Bioaccumulation of Heavy Metals in Cuttlefish *Sepiella Inermis* from Visakhapatnam Coastal Waters

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Abstract: ***This study investigates 9 elements both essential (Cr, Cu, Zn, Fe, Mn and Ni) and non essential (Cd, Hg and Pb) in the tissues and whole of the cuttlefish Sepiella inermis caught off Visakhapatnam coast (east coast of India, Bay of Bengal). The level of elements was determined by atomic absorption spectrophotometry (AAS). The concentration ranges found for these heavy metals, expressed on a wet weight basis, were as follows: Hg, Cd, Pb, Cu, Zn, Fe, Mn, Cr, and Ni concentrations in cuttlefish muscle samples were 0.01 - 0.07, 0.11-0.67, 0.11-1.14, 0.52-6.08, 4.82-19.32, 0.08-5.84, 0-0.49 and 0-2.11, 0-0.92 ppm respectively. As for other cephalopod species, the liver showed the highest concentrations of many elements highlighting their role in bioaccumulation and detoxification processes. The mean values of highly hazardous metals in the muscle of the Sepiella inermis, were: Hg = 0.04, Cd = 0.481, Pb = 0.525, Cr = 0.662, all within the international safety limits. The level of contamination in Sepiella inermis by these heavy metals is compared to those studied in other parts of the world and the legal standards set by international legalizations. The levels of heavy metal in Sepiella inermis was found to be within the safe limits for human consumption.***

Keywords: heavy metal, Bay of Bengal, accumulation; cuttlefish; *Sepiella inermis*

1. Introduction

Cephalopod (Squids and cuttlefishes) represents an increasing component of the world's fisheries (Boyle and Rodhouse 2006; FAO 2007). They are consumed throughout the world, both as food and as feed supplement and have a great commercial value (Navarro and Villanueva 2001). Indeed, they are actually much appreciated as a protein source and because of their gustative quality. Cephalopods are consumed not only fresh, but also manufactured into processed food in huge quantities such as dry, frozen and chilled products (Paredi and Crupkin 1997; Hurtado *et al*. 2001). Trace metals are accumulated by marine invertebrates from both water and food. Trace metal accumulation process vary within the same invertebrate species according to the metal concerned. Some metals, such as Cu and Zn, are essential for proper metabolic functioning, whereas others, like Cd and Hg, are highly toxic to aquatic organisms and have unknown biological activities. Copper is an active centre of both the metal enzymes and the oxygen-transporting proteins, e.g. hemocyanin superoxide dismutase. Zinc is a co factor of several enzymes, such as carbonic anhydrase, alcalin phosphatise and DNA and RNA polymerase. In spite of its biological function, both Zn and Cu could produce toxic problems at very high levels.

Trace metals, particularly cadmium (Cd), mercury (Hg), lead (Pb) 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.

The present paper reports the baseline concentrations of some trace metals in the economically important cuttlefish *Sepiella inermis* from the Visakhapatnam south east coast of India. These cuttlefishes 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 Pb, Cd or the bacterium *Salmonella*. Heavy metals in Cephalopods 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 and cuttlefishes 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 9 trace metals in cuttlefish *Sepiella inermis* from Visakhapatnam which is major fishing harbor in the east coast of India.

2. Materials and Methods

Fresh cuttlefish samples were purchased directly from fishermen from their commercial catches landed in the fisheries harbor, Visakhapatnam from August 2014 to December 2014. They were fished off the east coast of India. Samples were taken at monthly intervals. The average length and whole body weight of the samples were 94 ± 8.02 mm and weight 103 ± 18.5 g 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^{o}C) until analysis. The whole (after removing the cuttlebone) and whole cleaned soft parts were finely chopped and homogenized and aliquots were taken for wet digestion. Another lot was carefully dissected for various body components, such as muscle, liver, and gills, 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. Triplicate analysis was carried out in each case and the data were statistically analyzed.

De-ionized water was used to prepare all aqueous solutions. All mineral acids and oxidants used were of the highest quality (Merck, Germany). All the plastic and glassware were cleaned by soaking overnight in a 10% (w/v) nitric acid solution and then rinsed with deionized water.

