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Concentrations of Trace Metals in the Squids, *Loligo duvauceli* and *Doryteuthis sibogae* Caught from the Southwest Coast of India

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Abstract

Concentrations of trace metals like Hg, Cd, Pb, Cu, Zn, Fe, Mn, Cr, Co, and Ni were determined in the squids, *Loligo duvauceli* and *Doryteuthis sibogae* collected from Mangalore, Cochin and Quilon, the three major fish landing centers in the west coast of India, from August 1998 to December 1999. The mean values (highest at the three stations) of highly hazardous metals in the muscle of the two species, were: Hg < 0.05, 0.07; Cd = 0.55, 0.89; Pb = 0.99, 0.89; Cr = 0.72, < 0.45 and Ni = 0.45, 0.19 ppm, all within the international safety limits. However, elevated levels of some of the metals, particularly Cd, Zn and Cu were sometimes observed. The liver, the major site of metal accumulation, is probably as complex as metallothioneins. Gills and ink also significantly concentrate metals. Cadmium levels in the muscle increased with growth in *L. duvauceli* and liver concentrations of Cd, Cu, Zn significantly correlated with total length. Geographical variations in metal levels were observed in these species.

Introduction

Trace metals, particularly cadmium, mercury, lead etc are persistent pollutants bio-accumulated by marine animals, risking public health through seafood. There has been increasing concern over the safety of food items that may contain harmful chemicals. Many fish-producing, as well as fish-importing countries have therefore instituted regulations and stringent quality requirements and standards for many chemical hazards including toxic metals in fish and fishery products. The EU directive of 1991 (91/493/EEC) and the US regulations of 1997 have set safety limits for such hazards. It has become mandatory for all fish-exporting countries to monitor the levels of trace metals in their fishery products.

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The present paper reports the baseline concentrations of some trace metals in the economically important squids, *L. duvauceli* and *D. sibogae* from the south west coast of India. These squids occur in shallow coastal waters up to a depth of 80 m on the continental shelf of both coasts of India. From 1997 to 1998, India exported > 75,000 metric tons (mt) of cephalopods and earned US $163 million in foreign exchange. However, these products were sometimes detained or rejected from the EU markets on the ground that they contain high levels of cadmium or the bacterium *Salmonella*. Heavy metals in mollusks have been extensively studied in various parts of the world as in India (Sadig and Alam 1989, Dious and Kasinathan 1992, Prudente et al. 1997). Higher levels of cadmium and other metals in squids have been reported by Falandysz (1989, 1990, 1991). At present, only a few studies on metal concentrations in Cephalopods from the Indian waters (Lakshmanan 1988, Patel and Chandy 1988, Lakshmanan and Stephen 1993) exist. This paper reports the levels of 10 trace metals in two species of squids from three major fisheries harbors in the west coast of India, their distribution in the body, and geographic trends.

Materials and Methods

Fresh squid samples were purchased directly from fishermen from their commercial catches landed in the fisheries harbors, Cochin, Quilon and Mangalore from August 1998 to December 1999. They were fished off the west coast of India. Samples were taken at monthly intervals from Cochin and bimonthly from the other two centers. The average length and whole body weight of the samples were in the ranges of 20 to 40 cms and 50 to 200 gms respectively, which were of commercial grades. Samples were immediately iced and brought to the laboratory in insulated boxes. These were either analyzed fresh or kept frozen (-20°C) until analysis. The whole (after removing the pen) and whole cleaned soft parts were finely chopped and homogenized and aliquots were taken for wet digestion. *L. duvauceli* collected from the Cochin harbor were analyzed whole so as to get an idea regarding reduction in metal levels upon conversion into tubes or fillets. Another lot was carefully dissected for various body components, such as muscle, liver, gills, skin and tentacles and subjected to wet digestion using concentrated nitric acid and perchloric acid (AOAC 1990). Metal concentrations were determined using Atomic Absorption Spectrophotometer model (GBC 902) with an oxidizing air-acetylene flame or an Inductively Coupled Plasma Emission Spectroscopy. Samples for mercury analysis were wet digested using concentrated HNO_3 and H_2SO_4 in the ratio (4:1 v/v) in a Bethge's apparatus. Determination of mercury was carried out following the cold vapor Atomic Absorption technique, using a Mercury Analyzer (model MA 5843) after reducing with stannous chloride and hydrochloric acid. The fish samples separated from the mantle cavity of the squid were digested whole and metal levels were determined. Triplicate analysis was carried out in each case and the data were statistically analyzed.

