from Cochin region and gill samples from Quilon region exhibited highest Zn content of 160.99 ppm and 110.248 ppm respectively. Lowest levels of Zn were noted in the liver of Quilon region samples (40.558 ppm). In the gills, lowest levels of Zn were noted in Mangalore samples (2.289 ppm). In general, all the squid gill samples showed Zn content below 50 ppm. The geographic trends with respect to mean Zn content in the whole soft parts were of the order: Quilon > Mangalore > Cochin > Mumbai. In the muscle tissue Quilon samples exhibited mean Zn content slightly higher than the other 3 regions.

The liver of *L. duvauceli* was rich in Fe content particularly in samples from Mumbai and Cochin region, with a mean value of 83.298 and 80.662 ppm respectively. Fe content ranged from 23.305 to 189.23 ppm and 13.418 to 214.20 ppm in squid liver from Mumbai and Cochin regions respectively. The corresponding values of Fe in samples from Quilon and Mangalore regions were relatively lower (14.055 to 117.44 ppm and 16.233 to 55.105 ppm). It was interesting to note that the lower range of Fe was comparable in the species at the four regions. Distribution of Fe in whole *L. duvauceli* samples from Cochin and Mumbai regions were more or less similar; mean values being 13.887 and 12.490 ppm, respectively. At Quilon and Mangalore regions the muscle had homogenous levels of Fe (Table 3.2 and 3.3). The range of muscle values for Fe in *L. duvauceli* from Cochin, Quilon, Mangalore and Mumbai regions were: 0.121 to 12.309, 1.109 to 5.231, 0.018 to 4.175 and 8.398 to 10.772 ppm, respectively. The highest mean levels of Fe were noted in gill samples from Quilon region and lowest average levels were noted in Mumbai samples. The geographic trend observed in whole squids were of the order: Cochin> Mumbai> Mangalore> Quilon.

Mn, though an essential element was found only at low levels in squids. The highest mean values were noted in samples from Mumbai region and lowest average levels in muscle was found in Mangalore samples (Figs. 3.3 and 3.4). The lower range of Mn in the muscle from

all the four regions was zero. However, in whole soft parts of *L.duvauceli* the Mn content ranged from 0.369 to 9.903 at Cochin, 0.231 to 2.703 ppm at Quilon, 1.144 to 2.568 ppm at Mangalore and 0.292 to 0.682 ppm at Mumbai. In general, liver contained higher levels of Fe compared to other organs. The mean values being 9.955, 4.533, 7.001 and 2.074 ppm at Cochin, Quilon, Mangalore and Mumbai regions respectively. The average levels of Mn in gills showed a more or less homogenous distribution pattern; but with slightly higher average levels of Mn noted in Mangalore samples (Fig. 3.3). At the other regions average levels were 3.86 ppm (Cochin), 3.258 (Quilon), and 3.039 ppm (Mumbai). The geographic trend observed in the distribution of heavy metals in whole squids were of the order: Cochin > Mangalore > Quilon > Mumbai.

3.3.3. Levels of Chromium and Nickel.

Cr is the least toxic of the heavy metals and plays a vital role in glucose tolerance. Ni in various forms is relatively non-toxic when consumed; however Nickel exerts its toxic effects in many ways in marine organisms. The mean values of Cr from the four regions are plotted in Figs. 3.1 to 3.5 and the concentrations of Cr are presented in Tables 3.1 to 3.4. Cr content in whole *L.duvauceli* varied from region to region and were in the range 0.026 to 3.479 ppm at Cochin, 0.519 to 12.572 ppm at Quilon 0.830 to 2.124 ppm at Mangalore and 0.443 to

1.652 ppm at Mumbai. Around 11% of whole *L.duvauceli* showed Cr content above 12 ppm. However, Cr content in the muscle of *L.duvauceli* was below the tolerance limit (Table 3.6) at Cochin, Quilon, Mangalore and Mumbai regions. The mean values being 0.650, 0.600, 0.576 and 0.178 ppm, respectively. The lower range of Cr in the edible muscle in general, was zero except in Mumbai region samples (Table 3.4). Cr content in the liver also showed variation with region. The mean values being 2.108±2.181 at Cochin, 1.148±0.848 ppm at Quilon, 3.916±5.632 ppm at Mangalore and 3.153±3.943 ppm at Mumbai. As in the case of Cr content in muscle, in liver samples also the lower range, in general, was zero except in *L.duvauceli* from Mumbai region where Cr levels ranged from 0.576 to 7.692 ppm. The mean Cr value in liver was < 5 ppm in all the regions. Mean values of Cr in gills of *L.duvauceli* from Cochin, Quilon, Mangalore and Mumbai regions were 2.004, 2.959, 0.790 and 3.816 ppm respectively. The concentrations of Cr in the different organs of *L.duvauceli* varied among stations and followed the order: liver > gills > muscle at Cochin and Mangalore regions and gills > liver > muscle at Quilon and Mumbai regions. The geographic trend in the distribution of Cr in the whole soft tissues were in the order; Quilon> Cochin > Mangalore> Mumbai.

Comparatively higher levels were found in whole soft tissues than in muscle tissue at all the four regions (Tables 3.1 to 3.4). Ni levels ranged from 0 to 3.119 ppm along the west coast of India and

the mean values are presented in Fig. 3.5. Nickel was found at low levels in the muscle of *L.duvauceli* from Cochin, Quilon and Mangalore regions and varied from 0 to 1.032 ppm, 0.101 to 0.910 ppm, 0 to 0.868 ppm, respectively. Squids from Mumbai region however recorded elevated levels of Ni in the body components analysed. The levels ranged from 0.535 to 2.029 in muscle, 7.115 to 23.070 ppm in liver and 1.282 to 15.0 ppm in gills. Liver samples of *L.duvauceli* from Cochin region exhibited mean values of 2.211 ppm, while, Ni content in liver samples from Mangalore and Quilon regions were 0.669 and 3.181 ppm, respectively. The highest value observed for Ni in gill component of *L.duvauceli* from Cochin, Quilon and Mangalore regions was 5.228 ppm. The lower range of Ni in these three regions was zero (Tables 3.1 to 3.3). The distribution pattern of Ni in the body components from the four regions were of the order: liver > gills > muscle at Cochin and Mangalore regions, while, at Quilon and Mumbai region it was gills > liver > muscle.

Whole cleaned squid samples analysed showed lower mean values of all the nine metals analysed (Table 3.5). Toxic metals like Hg, Cd and Ni showed significant reduction in metal levels. Around 30% of reduction in Hg content was found in whole cleaned samples. Proper evisceration of the visceral parts were found to bring down Cd and Cr content by around 70% and 35% respectively (Table 3.5). Significant reduction of Cu, Zn and Ni levels were also noted.

Analysis of Variance (ANOVA) of the data followed by 't test' was employed to compare the means among the four regions, *viz.*, Cochin, Quilon, Mangalore and Mumbai regions. The results of ANOVA of some of the important metals in the muscle and whole soft tissues of *L.duvauceli* are presented in Tables 3.7 and 3.8. The results showed that the variance among the three groups were highly significant ($p<0.01$) with respect to Cd content in the muscle of *L.duvauceli*. No significant difference was observed in Zn levels in muscle samples between the four regions. However, significant difference was noticed among the four regions with respect to Hg, Cd, Cu, Zn, Cr, and Ni concentrations ($p<0.05$) in the whole soft tissue of *L.duvauceli.* Hg content in the whole soft tissues of *L.duvauceli* from Cochin region were significantly higher ($p<0.05$) than Mangalore and Quilon region samples. Mangalore squids showed significantly higher ($p<0.05$) levels of Hg than Quilon samples. Cd content in *L.duvauceli* from Cochin region was significantly higher ($p<0.01$) than Quilon and Mangalore region squids, while, Mumbai squids exhibited significantly higher ($p<0.05$) level than Mangalore and Quilon samples. Statistically, no significant difference was noted in Hg and Cd content between Cochin and Mumbai region samples. Pb content in whole soft parts of *L.duvauceli* from Cochin, Quilon and Mumbai regions were significantly higher ($p<0.05$) than in Mangalore squids. Zn content of whole *L. duvauceli* in Quilon samples were significantly higher

($p<0.01$) than in Cochin, Mumbai and Mangalore whole *L.duvauceli.* While, Cu content in Cochin samples were significantly higher ($p<0.01$) than Mangalore, Mumbai and Quilon samples.

In the muscle tissue of *L.duvauceli* ΣHg content in Cochin region squids was significantly higher ($p<0.01$) than Mangalore and Mumbai regions. Cd content in the muscle tissue of *L.duvauceli* from Mumbai, Quilon and Cochin regions were all significantly higher ($p<0.01$) than Mangalore region. Statistically, no significant difference was noted in the Cr and Ni levels in *L.duvauceli* muscle from the four regions.

3.4 Discussion

The highly toxic metal Cd often exceeded the permissible limit in around 20% of samples when whole *L.duvauceli* were analysed. However, their levels were below the tolerance limits in the edible muscle. Concentrations of ΣHg in squids were far below the limit of 1mg/kg permitted for seafoods by many fish importing nations like USFDA, Canada, E.U. Japan *etc.* Comparable levels of Hg have been reported in cephalopods caught from the Arabian sea (Ramamurthy, 1979; Patel and Chandy, 1988; Jasmine *et al.*, 1989; Lakshmanan and Stephen,1993; Lakshmanan *et al.*, 2001) In *Loligo* spp. only low levels were reported by Falandysz (1989,1990). Barska (1988 b) reported low levels of Hg in *Loligo patagonica.* The levels of Hg in the different body components were also low and the distribution followed the order

liver >gills >muscle in *L.duvauceli.* High levels of Cd (>3ppm) have been observed in around 20% of whole *L.duvauceli.* Higher levels of Cd in whole *Loligo* spp. had been reported from various parts of the world. Falandysz (1989) found high levels of Cd (2.9 to 10 mg /kg wet wt) in the edible parts of canned squid, *Loligo patagonica.* Raw whole squid contained on an average 4.0 mg Cd/kg as reported by Falandysz (1991). Lakshmanan and Stephen (1993) found whole soft parts of *L.duvauceli* to contain high levels of Cd (>2ppm) and higher levels were observed in the liver of *L.duvauceli* as in the present study. Cd content was low in finfish and shellfish caught from the same region indicating the selective bio-accumulation of Cd by cephalopods (Lakshmanan, 1988). Tomasevic (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. Oehlenschlaeger (1991) found elevated levels of Cd (570,760 and 680 ng/g wet wt) in the tube, viscera and whole Californian squid (*Loligo opalescens*). In addition, Barska (1988a) noted high levels of Cd in whole canned squid, *Loligo patagonica*; Cozzani *et al.,* (1990) found that 40 of 422 samples of squid (*Loligo* spp.) had higher levels of Cd and exceeded the tolerance limit of 3 ppm. 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.).

Bustamante *et al.* (1998a) suggested that cephalopods constitute an important source of Cd for cephalopod predators. 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 due to high cephalopod consumption in the diet. Law *et al.* (1997) and Sepulveda *et al.* (1997) suggested that squids play an important role in the bioaccumulation of Cd by their predators. The environmental factors and feeding habits of squids, wherein a variety of fish, shellfish and crustaceans are consumed, probably contribute to the high levels of the metals in squids. Cannibalism that has been noticed in *L.duvauceli* (Kore and Joshi,1975; Varghese ,1976) might have also contributed to high Cd levels. Very high levels of Cu in the liver of *L. opalescens* upto 200 to 300 mg/kg (wet wt) was reported by Falandysz (1991) and a moderate range of 12.79 to 178.94 mg/kg in *L.duvauceli* by Lakshmanan and Stephen (1993). The values observed in fresh skinless mantle of *L.duvauceli* are comparable to mean levels reported by Tariq *et al.* (1991) in *L.duvauceli* from the Arabian Sea. The Cu levels in muscle are also quite comparable to the levels in *L.opalescens* reported by Falandysz (1989).

The levels of Pb observed in the muscle of *L.duvauceli* are similar to the values reported in *L. opalescens* (Hall *et al.* 1978 and Falandysz, 1991). As observed in the present study a greater

concentration of Pb was found in the non edible parts of *L.duvauceli* . Also, 11% of whole samples had Pb content above 1.5 ppm, the tolerance limit.

Very high levels of Cu were noted in the liver of *L.duvauceli* as also reported by Martin and Flegal (1975) and Oehlenschlaeger (1991) in *L.opalescens.* The high levels of Cu found in the liver of *L.duvauceli* may be attributed to the very high concentration factor for this element: 2:1 million in squid (Anderlini, 1974). Since squid requires Cu for the synthesis of the respiratory pigment, haemocyanin, a high level in the body may be attributed to the functional necessity (Martin and Flegal ,1975 and Smith ,1984).

Zn content in the mantle and whole soft tissues of *L.duvauceli* are quite comparable to the values observed in *L.opalescens* (11,000 mg/g) and *L.patagonica* (12 mg/kg and 16 mg/kg wet wt) reported by Ohlenschlaeger (1991) and Falandysz (1989) respectively. Zn being a bio essential element is more widely distributed than Cd among the different organs and has greater importance in biological systems (Keillin and Mann,1940 and Vallee,1963).

The level of Fe found in the various body components of *L.duvauceli* was quite comparable to the values observed in *L. opalescens* (Falandysz 1991) and *L. patagonica* (Falandysz 1989). Whole animals had slightly higher content of Fe than muscle in squid

samples from all the four regions. Lower levels of Mn were reported in muscle by Falandysz (1988, 1989) in *Illex argentinus* and *L. patagonica* than observed in the present study.

Cr and Ni levels in the edible muscle of *L.duvauceli* was several folds lower than levels reported by Tariq *et al.* (1991). Bustamante *et al.* (1998a); Miramand and Bentley (1992) reported Ni levels of 16.3±7.8 and 1.3±0.4 µg/dry wt in cephalopods, *Nautilus macromphalus* and *Sepia officinalis* respectively.

In general, the metal levels in squids captured from different regions showed significant variability as a function of their geographical origin. Cd content in the whole squids often exceeded the tolerance limit of 3 ppm, in at least 20% of the whole samples analysed. Pb and Cr levels exceeded the tolerance limit in 11% of the samples analysed. In Cochin region, whole *L.duvauceli* recorded higher average levels of Hg, Cd, Pb, Cu, Zn, Mn and Ni than Quilon, Mangalore and Mumbai regions. Mangalore whole squids exhibited the lowest mean levels of metals like Cu, Zn Pb, Fe, and Ni. The average concentrations of all the metals were significantly lower in the edible parts of squids from all the regions and far below the legal limits (FDA, 1998). Concentrations of Hg was found to be <50 µg/kg in the edible muscle in 90% of the samples. However, liver was the major site of accumulation of Cd and other metals followed by gills. The bio-accumulation of different metals

at higher levels in squid have highlighted the key role of the liver in the metabolism of metals and suggest efficient detoxification processes in this organ as already observed in other molluscs (Coombs and George, 1978). Detoxification strategy involves storage mechanisms of these elements. This strategy appears to be efficient and probably applied to minimize energetic cost and is common among Coleoidae cephalopods. Moreover these high levels of toxic metals do not apparently disturb essential elements metabolism and squids are able to grow and reproduce with very high metal concentrations (Bustamante *et al.*, 1998b).

Toxic metals like Hg, Cd and Cr showed significant reduction of around 30%, 70% and 35% respectively in whole cleaned samples (with the visceral parts and skin removed). As squids are consumed in the whole form by some countries it is suggested that the liver and other visceral parts must be removed before consumption, as removal of the visceral parts and skin, reduces the metal levels to around 30-80% of that present in whole soft parts. Schulz-Schering (1995) suggested that as Cd concentrations of the squid products resulted from high concentrations in intestine, the visceral parts must be removed carefully. Barska *et al.* (1988b) observed high level of heavy metals (especially Cd) in whole canned *L. patagonica* and suggested diffusion of metals from the viscera to the mantle is possible during the products storage. Martin and Flegal (1975) noted that trace metals

migrate out of the liver into the flesh and it results in higher levels in the edible mantle. In order to reduce Cd and other trace metals in the edible muscle of squid, the visceral parts and skin should be removed before spoilage starts and consumption of liver must be avoided. Care must be taken to see that the edible muscle is not contaminated with any dissolved liver portion.

The present result has shown that variation in heavy metal concentrations exist between the squids collected from different regions. Therefore the base line data of the study will be useful for regular ecological monitoring considering the impact of pollution in the aquatic environment.

Table 3.1: Trace metal concentrations (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli* collected off Cochin

WHOLE / BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	47	0.076 ± 0.026 (0.035-0.098)	2.228 ± 1.378 (0.413-6.070)	0.961 ±-0.214 (0.564-1.985)	13.154 ± 8.473 (3.843 – 37.640)	9.933 ± 4.327 (5.314 – 20.371)	13.887±13.363 (2.354-37.061)	3.766 ±3.592 (0.369-9.903)	2.836 ± 0.912 (0.026 – 3.479)	3.027 ± 0.732 (0.588 – 2.456)
MUSCLE	62	0.042 ± 0.017 (0.014 – 0.071)	0.475 ± 0.403 (0.026-1.978)	0.596 ±0.349 (0 -1.414)	2.311 ± 2.915 (0– 13.798)	6.944 ± 4.345 (2.955 – 24.44)	3.502 ±3.203 (0.121-12.309)	1.411 ±1.307 (0-3.917)	0.650 ± 0.642 (0–2.660)	0.522 ± 0.722 (0– 1.032)
LIVER	43	0.156 ± 0.060 (0.053 – 0.231)	79.267 ± 104.396 (2.313-187.56)	3.970 ±3.538 (0 -12.316)	80.188 ± 61.343 (8.585 – 182.70)	48.885 ± 48.557 (6.462 – 160.99)	80.662 ±22.954 (13.418-214.20)	9.955 ±3.432 (0-22.750)	2.108 ± 2.181 (0–7.079)	2.211 ± 3.313 (0–12.447)
GILLS	53	0.052 ± 0.014 (0.032 – 0.073)	2.086 ± 2.035 (0.144–6.954)	1.959 ±1.732 (0 - 4.624)	28.938 ± 10.126 (14.88 – 55.230)	10.651 ± 7.041 (4.15 – 36.259)	46.665 ±58.005 (5.032-229.66)	3.860 ±3.854 (0-12.317)	2.004 ± 2.554 (0–7.820)	0.621 ± 0.721 (0–2.424)

*maximum number of samples analysed

Table 3.3: Trace metal concentrations (Mean ± S.D, Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli* collected off Mangalore

WHOLE / BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	24	0.046 ± 0.006 (0.027 – 0.056)	0.754 ± 0.077 (0.560– 0.839)	0.425 ±0.163 (0.721-1.969)	1.933 ± 1.166 (1.242 – 4.958)	10.032± 1.334 (6.048 – 12.816)	5.345 ±1.939 (3.232-11.064)	0.910 ±0.102 (1.144-2.568)	1.892 ± 0.450 (0.830– 2.124)	0.753± 0.040 (0.662 – 0.809)
MUSCLE	37	0.016 ± 0.030 (0.010 - 0.036)	0.233 ± 0.208 (0 – 0.917)	0.233 ±0.208 (0-1.277)	1.455 ± 1.369 (0– 4.644)	6.100 ± 2.415 (3.339 – 14.631)	1.849 ±1.071 (0-018-175)	0.744 ±0.831 (0-2.610)	0.576± 0.524 (0– 1.984)	0.264 ± 0.329 (0 – 0.868)
LIVER	34	0.086± 0.022 (0.054 – 0.114)	8.119 ± 7.676 (0.742 – 24.670)	1.695 ±3.005 (0-8.302)	16.697 ± 12.301 (0.479 – 44.520)	19.131 ± 19.413 (1.018 – 76.562)	42.732 ±16.585 (16.233-55.105)	7.001 ±9.675 (0-28.400)	3.916 ± 5.652 (0 – 12.936)	3.181 ± 3.607 (0– 10.000)
GILLS	37	0.041 ± 0.016 (0.017 – 0.065)	0.854 ± 0.247 (0..020 – 1.122)	1.268 ±0.976 (0-2.094)	14.832 ± 10.280 (1.477 – 43.450)	9.117 ± 5.848 (2.289 – 22.640)	35.276±27.997 (0.607-57.590)	4.906 ±3.664 (0-9.839)	0.790 ± 0.806 (0 – 2.760)	2.027 ± 1.586 (0 – 5.228)

*maximum number of samples analysed

Table 3.4: Trace metal concentrations (Mean ± S.D, Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli* collected off Mumbai

WHOLE / BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	12	0.057 ± 0.002 (0.055 – 0.059)	0.996 ± 0.267 (0.736–1.229)	0.758 ±-0.491 (0.134-2.014)	4.627 ± 2.369 (1.455 – 7.020)	8.378 ± 8.963 (2.040 – 14.716)	12.490 ±3.088 (9.242-15.621)	0.459 ±0.187 (0.292-0.682)	0.936 ± 0.429 (0.443 – 1.652)	1.313 ± 0.689 (0.535-2.029)
MUSCLE	23	0.009 ± 0.007 (0.010 – 0.017)	0.583 ± 0.604 (0.203–1.142)	0.592 ±0.618 (0-1.133)	1.890 ± 1.984 (0.368 – 5.924)	7.581 ± 2.458 (5.571 – 13.285)	5.569±1.321 (8.398-10.772)	0.309 ±0.047 (0-0.356)	0.178 ± 0.069 (0.133 – 0.313)	1.173 ± 0.912 (0– 2.561)
LIVER	17	0.039 ± 0.011 (0.031 – 0.047)	61.641 ± 23.739 (26.057–74.351)	0.861 ±1.008 (0-1.923)	68.584 ±56.118 (21.132-136.67)	59.934 ± 26.718 (30.576 – 76.15)	83.298 ± 6.718 (23.305-189.23)	2.074 ±0.623 (0-5.713)	3.153 ± 3.943 (0.576 – 7.692)	16.777 ± 7.247 (7.115 – 23.07)
GILLS	19	0.032 ± 0.031 (0–0.062)	2.417 ± 1.540 (0.673 – 5.00)	1.135 ±1.430 (0-2.821)	53.918 ± 69.293 (8.896 – 146.71)	36.434 ± 35.81 (10.60 – 88.570)	5.466 ±5.662 (0.541-10.857)	3.039 ±0.970 (0-3.725)	3.816 ± 4.197 (0.188 – 9..216)	7.883 ± 6.444 (1.282 – 15.00)

*maximum number of samples analysed

Table 3.5: Overall average levels of metals in whole soft parts and whole cleaned samples of *L. duvauceli.*

SQUID	MERCURY	CADMIUM	COPPER	ZINC	CHROMIUM	NICKEL
Whole	0.051	1.069	9.109	14.385	3.018	1.434
Whole cleaned	0.034	0.303	1.871	9.460	2.225	0.840
Percentage reduction	33.3	71.6	79.4	34.2	35.6	41.4

Table 3.6: 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	Crustacea	-
	86	Molluscan bivalves	
Cadmium	3	Crustacea	0.5-3.0*
	4	Molluscan bivalves	
Chromium	12	Crustacea	-
	13	Molluscan bivalves	
Lead	1.5	Crustacea	0.5-10*
	1.6	Molluscan bivalves	
Nickel	70	Crustacea	-
	80	Molluscan bivalves	
Mercury	1.0	All fish	1.0
Zinc	-	-	50

* varying between EU countries

Table 3.7: Analysis of variance (ANOVA) of heavy metals in muscle of *Loligo duvauceli* (Regional variation)

Mercury						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0080323	3	0.002677431	17.61102	7.58E-08	4.21795221
Within Groups	0.0072975	48	0.000152032			
Total	0.0153298	51				
Cadmium						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.3015695	3	2.100523152	8.236449	5.61E-05	3.971905471
Within Groups	27.032942	106	0.255027758			
Total	33.334512	109				
Lead						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14.274952	3	4.758317437	20.99975	3.16E-07	4.600906323
Within Groups	6.1179105	27	0.226589276			
Total	20.392863	30				
Copper						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	89.749497	3	29.91649895	4.778837	0.00464	2.752969408
Within Groups	388.13267	62	6.260204316			
Total	477.88216	65				
Zinc						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	455.32896	3	151.7763212	5.021925	0.003586	4.125894293
Within Groups	1813.3644	60	30.22274042			
Total	2268.6934	63				
Chromium						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.8564028	3	0.952134282	2.156959	0.108201	2.838746127
Within Groups	17.656973	40	0.441424326			
Total	20.513376	43				
Nickel						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.2414082	3	1.080469386	1.7761	0.166389	2.827050594
Within Groups	25.550198	42	0.608338038			
Total	28.791606	45				

Table 3.8: Analysis of variance (ANOVA) of heavy metals in whole of *Loligo duvauceli* (Regional variation)

Mercury						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0056245	3	0.001874817	7.021174	0.000976	4.483695193
Within Groups	0.0082777	31	0.000267023			
Total	0.0139022	34				
Cadmium						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.9173567	3	1.972452229	10.64432	3.01E-05	4.327375791
Within Groups	7.226918	39	0.18530559			
Total	13.144275	42				
Lead						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10.085035	3	3.361678492	6.431502	0.00117	4.312596502
Within Groups	20.907578	40	0.522689458			
Total	30.992614	43				
Copper						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2031.9525	3	677.3175116	19.51645	4.46E-08	4.285254818
Within Groups	1457.6083	42	34.70496029			
Total	3489.5609	45				
Zinc						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4551.032	3	1517.010673	23.94072	1.5E-09	4.227899808
Within Groups	2978.1692	47	63.36530165			
Total	7529.2012	50				
Chromium						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	100.9436	3	33.64786605	8.078584	0.000276	4.343007731
Within Groups	158.27266	38	4.165070022			
Total	259.21626	41				
Nickel						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	13.552508	3	4.517502518	3.265594	0.03234	2.866265447
Within Groups	49.801081	36	1.383363373			
Total	63.353589	39				

Fig 3.1: Distribution pattern of heavy metals (mean, ppm wet wt) in the body components of *L. duvauceli* collected off Cochin

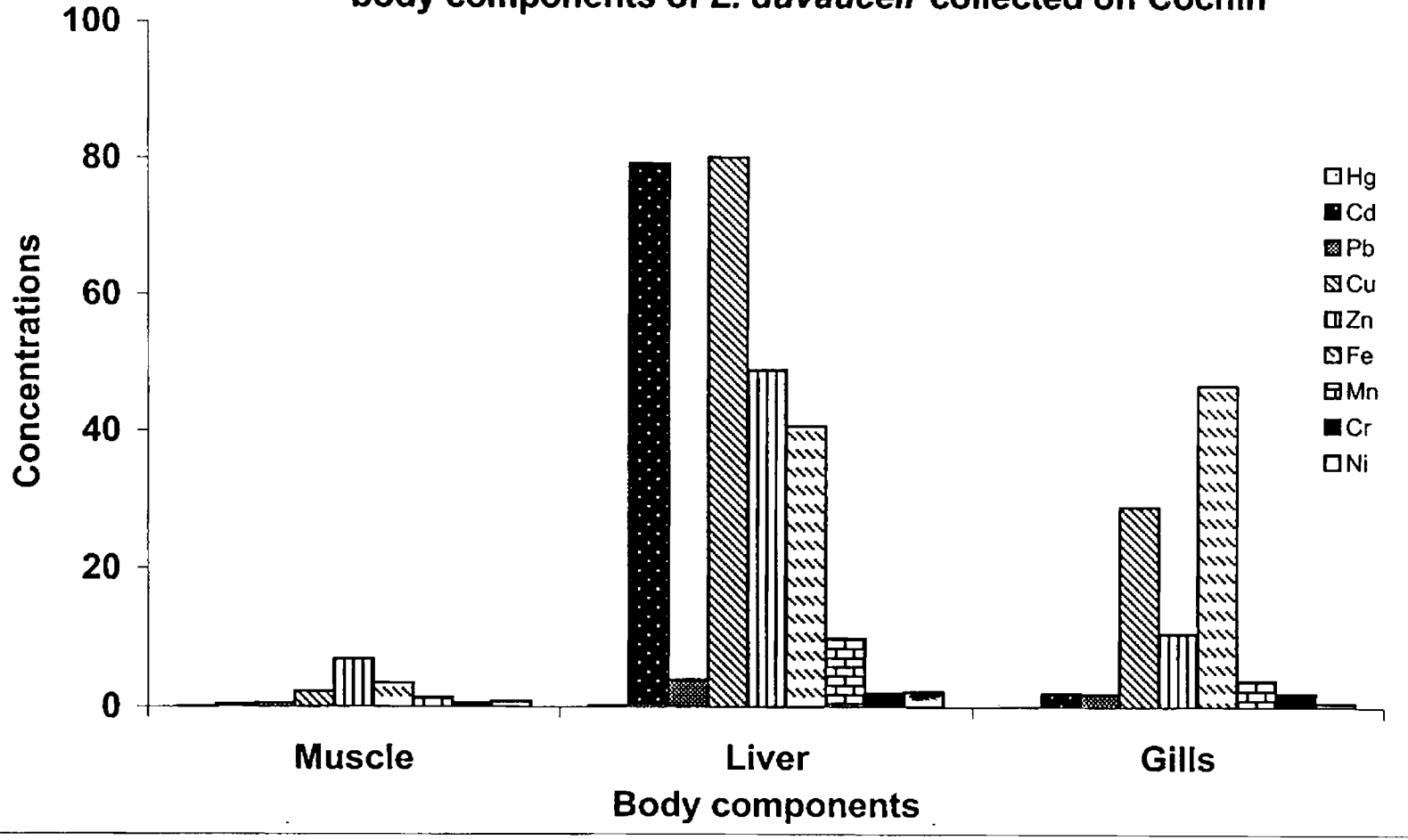


Fig 3.2: Distribution pattern of heavy metals (mean, ppm wet wt) in the body components of *L. duvauceli* collected off Quilon

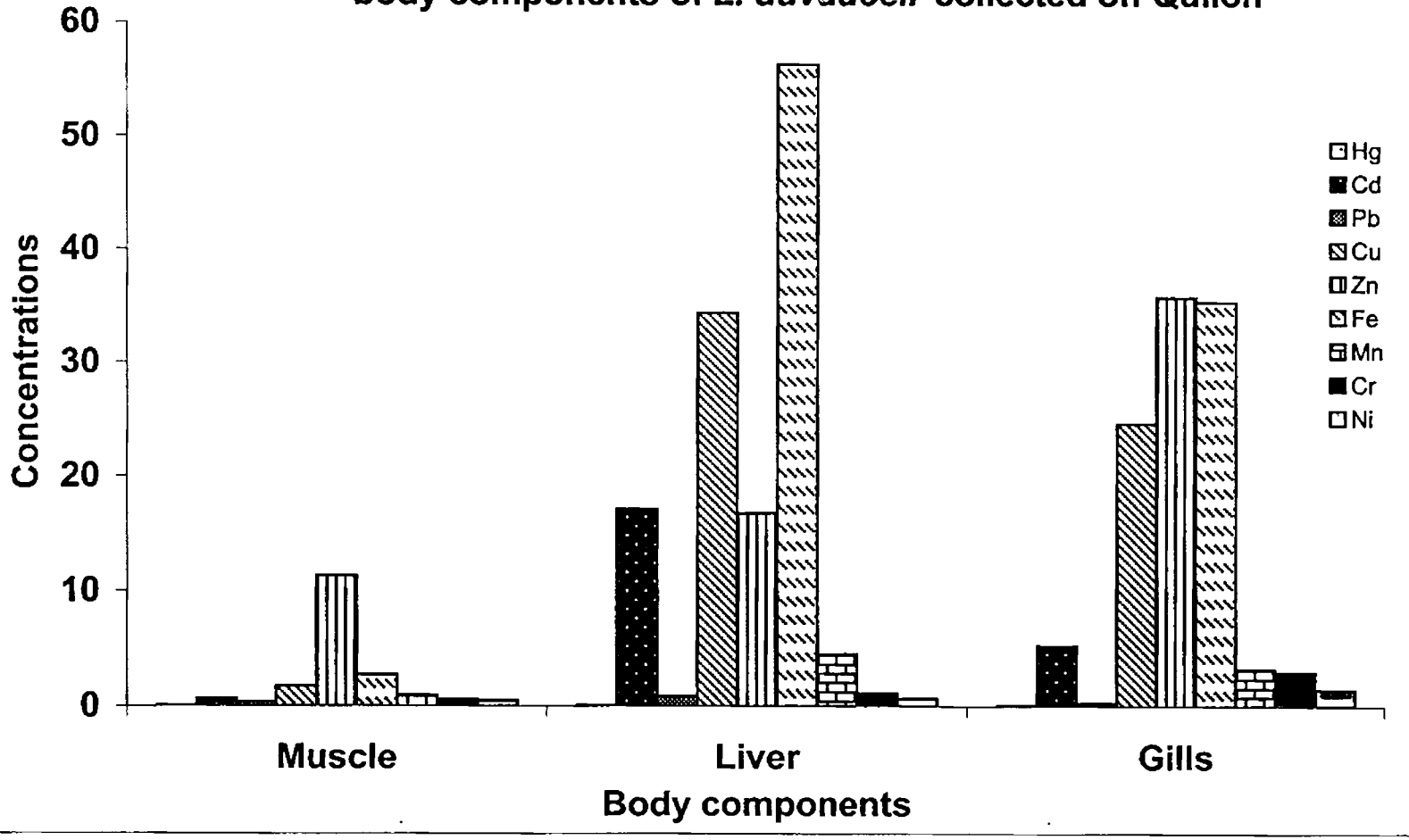


Fig 3.3: Distribution pattern of heavy metals (mean, ppm wet wt) in the body components of *L. duvauceli* collected off Mangalore