An independent *t* test analysis was applied to the observed data to check the level of significance site-wise. Pearson's Correlation Coefficient Statistical functions on Microsoft Excel were used to test the relation between the metal concentrations and the mantle length of the specimens.

3. Results and Discussion

Concentrations of 9 heavy metals found in the whole soft tissues of cuttlefish species, *Sepiella inermis* and in various body components are illustrated in figure 1 and table 2.

Levels of contaminants in marine animals are of particular interest because of the potential risk to humans who consume them. In particular, the tendency of heavy metals to accumulate in various organs of cephalopods, which in turn may enter the human metabolism through consumption causing serious health hazards. Accumulation of metals in marine animals is the function of their respective membrane permeability and enzyme system, which is highly species specifics and because this fact different metals accumulated in different orders in the studied cuttlefish samples. Therefore, the present study was undertaken to evaluate the heavy metals accumulation of Hg, Fe, Zn, Cu, Pb, Cr, Ni, Mn and Cd in the mantle tissues of the edible cuttlefish, *Sepiella inermis.*

3.1 Lead

Lead constitutes a serious health hazard to both children and adults. The adverse toxic effect caused by Pb on human was documented by Subramanian (1988). The high blood lead level can cause kidney dysfunction, brain damage, anaemia and can inhibits the normal functioning of many enzymes (Forstner and Wittmann 1983; USFDA 1993).

In the whole soft parts of cuttlefish (*Sepiella inermis*), Pb levels varied from 0.46 to 1.48 ppm, in the whole samples analysed 9 % showed Pb content above the tolerance limit of 1.5 ppm. However, mean Pb content in the edible muscle was below 1 ppm the range of values being 0.1 to 1.14 ppm at Visakhapatnam. The lower range of Pb, in the various body components analysed in general was zero. In the liver concentration of Pb ranged from 0 to 4.31 ppm. The mean Pb content was highest in gill samples of cuttlefish, the distribution pattern in general being gills> liver > muscle.

In the present study concentrations of Pb in muscle of cuttlefish (*sepiella inermis*) were less than the permissible limit (1.5 mg/kg, Table 1.) and were comparable to the values reported in *L. opalescens* (Falandysz, 1991). The levels of Pb observed in the liver of cuttlefish *sepiella inermis* were similar to the values reported in *Sepia pharaonis* (Lakshmanan and Stephen, 1993; Prafulla *et al*., 2001; Saleh Al Farraj *et al*., 2011). The lower range of Pb, in general was zero indicating more or less a cleaner environment. Another reason may be due to the sparingly soluble property of lead compounds and consequently non-availability to bio-mass in the marine environment. As observed in the present study a greater concentration of Pb was found in the non edible parts of cuttlefish. Also, 9% of whole samples had Pb content above 1.5 ppm, the tolerance limit.

3.2 Cadmium

Concerning Cadmium, the capability of cephalopods to concentrate high quantities of cadmium is well known (Miramand and Guary 1980; Miramand and Bentley 1992; Bustamante *et al*. 1998).The mantle of cuttlefish does not mentioned before as a specific organ for Cd accumulation,whereas, the digestive gland plays a primary role in the accumulation of Cd in cuttlefish. Such a role can be related both to the very efficient detoxification processes of the metal occurring in this organ and to the very high Cd assimilation efficiency (Bustamante *et al*. 2002a; Bustamante *et al*. 2006).

Dorneles *et al*. (2007) reported very high Cd concentrations; reach up 1000 µg g-1 wet wt, in the digestive gland of the short-lived species *Illex argentinus.* They stated that this value represents the highest cadmium level ever reported for a cephalopod. However, in this study, Concentrations of Cd in whole soft tissues of cuttlefish was 3.247 ± 1.553 ppm. Around 20% of the whole cuttlefish had Cd content > 3 ppm, the tolerance limit. Mean Cd content in the edible muscle was 0.481 ppm, liver was the major site of Cd accumulation in cuttlefish and it contributed significantly to the total body burden of Cd. Liver samples from Visakhapatnam region had Cd content in the range of 4.2 to 114.0 ppm. However, 25% of the gill samples from Visakhapatnam region showed Cd content above 2 ppm. The increasing order of abundance of Cd in the body components of cuttlefish was liver > gills >muscle.