Results

Concentrations of 10 heavy metals found in the whole soft tissues of two squid species, *L. duvauceli* and *D. sibogae* and in various body components for the three different regions are illustrated in figures 1 to 4. The levels of metals found in the whole body of food fishes separated from the mantle cavity of *L. duvauceli* are presented in figure 5. The distribution characteristics of metals in the body components of the two squids from the three locations are also presented. However, *D. sibogae* could only be obtained from the Cochin region.

Mercury

Mercury is the least abundant toxic metal found in squid muscle from all the three regions. The edible muscle had total mercury (ΣHg) in the range of 0.01 to 0.07 ppm; very low levels were invariably observed in samples from the Mangalore region (Fig. 3). Among the various body components analyzed only the liver exhibited a value > 0.10 ppm for ΣHg. The muscle and liver of *D. sibogae* had a mean value of 0.07 and ≅ 0.32 ppm, respectively. The highest value observed for ΣHg in whole *L. duvauceli* was 0.094 ppm and a reduction of around 30% in mercury content was seen in the edible muscle. Liver and skin of *L. duvauceli* caught off the Cochin region recorded the highest values viz., 0.221 ppm in the liver and 0.17 ppm in the skin, respectively, the (range being 0.053 to 0.227 ppm in liver and 0.03 to 0.17 ppm in skin.) The liver of *D. sibogae* also exhibited a higher value of 0.32 ppm.

Cadmium

The presence of cadmium in seafoods is of serious concern in recent years, due to its cumulative effect and toxicity to the consumer. The cadmium content in the muscle of squid *L. duvauceli* was < 2.0 ppm at all stations. As in the case of Hg, whole squid had a higher level of Cd than in the edible muscle; the highest value observed being 3.19 ppm. Around 11% of the whole squid had Cd content > 3 ppm, the tolerance limit. However, all the mantle and tentacles had relatively low values for Cd and was far below the tolerance limit. Liver is the major site of Cd accumulation in both species. Liver samples of squid from the Cochin area had Cd content in the range of 10.52 to 95 ppm, while the Cd content in liver samples from the Mangalore and Quilon regions, was in the range of 3.20 to 24.61 ppm and 3.53 to 37.54 ppm, respectively. In *Doryteuthis sp.* liver had Cd in the range of 9.27 to 40.8 ppm. Other organs had comparatively lower values of the metal (Figs. 1 to 4).

Average levels of Cd in the edible muscle of squid from the three regions were far below the tolerance limit (< 3.0 ppm). Ink had higher levels of Cd exceeding 3 ppm in all samples taken from the three landing centers. Further, the gills and skin also contributed significantly to the total body burden of Cd in squids. The increasing order of abundance of Cd in the body

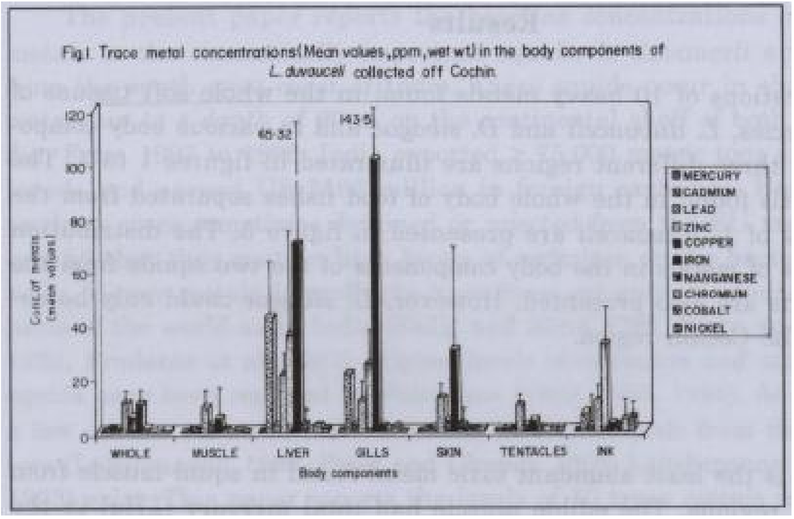

Fig. 1. Trace metal concentrations (mean values, ppm, wet wt) in the body components of *L. duvauceli* collected off Cochin.