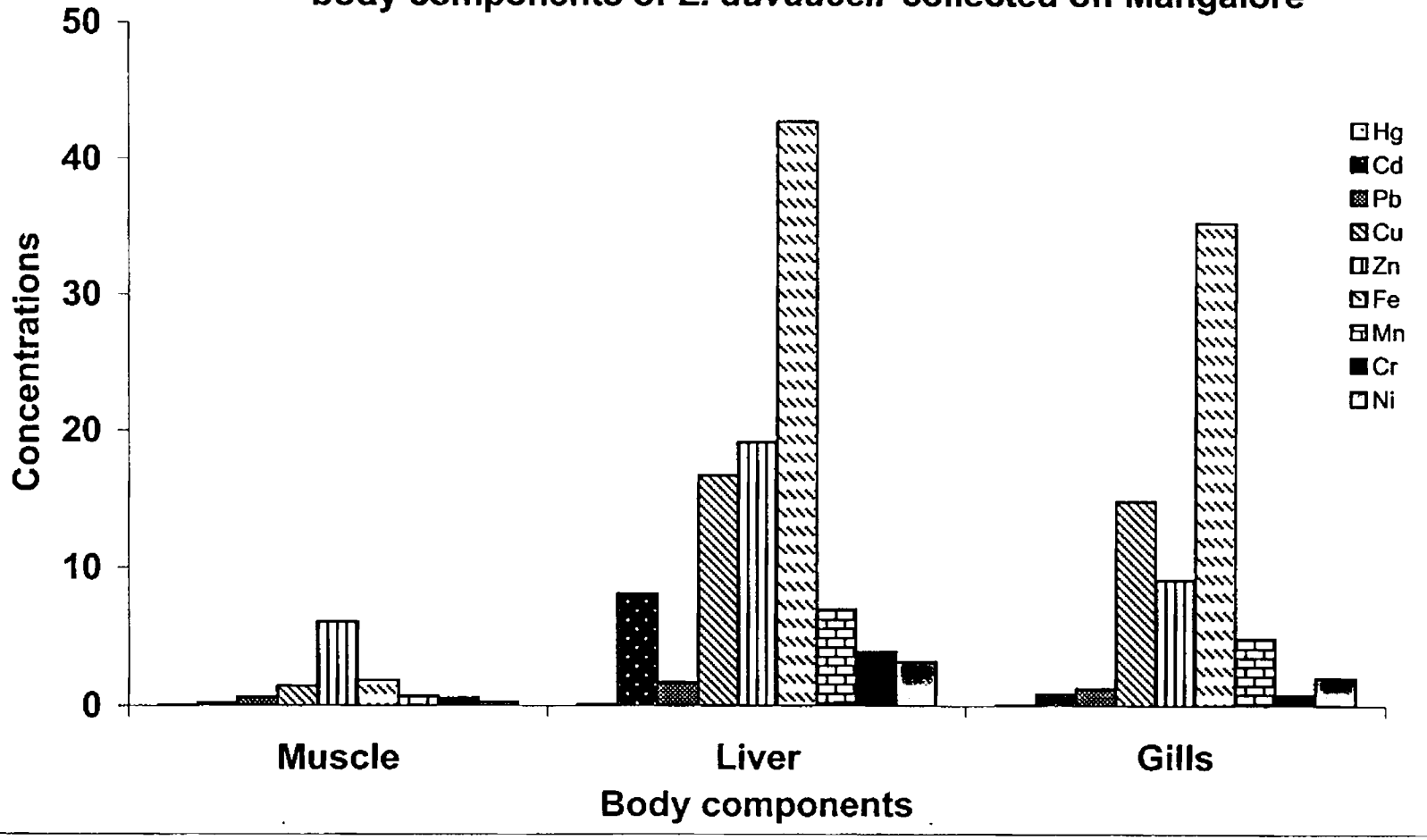


Fig 3.4: Distribution pattern of heavy metals (mean, ppm wet wt) in the body components of *L. duvauceli* collected off Mumbai

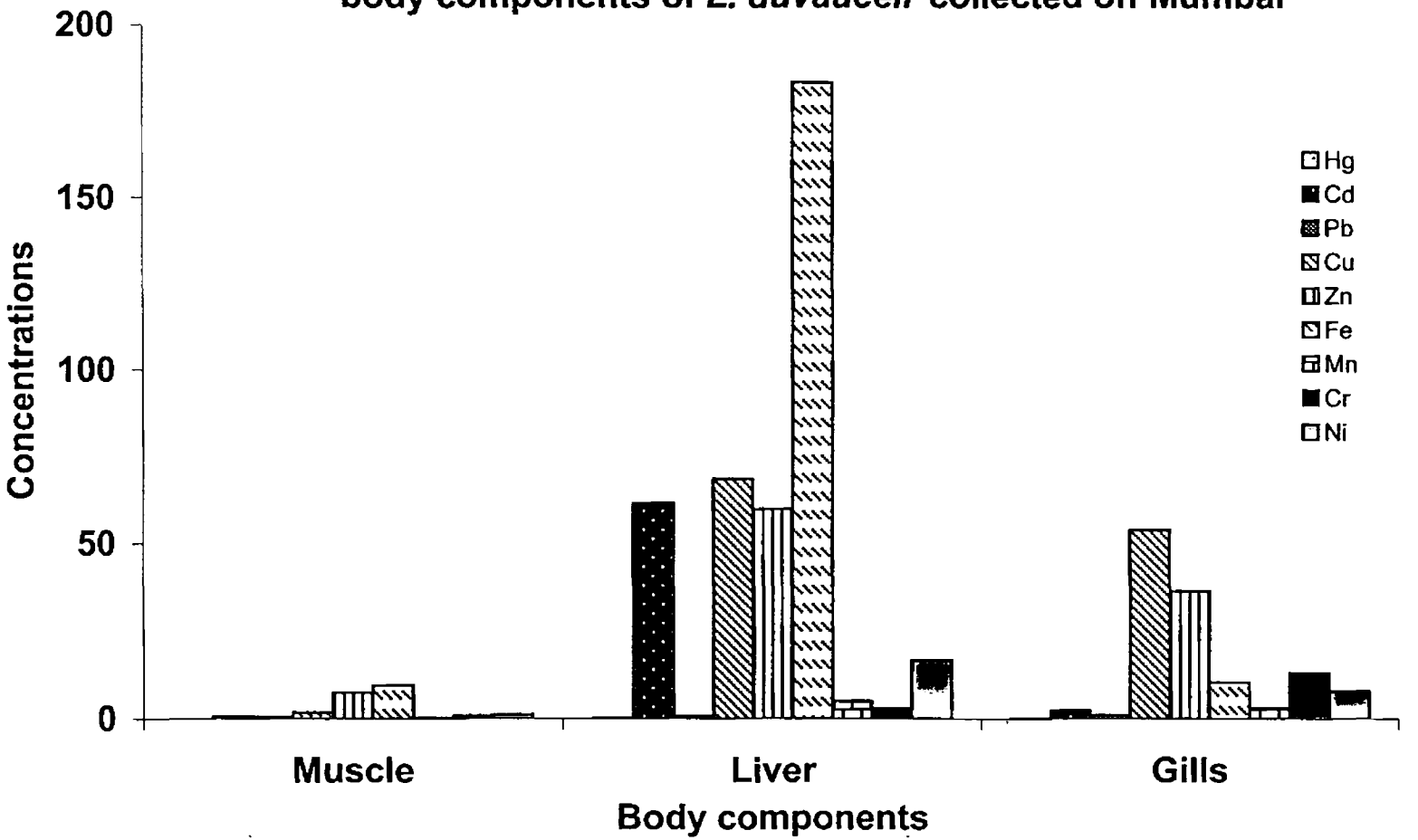
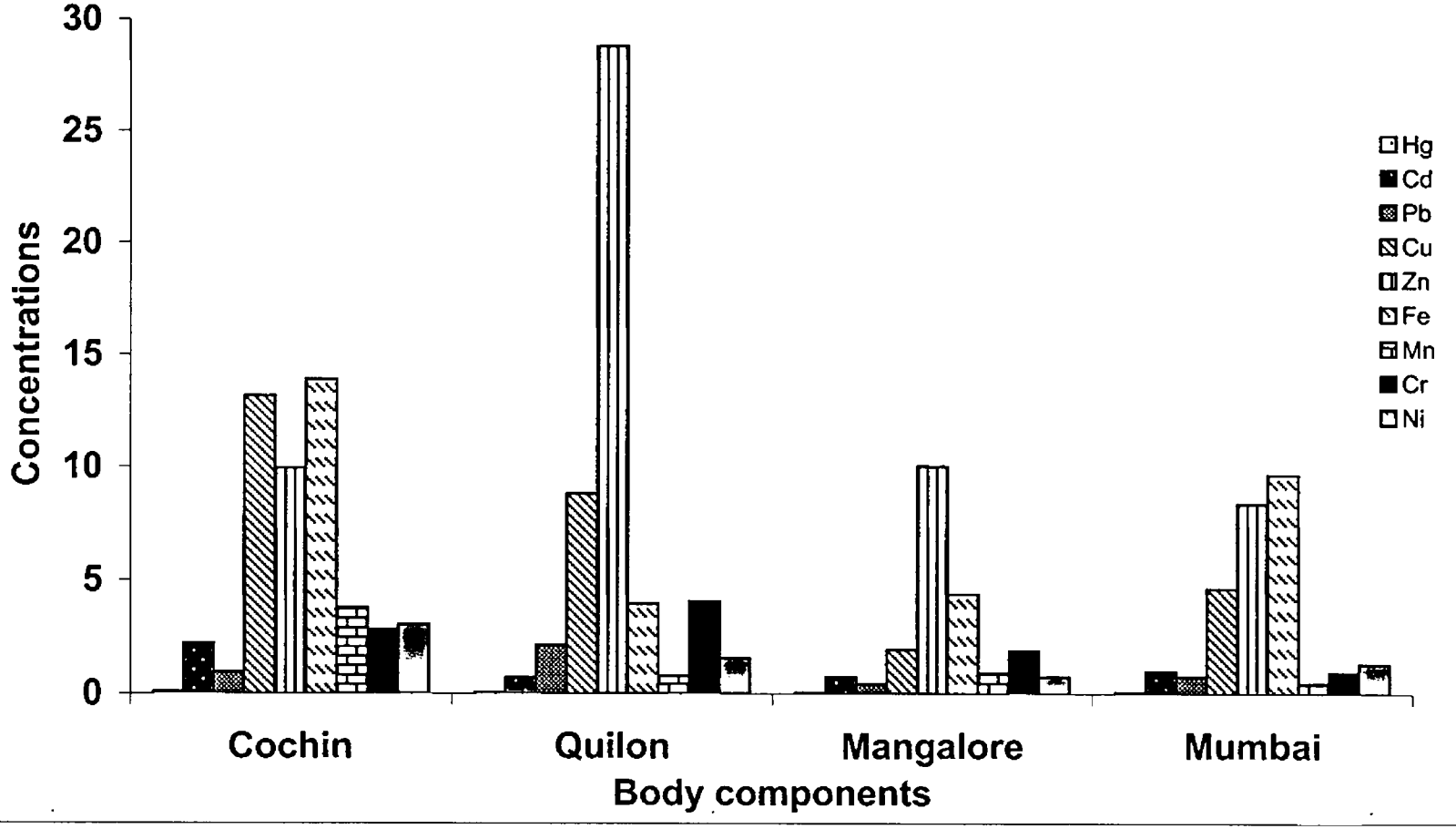


Fig 3.5: Distribution pattern of heavy metals (mean, ppm wet wt) in the whole soft parts of *L. duvauceli*



CHAPTER 4

Seasonal variations of heavy metals in *Loligo duvauceli*

4.1. Introduction

The tendency of metals to get accumulated in marine molluscs is one of the important properties of metals and bioavailability of trace metals is the key factor determining tissue trace metal concentrations. Due to increase in pollution, metals are concentrated to a certain extent by all marine organisms, but some species particularly molluscs, show exceptional affinity of bioaccumulation in certain tissues and bioacccumulation of heavy metals in cephalopods of the Indian waters have been reported by a few authors (Ramamurthy, 1979; Patel & Chandy, 1988; Lakshmanan, 1988a,b; 1989; Jasmine *et al.*, 1989; Dious & Kasinathan, 1992; Lakshmanan & Stephen, 1993; Prafulla *et al.*, 2000 and Lakshmanan *et al.*, 2001). Almost all metals are toxic at higher concentrations and some are lethal even at very low concentrations. Some of the trace metals play essential roles in biological processes, but at higher concentrations, they may be toxic to the biota.

The effect of heavy metals on different aquatic organisms is often complex and difficult to interpret. Dissolved oxygen, pH, salinity, temperature and hardness of water have been demonstrated to be factors that influence the physiology of an organism and the rate of uptake of heavy metals (Wittmann, 1979; Waiwood & Beamish, 1978; Winner,1985; Bradley & Sprague, 1985 and Everall, 1987). Bioaccumulation of heavy metals in marine invertebrates may be affected by factors such as the age, season, size, changes in the life

cycle and feeding rate (Phillips, 1980). As a result of the influence of these physico-chemical and biological parameters toxic heavy metal concentration can vary considerably among and within species. Squids have been shown to contain remarkably high concentrations of trace metals in whole body as well as exclusively in certain organs and individual variation from the same place is considerable. In view of this a broad spectral understanding of heavy metal levels in squid covering the whole season of the year assumes significance.

Therefore, an attempt has been made in this study to follow seasonal changes in the concentration of trace metals in *L. duvauceli,* to establish baseline concentrations for comparison with the same species in other sea areas where the availability of metals may be different, to identify specific organs that may be particularly selective and sensitive to accumulate heavy metals during the different seasons of the year and also to determine the ideal season of harvest of squids with a perspective on seafood safety.

Studies on the seasonal variation of heavy metals in molluscs have been reported worldwide. (Bryan, 1973; Fowler & Oregioni, 1976; Frazier, 1976; Kumagai & Saeki, 1980; Shiber, 1980; Gabbot, 1983; Lakshmanan & Nambisan 1983; Cain & Luoma, 1986, 1990; Carrascal *et al.*, 1996; De-Gregori *et al.*, 1996; Muralidharan & Raja, 1997; Motoe *et al.,* 1997; Rivonker & Parulekar, 1998 and Senthilnathan & Balasubramanian, 1998).

4.2. Materials and Methods

Preparation of squid samples and analysis of Hg, Cd, Pb, Cu, Zn, Fe, Mn, Cr and Ni in the whole soft tissues of *L. duvauceli* and body components (muscle liver and gills) were carried out as described in Chapter 2. Statistical analyses were carried out using one way ANOVA followed by student t-test to compare the means and to determine significant differences if any, in the distribution of heavy metals in squids collected during different seasons.

4.3. Results

Concentrations of heavy metals like Hg, Cd, Cu, Zn, Fe, Mn, Cr, and Ni in the whole soft tissue of *L. duvauceli* and in the various body components collected during premonsoon, monsoon and postmonsoon seasons for the different regions, *viz.*, Cochin, Quilon and Mangalore are presented in Tables 4.1a to 4.3c. Figs. 4.1 to 4.4 indicate the seasonal changes in the distribution of heavy metals at the three regions. Results of ANOVA (Analysis of Variance) in edible muscle and whole soft tissues of *L. duvauceli* are presented in Tables 4.4 and 4.5 for Cochin region, Tables 4.6 and 4.7 for Quilon region and Tables 4.8 and 4.9 for Mangalore region. The distribution characteristics of heavy metals in *L. duvauceli* covering the whole season of a year are discussed below.

4.3.1. Seasonal variations in Mercury, Cadmium and Lead

The distribution of ΣHg, Cd and Pb in *L. duvauceli* varied with season (Tables 4.1a to 4.3c and Figs. 4.1a to 4.4c). Mean ΣHg content in whole *L. duvauceli* from Quilon region varied from 0.041 to 0.052 ppm during premonsoon period, 0.023 to 0.048 ppm in monsoon period and 0.086 to 0.089 ppm during postmonsoon period. Elevated levels of ΣHg was found during monsoon period than premonsoon and postmonsoon period in the whole soft tissues of *L. duvauceli* from Cochin and Mangalore regions (Fig. 4.4). The values during monsoon period ranged from 0.092 to 0.098 ppm at Cochin region and 0.048 to 0.056 ppm at Mangalore region. In the edible muscle mean ΣHg content varied with season in the order: premonsoon < monsoon< postmonsoon at Cochin region, monsoon < premonsoon < postmonsoon at Quilon region and monsoon < post monsoon < premonsoon at Mangalore region. (Tables 4.2 a to 4.3c). However, the average muscle ΣHg content were quite low in *L. duvauceli* from the three regions during the three seasons. ΣHg levels did not exceed the limit of 1.0 mg/kg fresh weight as laid down by the U.S Food and Drug Administration (Figs. 4.1 to 4.3). Liver samples of squids caught during the premonsoon season off Cochin recorded highest mean ΣHg content (0.199 ppm) and lowest levels were recorded during the postmonsoon period (0.127 ppm). Mean ΣHg content in liver component of *L. duvauceli* from Quilon region varied from 0.079 to 0.143 ppm during premonsoon, 0.054 to 0.092 ppm during monsoon

and 0.028 to 0.067 ppm during postmonsoon season while from Mangalore region the seasonal distribution pattern followed the order: premonsoon > postmonsoon > monsoon (Fig. 4.3). ΣHg content in gills was found to be highest during postmonsoon season in *L. duvauceli* from Cochin region (0.061±0.016 ppm) and lower mean ΣHg content was found in premonsoon period (0.047±0.017 ppm) while at Quilon and Mangalore regions higher levels were found during premonsoon period, the respective values being 0.050±0.010 ppm and 0.050±0.014 ppm. However, lower values were found during monsoon season at Quilon region (0.021 to 0.028 ppm) and at Mangalore region lower levels were noted in postmonsoon period (0.017 to 0.049 ppm). Among the various body components analysed liver exhibited highest ΣHg content followed by gills and muscle in all the three regions during the premonsoon, monsoon and also postmonsoon period (Figs 4.1 to 4.3).

Effect of season on the distribution pattern of Cd was very much pronounced. In the whole soft tissues of *L. duvauceli* from Cochin region, Cd content varied from 0.684±0.180 ppm during premonsoon followed by Cd content of 2.919±1.584 ppm during monsoon period and Cd levels<1ppm during postmonsoon period. (Tables 4.1a to 4.3c). At Mangalore region, Cd content, varied from 0.701 to 0.839, 0.737 to 0.749 and 0.560 to 0.704 ppm during premonsoon, monsoon and postmonsoon periods respectively. Highest average levels of Cd was found in whole soft tissues of *L.duvauceli* from Quilon region during postmonsoon season (1.417 ppm) and lowest levels of Cd were

found during the premonsoon season (0.495 ppm). In the edible muscle mean Cd content varied with season and were in the order: monsoon < premonsoon < postmonsoon at Quilon. At Cochin region seasonal variations of heavy metals in the muscle were in the order: monsoon > postmonsoon > premonsoon. In Mangalore squid samples Cd content varied from 0 to 0.917 ppm during premonsoon period, followed by a more or less similar levels found in the other seasons and were in the range 0.146-0.226 ppm during monsoon period and 0.023 to 0.261 ppm during postmonsoon period. Liver samples of *L.duvauceli* from Cochin and Quilon region showed very high content of Cd during the postmonsoon period and were in the range 37.51 to 185.76 ppm and 8.937 to 45.045 ppm respectively. At Mangalore region, Cd content varied from 0.742 to 3.750 during premonsoon followed by Cd content of 11.875 to 24.67 ppm and 4.008 to 8.773 ppm during monsoon and postmonsoon period respectively. Gill component of *L.duvauceli* from the three regions exhibited a similar seasonal profile of higher mean Cd content during postmonsoon period than monsoon and premonsoon period (Fig 4.1 to 4.3). The highest recorded values were 6.954, 15.45 and 1.122 ppm from Cochin, Quilon and Mangalore regions respectively. The distribution pattern of Cd in the various body components during the premonsoon monsoon and postmonsoon season showed a similar seasonal profile and were in the order: liver > gills > muscle.

Whole squids showed comparatively higher mean Pb values during the postmonsoon periods from Cochin, Quilon and Mangalore regions (Fig. 4.4) than premonsoon and monsoon period. Pb content exceeded the tolerance limit in 11% of the samples analysed during the postmonsoon period. However, Pb content in muscle was very low during all the seasons and least Pb content was noted during the premonsoon period and the concentrations were 0.34±0.513, 0.045±0.084 and 0.048±0.126 ppm from Mangalore, Quilon and Cochin regions respectively. Highest mean Pb content in the muscle was noted during the monsoon period and were in the range 0 to 1.199, 0.436 to 1.112 and 0 to 1.277 ppm at Cochin, Quilon and Mangalore regions respectively. Liver samples of squids caught during the postmonsoon season off Cochin recorded highest mean Pb content of 5.334 ppm and lowest mean Pb content was noted during the premonsoon period (2.733 ppm). Mean Pb content in liver component of *L. duvauceli* from Quilon region varied from 0 to 0.079 ppm during premonsoon, 0.532 to 1.965 ppm during monsoon and 0 to 2.275 ppm during postmonsoon. At Mangalore region the seasonal distribution pattern followed the order: postmonsoon > monsoon > premonsoon. Distribution of Pb in gills varied with season and was of the order: postmonsoon > monsoon > premonsoon from Mangalore and Cochin regions and monsoon > postmonsoon > premonsoon (Quilon region).

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

Distribution of Cu, Zn, Fe and Mn in *L. duvauceli* seemed to be well influenced by season (Tables 4.1a to 4.3c and Figs. 4.1 to 4.4). Higher average values were observed during the postmonsoon season than premonsoon and monsoon seasons in whole *L.duvauceli.* The levels ranged from 8.822 to 37.640 ppm at Cochin, 5.151 to 29.965 ppm at Quilon and 2.40 to 4.958 ppm at Mangalore. Cu content in the different body components collected from the three regions indicated wide seasonal variation. Concentration of Cu in liver of *L.duvauceli* varied among the different seasons and followed the order: premonsoon > postmonsoon > monsoon in samples from Quilon area, monsoon > postmonsoon > premonsoon from Cochin area and monsoon > premonsoon > postmonsoon from Mangalore area. Gills of *L. duvauceli* showed comparatively higher mean values for samples collected from Mangalore region during the premonsoon season. In Quilon samples, higher values were observed during the postmonsoon period and from Cochin region higher concentrations were noted during the monsoon season (Figs. 4.1 to 4.3). In the edible muscle maximum average content of Cu were 2.313±1.156 and 4.008±3.946 ppm in the premonsoon season in samples collected from Quilon and Cochin regions respectively. At Mangalore region lower average Cu concentrations was exhibited during premonsoon season (Fig. 4.3).

Zn content in *L.duvauceli* seemed to be well influenced by season. In whole soft tissues of *L.duvauceli* from Cochin region, Zn

content varied from 5.769 to 20.371 ppm in premonsoon, 5.314 to 12.528 ppm in monsoon and 8.930 to 10.438 ppm in postmonsoon periods. In Quilon region whole samples, Zn levels ranged from10.598 to 39.80, 40.44 to 43.43 and 10.835 to 32.253 ppm during premonsoon, monsoon and postmonsoon periods respectively. Zn content in whole soft tissues of *L. duvauceli* from Mangalore region was >10 ppm during premonsoon and post monsoon season. It was comparatively lower during the monsoon period and was in the range 6.048 to 6.057 ppm (Fig. 4.4). Zn accumulation in the edible muscle were comparatively higher during premonsoon period than during monsoon and postmonsoon periods at both Cochin and Quilon regions and the range being 4.269 to 24.44 ppm (Cochin) and 7.117 to 32.410 ppm (Quilon). Distribution of Zn in gill component of *L. duvauceli* varied with season and were of the order: monsoon < premonsoon < postmonsoon at Cochin and Quilon regions and postmonsoon < monsoon < premonsoon at Mangalore region (Figs. 4.1 to 4.3). Among the various body components analysed the highest recorded Zn content was 160.99 ppm found in the liver of *L. duvauceli.*

Fe was the most abundant metal found in *L. duvauceli.* In the whole soft tissues of *L. duvauceli* from Cochin region Fe content varied from 4.422±0.279 ppm during premonsoon followed by 6.369±2.137 ppm during monsoon and 23.785±15.530 during postmonsoon period. Highest average levels were found in whole soft tissues of *L. duvauceli* from Quilon region during the postmonsoon

period and lowest levels were recorded during the monsoon period (Fig. 4.5). At Mangalore region the levels varied from 9.751 to 11.064, 4.384 to 6.393 and 3.232 to 7.328 ppm during premonsoon, monsoon and postmonsoon periods respectively. In the edible muscle mean Fe content varied with season and were in the order: postmonsoon > premonsoon > monsoon at Cochin region. In Mangalore squid samples mean Fe content was more or less similar during premonsoon and postmonsoon periods Figs. (4.1 to 4.3) while at Quilon region Fe levels were in the range 1.593 to 3.180, 1.109 to 1.126 and 2.377 to 5.231 ppm during premonsoon, monsoon and postmonsoon periods respectively. Fe content in liver showed a marked seasonal variation, highest mean levels were found during the premonsoon period in all the 3 regions (Figs. 4.1 to 4.3). In the gills Fe content varied from 10.194 to 111.8, 4.57 to 14.084 and 9.434 to 53.884 ppm during premonsoon, monsoon and postmonsoon periods, respectively at Quilon region. Gill component of *L. duvauceli* from Cochin region and Mangalore region exhibited a similar Fe profile of comparatively higher mean values during postmonsoon season than monsoon and premonsoon period (Table 4.1a to 4.3c). Distribution pattern of Fe in gills of squid samples from Mangalore region were of the order: monsoon > postmonsoon > premonsoon.

Higher average levels were observed during the postmonsoon season than premonsoon and monsoon period in whole *L. duvauceli* from Cochin and Mangalore regions and values ranged from 2.600 to

9.903 and 1.259 to 2.568 ppm respectively. At Quilon the Mn content varied from 0.627 to 2.703 ppm during premonsoon, 0.71 to 1.072 ppm during monsoon and 0.231 to 0.879 ppm during postmonsoon. Mn content in the different body components analysed indicated seasonal variation. In the muscle, Mn content varied in the order: postmonsoon > premonsoon > monsoon at Cochin and Quilon and premonsoon > postmonsoon > monsoon at Mangalore. Mn accumulation in the liver were comparatively higher during premonsoon period than during the other seasons at Quilon and Mangalore region. While, at Cochin region, average Mn levels varied from 0 to 22.750 ppm during premonsoon, 0 to 22.690 ppm during monsoon and 7.318 to 16.820 ppm during postmonsoon. Postmonsoon season recorded higher mean levels of Mn in gill samples of L. *duvauceli* from Cochin region. At Mangalore region more or less similar levels were noted during the monsoon and postmonsoon periods (Fig. 4.3). At Quilon region Mn content in gills were of the order: premonsoon>monsoon> postmonsoon.

4.3.3. Seasonal variation of Chromium and Nickel

The distribution pattern and seasonality of Cr and Ni in *L.duvauceli* are presented in Tables 4.1a to 4.3c and in Figs. 4.1 to 4.4. Cr content varied markedly with season in whole soft tissues of *L. duvauceli*. At Cochin region, Cr content in whole samples were 1.146±1.369, 1.265±0.442 and 0.969 ±0.514 ppm during premonsoon, monsoon and postmonsoon periods respectively. At Quilon region Cr

levels varied in the order: post monsoon > premonsoon > monsoon and at Mangalore region premonsoon > monsoon > postmonsoon (Fig. 4.4). Higher levels of Cr were noted in the edible muscle of *L. duvauceli* collected from Quilon region during the postmonsoon period than during monsoon and premonsoon periods (Table 4.1b to 4.1c) . Cr content was low during the monsoon period in Cochin squid samples with an average of 0.206 ppm but were more or less similar during premonsoon and postmonsoon period. The average levels being 0.472 and 0.491 ppm respectively. In the liver component the Cr content at Cochin region varied from 0 to 6.14 ppm during premonsoon, 0 to 7.079 ppm during monsoon and 0 to 5.144 ppm during postmonsoon. At Quilon and Mangalore regions mean Cr content in liver was comparatively higher during premonsoon period (1.566 and 7.482 ppm respectively) than during monsoon and postmonsoon period (Figs. 4.2 and 4.3). In gills, least Cr content was found in monsoon period in *L. duvauceli* from Cochin region (0.113±0.140 ppm) and at Quilon region (1.123±0.492 ppm) than in other seasons. Cr content in gills of *L. duvauceli* from Mangalore region varied from 0 to 2.094 ppm during premonsoon, 0 to 2.76 ppm during monsoon and 0 to 0.724 ppm during postmonsoon.

The prevalence of Ni in whole *L. duvauceli* varied with season and was comparatively lower during postmonsoon period than monsoon and premonsoon periods in all the three regions (Fig 4.4). In the edible muscle Ni content varied from 0 to 1.010, 0 to 1.032 and 0

to 0.444 ppm during premonsoon, monsoon and postmonsoon seasons respectively at Cochin region. At Quilon region the Ni levels varied in the order: premonsoon > postmonsoon > monsoon and the levels were 0.918±1.013, 0.393±0.265 and 0.106±0.007 ppm respectively. At Mangalore region, mean Ni content were the highest in premonsoon period (0.413 ppm) and very low levels were recorded during monsoon (0.015 ppm) and postmonsoon periods (0.032 ppm) respectively. Liver samples exhibited highest mean Ni content in monsoon season (3.621 ppm) and lowest in premonsoon season (0.820 ppm) at Cochin region whereas in Quilon samples concentrations were 0.619±0.679, 0.652±0.515 and 0.883±0.417 ppm during premonsoon, monsoon and postmonsoon periods respectively. At Mangalore region Ni content in liver varied from 0 to 10 ppm during premonsoon, 0 to 3.344 ppm during monsoon and 0 to 5.583 ppm during postmonsoon. The average concentration of Ni in gills from Cochin region varied with season and were of the order: premonsoon > monsoon > postmonsoon (Fig. 4.1). In Quilon and Mangalore samples mean Ni content were 1.508 and 0.575 ppm during premonsoon period, 0.282 and 2.06 ppm during monsoon and 1.071 and 2.371 ppm during postmonsoon periods respectively. The distribution pattern in the various body components analysed showed that higher levels were noted in liver and gill samples than in the edible muscle.

Statistical analysis carried out showed that significant seasonal difference (ANOVA; $p<0.01$, $p< 0.05$) in the distribution of Cu, Zn, and

Cr in the edible muscle and Cd, Pb, Cu, Zn and Ni in whole soft tissues of *L. duvauceli* between the three seasons from Cochin region were noted (Tables 4.4 and 4.5). At Quilon region, confidence level varied between 95% and 99% in the distribution of Hg, Cd, Pb and Zn in whole squids. Cd, Pb and Cu content in the muscle also showed significant difference ($p<0.05$) between the three seasons (Tables 4.6 and 4.7). At Mangalore region levels of significance varied between 1% and 5% levels in the concentrations of Pb, Cr and Ni (muscle tissue) and Cd, Pb, Cu, Zn, Cr and Ni (whole squids) between the three seasons (Tables 4.8 and 4.9).