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 10mg/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). Hussain and Mukundan (2005) reported the cadmium levels in edible parts like meat and tentacles of *Sepia pharaonis* and *Uroteuthis (Photololigo) duvauceli*, the level of cadmium was found to be below 1 ppm.

Lourenço, *et al*. (2009) found that Cd concentrations in the arms and the mantle of *Sepia officinalis* ranged between 0.03 and 1.0 mg kg-1 basis on wet wt. Saleh Al Farraj *et al*., (2011) found the average concentration of Cd in the mantle of *Sepia pharaonis* was 0.04 mg/ kg, and higher levels were observed in the liver of cuttlefish. The present results exceeded the maximum levels permitted (2 μg g-1) in molluscs that are recommended by FSA (2002) and disagree with Prafulla, *et al.* (2001) who stated that in the squid, mantle tissues accumulated relatively low values for Cd and was far below the tolerance limit. Generally, many studies have suggested that squid mantle could be consumed safely in most countries, considering the Cd concentration is two orders of magnitude lower than those in the digestive gland (Kim *et al*. 2008).

3.3 Mercury

Contamination of marine organisms with toxic metals such as mercury (Hg) is of ecological and health concern worldwide (Goldberg, 1995). The presence and behaviour of Hg in aquatic systems are of great interest and importance since it is the only heavy metal which bio accumulates and biomagnifies through all levels of the aquatic food chain (Lindqvist *et* al., 1991). Mercury has not necessary function in any living organism and is considered as a non-essential metal, is among the most toxic elements to man and many higher animals (Steinnes, 1995).

Mercury was the least abundant toxic metal found in cuttlefish (*Sepiella Inermis*). In whole soft tissues, Hg levels were in the range of 0.035 to 0.098 ppm . The highest value observed for Hg in whole cuttlefish was 0.098 ppm and a reduction of around 30% of Hg content was seen in the edible muscle. Highest mean values of Hg in edible muscle were noted was 0.042 ppm. Among the other body components analysed, only the liver exhibited a value of > 0.10 ppm for Hg and mean concentration was 0.186± 0.0419 ppm. Liver of cuttlefish caught off the Visakhapatnam region recorded the highest values, *viz* 0.291 ppm (Table 2). Only 12% of the liver samples showed Hg content above 0.2 ppm. Hg content in gills was in the range 0.032 to 0.070. The distribution pattern of Hg in the body components analysed was of the order: liver > gills > muscle.

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). In this study the levels of mercury in the different body components were also low, and the distribution followed the order liver > gills > muscle.

3.4. Chromium

Chromium is an essential trace element for human body for glucose tolerance (Dung 2001). Despite its high toxicity at high doses (Burger and Gochfeld 1995), mammalian bodies can tolerate 100 to 200 times their total natural body content of Cr without harmful effects (Moore and Ramamoorthy 1984).

The concentrations of Cr are presented in Table 2. The mean Cr content in whole cuttlefish (*Sepiella inermis*) was 3.616 ppm. Around 11% of whole cuttlefish showed Cr content above 12 ppm. However, Cr content in the muscle of cuttlefish was below the tolerance limit. The lower range of Cr in the edible muscle in general, was zero. The mean value of Cr content in the liver was 2.317 ± 2.312 at Visakhapatnam. The concentrations of Cr in the different organs of cuttlefish was followed the order: liver > gills > muscle.

Schumacher *et al.* (1992) found that the mean concentrations of chromium in the flesh of cephalopods were ranged from 0.090 to 0.220 μg/g of fresh weight. On the other hand, Prafulla *et al.* (2001) found that the mean muscle value for Cr in the two squids species *Loligo duvauceli* and *Doryteuthis sibogae* was < 1.0 ppm. After their study on some species of the cuttlefish *Sepia*, Ahdy *et al.* (2007) concluded that the average concentration of Cr was 2.2 μg/g. From other point of view, Cr concentrations were higher in juveniles than in adults, in the squid *Sthenoteuthis oualaniensis* (Ichihashi *et al.* 2001).