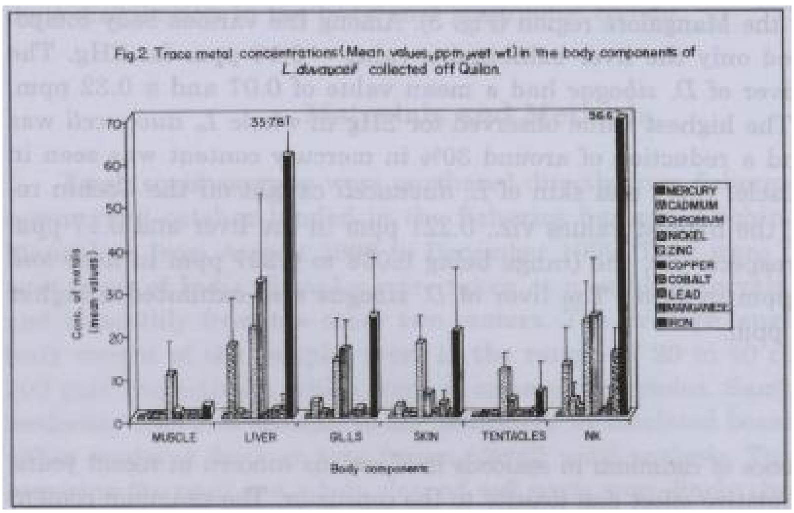

Fig. 2. Trace metal concentrations (mean values, ppm, wet wt) in the body components of *L. duvauceli* collected off Quilon.

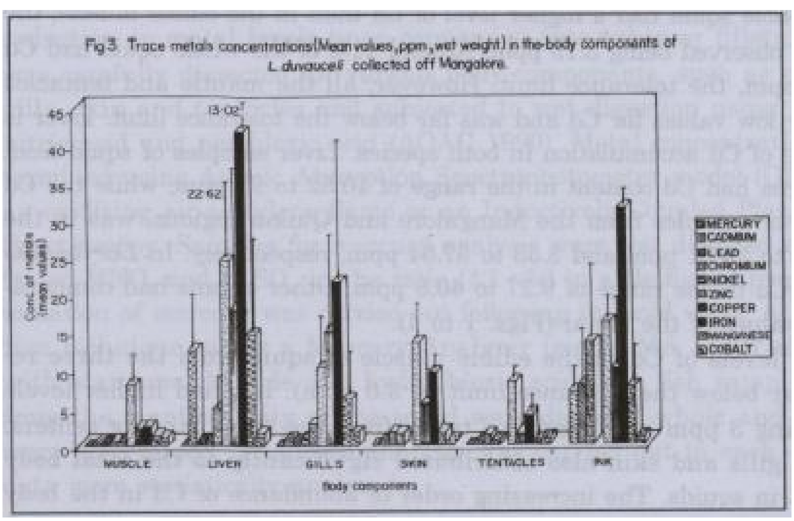

Fig. 3. Trace metal concentrations (mean values, ppm, wet wt) in the body components of *L. duvauceli* collected off Mangalore.

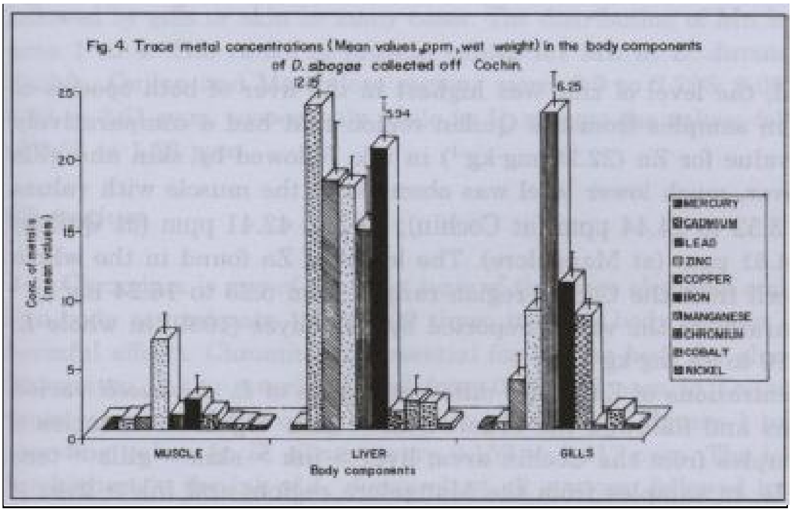

Fig. 4. Trace metal concentrations (mean values, ppm, wet wt) in the body components of *D. sibogae* collected off Cochin.