ANOVA followed by comparison of means of important metals ('t test') showed significant seasonal variations of heavy metals in the whole soft parts of *L. duvauceli*. At Cochin, Cd content was higher during the monsoon period as compared to premonsoon and postmonsoon periods whereas Pb and Cu content were higher during the postmonsoon and Zn and Ni during the premonsoon periods.

At Quilon, significantly higher levels of Hg Cd and Pb in the whole soft parts were observed during the postmonsoon period whereas Zn levels were higher during the monsoon period.

At Mangalore Cd, Cr and Ni showed significantly higher levels during the premonsoon periods. Significant higher Cu levels were however observed during the postmonsoon months.

4.4. Discussion

The concentration of the nine heavy metals studied, *viz.*, Hg, Cd, Cu, Zn, Fe, Mn, Cr and Ni varied markedly with season in *L. duvauceli* at Cochin, Quilon and Mangalore regions. The levels of Hg, Cd, Cu, Zn, Fe, Mn, Cr and Ni in *L. duvauceli* reported in this study were within the ranges found by other researchers in Indian waters (Lakshmanan 1988a, b; 1989; Jasmine *et al.*, 1989 and Lakshmanan & Stephen, 1993).

Hg levels in *L. duvauceli* was low during all seasons. A similar low mean concentration were observed during August month, (Falandysz, 1990) and in the edible and non edible tissues of *Illex argentinus* caught in autumn at the continental shelf of Argentina (Falandysz, 1988). ΣHg content was found to vary with season in *Ostrea edulis* (Carrascal, 1996) and a maximum content in winter was noted in the edible parts of short neck clam *Tapes taponica* (Kumagai and Saeki, 1980) as also observed in the present study in the edible muscle of *L. duvauceli* from Cochin and Quilon regions. The variations in ΣHg level in different organs of squids are also comparable to relatively large variations in mercury concentration often observed when organ levels are compared within as well as between pods (Caurant *et al.*, 1996; Wagemann *et al.*, 1996 and Hyatt *et al.*, 1999). Hg is known to be accumulative in higher tropic level organism of the food chain (Honda *et al.*, 1987). Since main food items of squids are

lower tropic organisms such as fish and crustaceans, accumulation of ΣHg is low during all seasons in *L. duvauceli.*

Although Cd concentration in the edible muscle was < 2ppm and highly elevated levels were noted in the liver during all the seasons. Significant seasonal variation in the mean concentration of Cd in the edible and non-edible tissues was observed with higher values during the monsoon (June–August) and postmonsoon (September–December) seasons. A similar higher concentration of Cd was noted in gill, viscera and mantle of *Mytilus galloprovincialis* (Serra *et al.*,1999). Seasonal variation of tissue Cd concentration with higher levels in monsoon season was noted in Oyster *Crassostrea madrasensis* Senthilnathan and Balasubramanian (1998). Phillips (1976) reported significantly higher concentration of Cd in whole soft parts of *Mytilus edulis* collected at different depth at Generator Beacon during winter (0.85 to 1.85 ppm) than during summer (0.301 to 0.501ppm). A similar seasonal profile was noted in whole soft parts of *L. duvauceli. L. duvauceli* showed higher Cd values which is probably due to Cd rich diet transferred from water and food (Jiro Koyama *et al.*, 2000) to the squid, and from the squid to their predators (Law *et.* al., 1997; Sepulveda *et al.*, 1997; Bustamante *et al.*, 1998a) through the food chain. Furthermore Lakshmanan (1988) suggested that cephalopods may selectively enrich Cd from the fish they feed on. Bustamante *et al.* (1998) noted high variability in the feeding habits of cephalopods and feeding intensity in Patagonian squid *Loligo gahi* was significantly

higher in August – September (Portela and Rasero, 1998) suggesting a pivotal role of feeding habits of squids in seasonal metal accumulation.

Pb levels obtained in skinless mantle in *L.duvauceli* were of the same magnitude as in *Illex argentinus* caught during autumn (Falandysz, 1988) (0.11, 0.15 ppm wet wt) A quite comparable maximum in summer (June-September) was found in molluscs from Beirut (Shiber, 1980). Oysters and clams of Taiwan also showed higher Pb content from May to September (Hsu & Wang, 1979) and it was speculated that seasonal Pb changes arise due to more available food and more land drainage in summer along the west coast. A seasonal profile of maximum Pb content in the digestive gland of Pecten in November and February was reported by Bryan (1973). However the Pb concentration in the mantle covering all the seasons of a year were all below the detection limit. (2 mg/kg dl) as reported by several authors in different species and in the present study.

Cu content in *L. duvauceli* varied markedly with season. The concentration of Cu in muscle of *Choritomaico* sp., *Almejs* sp. and *N. chilenas,* was found to vary among species during different season collected during winter, spring, summer, and autumn (De-Gregori *et al.*, 1996). In soft tissue of *Marcia recens*, Cu accumulation varied significantly in different seasons (Muralidharan & Raja, 1997). Higher ability to accumulate Cu by mussel *Perna viridis* (Rivonker & Paruleker, 1998) and soft parts of muscles *V. cyprinoides, M. casta* and *P. viridis* (Lakshmanan, 1982) during the post monsoon period was also

observed during the present study in the seasonal profile of whole soft tissues of *L. duvauceli.* Motoe *et al.* (1997) reported increase in Cu content by two folds between March and July in the liver of firefly squid. A similar pattern was also observed in the present study in Cochin, Quilon and Mangalore samples. Cu content in the muscle of *L. duvauceli* of postmonsoon season are comparable to Cu autumn concentrations found in *Illex argentinus* (Falandysz, 1988). Possible causes for higher accumulation of Cu in October could be due to availability of food (Bryan, 1973), local pollution (Fowler and Oregoini , 1976) moisture content and land drainage (Phillips, 1980). The elevated levels of Cu were primarily attributed to proximity to anthropogenic sources and secondarily to various biological and abiotic modifiers capable of modifying Cu uptake and retention in molluscs.

Zn concentration in the edible muscle of *L. duvauceli* showed a definite seasonal variation pattern with high Zn concentration during premonsoon season at Cochin, Quilon and Mangalore samples as also reported by Talbot (1986) in the oysters *Saccostrea cucullata* (highest in January) and in *Perna viridis* of Dona Paula Bay, Goa (Rivonker & Parulekar, 1998). In *Loligo patagonica* and in *Illex argentinus* caught in autumn (Falandysz, 1988) was also noted in *L.duvauceli.* The high level of Zn during monsoon is attributed to the pre monsoon showers which mobilize Zn from industrial and domestic sources (Sivadasan & Nambisan, 1988).

The Fe concentration were comparatively high in all the seasons, nevertheless, definite seasonal trend was noted in the liver of *L.duvauceli* and are quite comparable to the elevated Fe temporal concentrations noted in digestive gland of scallop *Chlamys* sp. (January–February) and *Pecten* sp. (February) as reported by Bryan (1976).

In *L. duvauceli* more than 50% of Mn lies in the liver and the concentration in these organs are almost equivalent to changes in the whole animal. The gills and digestive gland varied appreciably in Mn concentrations at different seasons in scallops (Bryan,1976). A similar trend of maximum mean values during monsoon and post monsoon in whole *L.duvauceli* at Quilon and Cochin region was noted in the edible parts of *Tapes japonica* with maximum in winter (Kumagai & Saeki, 1980) and mean autumn Mn concentration in skinless mantle and tentacles of *I.argentinus* (Falandysz,1988) were comparable to the present study.

Seasonal variation of Cr in *L.duvauceli* are comparable to Cr content in mussels of Richard Bay Harbour (Vermeulen and Wepener, 1999). Falandysz (1988) suggested that higher Cr content in the edible muscle may have resulted due to contamination with the ink of squid which is known to have higher contents of heavy metals.

Distribution pattern of Ni in *L. duvauceli* varied with season and in whole soft tissues and gills of *L.duvauceli* higher levels were noted during premonsoon season in all the three regions. Previously reported

seasonal trends of Ni in whole soft parts of mollusc from Beirut (Shiber, 1980) were quite comparable to the Ni content in whole soft tissues of *L. duvauceli.*

Several reasons have been cited for seasonal variations in the concentrations of heavy metal in molluscs. Phillips (1980) mentioned three interrelated factors that cause seasonality of metals in the biota. These are pollutants delivery (run off), organism physiology, particularly sexual cycle and changes in ambient water quality like temperature and salinity. Bryan (1973) studied the seasonality of trace metals in scallops. He found the highest concentration in autumn and winter and suggested that metal concentrations were inversely related to phytoplankton productivity and the food chain presumably play a major role in the bioaccumulation of trace metal as compared to the medium. Fowler and Oregioni (1976) studied trace metals in *Mytilus galloprovincialis* and found the maximum concentration in spring. They attributed this to the reproductive state of the organisms and to the high winter run-off. They found the ratio between seasonal maximum and minimum concentrations to be greatest for Cr (factor of 8.8) and minimum for Zn (factor of 2). Other studies have suggested temperature and salinity as causative agents for seasonality in metal concentrations. Strong seasonal variation in metal concentrations in *Macoma balthica* was found to be associated with seasonal changes in soft tissue weight (Cain & Luoma, 1990). Similarly, 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 & Phillips, 1981). Maximum metal concentrations in *M. edulis* from the North Sea occurred in late winter and minimum concentrations in autumn. The range of variation was nearly 2-3 fold (Borchardt *et al.*, 1988).

In the firefly squid (*Loligo* spp.) seasonal changes in Cu and Mn content were related to growth and spawning. Bryan *et al.* (1973) suggested food chain presumably play a major role in accumulation of trace metals as compared to medium. In the present study, it is not likely that run off could have affected metal concentration in the water and as a result in *L. duvauceli.* Variability in metal concentrations in squids could be as a function of their sexual maturity (as a greater number of mature squids were observed from October to January, (Oommen, 1977), their geographical origin, ecological behaviour (Bustamante *et al.*, 1998), reproductive cycle (as reproduction in molluscs is accompanied by variations in the biochemical composition - Lipids, Carbohydrates and proteins; Gabbot, 1983) causing changes in the affinity of these compounds to metals (Oesterberg, 1974) and perhaps significantly their diverse feeding habits. In Patagonian squid (*Loligo patagonica)* feeding intensity was significantly higher during Aug-Sept (Portela & Rosero, 1998). *L.duvauceli* is a highly predatory fast moving carnivore feeding on a variety of pelagic fishes, cephalopods of the same species (Kore & Joshi 1975; Varghese, 1976)

and also benthic creatures like prawn, crabs and squilla. The adults as well as juveniles may vary their diets seasonally in relation to availability of prey organisms (Boucher-Rodoni *et al.*, 1987) suggesting the energy requirements are not the same during the whole life cycle (O'Dor & Wells 1987) and these factors presumably play a major role in seasonal metal enrichment.

Table 4.1a: Distribution pattern of trace metals (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli*, collected from Cochin region (premonsoon period: Jan.-May.)

WHOLE/ BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	15	0.064± 0.015 (0.050 - 0.086)	0.684 ± 0.180 (0.413 - 0.953)	1.294±0.173 (1.025-1.495)	6.598 ± 3.800 (3.843 - 12.962)	13.035 ± 5.912 (5.769 -20.371)	4.422±0.279 (4.229-4.947)	1.913±0.120 (1.758-2.054)	1.146 ± 1.369 (0.099 - 3.479)	1.865 ± 0.665 (1.048 - 2.456)
MUSCLE	20	0.030 ± 0.010 (0.014 - 0.044)	0.372 ± 0.279 (0.11 4 - 0.942)	0.340±0.513 (0-1.414)	4.008 ± 3.946 (0.932 - 13.798)	10.683 ± 6.336 (4.269 - 24.440)	2.240±1.816 (0.121-5.450)	0.846±1.622 (0-3.770)	0.472 ± 0.823 (0.175 - 2.660)	0.517 ± 0.132 (0- 1.010)
LIVER	17	0.199 ± 0.052 (0.152 - 0.231)	37.432 ± 53.72 (2.313 - 157.7)	2.733±3.386 (0-11.360)	65.362 ± 63.371 (10.682 – 173.01)	18.619 ± 16.876 (6.462 - 72.50)	49.806±26.475 (15 – 214.20)	6.939±9.650 (0-22.750)	1.893 ± 2.099 (0 - 6.140)	0.820 ± 1.338 (0- 3.344)
GILLS	23	0.047 ± 0.017 (0.032 - 0.066)	0.892 ± 0.920 (0.144 - 3.172)	1.165±1.345 (0-3.355)	26.86 ± 6.985 (19.776 - 46.577)	10.164 ± 6.424 (4.150 - 31.153)	26.958±19.116 (5.032-58.274)	0.528±0.879 (0-2.10)	2.178 ± 3.074 (0 - 7.820)	0.774 ± 0.927 (0 - 2.424)

•maximum number of samples analysed

Table 4.1 b : Distribution pattern of trace metals (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli*, collected from Cochin region (monsoon period: Jun.-Aug.)

WHOLE/ BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	23	0.094 ± 0.003 (0.092 - 0.098)	2.919 ± 1.584 (1.584 - 6.07)	0.862±0.512 (0.564-1.454)	12.927 ± 6.616 (5.132 - 24.957)	7.969 ± 2.085 (5.314 - 12.528)	6.369± 2.137 (2.354–8.695)	3.253±2.212 (0.369- 6.358)	1.265 ± 0.442 (0.646 - 1.625)	1.279 ± 0.2810 (0.801 – 1.542)
MUSCLE	12	0.049 ± 0.017 (0.026 - 0.071)	0.597 ± 0.337 (0.026 - 1.003)	0.616±0.465 (0-1.199)	1.302 ± 0.787 (0.095 - 2.398)	5.971 ± 1.442 (4.117 - 8.589)	1.212±0.938 (0.103-2.384)	0.122±0.248 (0 - 0.564)	0.206 ± 0.259 (0 - 0.604)	0.529 ± 0.462 (0- 1.032)
LIVER	13	0.154 ± 0.042 (0.094 - 0.189)	47.302 ± 45.331 (7.193-119.89)	3.910±3.677 (0-7.416)	142.4 ± 69.077 (8.585-182.7)	102.15 ± 55.37 (25.60 - 160.99)	33.703±27.72 (13.41-69.39)	13.212±12.077 (0-22.690)	2.759 ± 2.597 (0- 7.079)	3.621 ± 4.865 (0-12.447)
GILLS	11	0.051 ± 0.006 (0.046 - 0.059)	2.099 ± 2.352 (0.150-5.875)	1.346±1.986 (0-4.587)	39.139 ± 12.571 (18.55 - 55.23)	9.202 ± 3.036 (4.688 - 16.139)	31.211±14.34 (19.41-56.74)	5.547±1.945 (3.258-8.354)	0.113 ± 0.140 (0 - 0.356)	0.670 ± 0.564 (0 - 1.475)

* maximum number of samples analysed

Table 4.1c : Distribution pattern of trace metals (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli,* collected from Cochin region (postmonsoon period: Sept.-Dec.)

WHOLE/ BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	09	0.067 ± 0.021 (0.035-0.082)	0.809 ± 0.070 (0.713-0.886)	1.642±0.340 (1.176-1.985)	23.213±16.561 (8.822-37.640)	9.653±0.828 (8.930-10.438)	23.785±15.530 (10.112-37.60)	6.798±3.756 (2.600-9.903)	0.969±0.514 (0.234-1.423)	0.818±0.197 (0.588-1.038)
MUSCLE	30	0.051 ± 0.015 (0.033-0.065)	0.481 ± 0.471 (0.057-1.978)	0.536±0.294 (0-1.025)	1.027±0.680 (0-2.086)	5.059±1.183 (2.955-7.727)	4.618±3.572 (0.755-12.309)	1.936±1.006 (0.047-3.917)	0.491±0.298 (0-0.964)	0.197±0.231 (0-0.444)
LIVER	20	0.127 ± 0.072 (0.065-0.221)	138.310 ± 137.10 (37.510-185.76)	5.334±3.382 (0-12.316)	77.544±51.066 (12.315-138.80)	30.968±10.859 (18.529-50.720)	33. 693±12.70 (23.65-59.50)	12.822±3.540 (7.318-16.820)	1.699±1.892 (0-5.144)	2.187±2.002 (0-5.909)
GILLS	19	0.061 ± 0.016 (0.043-0.073)	3.200 ± 2.034 (1.030-6.954)	2.988±1.362 (0-4.624)	24.905±7.610 (14.880-37.816)	12.135±9.288 (4.807-36.259)	62.955±75.400 (6.430-229.66)	6.410±3.103 (1.262-12.317)	1.993±1.737 (0.446-5.797)	0.231±0.311 (0-0.815)

* maximum number of samples analysed

Table 4.2a :Distribution pattern of trace metals (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli*, collected from Quilon (premonsoon period: Jan.-May.)

WHOLE/ BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	08	0.048 ± 0.005 (0.041- 0.052)	0. 495± 0.011 (0.482-0.510)	0.845±0.361 (0.321-1.238)	8.198 ± 3.324 (5.930-2.559)	22.307 ± 15.924 (10.598-39.800)	4.076±0.038 (4.049-4.103)	1.391±0.963 (0.627-2.703)	3.450 ± 0.791 (2.775 -4.463)	1.927 ± 0.016 (1.915 -1.938)
MUSCLE	15	0.018 ± 0.005 (0.011-0.024)	0.160±-0.152 (0.053-0.409)	0.045±0.084 (0-0.189)	2.313 ± 1.156 (0.783-4.139)	13.561 ± 7.435 (7.117 -32.410)	2.274±0.540 (1.593-3.180)	0.849±0.58 (0-1.775)	0.390 ± 0.223 (0-0.631)	0.918 ± 1.013 (0.116 - 2.117)
LIVER	17	0.110 ± 0.032 (0.079-0.143)	10.308±6.992 (4.058-26.54)	0.011±0.030 (0-0.079)	50.037±34.97 (14.906-107.66)	14.825 ± 2.903 (11.377-19.623)	71.389±31.453 (14.055-117.44)	5.446±3.281 (0-10.217)	1.566 ± 1.187 (0-2.816)	0.619 ± 0.679 (0-1.262)
GILLS	16	0.050 ± 0.010 (0.038-0.062)	4.235 ± 2.163 (1.489-7.282)	0.013± 0.017 (0-0.042)	20.002±10.484 (6.99-40.588)	15.788 ± 11.518 (6.390-42.352)	52.76±37.096 (10.194-111.8)	5.222±4.514 (0-14.893)	4.117 ± 2.852 (0.375-9.787)	1.508 ± 1.535 (0-3.129)

* maximum number of samples analysed

Table 4.2b : Distribution pattern of trace metals (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli*, collected from Quilon region (monsoon period: Jun.-Aug.)

WHOLE/ BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	08	0.033±0.011 (0.023-0.048)	0.509±0.017 (0.484-0.529)	1.517±0.307 (1.225-1.85)	4.82±1.526 (3.191-6.099)	42.007±1.445 (40.44-43.43)	3.912±0.303 (3.569-4.145)	0.862±0.184 (0.710-1.072)	0.541±0.025 (0.519-0.569)	1.566±1.350 (0.272-3.199)
MUSCLE	12	0.013±0.003 (0.011-0.016)	0.144±0.01 (0.132-0.153)	0.691±0.367 (0.436-1.112)	0.053±0.005 (0.049-0.059)	6.228±0.265 (6.044-6.532)	1.116±0.009 (1.109-1.126)	0.548±0.255 (0.352-0.836)	0.216±0.064 (0.125-0.285)	0.106±0.007 (0.101-0.114)
LIVER	10	0.073±0.019 (0.054-0.092)	14.441±8.026 (3.235-22.846)	1.063±0.623 (0.532-1.965)	20.685±5.220 (14.094-24.802)	8.995±2.980 (6.266-11.635)	13.050±6.208 (26.0-39.705)	3.643±2.721 (0.0-6.764)	1.177±0.976 (0-2.235)	0.652±0.515 (0.190-1.111)
GILLS	08	0.025±0.003 (0.021-0.028)	2.463±1.447 (0.340-4.750)	0.533±0.253 (0.288-0.866)	19.686±10.569 (9.601-29.665)	5.672±0.179 (5.482-5.839)	10.071-±3.304 (4.57-14.084)	1.445±1.133 (0.175-2.64)	1.123±0.492 (0.431-1.566)	0.282±0.025 (0.060-0.663)

* maximum number of samples analysed

Table 4.2 c : Distribution pattern of trace metals (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli,* collected from Quilon region (postmonsoon period: Sept.-Dec.)

WHOLE/ BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	10	0.088 ± 0.002 (0.086-0.089)	1.417 ± 0.747 (0.542-2.885)	1.731±0.336 (1.125-2.011)	13.295 ± 11.393 (5.151-29.965)	20.986 ± 9.333 (10.835-32.253)	6.280±2.355 (3.251-8.536)	0.646±0.242 (0.231-0.879)	5.937 ± 3.959 (1.596 -12.572)	1.045±0.848 (0-1.951)
MUSCLE	15	0.025±0.008 (0.017-0.036)	0.706±0.429 (0-1.288)	0.298±0.465 (0-1.185)	1.498±0.502 (0.855-2.418)	9.596±5.620 (2.139-20.060)	4.009±1.195 (2.377-5.231)	1.295±0.814 (0-2.279)	0.618±0.587 (0-1.950)	0.393±0.265 (0.167-0.910)
LIVER	19	0.044±0.020 (0.028-0.067)	24.217±12.24 (8.937-45.045)	0.488±0.920 (0-2.275)	26.576±15.440 (11.553-60.762)	20.659±9.996 (12.893-40.558)	52.094±19.384 (23.380-76.658)	3.761±2.216 (0-7.545)	0.929±0.543 (0-1.588)	0.883±0.417 (0.179-1.553)
GILLS	13	0.034±0.007 (0.027-0.041)	7.803±6.658 (1.575-15.45)	0.361±0.338 (0-0.824)	31.921±16.733 (8.689-52.733)	73.072±26.498 (32.532-110.248)	24.334±16.901 (9.434-53.884)	0.904±1.343 (0-3.448)	2.663±1.431 (0.325-4.838)	1.071±0.657 (0.666-2.270)

* maximum number of samples analysed

Table 4.3a :Distribution pattern of trace metals (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli,* collected from Mangalore region (premonsoon period: Jan.-May.)

WHOLE/ BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	08	0.044 ± 0.066 (0.035 - 0.049)	0.770 ± 0.061 (0.701 - 0.839)	0.768±0.036 (0.721- 0.803)	2.281 ± 0.027 (1.242 - 1.310)	10.166 ± 0.378 (9.810 - 10.816)	10.60±0.482 (9.751-11.064)	1.264±0.114 (1.144-1.388)	2.069 ± 0.042 (2.015 - 2.124)	0.767 ± 0.031 (0.725 - 0.809)
MUSCLE	16	0.021 ± 0.012 (0.010 - 0.036)	0.299 ± 0.298 (0- 0.917)	0.048 ± 0.126 (0 -0.333)	1.516 ± 1.294 (0.608 - 3.746)	7.047 ± 3.428 (3.339 - 14.631)	2.122±1.013 (0.992-4.175)	1.098±0.921 (0-2.610)	1.395 ± 1.068 (0.119 - 2.893	0.413 ± 0.347 (0- 0.868)
LIVER	11	0.080±0.026 (0.057 - 0.114)	1.862 ± 0.969 (0.742 - 3.750)	0.088±0.096 (0 -0.175)	16.81± 6.395 (8.904-26.250)	26.066± 23.945 (11.485-76.562)	55.603±10.538 (42.151-55.105)	11.496±10.331 (0-28.400)	7.482 ± 6.263 (0-12.411)	5.090 ± 3.300 (0-10.0)
GILLS	11	0.050 ± 0.014 (0.036-0.065)	0.042 ± 0.018 (0.02-0.062)	0.033±0.014 (0.017-0.049)	21.609±10.609 (15.129-43.45)	15.584 ± 5.864 (8.273-22.640)	0.874-±0.251 (0.607-1.185)	4.356±3.706 (0.877-8.669)	1.211± 1.107 (0-2.094)	0.575 ± 0.073 (0.502-0.675)

* maximum number of samples analysed

Table 4.3b : Distribution pattern of trace metals (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli,* collected from Mangalore region (monsoon period: Jun.-Aug.)

WHOLE/ BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	08	0.051± 0.004 (0.048-0.056)	0.743 ± 0.008 (0.737-0.749)	1.179±0.001 (1.178-1.18)	3.010 ± 0.005 (3.006-3.014)	6.052±0.006 (6.048-6.057)	5.388±0.006 (4.384-6.393)	1.244±0.008 (1.238-1.25)	0.834±0.006 (0.800-0.839)	0.665±0.004 (0.662-0.669)
MUSCLE	09	0.009±0.006 (0.010-0.017)	0.193±0.028 (0.146-0.226)	1.004±0.738 (0-1.277)	1.376±2.182 (0-4.644)	5.349±0.502 (4.660-6.030)	1.046±0.122 (0.867-1.194)	0.247±0.207 (0.0-0. 531)	0.363±0.207 (0.097-0.753)	0.015±0.018 (0-0.035)
LIVER	09	0.064±0.005 (0.059-0.069)	17.293±5.024 (11.875-24.67)	0.653±0.615 (0-1.280)	19.123±17.144 (0.479-44.520)	5.224±2.912 (1.018-7.981)	30.833±9.643 (16.233-37.784)	0.389±0.779 (0-1.557)	0.421±0.589 (0-1.250)	1.030±0.811 (0-3.344)
GILLS	11	0.041±0.018 (0.020-0.062)	0.856±0.203 (0.602-1.122)	1.211±1.106 (0-2.094)	10.807±4.573 (3.61-14.424)	7.096±2.369 (4.217-10.341)	31.441±19.846 (6.91-57.59)	5.587±4.393 (0.0-9.839)	1.321±1.048 (0-2.760)	2.06±2.823 (0-5.228)

* maximum number of samples analysed

Table 4.3 c : Distribution pattern of trace metals (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of *L. duvauceli,* collected from Mangalore region (postmonsoon period: Sept.-Dec.)

WHOLE/ BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	COPPER	ZINC	IRON	MANGANESE	CHROMIUM	NICKEL
WHOLE	09	0.038±0.008 (0.027-0.044)	0.638±0.050 (0.560-0.704)	1.196±0.357 (0.831-1.969)	3.849±1.107 (2.400-4.958)	10.204±1.858 (8.307-12.402)	5.996± 1.383 (3.232-7.328)	1.628±0.514 (1.259-2.568)	0.519±0.356 (0.114-0.783)	0.460±0.051 (0.414-0.532)
MUSCLE	12	0.016±0.010 (0.010-0.031)	0.144±0.098 (0.023-0.261)	0.336±0.322 (0- 0.638)	1.326±0.322 (1.063-1.890)	5.176±0.285 (4.860-5.562)	2.178±1.965 (0-3.817)	0.558 ± 0.491 (0-1.258)	0.366±0.249 (0-0.540)	0.032±0.011 (0.018-0.044)
LIVER	14	0.066±0.018 (0.054-0.087)	5.955±2.387 (4.008-8.773)	5.928±3.976 (0-8.302)	11.592±11.613 (3.061-25.943)	27.873±8.206 (22.389-34.339)	29.944±2.99 (23.296-29.42)	2.903 ±3.189 (0 – 8.695)	0.281±0.194 (0-0.433)	2.583 ± 2.600 (0-5.583)
GILLS	15	0.033±0.014 (0.017-0.049)	0.874±0.251 (0.607-1.085)	1.827±0.176 (1.589-1.995)	6.362±6.421 (1.477-13.711)	3.296±1.291 (2.289-5.051)	18.312±14.559 (12.562-35.25)	5.335±0.365 (5.077-5.593)	0.464±0.321 (0-0.724)	2.371± 2.085 (0-3.918)

* maximum number of samples analysed

Table 4.4: Analysis of variance (ANOVA) of seasonal variation of heavy metals in muscle of *Loligo duvauceli* from Cochin region

Mercury						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.0021522	2	0.001076085	4.061719	0.060608	4.458968306
Within Groups	0.0021195	8	0.000264933			
Total	0.0042716	10				
Cadmium						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.288169	2	0.144084519	0.88309	0.420396	3.19958815
Within Groups	7.5053372	46	0.163159505			
Total	7.7935062	48				
Lead						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.4484805	2	0.224240266	1.417243	0.254005	3.225679279
Within Groups	6.4871364	41	0.15822284			
Total	6.935617	43				
Copper						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	97.854246	2	48.92712317	7.257671	0.001822	5.098570455
Within Groups	310.10606	46	6.741436097			
Total	407.96031	48				
Zinc						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	128.74059	2	64.37029354	8.324515	0.000684	5.005517778
Within Groups	433.02662	56	7.732618153			
Total	561.7672	58				
Chromium						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4.4524971	2	2.226248548	7.025991	0.002533	5.211234111
Within Groups	12.040642	38	0.316858997			
Total	16.493139	40				
Nickel						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.3873466	2	0.193673313	0.244307	0.78595	3.591537734
Within Groups	13.476663	17	0.79274489			
Total	13.86401	19				

Table 4.5: Analysis of variance (ANOVA) of seasonal variation of heavy metals in whole of *Loligo duvauceli* from Cochin region

Mercury						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0018821	2	0.000941064	3.598	0.076814	4.458968306
Within Groups	0.0020924	8	0.000261552			
Total	0.0039745	10				
Cadmium						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	28.922214	2	14.46110681	16.95242	2.57E-05	3.402831794
Within Groups	20.472984	24	0.853041016			
Total	49.395198	26				
Lead						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.045769	2	0.522884511	6.690167	0.008376	3.682316674
Within Groups	1.1723575	15	0.078157165			
Total	2.2181265	17				
Copper						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	955.14791	2	477.5739569	9.369032	0.000476	5.194408459
Within Groups	1987.9732	39	50.97367218			
Total	2943.1211	41				
Zinc						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	213.19113	2	106.5955654	7.627632	0.001684	5.229026101
Within Groups	517.07215	37	13.97492286			
Total	730.26328	39				
Chromium						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.2023691	2	0.10118455	0.110214	0.896538	3.885290312
Within Groups	11.016843	12	0.918070219			
Total	11.219212	14				
Nickel						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	34.71933	2	17.35966519	9.823635	0.004366	4.102815865
Within Groups	17.671325	10	1.76713247			
Total	52.390655	12				

Table 4.6: Analysis of variance (ANOVA) of seasonal variation of heavy metals in muscle of *Loligo duvauceli* from Quilon region

Mercury						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0001229	2	6.14444E-05	2.957219	0.127712	5.143249382
Within Groups	0.0001247	6	2.07778E-05			
Total	0.0002476	8				
Cadmium						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3.552026	2	1.776013	13.98505	0.000106	3.422130135
Within Groups	2.920854	23	0.126993652			
Total	6.47288	25				
Lead						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.9405052	2	0.470252593	3.904208	0.040246	3.591537734
Within Groups	2.0476098	17	0.120447633			
Total	2.988115	19				
Copper						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	13.830688	2	6.915343768	8.929207	0.001117	5.526317182
Within Groups	20.136048	26	0.774463376			
Total	33.966735	28				
Zinc						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	72.0133	2	36.00665001	1.5879	0.222852	5.488118404
Within Groups	612.24233	27	22.67564188			
Total	684.25563	29				
Chromium						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.2936392	2	0.646819584	3.131306	0.063579	5.719016372
Within Groups	4.544439	22	0.206565409			
Total	5.8380782	24				
Nickel						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.4517864	2	0.725893217	2.296004	0.134925	6.358845894
Within Groups	4.7423256	15	0.316155038			
Total	6.194112	17				

Table 4.7: Analysis of variance (ANOVA) of seasonal variation of heavy metals in whole of *Loligo duvauceli* from Quilon region