3.5. Zinc

Zinc is an essential trace element required by all living organisms because of its critical role both as a structural component of proteins and as a cofactor in enzyme catalysis (Lall 1989; Sandstrom 1997; Leigh Ackland and Michalczyk 2006). Zinc is always present in seafood, but concentrations found in mollusks are generally higher (Lall 1995; Celik and Oehlenschläger 2004). Zn is involved in numerous protein functions such as the carbonic anhydrase and is efficiently absorbed and strongly retained in *Sepiella inermis* both from the food and seawater pathways (Villanueva and Bustamante 2006).

The range of values of Zn in the whole soft tissues of cuttlefish was 5.84 to 24.16. In general, the level of Zn was highest in the liver of cuttlefish (Figs. 1). Around 40% of the liver samples from Visakhapatnam region had Zn content above 50 ppm. However, much lower levels were observed in the muscle with values ranging from 4.82 to 19.32 ppm (Visakhapatnam). In general, all the cuttlefish gill samples showed Zn content below 50 ppm.

Moreover, it is lower than the values determined by Lourenço *et al*. (2009) in arms and mantles of some common Portuguese cuttlefish (14.0- 22.5 mg kg-1 wet weight). Craig and Overnell (2003) found that Zn present in the cytosol, as a percentage of total tissue metal, in squid *Loligo forbesi* tissues, in mantle was 11.5, while in the digestive gland was 43.0.

This agrees with the results of previous studies which reported an increase of zinc concentration stored in metal-containing enzymes and metalloproteins in the digestive gland of the cephalopods (e.g. Miramand and Bentley 1992; Gerpe *et al*. 2000; Villanueva and Bustamante 2006; Lacoue-Labarthe *et al*. 2008). Accordingly, although Zn is an essential element for most cephalopods, mantle tissues still accumulate the lower values.

3.6. Copper

Mollusks, particularly cephalopods use the soluble copper containing protein haemocyanin as a respiratory pigment, therefore copper is required in large concentrations (Decleir *et al*. 1978; Viant *et al*. 2002). In addition, Martoja and Marcaillou (1993) stated that Cu accumulates in membrane-limited vesicles (spherules) within the basal cells of the liver the *Sepia officinalis*. They postulated that copper was bound to a 'metallothionein-like' protein which has been detected in heat-treated liver cytosols from cephalopods (Bustamante *et al*. 2002a).

Among the various body components of *Sepiella inermis*, muscle had the lowest Cu content and ranged from 0.18 to 7.45 ppm in cuttlefish. Comparatively higher levels of Cu were found in the liver and gills of cuttlefish (Fig..1). The range of values of Cu in the liver of cuttlefish from Visakhapatnam, were 14.21 to 128.32, ppm,. 38% of Cu in liver samples from Visakhapatnam region showed Cu levels above 50 ppm. However, Cu content in the muscle samples reflected low levels of Cu. Hence, the mantle tissue looks as if it is not the target organ for Cu accumulation.

The involvement of this element in several metabolic functions, such as in metal-dependent enzymes (Bustamante *et al*. 2000; Craig and Overnell 2003) may explain its high concentration in the present species. The levels of Cu observed in the muscle of cuttlefish (*sepiella inermis)* are similar to the values reported in the mantle of *Sepiella inermis* in Portnovo samples by Dious and Kasinathan (1992). The copper levels in muscle are also quite comparable to the levels in L *duvauceli* reported by Tariq *et al.*, (1991) and Prafulla *et al*., (2001). Very high levels of Cu in the liver of *L opalescens* (200 to 300 mg/kg wet wt.) were reported by Falandysz (1991). Martin and Flegal (1975) and Oehlenschlaeger (1991) also reported high Cu content in the liver of *L opalescens.* Copper content in the liver of cuttlefish are quite comparable to the values reported by Lakshmanan and Stephen (1993) (56.16 ± 45.67 ppm). Since cuttlefish requires Cu for the synthesis of the respiratory pigment, haemocyanin, a high level in the body may be attributed to the functional necessity (Smith, 1984).

3.7. Nickel

A few years ago, the physiological functions of nickel (Ni) were unclear. Nowadays, it is thought that Ni plays an important role in hormone, lipid and cell membrane metabolism. Several studies on nickel indicated to the role of nickel in various enzymatic system as well as activating enzymes associated with the glucose breakdown and use (Nielsen *et al*. 1984; Lall 1995; Acu-Cell 2007).