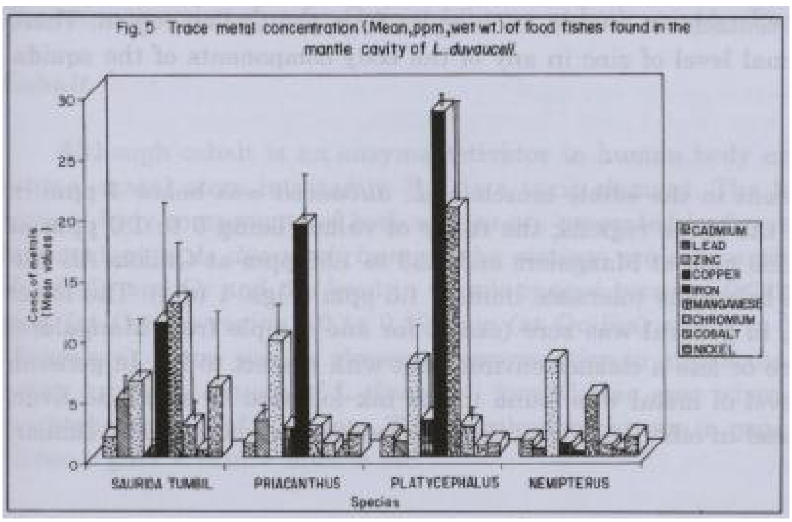

Fig. 5. Trace metal concentrations (mean, ppm, wet wt.) of food fishes found in the mantle cavity of *L. duvauceli*.

components of squid were liver >> ink > gills > skin > tentacles > muscle. This was true for samples from all the three regions.

Copper

Comparatively higher levels of copper were found in the liver, gills and ink of *L. duvauceli* collected from the three centers (Figs. 1 to 3). The mean Cu concentrations for all the three components is highest in the squids from the Cochin region and the lowest is in samples from Mangalore. The range of values of Cu in the liver of *L. duvauceli* from the Cochin, Quilon and Mangalore regions were 9.12 to 142.50, 13.95 to 86.49 and 10.60 to 44.52 ppm, respectively. Among the various body components, the muscle had the lowest Cu content. The muscle concentration of Cu ranged from 0.15 to 13.80 ppm in *L. duvauceli* and 0.62 to 1.66 ppm in *D. sibogae*, covering all the stations. However, the Cu content of skin is quite significant and had similar values at the three geographical locations.

Zinc

In general, the level of zinc was highest in the liver of both species of squid, except in samples from the Quilon region that had a comparatively higher mean value for Zn (22.28 mg·kg^{-1}) in ink, followed by skin and gills (Fig. 2). However, much lower level was observed in the muscle with values ranging from 3.52 to 24.44 ppm (at Cochin), 4.82 to 42.41 ppm (at Quilon) and 4.55 to 14.61 ppm (at Mangalore). The levels of Zn found in the whole raw *L. duvauceli* from the Cochin region ranged from 6.25 to 16.24 mg·kg^{-1} and are comparable to the values reported by Falandysz (1989) in whole *L. pantagonica*, 16 to 18 mg·kg^{-1}.

The concentrations of Zn in the different organs of *L. duvauceli* varied among stations and followed the order: liver > skin > gills > tentacles ≅ muscle, in samples from the Cochin area; liver > ink > skin > gills > tentacles > muscle, in samples from the Mangalore region; and ink > liver > skin > gills > tentacles ≅ muscle, in samples from the Quilon region. There was no abnormal level of zinc in any of the body components of the squids.

Lead

Lead content in the edible muscle of *L. duvauceli* was below 1 ppm in samples from the three regions; the range of values being 0 to 1.0 ppm at Cochin; 0 to 1.83 ppm at Mangalore and 0.33 to 1.58 ppm at Quilon. All values were well below the tolerance limit of 1.5 ppm (Figs. 1 to 4). The lower range of lead, in general was zero (except for one sample from Mangalore) indicating more or less a cleaner environment with respect to Pb. In general, the highest level of metal was found in the ink followed by skin and liver. The levels of lead in other body components were low and more or less similar.