Mercury						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.001682	2	0.000841	12.6519	0.002427	8.021515896
Within Groups	0.0005982	9	6.64722E-05			
Total	0.0022802	11				
Cadmium						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.1755064	2	0.0877532	7.491847	0.010273	4.102815865
Within Groups	0.1171316	10	0.01171316			
Total	0.292638	12				
Lead						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.9580943	2	1.479047139	14.17939	0.000238	3.591537734
Within Groups	1.7732637	17	0.104309631			
Total	4.731358	19				
Copper						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	251.50931	2	125.7546566	2.389979	0.123539	3.633715551
Within Groups	841.87961	16	52.61747534			
Total	1093.3889	18				
Zinc						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1623.7692	2	811.8846199	7.783898	0.005335	6.514937922
Within Groups	1460.2433	14	104.3030937			
Total	3084.0126	16				
Chromium						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	63.295621	2	31.64781067	3.628906	0.061616	3.982307817
Within Groups	95.931364	11	8.721033048			
Total	159.22698	13				
Nickel						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.5480688	2	3.274034395	0.906535	0.434697	4.102815865
Within Groups	36.115934	10	3.611593413			
Total	42.664003	12				

Table 4.8: Analysis of variance (ANOVA) of seasonal variation of heavy metals in muscle of *Loligo duvauceli* from Mangalore region

Mercury						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.521E-05	2	1.76033E-05	0.698946	0.533494	5.143249382
Within Groups	0.0001511	6	2.51856E-05			
Total	0.0001863	8				
Cadmium						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.0090824	2	0.004541185	0.316931	0.731805	5.780407264
Within Groups	0.3009013	21	0.014328631			
Total	0.3099836	23				
Lead						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.7282215	2	1.364110756	5.216551	0.020279	3.738890086
Within Groups	3.6609534	14	0.261496673			
Total	6.3891749	16				
Copper						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.3785033	2	0.189251632	0.084572	0.919178	3.402831794
Within Groups	53.705917	24	2.237746558			
Total	54.084421	26				
Zinc						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	20.105184	2	10.0525922	1.757043	0.194934	3.422130135
Within Groups	131.59017	23	5.721311555			
Total	151.69535	25				
Chromium						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.3276523	2	3.163826137	4.701543	0.020543	3.466794851
Within Groups	14.131605	21	0.672933556			
Total	20.459257	23				
Nickel						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.723504	2	0.361752	4.358609	0.030829	3.633715551
Within Groups	1.327954	16	0.082997125			
Total	2.051458	18				

Table 4.9: Analysis of variance (ANOVA) of seasonal variation of heavy metals in whole of *Loligo duvauceli* from Mangalore region

Mercury						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0003042	2	0.000152083	3.658781	0.104982	5.786148449
Within Groups	0.0002078	5	4.15667E-05			
Total	0.000512	7				
Cadmium						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.0595269	2	0.029763438	10.77187	0.002096	6.926597962
Within Groups	0.0331569	12	0.002763071			
Total	0.0926837	14				
Lead						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.6019929	2	0.30099645	4.03506	0.045686	3.885290312
Within Groups	0.8951435	12	0.074595292			
Total	1.4971364	14				
Copper						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	21.510635	2	10.75531758	17.54923	0.000274	6.926597962
Within Groups	7.3543848	12	0.612865403			
Total	28.86502	14				
Zinc						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	29.628828	2	14.81441388	8.297779	0.005461	6.926597962
Within Groups	21.424162	12	1.785346819			
Total	51.05299	14				
Chromium						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.0243723	2	3.512186138	67.91311	1.52E-06	7.559492587
Within Groups	0.5171588	10	0.05171588			
Total	7.5415311	12				
Nickel						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.2265784	2	0.113289208	80.01121	1.85E-06	8.021515896
Within Groups	0.0127432	9	0.001415917			
Total	0.2393217	11				

Fig 4.1: Seasonal variations of heavy metals in the body components of *Loligo duvauceli* collected from Cochin region (mean, ppm wet wt)

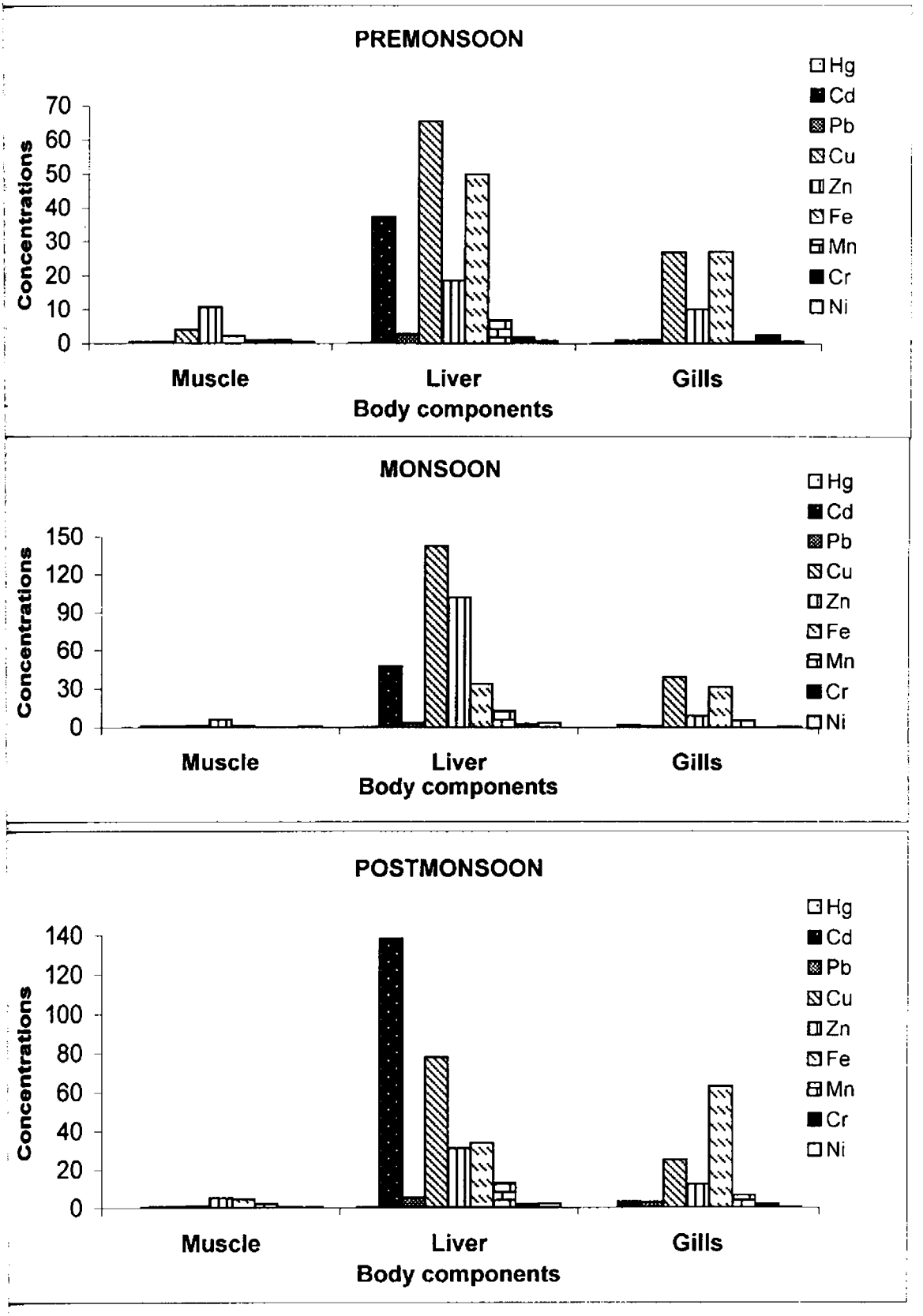


Fig 4.2: Seasonal variations of heavy metals in the body components of *Loligo duvauceli* collected from Quilon region (mean, ppm wet wt)

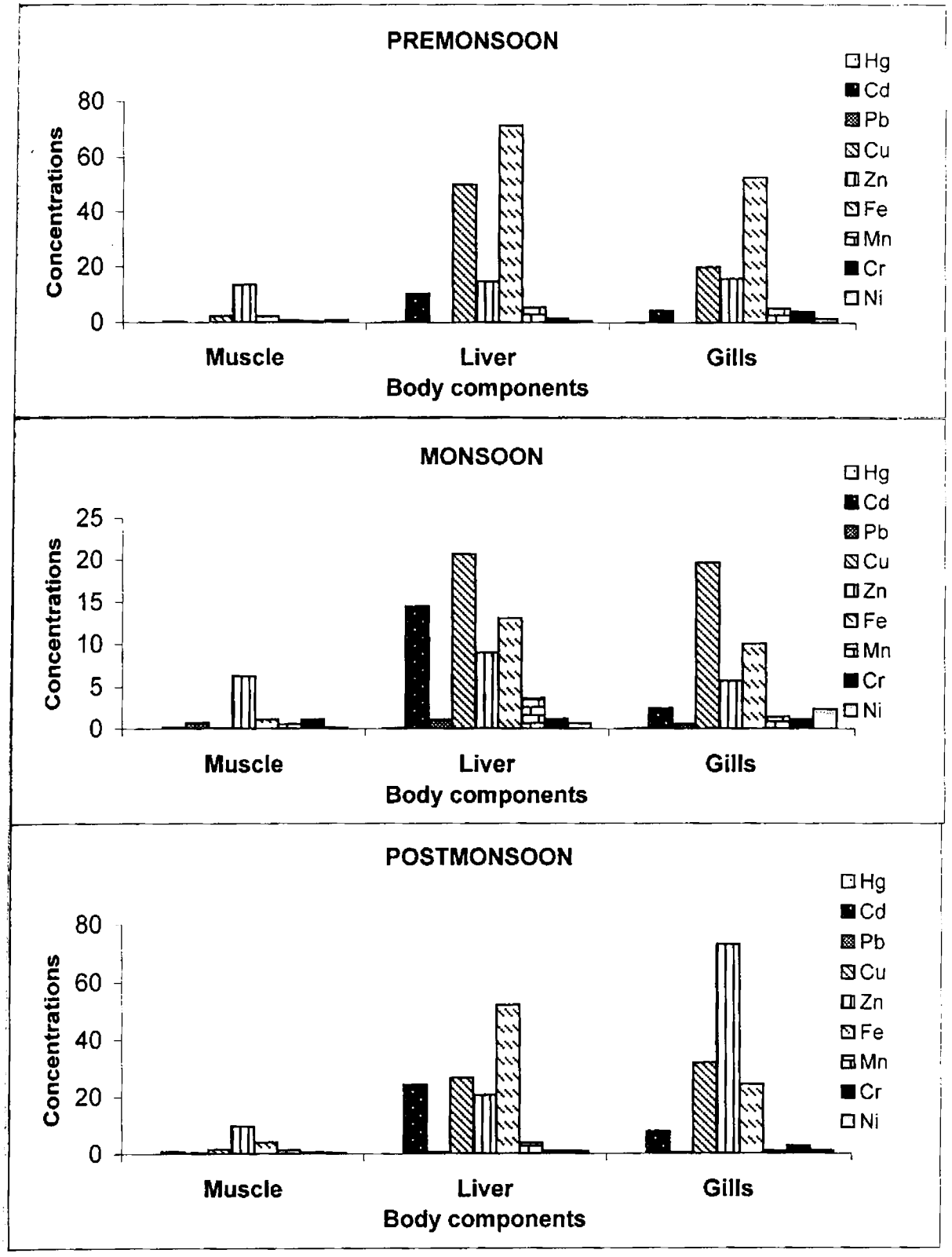


Fig 4.3: Seasonal variations of heavy metals in the body components of *Loligo duvauceli* collected from Mangalore region (mean, ppm wet wt)

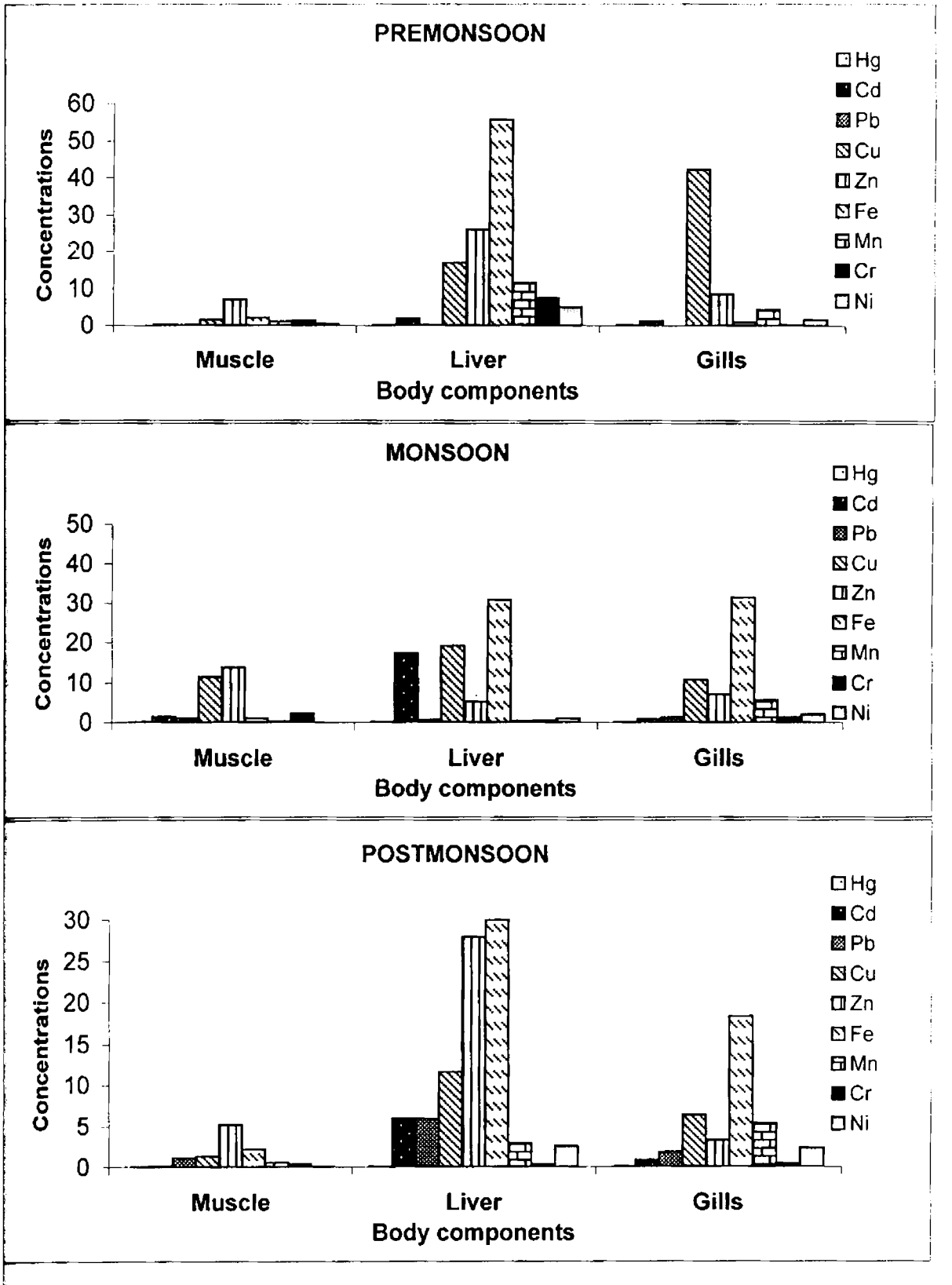
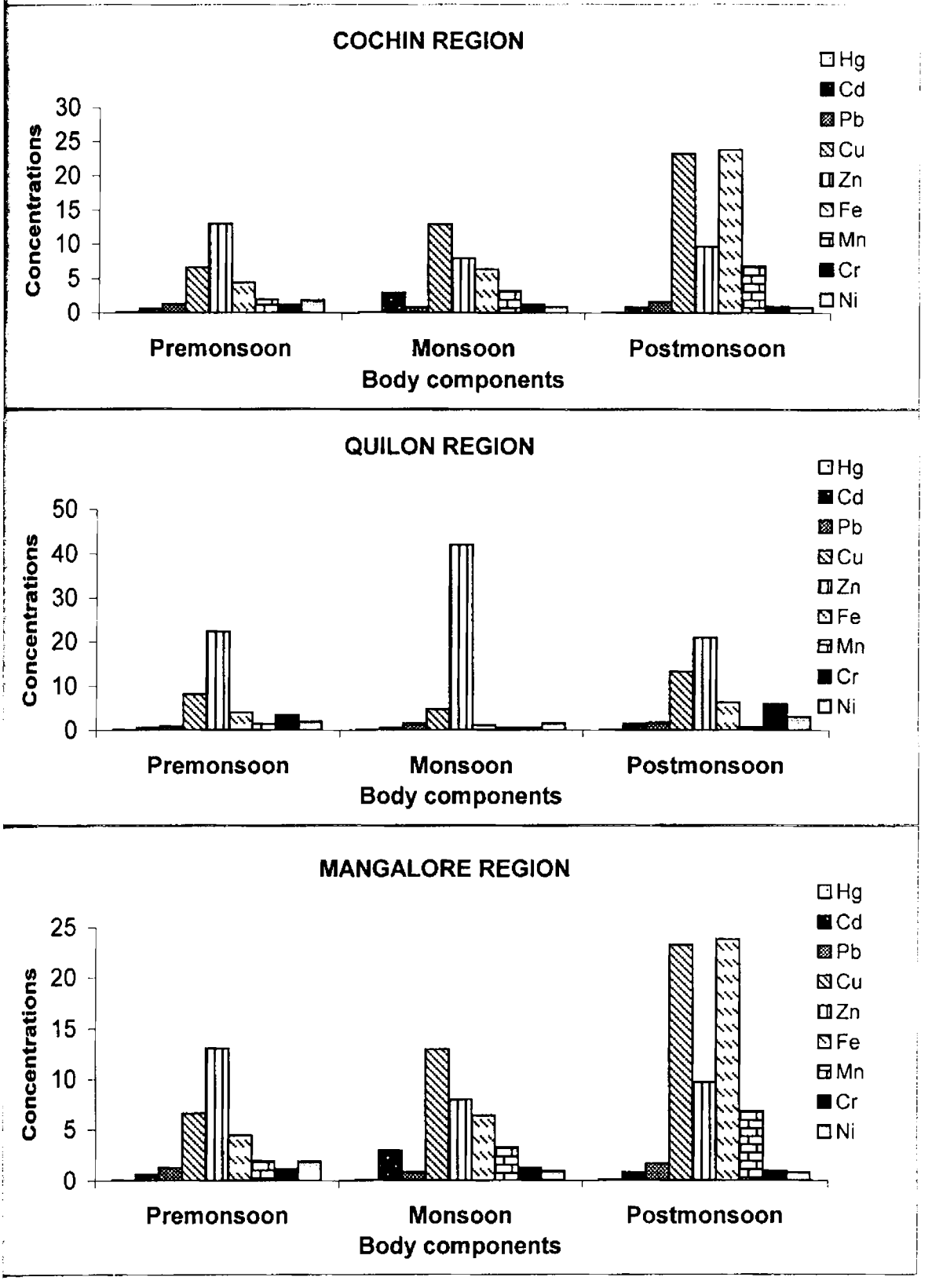


Fig 4.4: Seasonal variations of heavy metals in whole soft parts of *Loligo duvauceli* collected from different regions (mean, ppm wet wt)



CHAPTER 5

Comparative study of trace metal levels in neretic and oceanic squids

5.1. Introduction

Monitoring of metal pollution in the aquatic environment is becoming increasingly important with rapid industrial and technological development. Since the beginning of pollution in the aquatic environment, inshore organisms have been subjected to various pollutants (Swaileh, 1996). Offshore organisms on the other hand have been generally considered to be living in an environment away from the areas of pollution.

The use of offshore and inshore squids for metal pollution studies has many advantages since they integrate pollutants over a period of time in certain organs, provide a measurement of the bio availability and bio mobility of pollutants and concentrate pollutants to levels that are far higher than those in the abiotic environment (Phillips, 1976). Unfortunately, interpretation of the data concerning metal concentration can be complicated by biological as well as environmental processes that can cause variations in these metal concentrations and make comparisons of results between different sites difficult. Examples of these processes are: reproductive cycle, season of the year, growth, age, water temperature and salinity, run offs, presence of chelating agents and depth of sampling (Swaileh and Adelung,1995).

While mussels and oysters are among the best studied organisms for cosmopolitan bio monitoring, other species of local, national or international importance should be considered for

monitoring programmes. In addition most of the monitoring programmes are confined to the near shore areas that are more susceptible to pollution than offshore seas. There is derth of information from the offshore areas with regard to pollution. At present, dumping waste in the deep sea is a common way of getting rid of unwanted materials. This necessitates, establishing background levels of pollutants in some offshore organisms an element that might be needed for future monitoring programmes.

Most of the studies in cephalopod molluscs, concerning heavy metal levels have been carried out in neretic squids (Lakshmanan, 1988a, 1989, Falandysz, 1989, 1991, Barska, 1988a,b; Oehlenschlaeger, 1989; Tariq *et al.,* 1991; Bustamante *et al.,* 1998a; Storelli, 1999) and very little literature is available on the oceanic squids (Ueda *et al.,* 1979, Smith, 1984; Yamada *et al.,* 1997 Bustamante *et al.*, 1998a). However, no study has been carried out on *Ancistrocheirus* spp. an oceanic squid. So, in the present study, a comparative account of heavy metal levels in neretic squids (*Loligo* spp.) and oceanic squids (*Ancistrocheirus* spp.) collected offshore onboard the research vessels FORV *Sagar Sampada* and M V *Sagarika* during their cruises along the west coast of India have been studied. As there was no comprehensive studies on the levels of heavy metals in oceanic squids from Indian waters, heavy metal content in the tissues of oceanic squid caught onboard the fishing

vessels and results reported for neretic squid can be used for future base line reference.

5.2. Materials and Methods

Neretic squid (*Loligo* spp.) were caught at 30-70 m depth onboard MV *Sagarika* between May 1998 to October 1998 (lat. 9° 34' to 12° 08' N and long. 74° 50' to 76° 02'E) and onboard FORV *Sagar Sampada (*20°34' to 21°36' N lat. to 68°58' to 70°13' E long.). Oceanic squids were collected at 200-350 m depth, onboard FORV *Sagar Sampada* (Cruise No. 191) during January 2001 (6°58' to 13°30' N lat. and 74°15' to 77°50' E long.). Details of onboard sampling sites of neretic squids are shown in Table (2.1) and oceanic squids in Fig. (2.1). Samples were analysed for six heavy metals *viz.*, Hg, Cd, Cu, Zn, Cr and Ni in whole soft tissues and in the various body components. Preparation of samples analytical procedure adopted for digestion and metal determination followed are described in Chapter 2. Statistical analyses were carried out using student t-test to compare the means and to determine significant differences if any, in the distribution of heavy metals between neretic and oceanic squids.

5.3. Results

Concentrations of heavy metals like Hg, Cd, Cu, Zn, Cr and Ni in the whole soft tissues and body components of neretic and oceanic squids are presented in Tables 5.1 and 5.2. The distribution pattern in whole soft tissues and body components are illustrated in Figs. 5.1 to

5.3. The distribution characteristics of metals in the muscle, liver and gills and other body components of the two species are also presented.

5.3.1. Mercury

The results of Hg analysis in neretic and oceanic squids are presented in Tables 5.1 and 5.2 and the distribution pattern in whole soft parts and body components of neretic and oceanic squids are illustrated in Figs. (5.1 to 5.3). The overall average of ∑Hg levels in the whole soft parts of *L. duvauceli* varied from 0.059 to 0.099 ppm and in oceanic squids (*Ancistrocheirus* spp.) it ranged from 0.076 to 0.095 ppm. A reduction of around 45% in ∑Hg content was seen in the edible muscle of oceanic and neretic squids (Fig 5.1). ∑Hg was found to be the least abundant toxic metal found in both neretic and oceanic squids. The highest value observed for ∑Hg in muscle was 0.054 ppm and 0.065 ppm in neretic and oceanic squids respectively, the range being 0.028 to 0.054 ppm in *Loligo* spp. and 0.054 to 0.065 ppm in *Ancistrocheirus* spp. Among the various body components analysed only the liver in both neretic and oceanic squids exhibited a value > 0.10 ppm for ∑Hg. Liver of neretic squids and oceanic squids recorded the highest values *viz.*, 0.212 and 0.197 ppm respectively, the range being 0.085 to 0.212 and 0.095 to 0.197 ppm. The concentration of ∑Hg in the liver of oceanic squids was around 40% higher than in edible muscle and average levels of ∑Hg in the gills was more or less similar in the two types of squids (Figs. 5.1 and 5.2). Concentrations were 0.078 ± 0.010ppm in oceanic squids and 0.072 ± 0.012 ppm

neretic squids. The increasing order of abundance of ΣHg in the body components of both species of squids was liver > gills> muscle. Total mercury content observed in the edible as well as whole soft parts of neretic and oceanic squids never had values near to the International limits. The values were all < 0.2 ppm.

5.3.2. Cadmium

The results of the analysis and distribution pattern of Cd in neretic and oceanic squids are shown in Tables 5.1 and 5.2 and Figs. 5.1 to 5.3. Whole *Loligo* spp. (neretic) had Cd content in the range of 1.067 to 3.708 ppm and around 20% of neretic squid samples showed Cd content above 3ppm, the tolerance limit. However, all the samples of oceanic squids analysed had Cd content above 8 ppm and were more than five folds higher than in *Loligo* spp., the range being 9.841 to 12.947 ppm. The mean Cd content in the muscle of neretic squids (*Loligo* spp.) were <2 ppm (Fig.5.1) as compared to whole squid, indicating safety to the consumer. On the other hand, oceanic squids (*Ancistrocheirus* spp.) exhibited mean Cd content in the range of 3.777 ± 0.942 ppm in the muscle and around 85% exceeded the tolerance limit of 3 ppm. The maximum levels observed being 5.071 ppm. Liver was the major site of Cd accumulation in both species. Liver samples of neretic squids had mean Cd concentrations 38.741 ± 40.461, and in oceanic squids, the value was 88.802 ± 60.158-ppm indicating fairly high body burdens in both the squid species (Tables 5.1 and 5.2). However, in oceanic squids Cd content in liver was several magnitudes

higher than in neretic squids (Fig. 5.2). Gill samples of neretic and oceanic squids also recorded high Cd values, *viz.*, 11.176 ppm and 16.563 ppm respectively the range being 0 to 11.176 ppm in neretic squids and 1.492 to 16.563 ppm in oceanic squids. The order of abundance of Cd in the body components of neretic and oceanic squids were liver> gills > muscle.

5.3.3. Copper

Mean Cu content in the whole soft tissues and body components of neretic and oceanic squids are given in Figs. 5.1 to 5.3. Cu concentrations in the two types of squids are presented in Tables 5.1 and 5.2. Higher levels of Cu were detected in all neretic squids and oceanic squids caught onboard the fishing vessels. In the whole soft parts, Cu concentrations were 13.440 ± 2.461 ppm and 21.361 ± 5.520 ppm in neretic and oceanic squids respectively and a reduction of around 60% was seen in the muscle. The highest value in the muscle tissue of neretic and oceanic squids was 22.968 ppm and 14.965 ppm respectively. Mean Cu content in muscle of oceanic squids were about two folds higher than in neretic squids; the concentration being 4.464 ± 5.962 ppm in neretic squids and 8.201 ± 4.236 ppm respectively. The range of values of Cu in the liver of neretic squids (*Loligo* spp.) and oceanic squids (*Ancistrocheirus* spp.) varied from 6.630 to 135.440 and 10.870 to 482.17 ppm respectively. Average Cu levels in the gills were much lower than liver in both the species (Figs. 5.1 and 5.2). However, in whole oceanic squids mean Cu content was slightly higher than in

neretic squids. The concentration of Cu in the different body components of neretic squids and oceanic squids followed the order: liver > gills > muscle. Generally the Cu content in oceanic squids was slightly higher than that observed in neretic squids.

5.3.4. Zinc

Concentrations of Zn in whole squids and body components are presented in Tables 5.1 and 5.2. Figs. 5.1 to 5.3 shows the distribution pattern of Zn in whole neretic squids and oceanic squids and also in the various body components. Zn content was higher than Cu content in both types of squids. Greater abundance of Zn in whole soft parts of neretic squids (*Loligo* spp.) and oceanic squids (*Ancistrocheirus* spp.) caught onboard the fishing vessels were observed and the values varied from 88.455 to 109.910 ppm and 16.491 to 32.656 ppm respectively. Reduction of around 20% was seen in the muscle of oceanic squids. Mean Zn content in neretic squid was more than five folds higher than in oceanic squids. In the edible muscle Zn content varied from 6.119 to 12.761 ppm in neretic squids and 5.279 to 19.645 ppm in oceanic squids. Enrichment of Zn was comparatively lower than metals in both neretic and oceanic squids; (Figs. 5.1 and 5.2). In gills the Zn content varied from 1.590 to 38.342 ppm and 6.201 to 18.070 ppm in neretic and oceanic squids respectively, although both oceanic and neretic squids reflected more or less similar mean values (Figs. 5.1and 5.2). Concentration of Zn in different body components of oceanic squids followed the order liver > gills > muscle.

5.3.5. Chromium

Distribution pattern of mean Cr content in neretic (*Loligo* spp.) and oceanic squids (*Ancistrocheirus* spp.) are plotted in Figs. 5.1 to 5.3 and concentrations are presented in Tables 5.1 and 5.2. Cr content in whole soft tissues varied from 0.598 to 12.586 in *Loligo* spp. and 0.169 to 2.035 ppm in *Ancistrocheirus* spp. The Cr content of 20% of the samples was above the tolerance limit of 12 ppm. However, the prevalence of Cr in the mantle of neretic squid and oceanic squids were lower and concentrations varied between 1.025 $\pm$ 0.987 ppm and 0.180 $\pm$ 0.061 ppm respectively indicating lower body burden of Cr in muscle of oceanic squids. Among the other body components analysed, liver of neretic squids exhibited comparatively higher values (Tables 5.1 and 5.2) and were two folds higher than in oceanic squids. In gills, concentrations of Cr were more or less similar in both types of squids (Figs. 5.1 and 5.2). The concentrations varied from 3.612 $\pm$ 3.878 ppm (neretic) to 3.385 $\pm$ 2.263 ppm (oceanic). In general Cr content in whole muscle, liver and gills were higher in neretic squids than found in oceanic squids.

5.3.6. Nickel

Ni content exhibited in neretic and oceanic squids are presented in Tables 5.1 and 5.2 and distribution pattern of mean values in both the squids are plotted in Figs. 5.1 to 5.3. Average Ni values in whole soft parts of neretic squids were slightly higher than found in oceanic squids (Fig. 5.4). The concentrations varied between 1.688 $\pm$ 0.811

ppm and 1.393 ± 0.292 ppm in neretic and oceanic squids respectively. In the muscle tissue, Ni content varied between 0 to 5.054 ppm (neretic) and 0.187 to 2.293 ppm (oceanic), while, in the liver and gills Ni content in neretic squids were <10 ppm (Figs. 5.2 and 5.3). In oceanic squids mean Ni content was < 3 ppm, ranging from 0.227 to 1.968 ppm and 0.421 to 3.603 ppm in liver and gill components of oceanic squids respectively.

Statistical analysis was carried out using t test to compare the means between neretic and oceanic squids. Comparison of means showed that Hg, Cd, Cu and Cr content in muscle of oceanic squids was significantly higher (Cd and Cr; $p<0.01$ and Cu, $p< 0.05$) than found in neretic squids. There was no significant difference in the distribution of other heavy metals in the muscle. Cd and Cu levels in the gills and liver of oceanic squid were significantly higher ($p<0.01$) than in neretic squids. There was no significant difference ($p> 0.01$) in the distribution of Zn in the muscle, liver and gills. However, Cd and Cu levels of oceanic squids in all the three body parts analysed were significantly higher ($p< 0.01$) than in neretic squids. In whole soft parts, Zn content in neretic squids were significantly higher ($p < 0.01$) than in oceanic squids whereas Cd and Cu levels in oceanic squids were significantly higher ($p< 0.01$) than whole neretic squids.