Nickel (Ni) in excess is toxic to aquatic organisms and persistent in the aquatic environment. In the present study, the distribution of Ni is shown in Fig 1 and Table 1. Nickel content in whole cuttlefish was in the range 0.19 to 5.92 ppm. However, Ni content in the muscle of cuttlefish was below the tolerance limit the mean value being 0.297, slightly elevated levels of Ni were found in the liver of cuttlefish and Ni levels ranged from 0 to 5.32 ppm in gills. The distribution pattern of Ni in the body components were liver > gills > muscle (Table 2).

Ni levels in the edible muscle of cuttlefish *sepiella inermis* were several folds lower than levels reported by Prafulla *et al*., (2001). Lourenço, *et al.*, (2009) identified Ni content in the edible parts (arms and mantle) of the cuttlefish *Sepia officinalis* was 0.02-0.09 mg/kg wet wt. Villanueva and Bustamante (2006) observed low concentrations of Ni in the European cuttlefish *Sepia officinalis* as compared to the European squid *Loligo vulgaris* and the common octopus *Octopus vulgaris*. Concerning the relationship between Ni concentration and specimen sizes of squid, Ichihashi *et al.*, (2001) have noticed that the concentration of Ni was greater in adults of the squid *Sthenoteuthis oualaniensis* than in the juveniles. In the present study the Ni content is in permissible limits in all the samples from all the three regions.

3.8 Iron

Iron, (Fe) one of the most abundant metals on earth, is essential to most life forms and to normal human physiology. Iron is an integral part of many proteins and enzymes that maintain good health (Institute of Medicine, 2001). In humans, iron is an essential component of proteins involved in oxygen transport (Dallman, 1986). It is also essential for the regulation of cell growth and differentiation (Bothwell, 1979, Andrews, 1986). A deficiency of Fe limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity (Institute of Medicine, 2001, Bhaskaram, 2001). On the other hand, excess amounts of iron in human can result in toxicity and even death (Corbett, 1995).

The liver of cuttlefish was rich in Fe content with a mean value of 71.981. The Fe content in liver ranged from 3.24 to 141.56 ppm in cuttlefish *sepiella inermis*. Distribution of Fe in whole cuttlefish samples from Visakhapatnam was 5.201. The range of Fe values in muscle of cuttlefish from Visakhapatnam, were: 0.08 to 5.84 (Table 2).

The level of Fe found in the various body components of cuttlefish (*sepiella inermis*) was quite comparable to the values observed in *L opalescens* (Falandysz 1991) and *L duvauceli* (Prafulla 2001). Liver of *Todarodes pacificus* from Japan showed higher level of Fe in visceral organs than in the edible parts (Ueda *et al.,* 1979). Lozano Soldevilla (1989) found Fe content to be high in whole bodies of *Todarodes sagittatus* when compared to those in mantles and tentacles.

3.9 Manganese

Manganese (Mn) is essential in animal body and its deficiency is impaired in the glucose utilization. Mn was found only at low levels in Cuttlefish. However, in whole soft parts of cuttlefish the Mn content ranged from 0.11 to 5.46 ppm at Visakhapatnam. In general, liver contained higher levels of Mn compared to other organs. The mean values being 3.18, ppm at Visakhapatnam. The average levels of Mn in gills showed a more or less homogenous distribution pattern;

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Miramand *et al*., (2006) found that the highest significant average Mn concentration approximately 4.0 mg/kg was found in cuttlefish and squid. The level of Mn in the whole of cuttlefish observed in the present study compares well with the values for whole *L duvauceli reported* by Prafulla *et al*., *(*2001). Wide ranges in the concentrations of the elements Fe, Mn, Pb and Zn were observed in the digestive gland of squid, *Nototadarus gouldi* by Smith *et al*., (1984).

4. Conclusion

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 cuttlefish *sepiella inermis.* 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 ng/kg in the edible muscle in 90% of the samples and Hg content was far below the limit of 1 mg/kg permitted for seafoods by many fish importing nations and USFDA. Cd content in muscle was < 3 ppm in cuttlefish from all the regions. Cuttlefish liver was the major site of accumulation of Cd and other toxic metals. The increasing order of abundance of most of the metals in cuttlefishes was: Liver > Gills > Muscle. As liver is the major site of accumulation, in cuttlefishes consumption of liver should be avoided at any cost. Gutting stage should be included as a Critical Control Point (CCP) during cuttlefish processing ensuring seafood safety.