Iron

The liver of *L. duvauceli* is rich in iron content, particularly in samples from the Cochin and Quilon regions, with a mean value of 69.89 and 61.20 mg·kg^{-1}, respectively. Iron content ranged from 26.18 to 214.20 mg·kg^{-1} and 15.47 to 117.4 mg·kg^{-1} in squid liver from the Cochin and Quilon regions, respectively. While the corresponding values of Fe in samples from the Mangalore region was relatively lower: 21.70 to 60.42 mg·kg^{-1}, it must be noticed that the lower range of Fe was comparable in the species at the three stations, probably due to the essential nature of the element. Muscle and tentacles had homogeneous levels of iron.

Manganese

Manganese, although an essential element was found only at low levels in the squids; the value in muscle was comparable to the levels of the toxic elements lead and cadmium (Figs. 1 to 4). Liver and ink of the two species, in general, contained higher levels of Mn compared to other organs. These were followed by gills or skin in many cases. The distribution of Mn is shown in figures 1 to 4. The range of muscle values for Mn in *L. duvauceli* from the Cochin, Quilon and Mangalore regions were: 0.0 to 2.786; 0.057 to 2.28 and 0.39 to 2.61 ppm, respectively while in *D. sibogae* the values fell in the range of 0.53 to 1.58 ppm.

Chromium

Chromium is one of the least toxic of the trace elements and the mammalian body can tolerate 100 to 200 times its total body content of Cr without harmful effects. Chromium is essential for human body for glucose tolerance. Chromium in the muscle ranged from 0 to 1.98 ppm in *Loligo* squids from these regions and the mean values are presented in figures 1 to 3. The corresponding values in *D. sibogae* were 0.152 to 0.617 ppm. The level of Cr was the highest in the ink of *L. duvauceli* at all stations, followed by skin or liver. The mean muscle value for Cr is < 1.0 ppm in both squids (Figs. 1 to 4).

Cobalt

Although cobalt is an enzyme activator in human body and forms, the central metal atom in vitamin B_{12}, is a toxic element. The level of Co in various body components of both species are presented in figures 1 to 4. Cobalt content in *L. duvauceli* from all the stations are comparable to the levels of Mn and Cr and the level in muscle ranged between 0.042 ppm to 2.86 ppm (at Cochin region), 0 to 0.32 ppm (at Quilon) and 0 to 3.82 ppm (at Mangalore). There was no abnormal accumulation in any of the body components, except in the ink of *L. duvauceli* from Cochin area where it registered the highest value of 9.81 ppm. The distribution pattern in general being ink >liver > gills > skin > muscle etc.

Nickel

Nickel is included in the category of highly toxic and non-essential element to biological systems. The level of Ni in squid body tissues were generally low in all the samples (Figs. 1 to 4). Nickel is found at low levels in the muscle of the squid from these three regions (Figs. 1 to 3). A slightly elevated level of Ni (4.70 ppm) is found in the liver of *L. duvauceli* from Mangalore region (Fig. 3). The distribution of Ni in other body components were more or less similar. None of the squid muscles or other body components had Ni above the FDA limit.

Metal levels in food fishes

Food fishes separated from the mantle cavity of *L. duvauceli*, viz., *Platycephalus tuberculatus*, *Saurida tumbil*, *Priacanthus hamrur* and *Nemipterus japonicus* were analyzed for Cd and other metals with a view to identify the source of higher metals in squid. The levels of some of the metals are presented in figure 5. The results indicated that these fishes of squid had higher levels of Cd (0.109 to 3.21 ppm), Pb (2.43 to 14.67 ppm) and Mn (2.59 to 19.58 ppm) and the relatively higher levels of metals observed in squid may be attributed to the contribution made by these food fishes. Thus, the fish consumption pattern of squid could explain one source of Cd accumulation in these species. Due to derth of data on food fishes, statistical analysis was not carried out at this stage.

Discussion

The highly toxic metals, mainly Cd, Cr and Pb often exceeded the permissible limit in around 11% of samples when whole squid was analyzed. However, their levels were far below the tolerance limits in the edible muscle.

Concentrations of mercury in squids are far below the limit of 1 mg·kg^{-1} permitted for seafoods by many fish importing nations and USFDA indicating that mercury did not cause any health hazard from sea foods. Comparable levels of Hg have been reported in cephalopods caught from the Arabian Sea (Patel and Chandy 1988m Lakshmanan and Stephen 1993). Only low levels of mercury were reported in *Loligo opalescens* during the past several years (Falandysz 1989, 1990). The levels of mercury in the different body components were also low, and the distribution followed the order liver > skin > gills >tentacles ≅ muscle in both the squids.