5.4. Discussion

The present study has shown that oceanic squids (*Ancistrocheirus* spp.) caught at a depth of > 200 m had higher levels

of most of the metals than neretic squid (*Loligo* spp.) both of which were collected onboard the fishing vessel MV *Sagarika* and FORV *Sagar Sampada* during their cruise along the west coast of India. The metals namely Cd and Cr exceeded the permissible limit in 20% of the samples when whole *Loligo* spp. were analysed, whereas in whole oceanic squid (*Ancistrocheirus* spp.) an unexploited species, all the samples analysed showed Cd content above 3 ppm. In the edible muscle about 85% of oceanic squids had Cd content > 3ppm; far above the tolerance limit. However in the commercially exploited squids (*Loligo* spp.). concentrations of the heavy metals studied complied within the limits indicating safe consumption with respect to the consumer.

The concentration of cadmium in whole soft tissues of neretic squids observed in the present study were quite comparable to the levels reported by Falandysz (1989) in raw whole *L. patagonica* and in *L.opalescens* by Oehlenschlaeger (1991). Elevated Cd contents were also observed in whole *L.patagonica* (Barska, 1988), in 40 out of 422 samples of *Loligo* spp. (Cozani, 1990), *L. opalascens* (Falandysz, 1991), *L. duvauceli* (Lakshmanan & Stephen 1993 and Tariq *et al.*, 1991) and in Lolignidae squid (Tomasevic, 1988). However their levels were several folds higher than that encountered in the Lolignidae squid, *L. forbesi* (0.10±0.04) and *L. vulgaris* (0.10±0.03) ppm wet wt from French and Irish waters (Bustamante et al., 1998). Cd concentrations in oceanic squids in the present study were comparable

to levels reported by Bustamante *et al.,* (1998) in *Todarodes sagittatus* of West Irish Shelf (8.41±5.99 ppm) and Faroe Islands. A more or less similar Cd level (14.16±1.75 ppm) was noted in *T. sagittatus* of Canary Islands (Lozano, 1980). The higher Cd content in whole oceanic squids could be probably because they were larger individuals (Bustamante *et al.*, 1998). However, Cd accumulation in the muscle and most significantly in the liver of oceanic squids (*Ancestirocherius* spp.) was several folds higher than *Loligo* spp. but comparable to the oceanic squid, *Illex condetii* where concentration in edible muscle exceeded the permissible limit (Storelli, 1991). Similar levels were reported in *Ommastrephes bartami* (71.694 μg/g). Nevertheless, the values were higher than levels reported in *N. gouldi* (19 -110 μ/g) and lower than in *Symplectoteuthis oulanensienses* (427–1100 μg/g). Cu levels on dry/wet wt. levels reported in this study were similar to the results published earlier. Mean Cu concentrations decreased in the order liver > gills > muscle in both species. The Cu concentration in the muscle of *Loligo* spp. in the present study is comparable to levels reported by Lakshmanan & Stephen (1993); Oehlenschlager, (1991) and Falandysz (1989) in the edible muscle of *L. duvauceli, L. patagonica* and *L. opalascens* respectively. The Cu burden in *Ancistrocheirus* spp. however was 1-3 magnitudes higher. As observed in the present study in the oceanic squid, comparatively higher Cu content of (540 μg/kg) was also noted in *S. bartami* (Ueda et al., 1979) and other oceanic squids (Finger and Smith, 1987; Smith *et*

al., 1984; Martin & Flegal 1975). Such high concentrations are a reflection of the functional necessity as squids require Cu for the synthesis of haemocyanis (Martin & Flegal; 1975 and Smith, 1984). Zn contents observed in the mantle and whole soft tissues of *Loligo* spp. In the present study are comparable to the values reported by Oehlenschlaeger (1991) and Falandysz (1989) in *L. opalascens* and *L. patagonica* and by Martin and Flegal (1975) in liver of *L. opalescens.* Zn concentrations in all the samples were below the permitted levels, similar to as reported in *L. duvauceli* by Lakshmanan & Stephen (1993) and Tariq *et al.* (1991). Compared with values reported for other cephalopods whole *Ancistrocheirus* spp. had generally low levels of Zn. However, in the edible muscle Zn content was similar to that reported in *l.argentinus* by Falandysz (1988). Elevated levels of Zn were noted in the liver of other oceanic squids of family *Ommastrephidae* like *T. pacificus* (Ueda *et al.,* 1979), *S.bartami* (Smith *et al.,* 1984) than in the present study.

Zn being a bio essential element is more widely distributed than Cd among the different organs. Being an enzyme activator and constituent of several important metalloprotein enzymes (Keillin & Mann, 1940 and Vallee, 1963) it has greater importance in biological systems.

Cr concentration in the liver of *Loligo* spp. and *Ancistrocheirus* spp. In the present study were higher compared to Cr levels in *E. cirrhosa*, and *S. officianalis* from the English channel Miramand &

Bentley (1992). However, the levels of Cr in the present study are similar to levels reported by Tariq *et al.,* (1991) in *Loligo* spp.

Ni concentrations in *Loligo* spp. are comparable to levels reported by Bustamante *et al.*, (1998) in *Nautilus macrocephalus* but lower than levels found in *Sepia* spp. (Miramand & Bentley, 1992).

High variability in metal levels have been observed by Bustamante *et al.,* (1998) with respect to families, geographical origin and feeding habits in North Atlantic cephalopods A similar trend was also noted in the present study. Oceanic squids (*Ancistrocheirus* spp.) exhibited higher mean concentrations than neretic squids (*Loligo* spp.) with regard to Cd and other toxic metals. In addition to spatial variations, and other environmental factors, variability in metal levels between neretic and oceanic squid is probably because oceanic squids are larger individuals (Bustamante *et al.*, 1998a). Squids also show diurnal and seasonal changes in feeding intensity (Sauer & Lipinski, 1991) and feed on a wide variety of fishes, crustaceans and cephalopods (Kore & Joshi 1975; Varghese, 1976; Boucher – Rodoni *et al.,* 1987 and Nixon, 1987) and move in different depths of water to catch its prey. Thus, the highly voracious and diverse feeding habits of neretic and oceanic squids could have contributed to the high levels of metals in both species of squid. Cannibalistic behaviour has been found to increase with the age of squids (Kore & Joshi, 1975; Sauer & Lipenski, 1991), indicating greater accumulation in larger individuals.

Table 5.1 : Trace metal concentrations (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of neretic squids caught onboard MV *Sagarika* and FORV *Sagar Sampada* at 30-70m depth, off west coast of India.

BODY COMPONENT	n*	MERCURY	CADMIUM	COPPER	ZINC	CHROMIUM	NICKEL
WHOLE	15	0.077 ± 0.017 (0.059–0.099)	1.896 ± 0.990 (1.067– 3.708)	13.440 ± 2.461 (9.263–6.871)	95.611 ± 7.036 (88.455–109.910)	3.655 ± 4.941 (0.598–12.586)	1.688 ± 0.811 (0.352–2.532)
MUSCLE	31	0.041 ± 0.009 (0.028–0.054)	0.545 ± 0.509 (0–1.795)	4.464 ± 5.962 (0.306–22.968)	9.585 ± 1.987 (6.119–12.761)	1.025 ± 0.987 (0–4.049)	1.358 ± 1.168 (0–5.054)
LIVER	29	0.119 ± 0.031 (0.095–0.197)	38.741 ± 0.461 (2.011–27.650)	48.558±36.497 (6.630–135.440)	27.830 ± 23.562 (10.510–111.330)	6.885 ± 7.524 (0–25.00)	9.172 ± 10.386 (0–32.901)
GILLS	28	0.072 ± 0.012 (0.058–0.085)	1.905 ± 2.724 (0–11.176)	24.529 ± 7.712 (15.00–45.277)	13.417 ± 8.934 (1.590–38.342)	3.612 ± 3.878 (0–17.959)	5.880 ± 6.372 (0–21.760)

*maximum number of samples analysed

Table 5.2: Trace metal concentrations (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of oceanic squid caught onboard FORV *Sagar Sampada* at 200-350m depth off west coast of India.

BODY COMPONENT	n *	MERCURY	CADMIUM	COPPER	ZINC	CHROMIUM	NICKEL
WHOLE	12	0.084 ± 0.069 (0.076 – 0.095)	11.228 ±1.199 (9.841 –12.947)	21.361 ± 5.520 (16.491 – 32.656)	13.891 ± 9.918 (6.185 – 29.923)	0.864 ± 0.759 (0.169 – 2.035)	1.393 ± 0.292 (1.103 – 1.185)
MUSCLE	18	0.060 ± 0.005 (0.054 – 0.065)	3.777 ± 0.942 (1.979 – 5.071)	8.201 ± 4.236 (2.605 – 14.695)	10.972 ± 4.513 (5.279 – 19.645)	0.180 ± 0.061 (0.099 – 0.233)	0.954 ± 0.640 (0.187 – 2.293)
LIVER	15	0.129 ± 0.047 (0.085 – 0.212)	88.802 ±60.058 (37.056 –200.4)	164.657 ± 185.265 (10.870 – 482.170)	26.488 ± 14.081 (10.409 – 57.968)	3.112 ± 1.936 (0 – 6.519)	1.043 ± 0.661 (0.227 – 1.968)
GILLS	12	0.078 ± 0.010 (0.065 – 0.086)	7.294 ± 6.539 (1.492 – 16.563)	38.344 ± 43.851 (13.867 – 121.637)	12.977 ± 4.928 (6.201 – 18.070)	3.385 ± 2.263 (0.359 – 5.529)	2.040 ± 1.532 (0.421 – 3.603)

* maximum number of samples analysed

Fig. 5.1: Distribution pattern of heavy metals in body components of neretic squids (mean, ppm, wet wt)

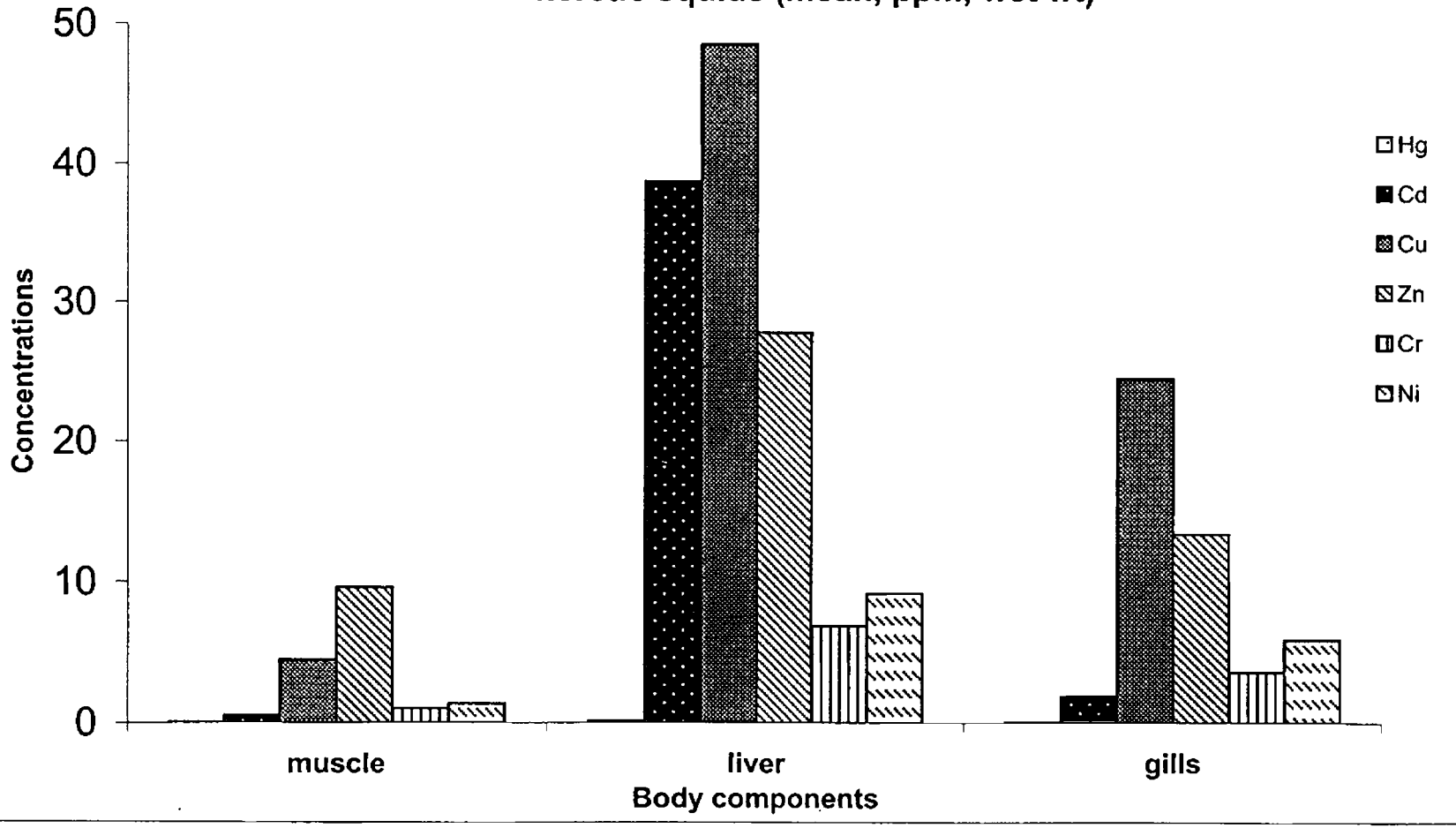


Fig. 5.2: Distribution pattern of heavy metals in body components of oceanic squids (mean, ppm, wet wt)

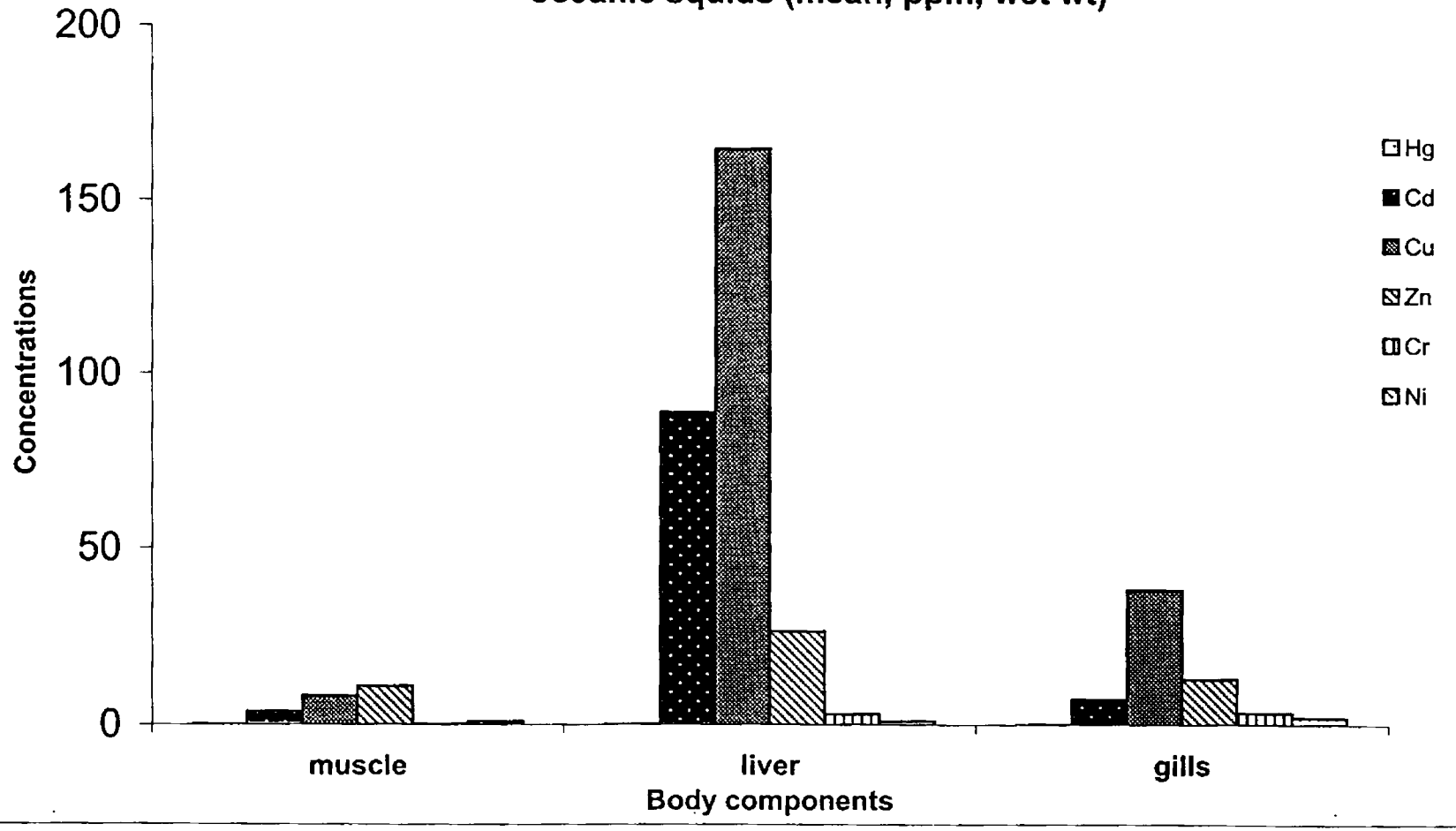
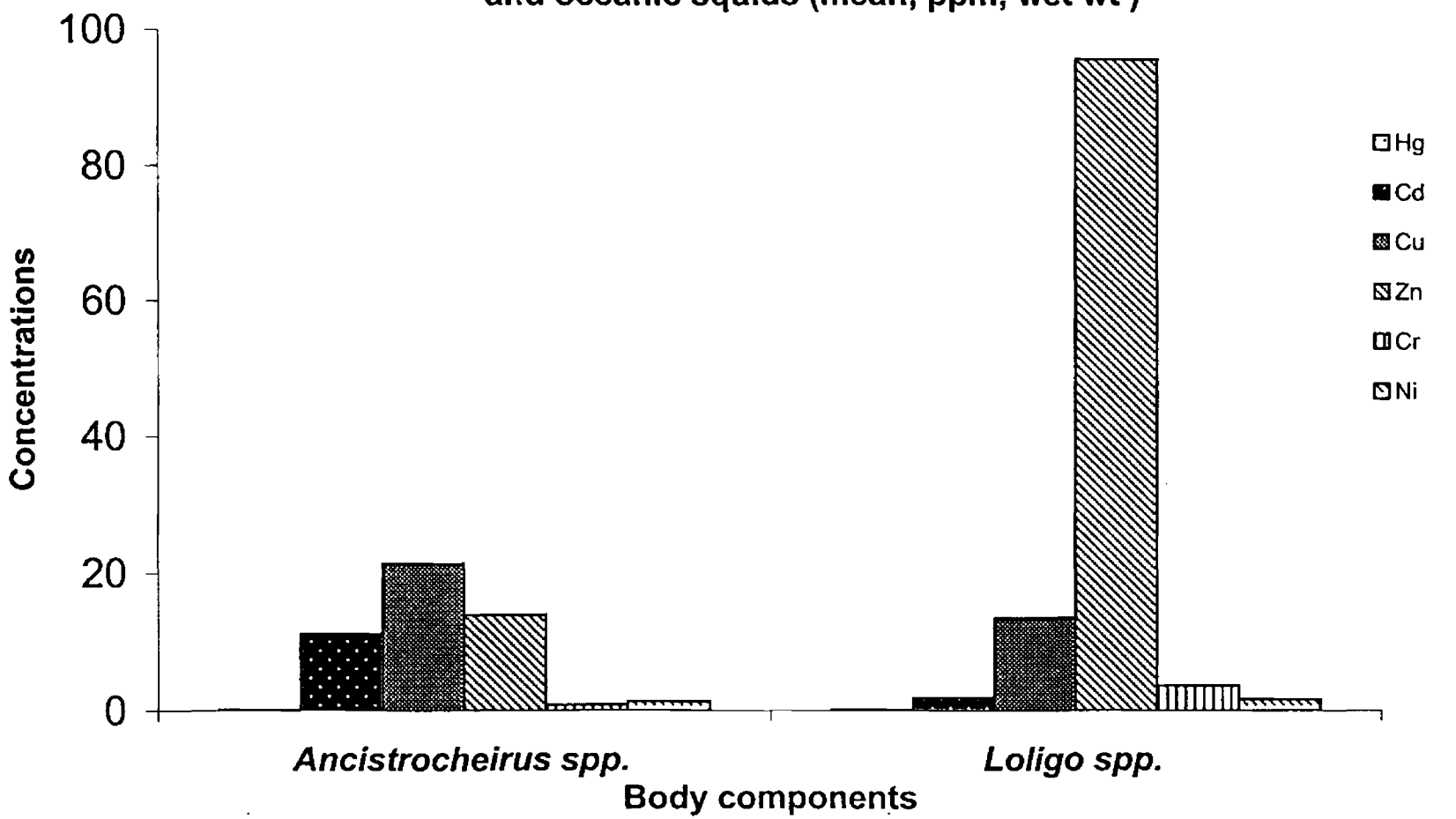


Fig.5.3: Distribution pattern of heavy metals in whole soft parts of neretic and oceanic squids (mean, ppm, wet wt)



CHAPTER 6

Metal accumulation in squids in relation to levels in food fishes

6.1. Introduction

The squids are the most active and specialized group of cephalopods living in the open waters of the ocean on 'more or less' in equal terms with such creatures as fish and aquatic mammals (Varghese,1976). However, squids tend to concentrate remarkably higher levels of both essential metals and toxic heavy metals than observed in fishes. As squids are important members of the food chain, they are among the main prey to a large variety of fishes, sea birds, cetaceans and marine mammals and cephalopods themselves (Silas *et al.* 1963) thereby constituting an important source of cadmium and other heavy metals in cephalopod predators. Indeed, a large number of top predators regularly include cephalopod in their diets, or at least ingest them opportunistically. Cephalopods especially squids are the favorite food of sperm whales and form an important forage for seasonal species of fishes and a very significant constituent of the food of tunas and bill fishes. Squids also are fairly consumed by 80% of Odontocetes species; Ziphidae and Physteridae families being the principal consumer squid family. Ommasterephidae in oceanic waters and family Loliginidae in neretic waters are the main squid groups consumed by Odontocetes. Ommastrephidae, Lolignidae, Onchyoteuthidae, Gonatidae and Octopodidae are the most common cephalopod families recorded in the diet of seals. Likewise a great numbers of sea birds such as Procellariforms, Pelicaniformes and Alicids are known to feed on cephalopods, mainly squid. Numerous

seabird species such as albatrosses and petrel prey upon cephalopods, which are as important as fish or crustaceans. Penguins, auks and terns also eat significant quantities of squid during certain seasons. In turn squids are active predacious carnivores feeding on living prey during the whole period of their life cycle.

Squids are voracious feeders with high food consumption as well as an efficient digestive system which enable them to simultaneously digest 2 meals. The prey captured by the tentacles of squids *L. duvauceli* reaches the stomach through the long straight oesophagus where preliminary digestion takes place and at the caecum final digestion and absorption takes place. Undigested matter coupled with rapid digestion, makes identification of prey and quantification of diet from gut contents particularly difficult. In cephalopods the difficulties include partial ingestion, fragmentation and rapid digestion of prey, ingestion of fish or squid trapped in the net during trawling, predation on other squid trapped in nets and ingestion of stomach contents of prey animals. Nevertheless, a large number of studies have been carried out on the stomach contents of squids mostly by volumetric analysis (Pierce *et al.*, 1994; Collins *et al.,* 1994 & Santos 1998). Thus the presence of fish was often determined from scales or sometimes otoliths, vertebrae, bones, eyeballs and fish rays. Crustaceans which include prawns, crabs and squilla form diagnostic exoskeleton fragments, mainly mandible morphology, telson and rostrum spination. Cephalopods are identified due to the presence of

pigmented portion of skin, suckers, chitineous rings of identified forms, statolith and beaks of soft tissue. As identification thus, becomes rather difficult and does not reflect the true picture, in this study fishes separated from the mantle cavity of whole raw squids and fishes caught in the same habitat area have been treated as the food fishes as treated by Oommen (1977). So in the present study an attempt has been made to correlate metal levels in the food fishes to that in squid and thereby track down the source of Cd and other metal accumulation in squids.

6.2. Materials and methods

Whole fishes protruding from the mantle cavity of *L. duvauceli* or found entangled within the visceral mass of *L. duvauceli* caught off Cochin region during 1998 - 2000 were subjected to metal analysis. Fishes and crustaceans separated from the mantle cavity of *Ancistrocheirus* spp. collected on board FORV *Sagar Sampada* Cruise No. 191 at 200-350m depth 6°58' to 13°30' N lat and 74°15' to 77°50' E long and fishes caught along with *L. duvauceli* in the fishing vessel, *MV Sagarika* in two different cruises from May to June 1998 at depths 30-58m lat 9° 47' to 12° 08'N and long 75° 02' to 75° 50' E were also subjected to metal analysis. Water samples collected during the cruise from the above mentioned depths were also analysed for metals. The details of preparation of samples and analytical procedures adopted in the determination of heavy metals are described in Chapter 2. The

length and weight range of the fishes caught along with squids and separated from the mantle cavity of squids were 9 -15 cm and weight 40-150 g. Statistical analysis involved regression analysis of heavy metal levels in squids with that of metal levels in fishes and crustaceans.

6.3. Results

The concentrations of metals, *viz.*, Cd, Pb, Cu, Zn, Cr and Ni in the whole fishes separated from the mantle cavity of *L. duvauceli* and *Ancistrocheirus* spp. are presented in Tables 6.1 and 6.2. Concentration of heavy metals in fishes caught along with neretic squids are presented in Table 6.3. Trace metal concentrations in water samples collected on board FORV *Sagar Sampada* at 30-65m depth and 200-350m depth are presented in Tables 6.4a and 6.4b. The fitted linear regressions are shown in the Figs. 6.1a to c, 6.2 a to 6.3 b. The goodness of fit were tested using ANOVA technique and they are presented in Tables (6.5 to 6.7). The distribution pattern of heavy metals, *viz.*, Cd, Pb, Cu, Zn, Cr and Ni in the body of whole fishes and crustaceans and their correlations with squids are discussed below.

6.3.1. Metal levels in fishes separated from the mantle cavity of *L. duvauceli*

The fishes found to occur recurrently in the mantle cavity of *L. duvauceli* were namely *Saurida tumbil, Platycephalus tuberculatus, Nemipterus japonicus, Priacanthus hamrur, Cynoglossus* sp.,

Apogonichthys sp. and *Anchovies.* The concentrations of metals, *viz.*, Cd, Pb, Cu, Zn, Cr and Ni in the whole fishes separated from the mantle cavity of *L. duvauceli* are presented in Table 6.1. Elevated levels of highly toxic metals, Cd (>1 ppm), Pb and Cr (>2 ppm) were noticed in more than 50 % of *Saurida tumbil* samples separated from the mantle cavity of *L. duvauceli.* The prevalence of Cd was in the range 0.709 to 2.567 ppm. The concentrations of Pb and Cr were 2.985 ± 1.524 ppm and 2.720 ± 1.622 ppm, respectively. Cu was the least abundant metal and lower values of Cu, in general, was zero (Table 6.1). *S. tumbil* exhibited the highest concentration of the essential metal Zn (17.416 ± 18.33 ppm). In *Platycephalus tuberculatus,* mean Cd content was 2.691 ± 2.998 ppm; with highest level of 7.126 ppm. The toxic metals, *viz.*, Pb and Ni were also high and the highest values being 5.010 and 3.564 ppm, respectively. In 50% of the samples Pb content in this species exceeded 2 ppm. Ni levels ranged from 1.299 to 3.564 ppm. Cu concentration ranged from 0 to 5.912 ppm and Zn levels ranged from 4.901 to 14.240 ppm. The distribution pattern of heavy metals in *Priacanthus hamrur* showed that Zn was the most abundant metal. The concentrations being 8.096 ± 4.714 ppm. Elevated levels of Cd in the range 1.041 to 1.182 ppm was observed in *P. hamrur* and more than 90% of the samples had Cd content > 1ppm. Cr levels varied from 1.022 to 1.220 ppm. In *Nemipterus japonicus* separated from the mantle cavity of *L. duvauceli* the highest recorded value of Cd was 2.588 ppm. Mean Pb, Cu and Cr content in *N. japonicus* was <1 ppm (Table 6.2). Zn content ranged

from 0.579 to 7.850 ppm. Average Ni levels was 1.707 ppm and the highest value recorded was 2.391 ppm. About 20% of *Apogonichthys* sp. had Pb content >1.5 ppm and the highest recorded value was 3.531 ppm; the mean value being 1.758 ppm. Although Cu and Zn are essential metals low levels of these metals were noted in this species. However, the highly toxic metal Cr was comparatively higher. (Table 6.1). High levels of Cd was noted in *Cynoglossus* sp. with more than 50% of samples showing Cd levels above 1 ppm (Table 6.2). Cr content in this species was relatively high with mean values of 4.003 ppm and Cr levels ranged between 1.943 to 5.925 ppm. Anchovies showed low levels of most of the metals analysed (Table 6.1)

6.3.2. Metal levels in fishes separated from the mantle cavity of *Ancistrocheirus* spp.

Crustaceans separated from the mantle cavity of oceanic squid *Ancistrocheirus* spp. include *Heterocarpus woodsomani, and Plesionika ensis.* The most abundant and recurrently occurring fishes found in the mantle cavity of *Ancistrocheirus* spp. were *Bembrops* sp., *Uranoscopidae* and *Bothus* sp. Trace metal distribution pattern in these fishes are presented in Table 6.2. It was interesting to note that Cd content in whole *Bothus* sp. was very high and ranged between 2.203 to 6.358 ppm, the mean value being 3.652 ppm. Levels of Cd were also noted in crustaceans *Plesionika ensis* and in *Heterocarpus gibbosus* (2.895 and 2.125 ppm), respectively. In *Bembrops* sp. and *Uranoscopidae* caught along with oceanic squids, the Cd content

ranged from 0.989 to 3.026 ppm and 1.177 to 2.809 ppm, respectively. Pb levels in *Bembrops*, *Uranoscopidae* and *Bothus* sp. separated from the mantle cavity of *Ancistrocheirus* spp. ranged from 0.855 to 2.047, 0.432 to 0.683 and 0.634 to 1.522 ppm, respectively. The essential metals Cu and Zn levels in the crustaceans and fishes were low except for a higher mean value of 16.859 ppm in *Heterocarpus gibbosus*. Elevated levels of Cr was noted in *Plesionika ensis* and Cr content ranged from 1.408 to 5.468 ppm and 20% of the samples had Cr content above 2ppm. Nevertheless, low content of Cr was found in *Heterocarpus gibbosus* (Table 6.2). Interestingly, Ni levels in the crustaceans and fishes separated from the mantle cavity of the oceanic squid were comparatively lower, with the highest value of 1.023 ppm found in *Plesionika ensis* and lowest value of 0.121 ppm noted in *Bothus* sp.

6.3.3. Metal levels in fishes collected in the same habitat area as neretic squids

The most abundant and recurrently occurring fishes along with neretic squids were *Priacanthus hamrur, Dactyloptena orientalis, Epinephelus diacanthus, Saurida tumbil, Upeneus* sp., *Alectus indica* and *Lutjanus lutjanus*. Trace metal distribution pattern in these fishes are presented in Table (6.3). Among the various fishes analysed Cd content in *Saurida tumbil* was comparatively higher (3.784±3.499 ppm). The highest value recorded was 6.854 ppm. In *Upeneus sp.* mean Cd content was in the range of 0 to 0.876 ppm. Mean Pb levels were < 1

ppm in *P. hamrur D. orientalis, E. diacanthus and Upeneus sp.* However, higher levels were noted in *S. tumbil* and *A. indica*. The highest value recorded were 3.967 and 3.263 ppm, respectively. The concentration of Cu ranged from 0.740 to 6.838 ppm covering all the fishes caught during the period of study. Highest Zn content was noted in *Saurida tumbil* (10.502 ppm). Cr content in *Saurida tumbil, Epinephelus diacanthus* and *Alectus indica* was in the range 0.581 to 2.513 ppm, 0.205 to 5.588 ppm and 0.595 to 2.236 ppm, respectively. whereas in other fishes (*Priacanthus hamrur, Dactyloptena orientalis, , Upeneus sp.*, and *Lutjanus lutjanus*) the lower range value in general was zero. Average Ni levels were in the range of 3.526 to 24.146 ppm and 3.256 to 24.186 ppm. in *Dactyloptena orientalis* and *Epinephelus diacanthus* respectively.