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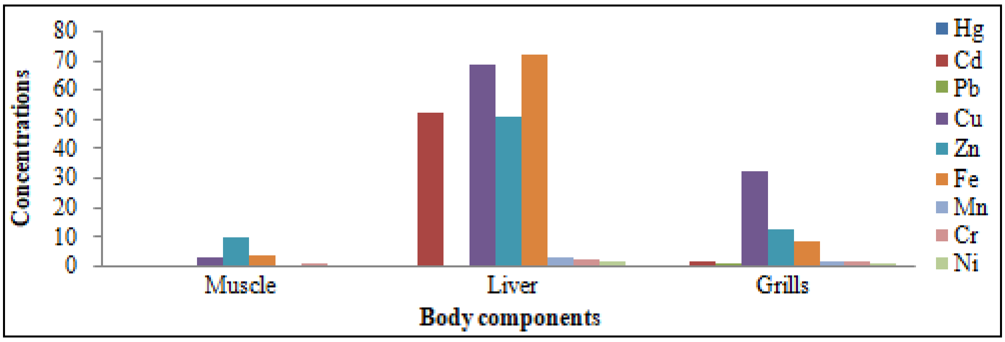

Figure 1: Distribution pattern of heavy metals (mean, ppm wet wt) in the body components of *Sepiella inermis* colected off Visakhapatnam

Table 1: Tolerable Daily Intake of Heavy Metals by Human as Prescribed by JECFA (2000)

Heavy metals	Provisional Maximum Tolerable Daily Intake (PMTDI) (mg/kg)
Pb	0.025
Cr	0.1-1.2
Co	0.005-1.8
Ni	0.1-0.3
Hg	0.025
Mg	250-380
Fe	0.8
Zn	0.3-1.0
Cu	0.05-0.5
Mn	0.4-10

Table 2: Trace metal concentrations (Mean ± S.D., Range, ppm wet wt) in the whole soft parts and body components of Cuttlefish Sepiella inermis collected off Visakhapatnam Region

Whole/Body Component	n*	Mercury	Cadmium	Lead	Copper	Zinc	Iron	Manganese	Chromium	Nickel
WHOLE	22	0.079 ± 0.013 (0.03-0.09)	3.247± 1.553 (0.416-6.08)	0.925 ± -0.279 (0.46-1.48)	12.023 ± 2.960 (4.92-15.83)	14.673± 4.341 (5.84-24.16)	5.201 ± 1.317 (2.32-10.11)	1.868± 1.396 (0.11-5.46)	3.616± 1.823 (0.05-6.43)	3.248± 1.406 (0.19-5.92)
Muscle	27	0.04 ± 0.017 (0.01-0.07)	0.481 ± 0.140 (0.11 – 0.67)	0.525 ± 0.295 (0.11 – 1.14)	2.489 ±1.451 (0.52-6.08)	9.449± 4.056 (4.82-19.32)	3.182± 1.595 (0.08-5.84)	0.310± 0.092 (0 - 0.49)	0.662 ± 0.479 (0 -2.11)	0.297± 0.243 (0-0.92)
Liver	28	0.186± 0.041 (0.16-	52.291 ± 36.025 (4.21-	0.533± 0.330 (0.1-1.18)	68.530 ±33.354 (14.21-	5.0332± 25.207 (28.41-	71.981± 42.249 (3.24-	3.181 ± 1.876 (0-9.13)	2.317 ± 2.312 (0-7.11)	1.854± 1.478 (0.21-5.86)

		0.29)	114.01)		128.32)	149.87)	141.56)			
Gills	42	0.047 ± 0.013 (0.03 - 0.07)	2.093 ± 0.928 (0.89- 4.11)	1.210± 0.966 (0 – 4.31)	31.896± 12.505 (13.22- 58.12)	12.080± 7.141 (4.08- 28.45)	7.96 ± 1.317 (4.16- 8.94)	1.373 ± 0.375 (0.22- 1.64)	1.12 ± 1.360 (0-5.74)	0.920 ±0.692 (0.2-2.54)

* number of samples analysed