High level of Cd (> 3 ppm) had been observed in around 11% of whole squid, but low levels were found in the edible muscle. Higher levels of Cd in cephalopods have been reported from various parts of the world. Falandysz (1989) found high levels of Cd (2.9 to 10 mg·kg^{-1} wet weight) in the edible parts of canned squid, *Loligo patagonica*. Raw whole squid contained on the average 4.0 mg Cd·kg^{-1}(Falandysz 1991) and the highest level was found in the liver as in the present study. Lakshmanan and Stephen (1993) observed higher levels of Cd and Cu in the liver of *L. duvauceli*. However, Cd content was low in fin fish and shellfish caught from the same region indicating the selective bio-accumulation of Cd by cephalopods (Lakshmanan 1988). High levels of Cd reported in Arctic marine mammals (Dietz et al. 1998) and also in Juan Fernandez fur seals (Ochoa-Acuna and Francis 1995), had been attributed to high squid consumption by the seal.

The environmental factors and feeding habits of squids, being fed on a variety of fish, shellfish and crustaceans, probably contributed to the high levels of the metal in squid. Cannibalism that has been noticed in *L. duvauceli* (Varghese 1976, Santos and Haimovici 1997) might have also contributed to high Cd level. The levels of metals in the fishes separated from the mantle cavity of squids in the present study indicated elevated levels of Cd probably indicating an important source of Cd in squids. However, a correlation of these data with metal levels in squid is not attempted at. A detailed investigation on this aspect is quite warranted. The most significant finding in this study was the enormous concentrations of the non-essential element Cd in the liver of *L. duvauceli*.

Very high levels of Cu in the liver of *L. opalascens*, i.e. 200 to 300 $mg \cdot kg^{-1}$ (wet wt.) was reported by Falandysz (1991) and in *L. duvauceli* a moderate range of 12.79 to 178.94 $mg \cdot kg^{-1}$ by Lakshmanan and Stephen (1993). The value observed in fresh skinless mantle of *L. duvauceli* (ie. 0.73 to 13.8 $mg \cdot kg^{-1}$ are quite comparable to the levels in *L. opalescens* (Falandysz 1991). A similar pattern of distribution of Cu was observed in *D. sibogae* also in the present study. 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 copper for the synthesis of the respiratory pigment, haemocyanin, a high level in the body may be attributed to the functional necessity. Among the essential trace elements, copper forms the second most abundant element in squid after iron.

The levels of Pb observed in the muscle of *L. duvauceli* are similar to the values reported in *L. opalascenes* (Hall et al 1978, Falandysz 1991). As observed in the present study a greater concentration of Pb was found in the ink of *I. argentinus* and *L. opalascenes* (Falandysz 1988, 1991). It can be seen that in the case of *L. duvauceli* only the non-edible body parts of squid sometimes exceeded the tolerance limit.

The level of iron 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). Comparatively lower levels of iron were found in the body components, including liver of *D. sibogae* (Fig. 4). Whole animal had slightly higher content of iron than muscle or tentacles, in both the species.

The higher values of Mn, in general, were found in liver and ink of *L. duvauceli*. In *D. sibogae* the order was: gills > liver > muscle. The overall mean values for Co in the various body parts of *L. duvauceli* from Quilon area was significantly lower than the values found in the species from Cochin and Mangalore (Figs. 1 to 3). The level of Co observed in *D. sibogae* was comparable to the values observed in *L. duvauceli* from the other three regions. Bebianno and Machado (1997) obtained Ni in the range of 0.37 ppm to 0.77 $mg \cdot g^{-1}$ in the mussel, *Mytilus galloprovincialis* from the South coast of Portugal which could be compared with the present values.