6.3.4. Metal levels in water samples collected from the same habitat area as squids

Metal levels in water samples collected from the same habitat area of neretic and oceanic squids are presented in Table 6.5a and b. Cd, Cu and Zn levels were found to be very low in both the sites and were at normal levels. The level of Cd in sea water samples were in the range of 0.0005 to 0.0013µg/L Cu levels were 0.008 to 0.021 µg/L and Zn was in the range 0.013 to 0.078 µg/L, respectively. At 200-350m depth, Cd, Cu, Zn, Pb and Cr levels were comparatively low (Table 6.5b). Cd content in the surface and deep waters ranged from 0.0003 to 0.005 µg/L, respectively. Not much of a difference was noted in Cd

levels at surface and deep waters. However, Cu, Zn and Pb levels in deeper waters were found to be slightly higher and were in the range 0.003 to 0.007, 0.028 to 0.042 and 0.022 to 0.046 μg/L respectively. The highest values recorded at surface waters were 0.005 μg/L for Cu, 0.032 μg/L for Zn and 0.031 μg/L for Pb. The lower levels of Cr in surface and deep waters were similar and the levels ranged between 0.006 to 0.009 μg/L and 0.006 to 0.008 μg/L.

6.3.5. Metal levels in squids and relation to food fishes

An attempt has been made to determine the source of cadmium and other metals in squids. Regression analysis was carried out to determine relationship between metal levels in squids with that in fishes. The regression co-efficient (b) was computed using the equation

$$b = \frac{\Sigma xy - (\Sigma x)(\Sigma y)/N}{\Sigma x^2 - (\Sigma x)^2/N}$$

where N = number of samples, x = metal levels in fish and y = metal levels in squid. The intercept 'a' was determined by the formula $a = \bar{y} - b\,\bar{x}$. Using these values the linear equation ($y = a+bx$) of squid – fish relationship was determined.

The regression plots are presented in Figs. 6.1a to c and 6.2a to 6.3b. The regression equation for metal levels in various species of fish versus metal levels in squids are as follows:

Fishes separated from the mantle cavity of *L. duvauceli*

Cadmium			
Saurida tumbil	y = 0.06 + 0.604 x	r =0.564	p<0.10
Priacanthus hamrur	y = 1.047+0.055 x	r = 0.781	p<0.05
Cynoglossus sp.	y = 1.43 – 0.172 x	r = 0.947	p<0.01
Lead			
Platycephalus tuberculatus	y= 0.485 + 0.393x	r = 0.582	p<0.10
Apogonichthys sp.	y =0.220 +0.760 x	r = 0.734	p<0.05
Zinc			
Platycephalus tuberculatus	y = -0.385 +1.419 x	r = 0.617	p<0.10
Priacanthus hamrur	y =-4.94 + 1.745 x	r = 0.698	p<0.05
Chromium			
Priacanthus hamrur	y = -16.4 +15.98 x	r = 0.734	p<0.10
Nickel			
Platycephalus tuberculatus	y = 3.268 – 0.698 x	r = 0.764	p<0.05

Fishes and crustaceans separated from the mantle cavity of *Ancistrocheirus* spp.

Cadmium			
Bothus sp.	y = 13.163–0.529 x	r=0.725	p<0.05
Copper			
Plesionika ensis	y = 52.587–7.226 x	r=0.727	p<0.05
Bothus sp.	y = 4.91 – 0.120 x	r=0.596	p<0.10
Chromium			
Plesionika ensis	y = -0.343+0.339 x	r=0.769	p<0.10
Nickel			
Bembrops sp.	y =1.323 - 0.371 x	r=0.720	p<0.10

Fishes caught along with neretic squids

Epinephelus diacanthus	y= 0.05 - 0.761 x	r=0.887	p<0.01

Saurida tumbil	y = 0.049 + 0.069 x	r=0.746	p<0.01
Alectus indica	y = 1.840 - 0.179 x	r=0.729	p<0.01
Zinc			
Dactyloptena orientalis	y = 8.632 + 0.448 x	r=0.976	p<0.01
Upeneus sp.	y = 8.974 + 0.646 x	r=0.90	p<0.05
Chromium			
Alectus indica	y = 0.354 + 0.723 x	r=0.723	p<0.01
Saurida tumbil	y = 1.710 + 0.087 x	r=0.912	p<0.01

6.4. Discussion

The present study has shown that significant correlations exist between toxic metals levels in fishes and in squids. Significant positive correlations were noted between Cd levels in squids with that in *Saurida tumbil* ($p<0.10$), and *Priacanthus hamrur* ($p<0.05$) separated from the mantle cavity of *L. duvauceli.* Significant positive correlation was also noted between Pb, and Zn content in *Platycephalus tuberculatus* with that observed in squids. Positive correlation was also noted between Cr and Zn content in *Priacanthus hamrur*, and Pb content in *Apogonichthys* sp.

Fishes collected in the same habitat area as that of squids also showed statistically significant relationship. Cr content in *Alectus indica*, Zn levels in *D. orientalis* and *Upeneus sp.*, Cd and Ni levels in *Saurida tumbil* showed significant positive correlation with metal levels in squids. Significant positive correlation was noted between Cd, Cr

and Zn levels in oceanic squid, *Ancistrocheirus* spp. and Cd levels in *Bothus* sp. ($p< 0.05$), Cr content in *Plesionika ensis* ($p<0.10$) and Zn in *Bembrops* sp. These results indicate that as metal levels in these fishes increase, metal levels in squid also increases. Hence, the presence of these fishes in the diet of squid could be one probable source of metal accumulation in squids. Varghese (1976) reported on the feeding habits of cephalopods from the west coast of India and found among the stomach contents the presence of *Saurida tumbil, Platycephalus* sp., *Nemipterus japonicus, Priacanthus hamrur, Cynoglossus* spp., and *Anchovies,* in addition to other fishes observed in mantle cavity of *L.duvauceli* in the present study. As the food contents in the stomach are usually in the advanced stage of digestion and identification of the species is very difficult (Nixon, 1987, Dawe, 1992). Varghese (1976) treated fishes found in the trawl nets along with squids as its food fish. In the present study also fishes found in the same habitat area as of squids were treated as food fishes. *Saurida tumbil, Upeneus spp.* caught along with squids in the trawl nets along with *L. duvauceli* by Varghese (1976) were noted in the present study as well. The relatively higher level of metals observed in *L. duvauceli* may be attributed to the contributions made by these fishes.

The prey of neretic and oceanic squids have been identified by several workers (Kore & Joshi, 1975; Ivanovic *et al.*, 1994; Coelho *et al.* 1997). The Cd concentration in the preys of cephalopod have been recorded at 0.009-0.9 mg/kg dw in small fishes, (Hornung and

Ramelow, 1987), in whitebait, 0.012-0.013 mg/kg dw (Prudente *et al.*, 1997), in northern anchovy, 0.73-0.99 mg/kg dw (Sydeman and Jarman, 1998) and 2.2 -2.7 mg/kg dw in krill, (Sydeman and Jarman, 1998). Jiro Koyama *et al.* (2000) reported on the relative contribution of water and food to Cd uptake by oval squid *Sepioteuthis lessoniana* and noted Cd derived from prey fish mummichog, *Funulus heteroclitus made* up about 55% of the total squid Cd residue.

In the present study, the prey of squids namely *Saurida tumbil, Platycephalus tuberculatus, Cynoglossus sp., Alectus indica, Dactyloptena orientalis, Priacanthus hamrur, Upeneus sp. Bembrops* sp, *Plesionika ensis, Uranoscopidae, Bothus sp.* all showed significant relationship with metal levels in squids. Metal levels in the habitat water was also very low (Table 6.5 a and b). It is suggested that the fishes and crustaceans could be one probable source of Cd and other heavy metal accumulation in squids although only a very small range of food spectrum of these highly predatory and voracious squids have been analysed. In addition cannibalistic behaviour has been noted in several squid species (Verrill, 1882; Bidder, 1950; Okutani, 1990) and were found to increase with age of squids (Kore & Joshi, 1975) indicating that the dietary habits of squids may play a major role in metal accumulation.

Table 6.1: Distribution pattern of heavy metals (Mean ± S.D., Range, ppm wet wt) in fishes separated from the mantle cavity of *L. duvauceli* collected off Cochin

FISHES	n*	CADMIUM	LEAD	COPPER	ZINC	CHROMIUM	NICKEL
Saurida tumbil	10	1.723±0.652 (0.709-2.567)	2.985±1.524 (0.540-4.538)	0.016±0.032 (0-0.081)	17.416±18.330 (0.055-43.740)	2.720±1.622 (0.409-4.940)	5.832±5.939 (0.235-14.920)
Platycephalus tuberculatus	12	2.691±2.998 (0.016-7.126)	2.623±1.287 (0-5.010)	2.938±2.147 (0-5.912)	8.41±3.371 (4.901-14.240)	1.083±0.401 (0.352-1.452)	2.536±1.130 (1.299-3.564)
Priacanthus hamrur	10	1.125±0.055 (1.041-1.182)	3.491±0.933 (2.430-4.183)	2.330±0.086 (2.215-2.405)	8.096±4.714 (1.760-11.579)	1.132±0.063 (1.022-1.220)	2.178±0.374 (1.759-2.477)
Nemipterus japonicus	08	1.503±0.904 (0-2.588)	1.753±0.700 (0-1.753)	0.849±0.627 (0.398-1.956)	3.427±3.652 (0.579-7.850)	0.894±0.478 (0.401-1.590)	1.707±0.617 (1.167-2.391)
Apogonichthys sp.	07	3.568±0.733 (2.758-4.266)	1.758±0.870 (0.550-3.531)	1.502±0.233 (1.191-1.810)	2.390±0.296 (1.987-2.641)	2.556±0.721 (0.657-3.278)	0.525±0.172 (0.321-0.768)
Cynoglossus sp.	10	1.199±0.155 (0.989-1.324)	1.124±0.339 (0.572-1.404)	0.858±0.310 (0.549-1.204)	0.573±0.353 (0.224-0.977)	4.003±2.209 (1.943-5.925)	0.702±0.208 (0.536-0.989)
Anchiovies	06	0.224±0.059 (0-0.456)	0.335±0.069 (0.689-0.856)	0.798±0.332 (0.587-0.937)	2.831±0.386 (2.633-3.215)	0.538±0.678 (0.0-1.358)	0.968±0.125 (0.352-2.365)

* maximum number of samples analysed

Table 6.2: Distribution pattern of heavy metals in fishes separated from the mantle cavity of oceanic squids (*Ancistrocheirus* spp.) caught onboard FORV *Sagar Sampada* (Cruise No. 191) during January 2001 at 200-350m depth.

Fish/Crustacean	n*	CADMIUM	LEAD	COPPER	ZINC	CHROMIUM	NICKEL
Bembrops sp.	10	1.821±1.079 (0.989-3.026)	1.090±0.432 (0.855-2.047)	1.139±0.174 (0.840-1.272)	0.930±0.717 (0.277-1.688)	1.247±0.652 (0.478-3.952)	0.580±0.362 (0.231-0.984)
Uranoscopidae	11	1.986±0.923 (1.177-2.809)	0.580±0.120 (0.432-0.683)	1.502±0.478 (0.978-1.915)	2.932±2.075 (0.664-4.957)	2.375±2.473 (1.215-3.558)	0.456±0.212 (0.215-0.896)
Bothus sp.	12	3.652±1.640 (2.203-6.358)	0.879±0.470 (0.634-1.522)	2.226±1.483 (0.816-3.709)	0.749±0.196 (0.521-0.989)	2.149±1.585 (1.586-4.806)	0.353±0.059 (0.121-0.779)
Plesionika ensis	08	2.895±0.536 (1.248-3.488)	0.775±0.121 (0.476-1.254)	4.275±0.660 (3.297-5.117)	2.196±0.435 (1.457-2.707)	3.927±1.672 (1.408-5.468)	0.402±0.223 (0.321-1.023)
Heterocarpus gibbosus	09	2.125±0.441 (1.154-4.532)	2.213±0.357 (1.898-2.560)	16.859±0.237 (16.602-17.164)	9.344±0.812 (8.419-10.397)	0.934±0.730 (0.347-1.912)	0.390±0.121 (0.265-0.536)

*maximum number of samples analyzed

Table 6.3: 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	10	0.326±0.131 (0 - 0.501)	0.510±0.414 (0-0.950)	0.808±0.280 (0.451-1.119)	5.485±0.894 (4.070-6.355)	0.843±1.029 (0-2.380)	2.543±1.654 (0.492-4.095)
Dactyloptera orientalis	12	0.955±0.841 (0-1.585)	0.970±0.925 (0-1.841)	1.815±1.428 (0.548-3.060)	6.789±2.305 (4.715-8.902)	1.183±0.664 (0.368-1.951)	15.894±13.768 (3.526-24.146)
Epinephulus dicanthus	08	0.357±0.262 (0-6.86)	0.198±0.289 (0-0.776)	0.485±0.291 (0.073-0.858)	4.606±1.860 (2.185-7.488)	2.251±2.046 (0.205-5.588)	28.340±16.760 (3.256-24.186)
Lutjanus lutjanus	12	0.309±0.216 (0-0.500)	2.519±2.911 (0-5.173)	0.569±0.373 (0.057-0.940)	4.949±1.571 (3.074-6.605)	0.637±0.745 (0-1.656)	4.053±4.526 (0-9.538)
Saurida tumbil	08	3.784±3.499 (0.714-6.854)	3.318±3.499 (2.714-3.967)	3.795±3.511 (0.740-6.838)	6.415±3.720 (3.629-10.502)	1.828±0.881 (0.581-2.513)	11.021±3.856 (7.52-14.491)
Upeneus sp.	12	0.548±0.342 (0-0.876)	0.235±0.462 (0-0.928)	0.511±0.288 (0.174-0.794)	4.089±1.159 (2.567-5.032)	3.311±2.117 (0-5.742)	14.370±17.714 (9.865-34.440)
Alectus Indica	10	0.302±0.245 (0-0.597)	1.165±0.452 (0-3.263)	1.400±1.094 (0.118-2.881)	5.787±1.202 (4.430-7.201)	1.458±0.614 (0.595-2.236)	3.719±2.287 (0-6.032)

*maximum number of samples analysed

Table 6.4: Anova of significance after fitted regression between metal levels in *L.duvauceli* with metal levels in fishes separated from the mantle cavity

ANOVA	Squid Cd on *Saurida tumbil* Cd				
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.39817	1.39817	3.739244	0.089208064
Residual	8	2.991344	0.373918		
Total	9	4.389514			
ANOVA	**Squid Cd on *Priacanthus hamrur* Cd**				
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.811129	1.811129	6.256266	0.066677224
Residual	4	1.157961	0.28949		
Total	5	2.96909			
ANOVA	**Squid Cd on *Cynoglossus s* p. Cd**				
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.622431	2.622431	25.93567	0.014635329
Residual	3	0.303339	0.101113		
Total	4	2.92577			
ANOVA	**Squid Pb on *Platycephalus tuberculatus* Pb**				
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.302547	2.302547	4.104488	0.07734119
Residual	8	4.487863	0.560983		
Total	9	6.79041			
ANOVA	**Squid Pb on *Apogonichthys* sp.Pb**				
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.576849	3.576849	8.194009	0.024250088
Residual	7	3.05564	0.43652		
Total	8	6.632489			
ANOVA	**Squid Zn on *Platycephalus tuberculatus* Zn**				
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	183.1967	183.1967	4.306037	0.076637631
Residual	7	297.8091	42.54416		
Total	8	481.0058			
ANOVA	**Squid Zn on *Priacanthus hamrur* Zn**				
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	234.2313	234.2313	6.644202	0.036598251
Residual	7	246.7745	35.25349		
Total	8	481.0058			
ANOVA	**Squid Cr on *Priacanthus hamrur* Cr**				
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	6.014635	6.014635	5.838131	0.060398298
Residual	5	5.151165	1.030233		
Total	6	11.1658			
ANOVA	**Squid Ni on *Platycephalus tuberculatus* Ni**				
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.520935	2.520935	7.00557	0.045601367
Residual	5	1.799236	0.359847		
Total	6	4.320171			

Table 6.5: ANOVA of significance after fitted regression between metal levels in *Ancistrocheirus* spp. with metal levels in fishes separated from the mantle cavity

ANOVA **Squid Cd on *Bothus* sp. Cd**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	6.797256	6.797256	8.84041	0.017781792
Residual	8	6.151077	0.768885		
Total	9	12.94833			

ANOVA **Squid Cu on *plesionika ensis* Cu**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	159.4072	159.4072	6.73832	0.040890647
Residual	6	141.9409	23.65681		
Total	7	301.3481			

ANOVA **Squid Cu on *Bothus* sp. Cu**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	110.5224	110.5224	2.46003	0.108528907
Residual	4	179.7088	44.9272		
Total	5	290.2312			

ANOVA **Squid Zn on *Bembrops* sp. Zn**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	211.1389	211.1389	2.59315	0.108614882
Residual	4	325.6873	81.42184		
Total	5	536.8263			

ANOVA **Squid Cr on *Plesionika ensis* Cr**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.610993	1.610993	5.7725	0.074146829
Residual	4	1.116324	0.279081		
Total	5	2.727317			

ANOVA **Squid Ni on *Bembrops* Ni**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.05329	0.05329	5.38364	0.068030942
Residual	5	0.049493	0.009899		
Total	6	0.102783			

Table 6.6: Anova of significance after fitted regression between metal levels in neretic squid with metal levels in fishes from same habitat area

ANOVA **Squid Cd on *Alectus indica* Cd**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.277965	0.277965	18.38416	0.007806304
Residual	5	0.075599	0.01512		
Total	6	0.353563			

ANOVA **Squid Cd on *Saurida tumbil* cd**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.196872	0.196872	6.28217	0.054063638
Residual	5	0.156691	0.031338		
Total	6	0.353563			

ANOVA **Squid Cu on *Alectus indica* Cu**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.191828	0.191828	4.540709	0.100107212
Residual	4	0.168985	0.042246		
Total	5	0.360813			

ANOVA **Squid Zn on *Dactyloptera orientalis* Zn**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.239463	3.239463	80.30362	0.000857946
Residual	4	0.161361	0.04034		
Total	5	3.400824			

ANOVA **Squid Zn on *Upeneus* sp. Zn**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.797767	2.797767	18.55722	0.012567781
Residual	4	0.603057	0.150764		
Total	5	3.400824			

ANOVA **Squid Cr on *Alectus indica* Cr**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.812073	0.812073	7.132567	0.044312004
Residual	5	0.569271	0.113854		
Total	6	1.381344			

ANOVA **Squid Ni on *Saurida tumbil* Ni**

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	7.109	7.109	9.869673	0.088131936
Residual	2	1.440574	0.720287		
Total	3	8.549574			

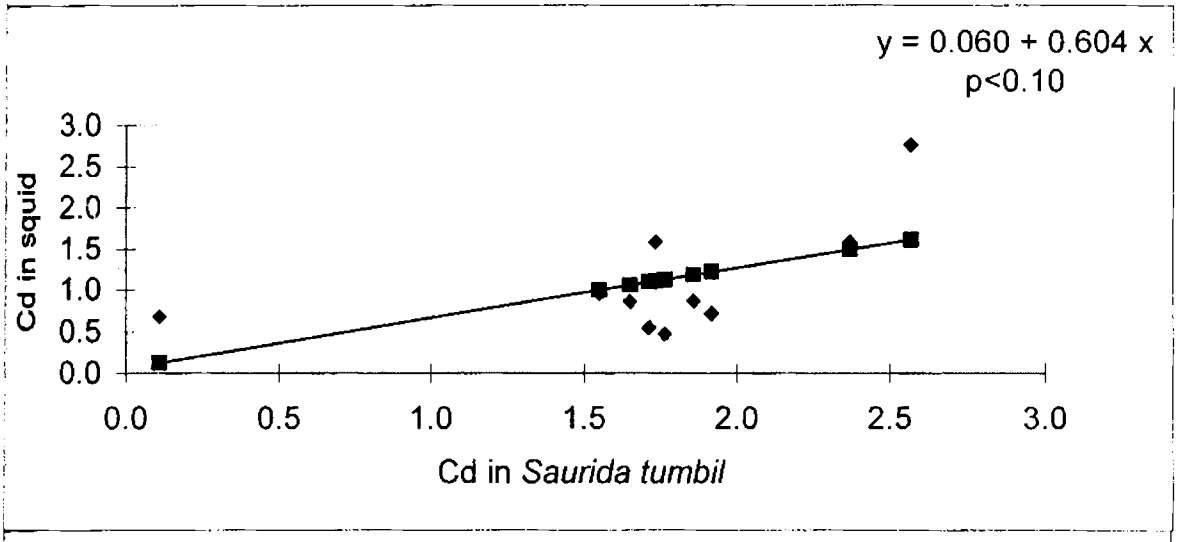
Table 6.7 a: Trace metals concentration in water samples collected onboard MV *Sagarika* at 30-58m depth. (Range expressed in μg/L)

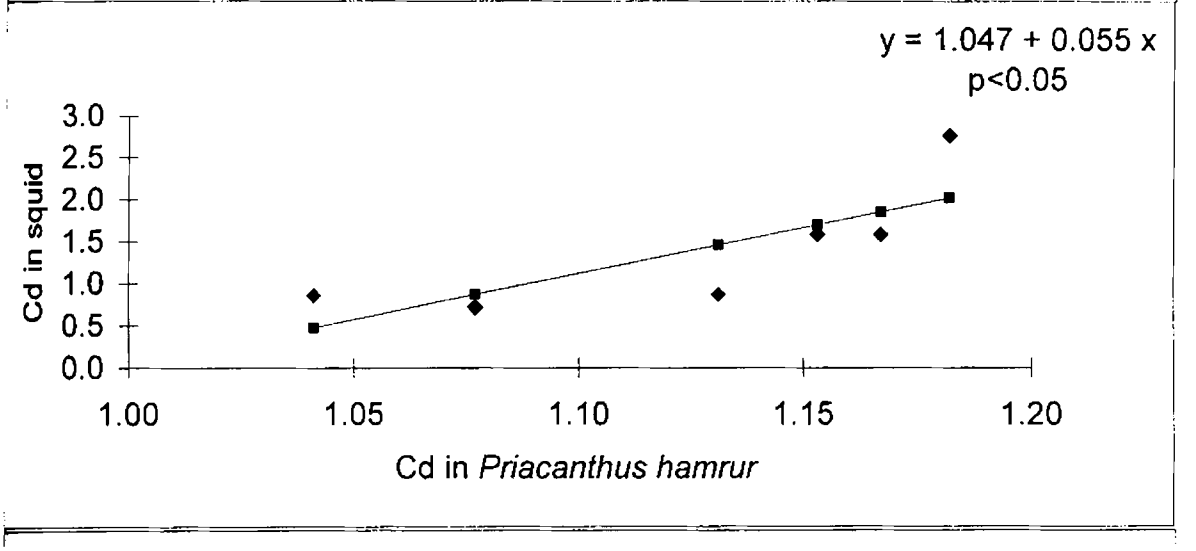
METAL	CONCENTRATION (bottom water)
Cadmium	0.0005-0.013
Copper	0.008-0.021
Zinc	0.013-0.078

Table 6.7 b: Trace metals concentration in water samples collected onboard FORV *Sagar Sampada* at 200-350m depth. (Range expressed in μg/L)

METAL	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

Fig. 6.1a : Regression plot of metal levels in fishes seperated from the mantle cavity of *Loligo duvauceli* vs. metal levels in food fishes expressed (in ppm)





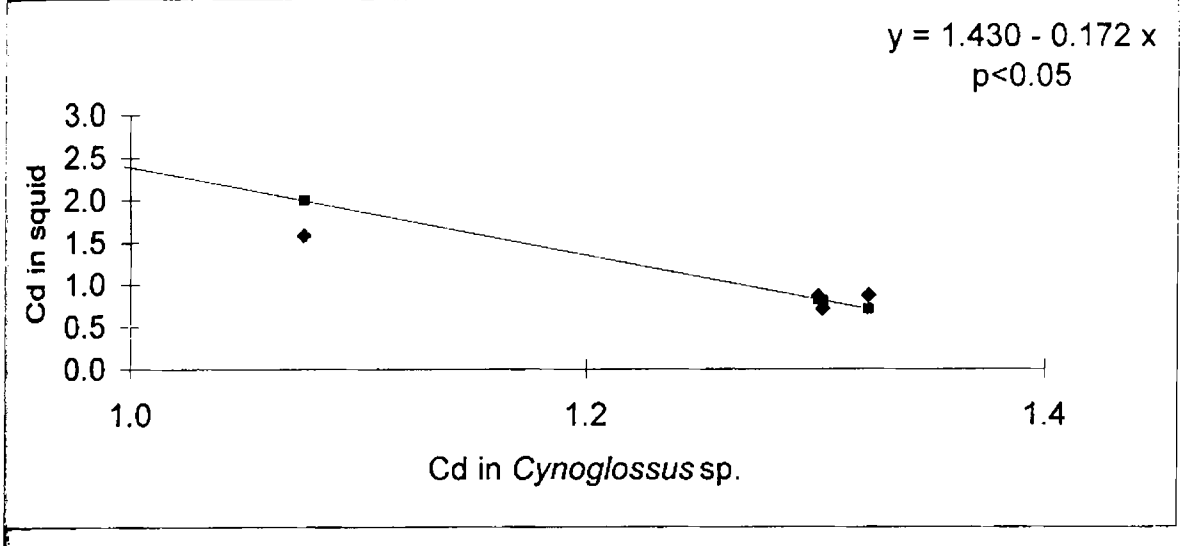


Fig. 6.1b : Regression plot of metal levels in fishes seperated from the mantle cavity of *Loligo duvauceli* vs. metal levels in food fishes expressed (in ppm)

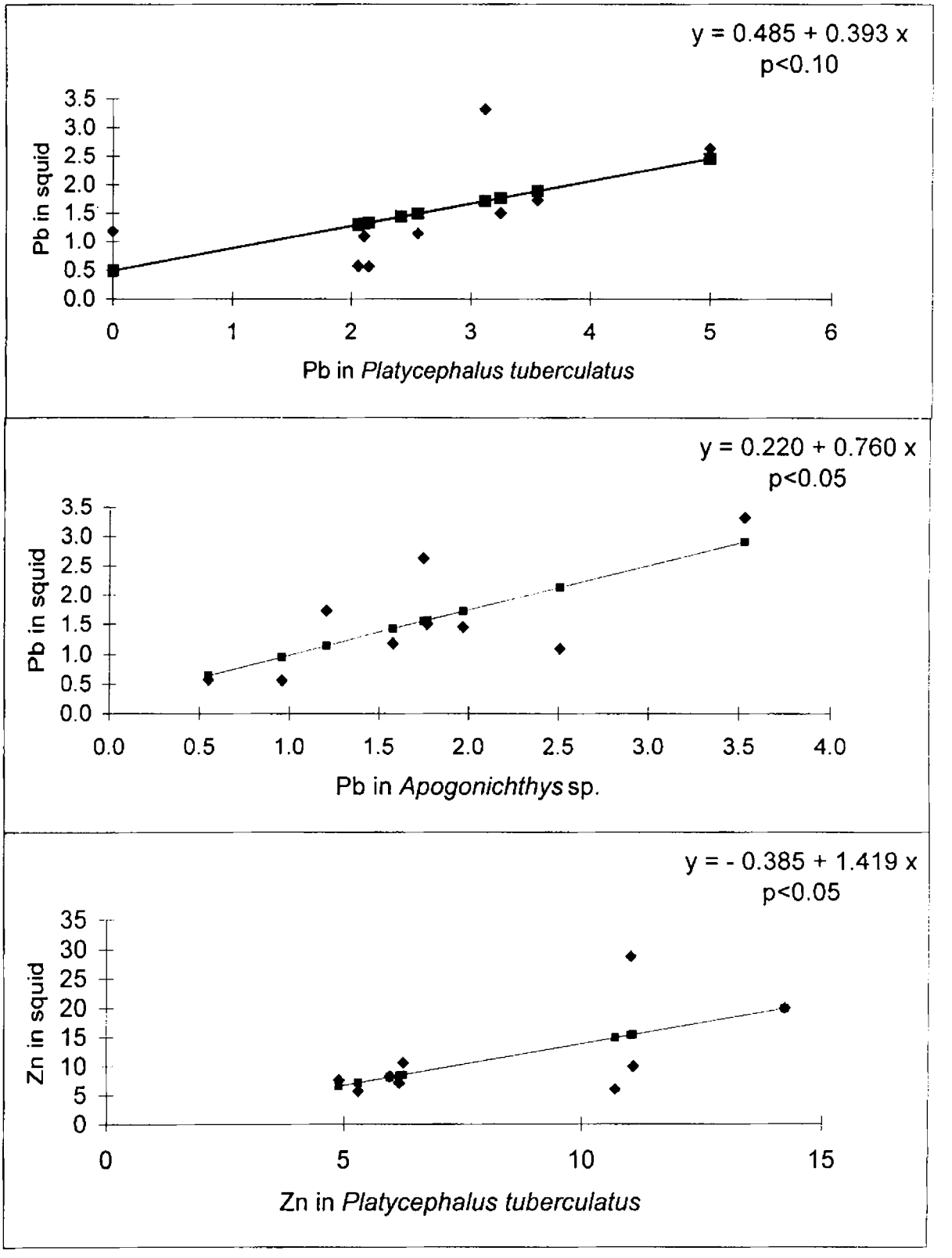


Fig. 6.1c : Regression plot of metal levels in fishes seperated from the mantle cavity of *Loligo duvauceli* vs. metal levels in food fishes expressed (in ppm)

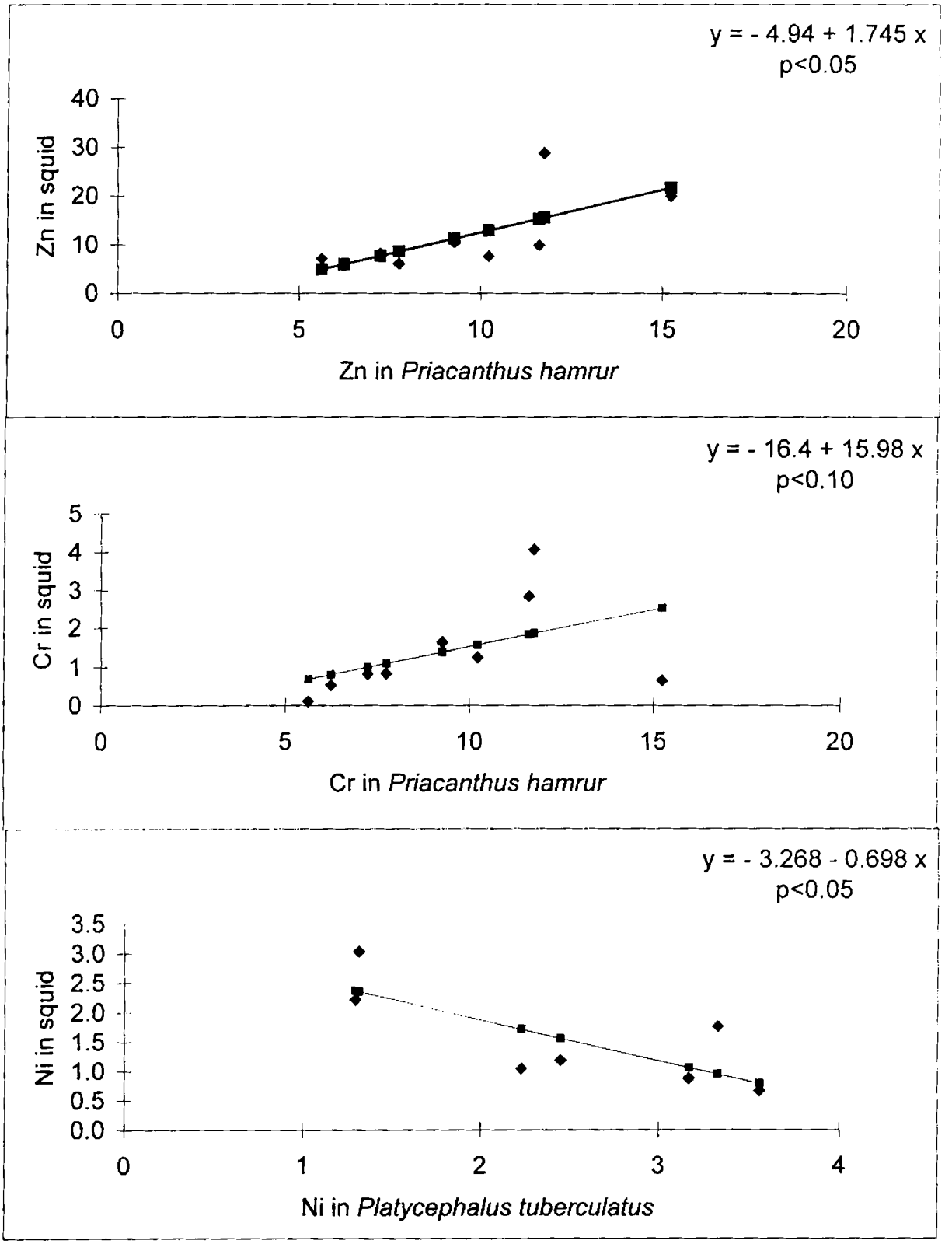
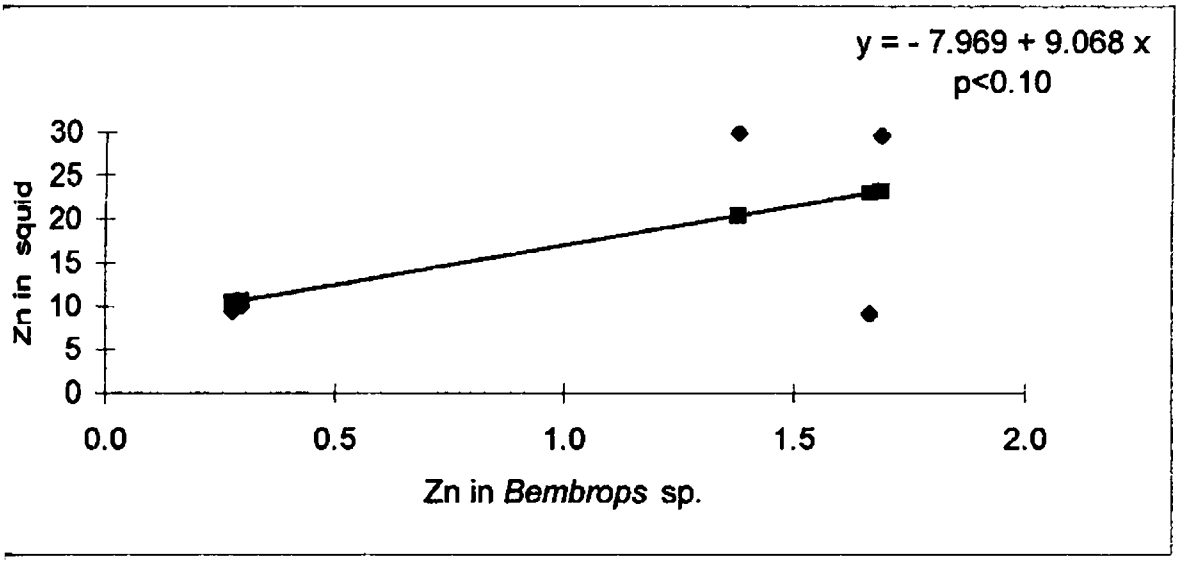
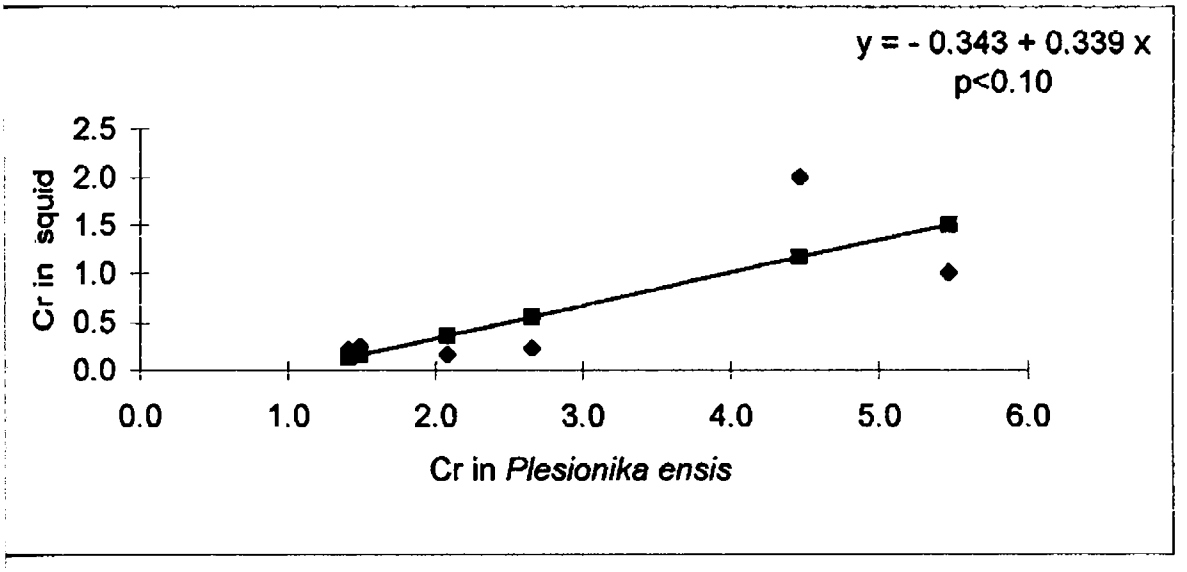


Fig. 6.2 a : Regression plot of metal levels in food fishes separated from the mantle cavity vs. metal levels in oceanic squid expressed (in ppm)





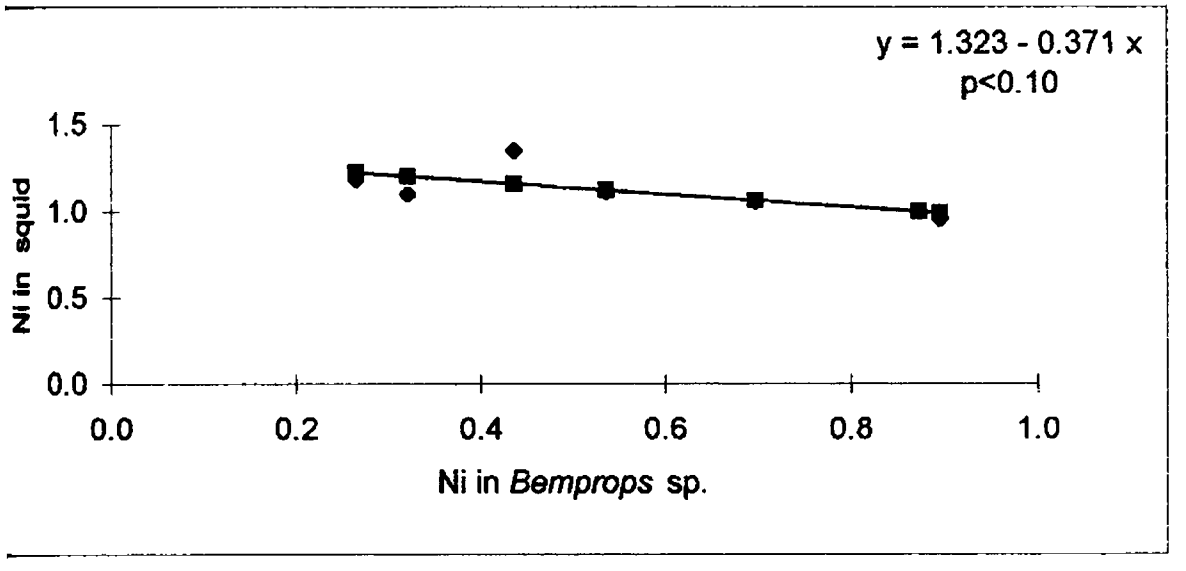
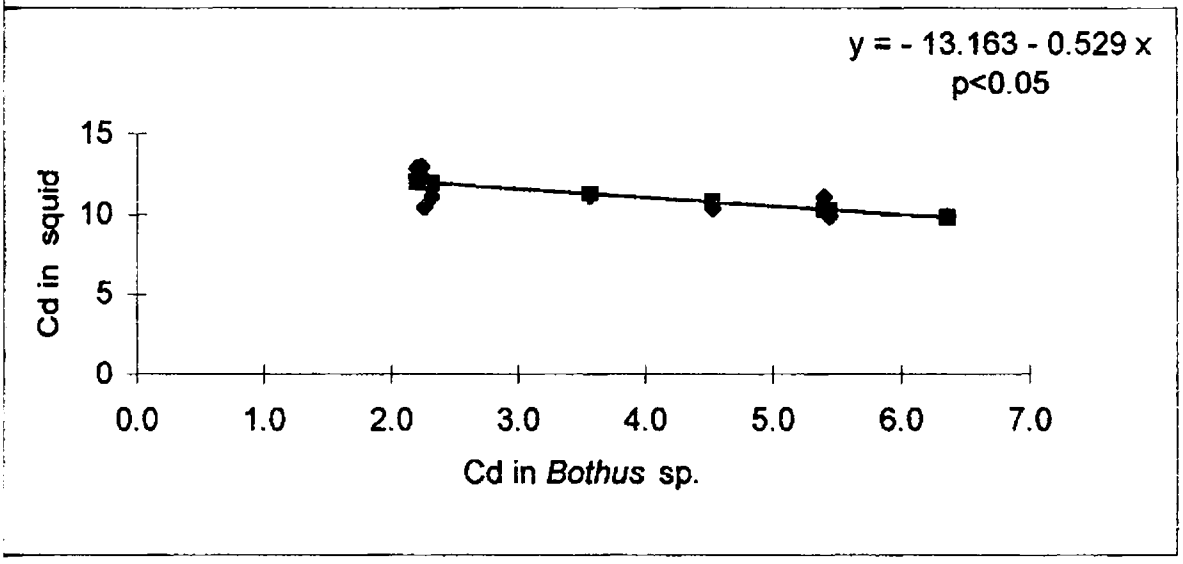
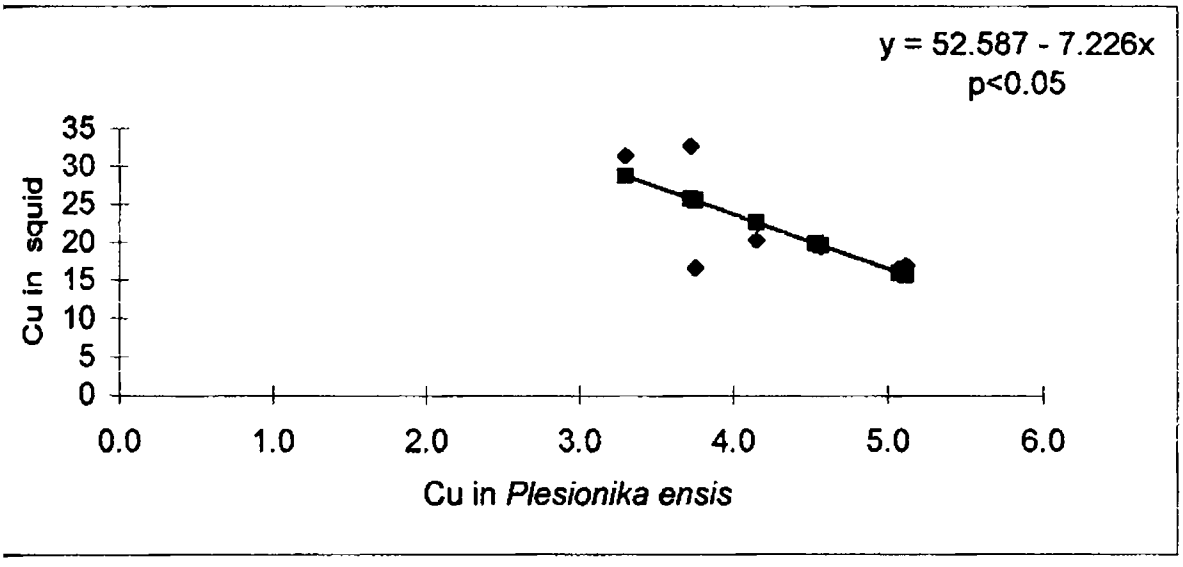


Fig. 6.2 b : Regression plot of metal levels in food fishes separated from the mantle cavity vs. metal levels in oceanic squid expressed (in ppm)





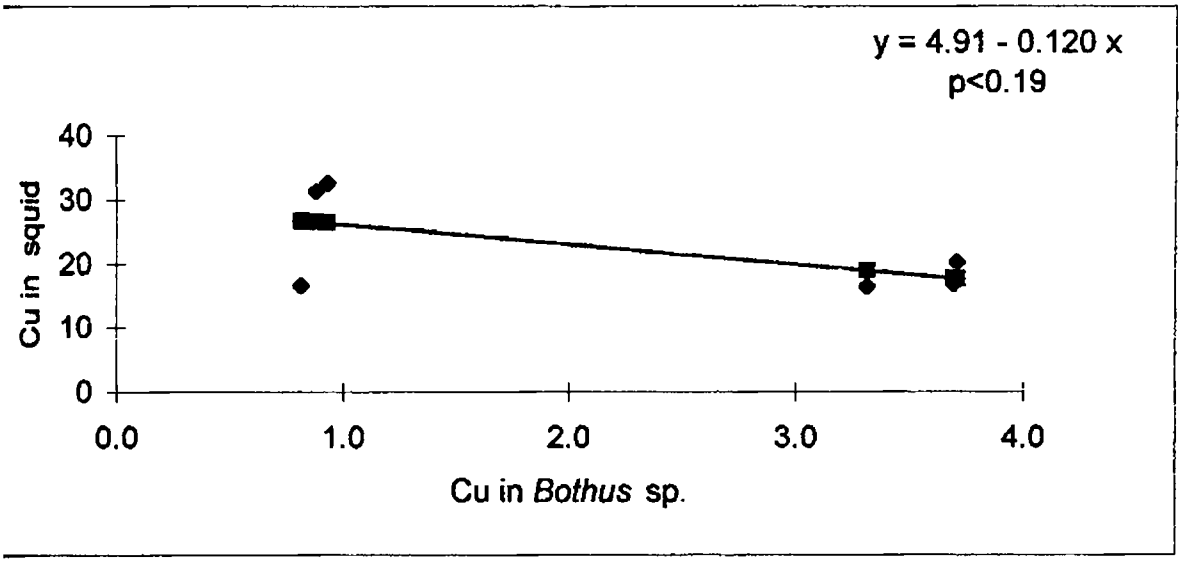


Fig. 6.3a : Regression plot of metal levels in fishes caught in the same habitat area of squids vs. metal levels in neretic squids expressed (in ppm)

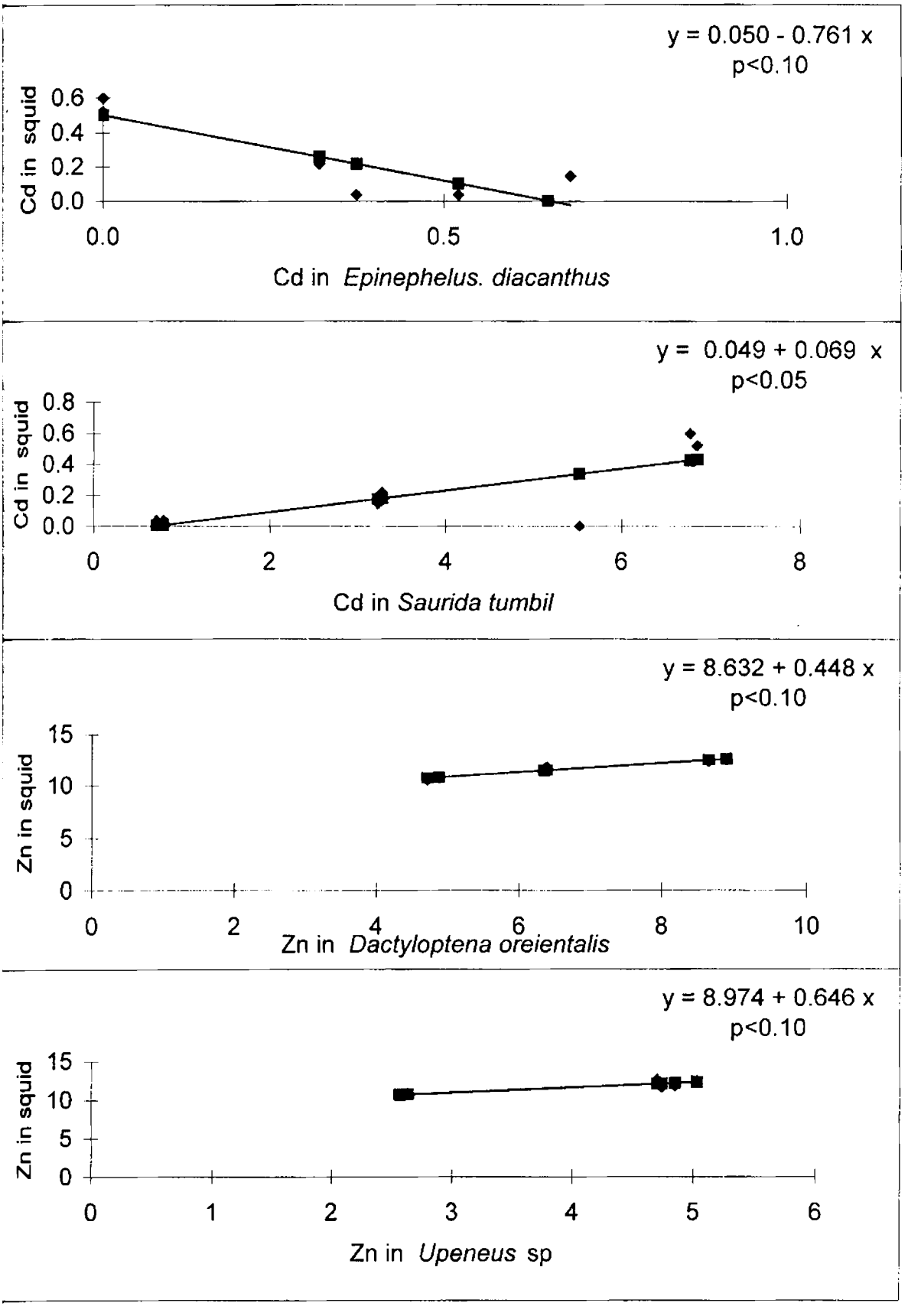
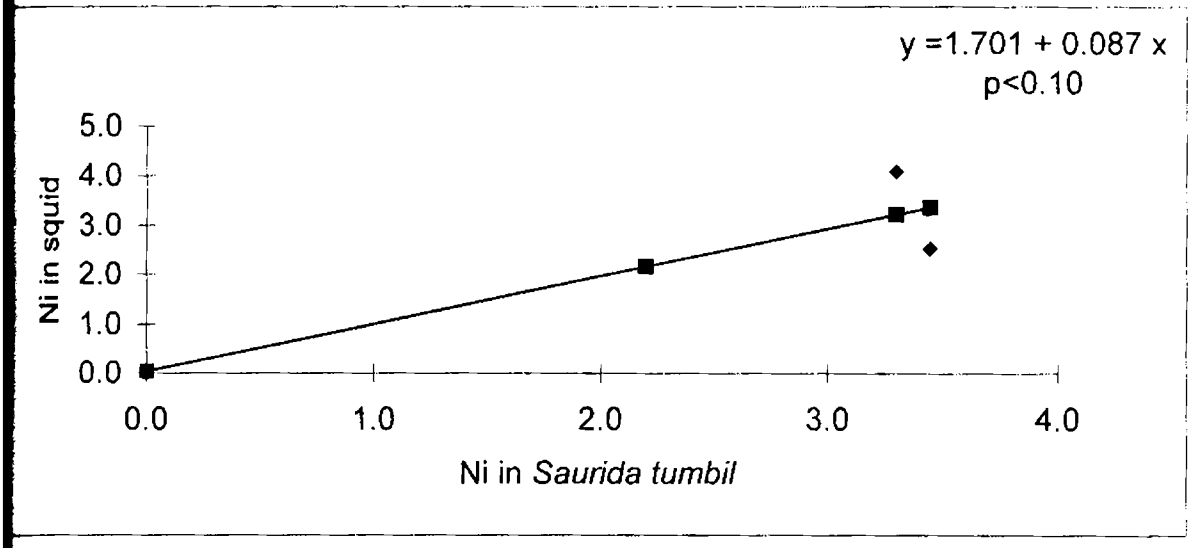
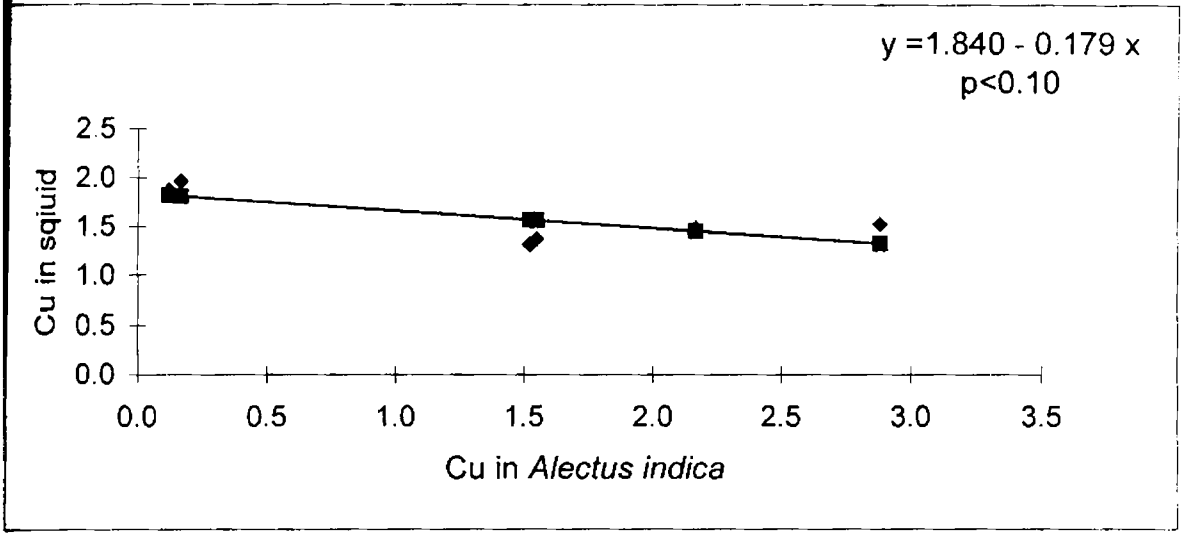
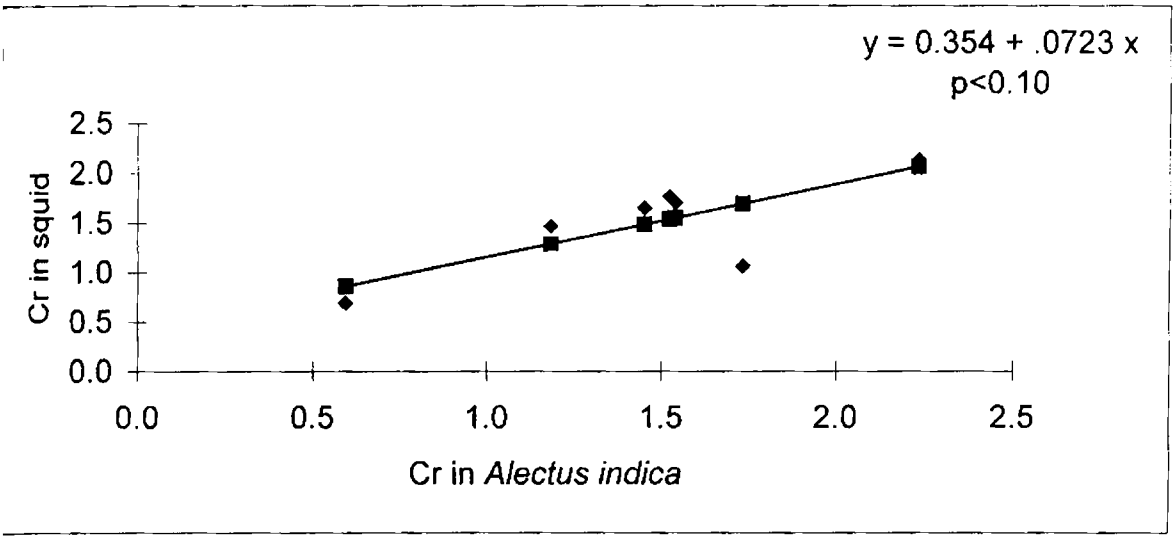


Fig. 6.3b : Regression plot of metal levels in fishes caught in the same habitat area of squids vs. metal levels in neretic squids expressed (in ppm)







CHAPTER 7

Studies on the toxic effect of liver bound cadmium in Albino rats

7.1. Introduction

Cadmium is a toxic element that has no biological function. It is an important industrial and environmental pollutant that causes severe damage to a variety of organ systems (Friberg *et al.*, 1985). The major source of environmental exposure to Cd for the general population is the intake in food (Foulkes, 1986). A few specific foods have long been known to contain amounts of Cd that are higher than in grains, cereals, fish, poultry, fruits and beverages. These include oysters, clams, other molluscs, liver and kidney (Fox, 1974). The cephalopod molluscs have been known to accumulate Cd and other metals in greater concentrations in their body and the most striking feature is that the liver stores most of the total cadmium as observed in the present research study and also as noted by other workers (Lakshmanan, 1988a,b; 1989, Falandysz,1989,1990,1991; Bustamante *et al.*, 1998a, b). Hence, in order to study the effects of Cd to the consumer it is essential to carry out animal feeding studies. Generally, cadmium levels as low as 5 mg Cd/kg diet produces adverse physiological effects (Fox, 1974). This is over 50 times more than the typical dietary concentration of cadmium for man. However, much higher levels are frequently used in toxicity studies, as the turnover of Cd in experimental animals is very slow (Cotzias *et al.* 1961).

The effects of inorganic $CdCl_2$ have been extensively studied in laboratory animals (Chapatwala *et al.*, 1982, Elinder, 1986; Anderson *et al.*, 1988; Groten *et al.*, 1989; Chatterjee *et al.*, 1996). Previous

studies in rats have evaluated cadmium toxicity after exposure to inorganic cadmium. However, the effect of muscle bound Cd toxicity in experimental animals is not extensively studied and very few studies have compared the early signs of toxicity after continuous exposure of cadmium through the diet. As Cd is often found in cephalopod products and present at very high levels in liver, a liver incorporated diet was fed to albino rats in order to assess the toxic effect of muscle bound Cd on Albino rats and for comparison, $CdCl_2$ was also incorporated in the diet of Albino rats. Therefore the effect of a continuous oral intake of Cd containing diet in albino rats was conducted in order to find out the toxic effect in consumer, if any.

7.2 Materials and Methods

7.2.1. Test substance

Cadmium chloride with purity, of at least 99%, AnalaR grade was obtained from Glaxo, BDH.

7.2.2. Preparation of squid livers

Squids of medium size weighing 200-300 g were washed free of debris with deionised water. They were cut open using clean scissors and forceps and the liver was dissected out carefully. The livers from several squids were pooled, homogenized and vaccum dried in an oven at 50^0 C. These were stored in a vaccum desiccator. The squid liver was incorporated in the diet so as to give a final Cd content of 40 ppm in the diet.

7.2.3. Basal Diet

A semi synthetic powder basal diet was prepared. One diet consisted of the basal diet supplemented with the vaccum dried squid liver homogenate described above providing 40mg Cd/kg diet. A second diet contained the basal diet supplemented with $CdCl_2$ to a dietary level of 40mg Cd/kg. The third diet contained the basal diet only and was used as the control diet. The composition of the feed was analysed and provided in Table 7.1.

7.2.4. Animals and maintenance

The toxicity evaluation study conducted in 4 week old male Albino rats maintained in the animal house of Central Institute of Fisheries Technology, Cochin. Young growing animals were used to study the effects of cadmium for 6 weeks period. The rats were housed under conventional conditions in suspended stainless steel cages fitted with a wire mesh floor and front. The room temperature was maintained at 22+2°C and the relative humidity at 40-70% Air Conditioned room. They were kept under 12 hour light and 12-hour dark photoperiod. The animals were acclimatized in the cage for a period of 1 week. Before the commencement of the experiment all rats were fed, the basal diet without any additions. 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 cup in the enclosure area of the respective cages, with proportional increase depending upon the feed consumption.

7.2.5. Experimental design and treatment

The healthy animals were selected and their individual body weight recorded. Animals having the same weight range 82-85g were selected and housed individually in six metabolic cages. The Albino rats of Group I served as the control, Group II housed Albino rats were given diet containing squid liver bound Cd. Group III Albino rats were fed diet containing $CdCl_2$. Average body weight of the animals was recorded every week.

7.2.6. Observation

The food intake, water consumption and general condition of the animals were recorded daily.

7.2.7. Haematology

At the end of the 6 week period blood was drawn from all the Albino rat groups through retrorbital puncture into heparinized tubes meant for the purpose of hematological studies. Blood samples were examined for Haemoglobin (Hb) content, Packed Cell Volume (PCV), Red Blood Cell (RBC) Count, Total Count (TC) and Differential Leucocyte Count (DC) and Platelet Count (PC) at Diagnostic centre, Cochin.

7.2.8. Pathology

After 6 weeks study, the Albino rats were killed by exsanguinations from abdominal aorta, whilst under light ether anesthesia and autopsied. Immediately after evisceration the liver,

kidney and a small portion of the muscle were taken. Liver and kidney tissue of all Albino rats were subjected to histochemical analysis. (Details in Chapter 2). The slides were examined by the light microscopy and photographed using a binocular microscope and Nikon camera combination.

7.2.9. Metal analysis

Cd, Cu and Zn content in the liver and kidney tissues were determined by flame Atomic Absorption Spectrophotometer following wet digestion. (Details as mentioned in Chapter2).

7.3. Results

Mean values of body weight in control and Cd-fed Albino rats are presented in Table 7.2. The metal concentrations in the body components of control and Cd-fed Albino rats are presented in Table 7.3. Results of the haematological analysis of the Albino rats fed by diets containing squid liver bound cadmium and $CdCl_2$ at 40 ppm are presented in Table 7.4 and photographs of liver and kidney tissues of control Albino rats and Cd fed Albino rats are shown in Plates 7.1 to 7.6.

All rats appeared to be healthy throughout the 42 day (6 weeks) study. Food consumption and water intake by Cd-fed Albino rats did not vary considerably from that of the control group. However, net gains in body weight of rats fed with Cd diets were lower than that of control animals. It is evident from Table 7.1 that the percentage net gain in body weight in rats receiving $CdCl_2$ alone was lower than squid

liver bound cadmium fed albino rats. The albino rats fed squid liver bound cadmium gained more weight than those fed $CdCl_2$ especially during the last 2 weeks of the study (Table 7.2).

7.3.1. Metal levels in body components of experimental Albino rats

The body components – muscle, liver and kidney were analysed for metals at the termination of the experiment. Results of the study showed that mean cadmium content in the muscle tissue of rats fed with squid liver bound cadmium was more than two folds higher than $CdCl_2$ fed groups (Table 7.3). Copper and zinc content in the muscle of Albino rats of both Cd fed groups did not show significant difference. Feeding diet containing 40 ppm $CdCl_2$ for 6 weeks resulted in a mean Cd concentration of 4.187 ppm in the liver which is more than twice the amount found in albino rats fed squid liver bound cadmium (Table 7.3). The Cu level of the liver increased in both Cd-fed groups. The values were 2.951± .852, 3.520±1.974 and 3.596±0.246 ppm respectively in control Albino rats, Albino rats fed with squid liver incorporated diet and diet containing $CdCl_2$. Average Zn content in the liver of $CdCl_2$ treated animals was several magnitudes higher (240.767 ppm) than in animal fed with squid liver incorporated diet (31.358ppm) and control group (18.746ppm). In contrast to the liver, the kidneys show a much less marked difference in Cd accumulation in the Cd-fed groups. Feeding squid liver bound Cd resulted in only a slightly higher mean Cd level (6.96 ppm) in the kidney than feeding $CdCl_2$ (5.89 ppm). In contrast to the cadmium content in the kidney, Cu content in the kidney in both Cd

fed groups did not vary significantly from that of the control group. Average levels were 5.741 ppm in squid liver bound cadmium, 7.097ppm in $CdCl_2$ and 5.183 ppm in control group. However, Zn content in both the cadmium treated groups varied markedly from the control group with higher mean values (34.479 ppm) noted in Albino rats fed with squid liver bound cadmium. In general, the Cd, Cu and Zn levels in the muscle, liver and kidney were comparatively higher in the Cd fed groups than found in control Albino rats (Table 7.2). Enrichment of Cd and Cu in the Albino rats occurred in the following order kidney > liver > muscle. However, distribution pattern of Zn showed a different trend and followed the pattern : liver > kidney > muscle.