It can be seen that the whole soft parts of the squid, *L. duvauceli* invariably exhibited higher levels of all the metals than those found in the muscle, which may be attributed to the contributions made by other body components like liver, gills, ink etc. that contained higher levels of the metals. Thus, by removing the gut, the metal levels could be reduced to around 1/10 of that present in whole soft parts in many cases. The metal content seemed to increase with age in the squid, particularly in the liver. Significant positive correlations have been observed between liver metal levels (Cd, Cu, and Zn) and the length of whole squid, *L. duvauceli* ($r = 0.8936$, $P \leq 0.001$ for Cd; $r = 0.9089$, $P \leq 0.001$ for Cu and $r = 0.8897$, $P \leq 0.001$ for Zn) which are presented in figure 6. A similar relation was also observed between cadmium content in the edible muscle and whole body length of *L. duvauceli* ($r = 0.812$; $P \leq 0.001$) (Fig.7). However, no significant correlation could be seen in the muscle between copper or zinc and length of the animal. Analysis of variance (ANOVA) of the data was employed to compare the group means among the three sampling sites, viz., Cochin, Quilon and Mangalore regions in respect to Cd, Cu and Zn. Preliminary studies showed that the variance among the three groups was highly significant ($P \leq 0.01$) with respect to Cd content in the muscle and liver of *L. duvauceli*. No significant correlation was observed in the distribution pattern of Zn in the body components of muscle and liver from the three regions. However, significant difference was noticed among the three regions with regard to Cu concentrations ($P \leq 0.01$) in the liver of *L. duvauceli*.

Feeding habits of squids play an important role in the accumulation of Cd and other metals. Varghese (1976) who studied the food and feeding habits of *L. duvauceli* observed carnivorous and cannibalistic behavior in this species from Arabian Sea. He treated the fishes obtained in the trawl catches of squid as its food fishes. In the present study, the fish obtained from the mantle cavity of *L. duvauceli* are treated as its food fishes.

The results indicated that these food fishes of squid had higher levels of Cd, Pb and Mn in their body and their relatively higher levels of metals observed in squid may be attributed to the contribution made by these food fishes.

Conclusion

The metal levels in the squids varied greatly between the species and in the same species (*L. duvauceli*) from different regions. The cadmium content in the food fishes separated from the mantle cavity of *L. duvauceli* is quite significant and may be one probable source of the metal in this species. The extent of contributions made by the food fishes to the total body burden of metals in the squid has yet to be studied in detail.

The present study shows that the average concentrations of all metals are significantly lower in the edible parts (tubes, fillets, fins and tentacles) of both the squids from all the regions and are far below the legal limits (FDA 1998). They do not seem to cause any health hazard by consuming the edible parts of

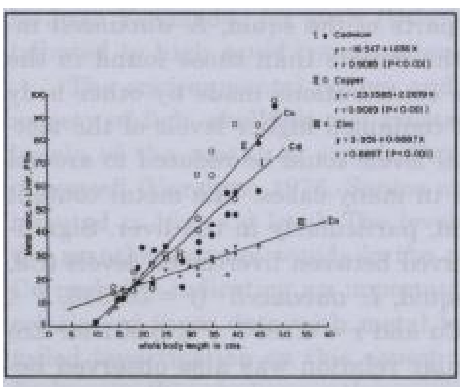

Fig. 6. Correlation of liver metal content with growth in the squid, *L. duvauceli*.

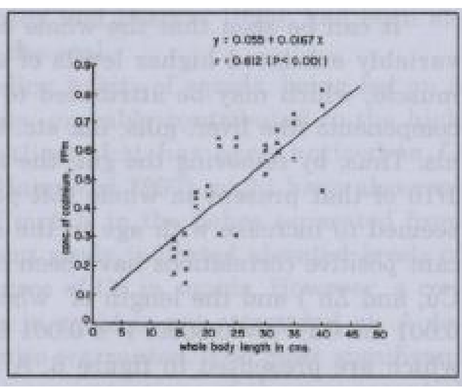

Fig. 7. Correlation of muscle cadmium with body length in the squid, *L. duvauceli*.

cephalopods. Concentrations of total mercury is < 50 $\mu g \cdot kg^{-1}$ in the edible muscle in > 90% of the samples; however, higher levels were found in the liver and skin of both the species. The highest concentration observed in the liver was only 0.227 ppm. In general, liver was found to be the major site of trace metal accumulation in both species of squids. Higher levels of Cd, Cu and Pb were observed in the liver tissue of both species of squids. Cadmium content in the whole squid often exceeded the tolerance limit of 3 ppm, at least in 11% of the samples analyzed and liver often exhibited high level of Cd. The ink like solution in squid (melanin) is another important source of metals in squid. The results of the study clearly emphasized the need to remove the liver and visceral part of squid before processing for human consumption. The data generated in the present study may form base line values for trace metals in squids in these regions. Significant correlations between metal levels in the liver and the body length of the squid (*L. duvauceli*) were noticed for the metals Cd, Cu, and Zn and also between muscle Cd and body length.

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