7.3.2. Haematological Evaluation

Results showed a lower Haemoglobin content (Hb), Packed Cell Volume (PCV), Red Blood Cell content (RBC), Total Leucocyte Count (TC), Differential Leucocyte Count (DC) and Platelet Count (PC) in both the Cd-fed groups compared to control animals (Table7.4). The average percentage of PCV in the control animals were slightly higher (44%) than in the Cd-fed Albino rats (38%). RBC, Hb content, TC, DC, and PC were all in general more pronounced in the animals fed squid liver diet cadmium. The average Hb content in Experimental albino rats were 12.2gm/dl (squid liver) and 12.6 gm/dl in the Albino rats fed with diet containing $CdCl_2$ and 14.2 gm/dl (control). The slightly lower Hb content in the group fed with squid liver diet was accompanied by a

similar decrease in the average RBC count ($4.6x\ 10^6$),TC ($2000/mm^3$) and PC (1.0 lakh mm^3).

7.3.3. Histopathological study

Histopathological examination of the liver and kidney tissues of the experimental and control Albino rats were made. Microscopic examination of these tissues revealed substantial changes in both these organs compared to the control Albino rats. The kidney and liver sections of control group showed normal architecture of tissues. (Plate 7.1 and 7.2) whereas, liver and kidney sections of Albino rats fed with Cd incorporated diets showed histomorphological alterations. The section of tissues of kidney of Cd-fed Albino rats showed shrinkage of glomeruli, adhesion to the bowman's capsule (Plate 7.3). Renal tubule showed epithelial cell swelling, degenerative changes and necrosis of tubules was also observed (Plate 7.4). Similarly, liver tissue of Cd-fed Albino rats showed the presence of pyknotic nuclei and mild epithelial proliferation (Plate 7.5). Focal area of necrosis and accumulation of leucocytes around necrosed area was seen (Plate 7.6).

7.4. Discussion

In the present study several well known signs of Cd toxicity occurred after continuous oral exposure to $CdCl_2$ and squid liver bound cadmium. Both Cd-fed Albino rats showed marked differences from that of control rats in their body weight, in Cd accumulation in the body components, haematological and histopathological effects. Although the exposure level of the Albino rats was similar for both

cadmium compounds, the severity of the toxic effect was different. It is evident from Table 7.2 that there was greater reduction in body weight gain in rats receiving $CdCl_2$. This may be due to the utilization of fat deposits by the animals for the synthesis of glucose as $CdCl_2$ increases the activities of gluconogenic enzymes (Chapatwala *et al.,* 1982). However, reduced Hb count, RBC count, TC count, DC count, PC count were all more pronounced in the rats fed squid liver bound cadmium. A similar reduction in Hb, RBC, PCV and DC count was reported by Mary *et al.* (1997) in mice administered lead nitrate for 21 days.

The distribution of heavy metals Cd, Cu and Zn in the muscle, liver and kidney tissues of Cd-fed rats increased markedly from that of the control animals. Also, differences in the extent of accumulation of heavy metals in liver and kidney tissues of Albino rats fed with squid liver bound cadmium and $CdCl_2$ were noted. In the present study it was noted that the difference in the extent of signs of adverse effects between inorganic and the tissue bound cadmium is accompanied by differences in the Cd concentration in the liver and kidneys. Similar findings have been reported by Maitani *et al.* (1984) and Groten *et al.* 1990. The observed difference is almost solely due to the difference in accumulation in the liver and not to any marked difference in the uptake by kidneys (Groten *et al.,* 1990). There are two possible explanations of the difference in the extent of effects of $CdCl_2$ and tissue bound cadmium. First there is a difference in the organs and

secondly there may be a difference in the sensitivity of the target organs to the two compounds (Groten *et al.,* 1990).

It was interesting to note that both Cd-fed groups showed similar treatment related histopathological changes in the liver and kidney. Histopathological signs of toxicity have been reported by several workers in the liver and kidney of rats (Hoffman *et al.*, 1975; Itokawa *et al.,* 1978; Dudley *et al.*, 1982; 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 4 weeks as also observed in the present study. Andersen et al. (1988) found histopathological changes in the livers of mice 10 days after oral exposure 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 on the liver, liver morphology is a more sensitive parameter than the liver-enzyme activities in the blood. Dudley *et al.* (1985) noted morphological signs of toxicity only after four weeks of exposure to cadmium.

Thus, the differences in the extent of signs of adverse effects between $CdCl_2$ and squid liver incorporated cadmium are accompanied by differences in the cadmium concentrations in the liver and kidneys. A dosage of 40ppm Cd either as $CdCl_2$ or tissue bound Cd would lead to histopathological effects. Hence, higher levels of Cd in squids can cause hazard to the consumer.

Table. 7.1 : Proximate and nutrient composition of feed for experimental albino rats

Proximate composition (%)			
Constituents	**Control animals**	**Experimental animal diet with squid liver (40 ppm Cd)**	**Experimental animal diet with $CdCl_2$ (40 ppm Cd)**
Moisture	11.42	13.51	13.38
Protein	60.96	60.91	60.04
Fat	4.72	3.66	5.01
Carbohydrate	15.88	14.85	14.13
Ash	7.02	7.07	7.44
Nutrient composition (μg/g)			
Calcium	2.208	2.194	2.360
Sodium	0.200	0.203	0.213
Potassium	0.138	0.120	0.132
Trace metal composition (ppm dry wt. basis)			
Cadmium	4.492	39.07	33.81
Copper	39.39	35.81	13.94

Table. 7.2: Mean values of body weight of albino rats fed with a diet containing Cd (squid liver) and $CdCl_2$ at 40ppm

Body weight in (g)			
Duration of exposure (weeks)	**Control**	**Squid- liver bound cadmium**	**$CdCl_2$**
1	72	74	76
2	82	84.5	82.75
3	122	120	117
4	153.1	149.8	145.35
5	180	176.5	167.2
6	200	199.7	185.4
Net gain	128	125.7	109.4

Table 7.3: Metal concentrations in the body components of experimental Albino rats fed with a diet containing Cd (squid liver) and $CdCl_2$ at 40 ppm, level for 6 weeks period (Mean ± S.D., ppm wet wt)

BODY COMPONENTS	Metals	Control	Squid liver bound Cd	$CdCl_2$
MUSCLE	Cadmium	0.092 ± 0.081	0.441 ± 0.028	0.177 ± 0.062
	Copper	0.238 ± 0.196	0.448 ± 0.007	0.531 ± 0.200
	Zinc	5.156 ± 0.124	4.165 ± 0.340	5.888 ± 1.257
LIVER	Cadmium	0.053 ± 0.047	1.895± 0.593	4.187 ± 0.129
	Copper	2.951 ± 1.852	3.520± 1.974	3.596 ± 0.246
	Zinc	18.746 ± 3.958	31.358± 3.452	240.767 ± 34.132
KIDNEY	Cadmium	0.126 ± 0.127	6.960± 0.180	5.890 ± 1.053
	Copper	5.183 ± 10.238	5.741± 6.915	7.097 ± 2.554
	Zinc	10.238 ± 2.491	34.479± 11.825	15.246 ± 1.226

Table:7.4: Haematological analysis of experimental Albino rats fed 40 ppm cadmium in the diet for 6 weeks.

Parameter tested	Std. Value*	Control Animal	Animal fed with squid liver bound cadmium	Animal fed with diet containing inorganic Cd
Packed cell Volume Average % Range	46 39 – 53	44	38	38
Haemoglobin gm/dl Average Range	14.6 12 – 17.5	14.2	12.2	12.6
Red Blood Cell count Average Range	$8.9x10^6mm^3$ $7.2 – 9.6x10^6$	$5.7x10^6$	$4.6x10^6$	$5.1x10^6$
White Blood Cells Total leucocyte count Differential leucocyte count	$6\text{-}12x10^3mm^3$	5000 P–17%, L-83%	2000 P-10%, L-90%	3000 P-12%, L-88%
Platelet Count (in mm^3)	$340x10^3$	1.5 lakh	1.0 lakh	1.4 lakh

* Source: Indian Council of Medical Research (1980)

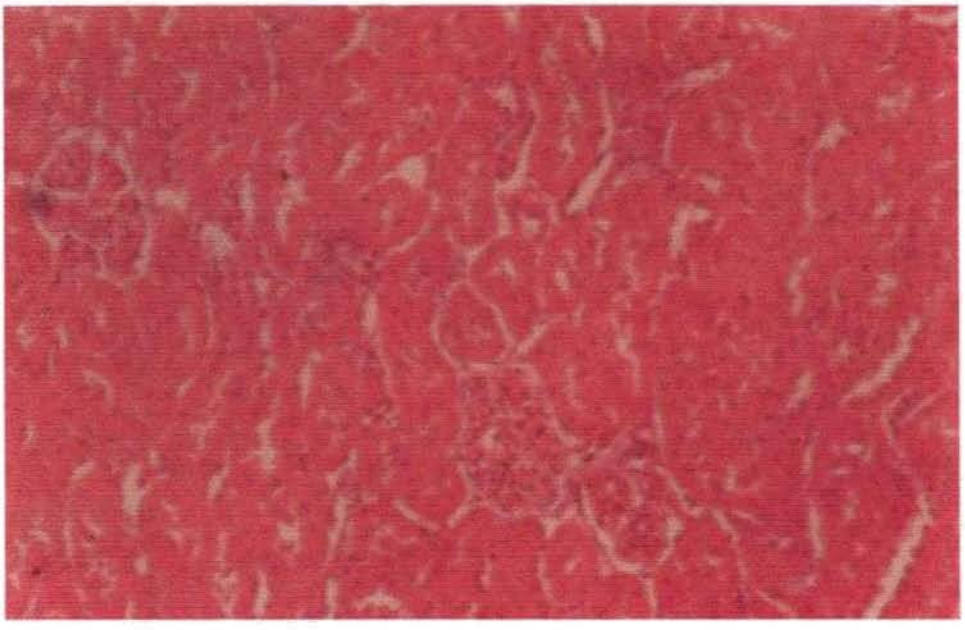


Plate 7.1. ***Microphotograph of kidney tissue of control albino rats*** **(H.E. x 20)**

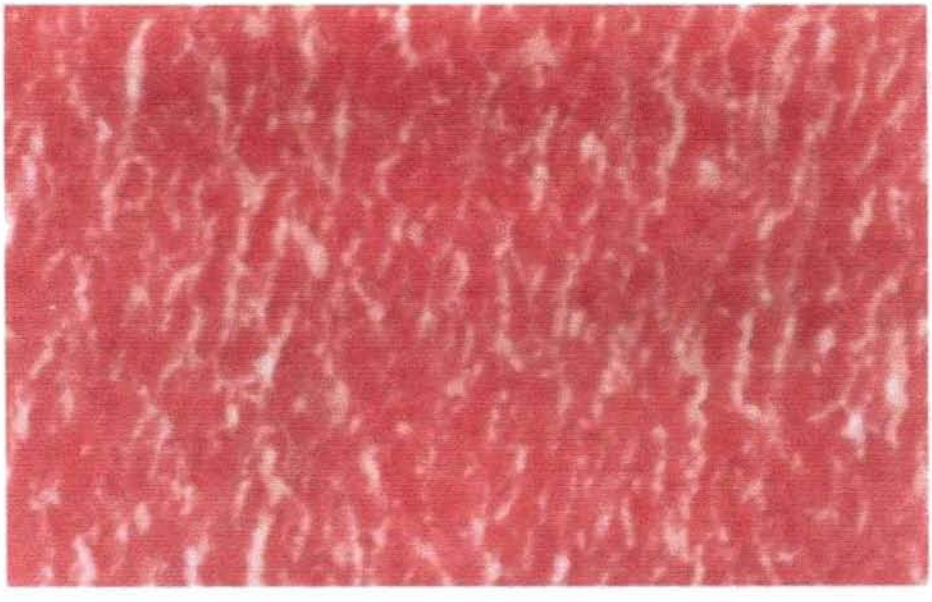


Plate 7.2. ***Microphotograph of liver hepatocytes of control albino rats*** **(H.E. x 20).**

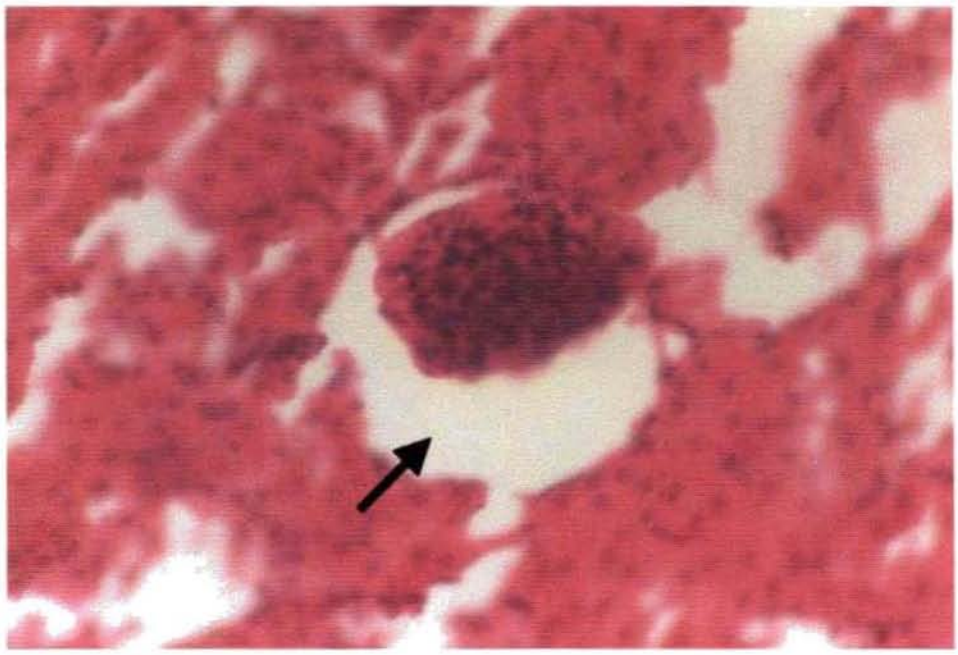


Plate 7.3. ***Microphotograph of kidney tissue of experimental albino rats fed with diet containing Cd at 40 ppm level showing shrinkage of glomeruli*** **(H.E. x 20)**

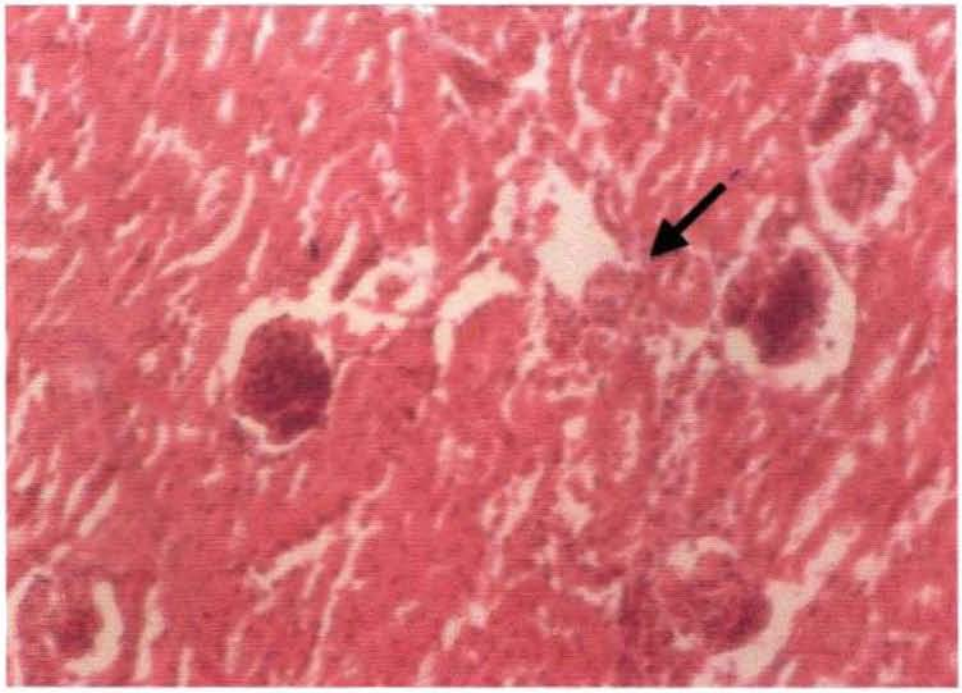


Plate 7.4. ***Microphotograph of kidney tissue of experimental albino rats fed with diet containing Cd at 40 ppm level showing focal area of necrosis and accumulation of leucocytes and necrosed area*** **(H.E. x 20).**

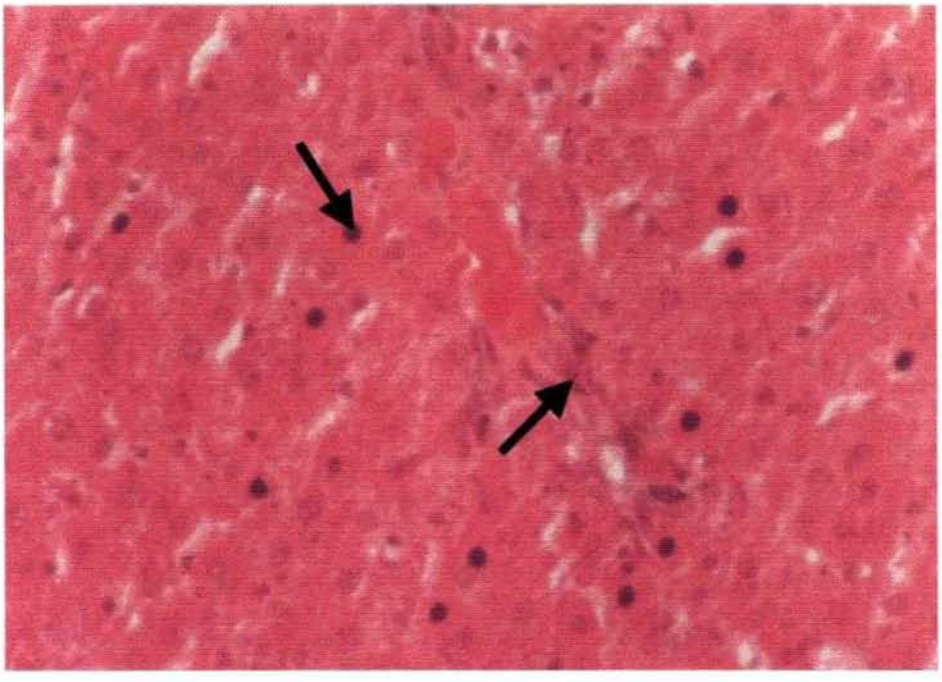


Plate 7.5. ***Microphotograph of liver tissue of experimental albino rats fed with diet containing Cd at 40 ppm level showing pyknotic nuclei and mild bilary epithelial proliferation*** **(H.E. x 20)**

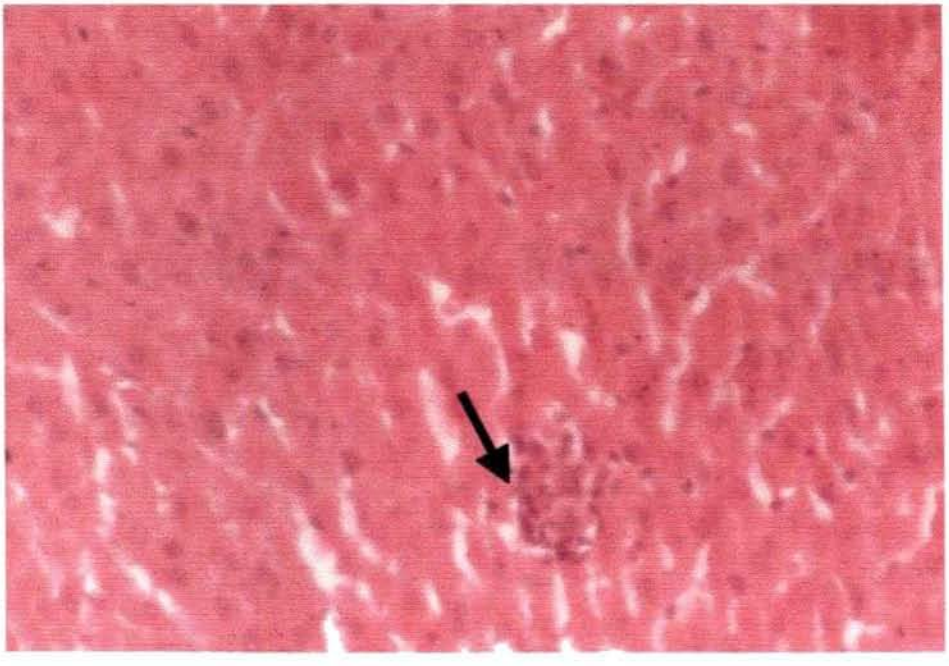


Plate 7.6. ***Microphotograph of kidney tissue of experimental albino rats fed with diet containing Cd at 40 ppm level showing haemorrhage*** **(H.E. x 20)**

Summary and Recommendations

Summary and Recommendations

The presence of higher levels of cadmium and other toxic metals in the commercially important squid species has caused concern during recent years. Many consignments of squids exported from India were rejected/detained by some members of European Union on the ground that the samples had higher levels of cadmium or *salmonella* contamination. The present study entitled "INVESTIGATIONS ON THE DISTRIBUTION CHARACTERISTICS OF HEAVY METALS IN SQUID (*LOLIGO* SPP.) IN RELATION TO LEVELS IN FOOD FISHES FROM THE WEST COAST OF INDIA WITH A PERSPECTIVE ON SEAFOOD SAFETY" attempts to establish the base line data on metal levels in squids along the west coast of India. The study is of great relevance in the present context when utmost importance is being given for producing wholesome seafoods especially in the export market with a perspective on seafood safety.

The main objectives of the study were:

i. To provide base line data on the concentration of heavy metals, *viz.*, Hg, Cd, Cu, Zn, Fe, Mn, Cr and Ni in whole soft tissues as well as the edible (muscle) and the non-edible body components (liver and gills) of the most abundant squid species, *L. duvauceli*, found along the west coast of India.

ii. To study regional trends in the distribution characteristics of heavy metals in *L. duvauceli* caught off Cochin, Quilon, Mangalore and Mumbai regions.

iii. To study seasonal variations of heavy metals in *L. duvauceli* caught off Cochin, Quilon and Mangalore regions.

iv. To study the comparative distribution of heavy metals in neretic and oceanic squids

v. To study the comparative levels of heavy metals in squids, associated fish fauna and environment.

vi. To evaluate Cd toxicity in experimental albino rats following haematological and histopathological investigations.

The thesis presents a comprehensive account of the base line data on important heavy metals, viz., Hg, Cd, Pb, Cu, Zn, Fe, Mn Cr and Ni in the edible and non-edible body components of the most abundant Loligo species, viz., *L. duvauceli* caught along the west coast of India.

The thesis is presented in seven chapters. Chapter 1 and Chapter 2 are general and common for the entire study. The rest of the chapters are more specific on the different aspects of study taken up.

Chapter 1:

Includes introduction to the topic of study and the need for taking up such a study along the west coast of India. It also gives a brief insight into the cephalopod fishery of India. The objectives of the present study and a detailed review of related work on this subject form the rest of this chapter.

Chapter 2:

Details of all the materials and methods used for the determination of heavy metal accumulation in squids along the west coast in the present study are presented in Chapter 2. Standard methods of AOAC (1990) and Grasshoff *et al.* (1976) were adopted for heavy metal analysis and that of Pearse (1968) for histopathological investigations.

Chapter 3:

The metal levels in squids collected from different regions, viz., Cochin, Quilon, Mangalore and Mumbai regions showed significant variability as a function of their geographic origin. Whole squid samples from Cochin region showed significantly higher levels of Hg, Cd, Cu, Pb, and Mn than samples from Quilon, Mangalore and Mumbai regions. Squids from Mangalore region recorded the lowest levels of Cu, Zn, Ni, Pb, and Fe. The mean content of the essential and non-essential metals in whole *L. duvauceli* varied from region to region and were in the order: ∑Hg: Cochin>Mumbai> Mangalore> Quilon, Cd: Cochin> Mumbai >Mangalore> Quilon, Pb: Cochin> Quilon > Mumbai> Mangalore, Cr: Quilon> Cochin >Mangalore> Mumbai, Ni: Cochin> Quilon> Mangalore> Mumbai. The highly toxic metals, viz., Cd, Pb, and Cr often exceeded the tolerance limit in around 20% (Cd), and 11% (Pb and Cr) in whole *L. duvauceli.* However, the mean content of all the metals analysed were significantly lower in the edible parts and far below the tolerance limits. Concentration of ∑Hg was

found to be <50 μg/kg in the edible muscle in 90% of the samples and ΣHg content was far below the limit of 1 mg/kg permitted for seafoods by many fish importing nations and USFDA. Muscle Cd content was <3 ppm in squid from all the regions. Liver of squids was the major site of accumulation of Cd and other toxic metals. The increasing order of abundance of most of the metals in squids were: Liver > Gills > Muscle.

Chapter 4:

Investigations on the seasonal variation of heavy metals in *Loligo duvauceli* collected during all the seasons, *viz.*, premonsoon (Jan-May), monsoon (June–August) and postmonsoon (September-December) off Cochin, Quilon and Mangalore regions showed significant seasonal difference in the distribution of heavy metals among the three seasons at the three regions ANOVA (Table 4.4 & Table 4.5.).

Seasonal variation in Cd, Pb, Cu, Zn and Ni was observed In whole squids caught off Cochin, ($p<0.01$). Similarly significant seasonal variation in Hg, Cd, Pb and Zn was also observed in samples from Quilon ($p<0.01$). Whole squids caught off Mangalore exhibited significant seasonal variation in Cd, Cu, Zn and Cr ($p<0.01$) and Pb ($p<0.05$). In general, higher levels of metals were noted during the monsoon and postmonsoon periods.

Chapter 5:

A comparative study of heavy metals in neretic squids (*Loligo* spp.) and oceanic squids (*Ancistrocheirus* spp.) were also made. Results indicated that oceanic squids had higher average levels of Hg, Cd and Cu than in neretic squids. Significant difference was noted in the distribution of Cd ($p<0.01$). In oceanic squids Cd content showed levels of 11.228±1.199 in whole soft tissues, and 88.802±60.058 ppm in liver tissue where as in Neretic squids the level was 1.896±0.990 (whole) and 38.741±0.461 (liver). Cd content exceeded the tolerance limit of 3 ppm in around 85 % in whole soft parts of oceanic squids and around 20 % in whole soft parts of neretic squids. However, the level of Cd in the edible muscle of neretic squids was within the tolerance limit, the range being 0 to 1.795 ppm while the levels ranged from 1.979 to 5.071 ppm, in oceanic squids.

Chapter 6:

With a view to identify the probable source of Cd and other toxic metals in squids, fishes separated from the mantle cavity of neretic and oceanic squids or fishes caught in the same habitat area as that of squids were studied. Significant correlations existed between some of the metals levels in fishes and that in squids. Significant positive correlations were observed between Cd levels in squids and Cd levels in *Saurida tumbil* ($r=0.564$, $p<0.10$) and *Priacanthus hamrur* ($r=0.781$, $p<0.05$), separated from the mantle cavity of *Loligo duvauceli*. Similarly, significant positive correlation was also noticed between

levels of Pb, and Zn content in *Platycephalus tuberculatus* (r=0.617,p<0.0) with that observed in squids. Positive correlation was also noted between Cr and Zn content in *Priacanthus hamrur*, (r=0.734, p<0.10; r=0.617, p<0.05 respectively) and Pb content in *Apogonichthys* sp. (r=0.734, p<0.05) with respective levels in neretic squids.

Significant positive correlations existed between the levels of Cr, Cd, Ni and Zn, in fishes, viz., *Alectus indica,* (r=0.729, p<0.01) *Dactyloptena orientalis*, (r=0.976, p<0.01) *Saurida tumbil* (r=0.746, p<0.01) and *Upeneus* sp. (r=0.90, p<0.05) caught in the same area with respective metal levels in neretic squids. Significant positive correlation was also noted between Cd, Cr and Zn levels in oceanic squid, *Ancistrocheirus* spp. and Cd levels in *Bothus* sp. (r=0.725, p<0.05), Cr content in *Plesionika ensis* (r=0.769, p<0.10) and Zn in *Bembrops* sp., (r=0.720, p<0.10) respectively. These results indicate that metal levels in squids are very much dependent on metal levels in these fishes. Thus, the dietary intake of fishes from the environmental waters could partly explain one probable source of comparatively elevated Cd content and other metals in squids. This is supported by the fact that the levels of heavy metals in habitat area of the animals were low and being 0.0005 to 0.0013 μg/L for Cd, 0.008-0.021 μg/L for Cu and 0.013-0.078 μg/L for Zn.

Chapter 7:

Toxicity studies of liver bound cadmium in experimental albino rats indicated that organically bound Cd was toxic to the animal. Haematological analysis showed that rats fed with Cd incorporated diets had low Haemoglobin (Hb) content, Packed Cell Volume (PCV), Total Count (TC) and Platelet Count (PC) compared to control animals. Histopathological investigations of the liver and kidney tissues of experimental albino rats showed deviations from the normal architecture of these tissues. Liver and kidney tissues of these albino rats were affected by Cd incorporated diets. Cd in the liver (organic bound Cd) as well as inorganic Cd at 40 ppm level had toxic effects in albino rats as indicated by alterations in the liver and kidney tissues. This indicates that higher levels of dietary Cd could be a potential health hazard for human consumers.

Conclusions

- Liver of squids was the major site of accumulation of Cd and other toxic metals. The increasing order of abundance of most of the metals in squids were: Liver > Gills > Muscle.
- The highly toxic metals, viz., Cd, Pb, and Cr often exceeded the tolerance limits. Cd content exceeded the tolerance limit of 3 ppm in around 20% of the whole squid samples and Pb and Cr in around 11% each of the samples. However, the mean content of all the metals analysed were significantly lower in the edible parts and far below the tolerance limits.

- Concentration of ΣHg was found to be <50 μg/kg in the edible muscle in around 90% of the samples and ΣHg content was far below the limit of 1 mg/kg permitted for seafoods by many fish importing nations and USFDA.
- In general, Cochin region showed significantly higher levels of most of the metals analyzed than Quilon, Mangalore and Mumbai regions.
- Higher levels of most metals were noted during the monsoon and postmonsoon seasons.
- Oceanic squids showed higher levels of most metals than neretic squids.
- The dietary intake through forage organisms could partly explain elevated levels of Cd and other toxic metals in squids.
- Higher levels of Cd in cephalopods could be hazardous to the human consumer as indicated by animal feeding studies.
- Heavy metal concentrations in aquatic environment covered in the study is very low.

Recommendations

- Proper evisceration of the squid would help to bring down the metal content level by 30-80% of the original level. Hence it is recommended to popularize this practice in it the processing of squids for human consumption. As liver is the major site of

accumulation, in squids: consumption of liver should be avoided at any cost.

- Gutting stage should be included as a Critical Control Point (CCP) during squid processing ensuring seafood safety.
- The base line data generated in this study could be used for convincing the seafood buyer nations about safety of squids originating from Indian waters.
- The base line data generated in heavy metal content could be used as references for monitoring their future trends in seafoods originating off west coast of India.

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