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DISTRIBUTION OF TRACE METALS IN CUTTLEFISH (*SEPIELLA INERMIS)* FROM COASTAL ZONE OF ANDHRA PRADESH

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ABSTRACT

Degradation of the quality of the marine environment with chemical contaminants, especially heavy metals is of global concern. There is increasing pressure on the quality and safety of seafood products from the importing countries and hence monitoring of the levels at various points is quite warranted. One of the peculiar features of cephalopods is that they concentrate several trace elements such as Ag, Hg, Pb, Cd, Cr, Cu and Zn at sometimes very high concentrations. In the present study, the levels of five trace metals, viz., Hg, Cd, Pb, Cr and Ni in the cuttlefish (*Sepiella inermis*) from the three major fisheries harbor (Visakhapatnam, Bheemunipatnam and Kakinada) along the east coast of Andhra Pradesh, and their distribution in the body components and geographical trends are presented. The present study's results were subjected to statistical analysis by one-way ANOVA to find geographical variation in metal levels, if any, in the distribution of heavy metals in cuttlefish collected from the east coast of India. The metal levels in cuttlefish (*sepiella inermis*) captured from different regions showed significant variability as a function of their geographical origin. Cd content in the whole cuttlefish (*sepiella inermis*) 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. However, the mean content of all the metals analysed was significantly lower in the edible parts and far below the tolerance limits. The 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 seafood by many fish importing nations and USFDA. Cd content in muscle was <3 ppm in cuttlefish (*sepiella inermis*) from all the regions. Cuttlefish (*sepiella inermis*) 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. The study also identifies specific organs that may be particularly selective and sensitive to heavy metal accumulation.

Index Terms: **Cephalopods, Mercury, Cuttlefish, Heavy metals, Pollution, East coast**

INTRODUCTION

Cephalopods (Cuttlefish and Squid) are powerful swimmers and undertake long feeding and spawning migrations, thus influencing ecosystem functioning and community structure on a regional and seasonal basis. Among environmental factors, the geographical origin often has a considerable impact on trace element concentrations in cephalopods. The environment dictates the quantity of trace elements available, either directly by water absorption or indirectly through the ingestion of impregnated prey items (Jessica *et al.*, 2011). Cuttlefishes are important elements in marine food webs and interact significantly with commercially exploited finfish species. They are consumed throughout the world, both as food and as feed supplements and have great commercial value (Navarro and Villanueva, 2001; Koueta and Boucaud-Camou, 2001; Vairamani, 2010).

Degradation of the quality of the marine environment with chemical contaminants, especially heavy metals is of global concern. Considering the importance of global trade, the safety of products is of great concern. There is increasing pressure on the quality and safety of seafood products from the importing countries and hence monitoring of the levels at various points is quite warranted (Narasimha Murthy *et al.*, 2009). However, variations were observed in the levels of metals at different geographic locations. In the present study, the geographical variation of various toxic metals in cuttlefish (*Sepiella inermis*) from the important landing centers of the east coast of Andhra Pradesh is thought of monitoring with the objective of understanding spatial variations in metals at these different centers, viz., Visakhapatnam, Bheemunipatnam and Kakinada.

Many studies have been achieved on the contamination of different cephalopod species with heavy metals in different parts of the world (e. g. Bryan, 1968; Miramand and Bentley, 1992; Bustamante *et al*., 2002a, b; Suhaimi *et al*., 2005; Pierre Miramand *et al*., 2006; Suwann a *et al*., 2008; Jessica *et al.*, 2011; Saleh Al Farraj *et al*., 2011; Nurjanah *et al*., 2012; Onder *et al*., 2013 and Jamil *et al*., 2014). Not much information is available on the trace metal distribution in cuttlefish (*Sepiella inermis*) at different geographic locations in India. The levels of a range of trace metals in the cephalopods (both squid and cuttlefish) were studied in detail by Lakshmanan and Stephen, 1993; Prafulla *et.al*., 2002, had reported the levels of some of the heavy metals in the body components of squid and cuttlefish from the west coast of India. No information is available on the trace metal distribution in cuttlefish (*Sepiella inermis*) at different geographic locations in the east coast of India.

In the present study, the levels of five trace metals, viz., Mercury (Hg), Cadmium (Cd), Lead (Pb), Chromium (Cr) and Nickel (Ni) in the cuttlefish, *Sepiella inermis* collected from the three major fisheries harbor (Visakhapatnam, Bheemunipatnam and Kakinada) along the east coast of Andhra Pradesh and their distribution in the body components and geographical trends are presented. The study also identifies specific organs that may be particularly selective and sensitive to heavy metal accumulation.

MATERIALS AND METHODS

The cuttlefish (*Sepiella inermis*) samples were collected at monthly intervals from Visakhapatnam and bimonthly intervals from Kakinada and Bheemunipatnam regions. The samples were brought to the laboratory and immediately analysed following wet digestion or kept frozen until analysis. Samples were digested whole and also after separating into various body components like muscle, liver and gills.

Determination of Hg was carried out in a Mercury Analyser, MA-5840, and digestion was carried out by wet oxidation under reflux with con. nitric acid and con. sulphuric acids in the ratio 4:1 (v/v) using the Bethge's apparatus. Metals such as Cd, Pb, 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), metal levels were determined by flame AAS. The study's results were subjected to statistical analysis by one-way ANOVA to find geographical variation in metal levels.

RESULTS AND DISSCUSSION

The concentration of heavy metals like Hg, Cd, Pb, Cr and Ni found in the whole soft parts, edible muscle and important body components like muscle, liver and gills from the three regions are presented in Tables 1 to 3. Results of the Analysis of Variance (ANOVA) of some of the metals (Hg, Cd, Pb, Cr and Ni) in muscle and whole soft parts are presented in Tables 5 and 6.

Levels of Mercury, Cadmium and Lead: The results of concentrations of Hg, Cd and Pb from the three regions and their geographic trends are presented in Tables 1 to 3. These three metals are included in the category of highly toxic metals. Mercury was the least abundant toxic metal found in cuttlefish (*Sepiella Inermis*) from all three regions. In whole soft tissues, Hg levels were in the range of 0.035 to 0.098 ppm (Visakhapatnam region), 0.023 to 0.069 ppm (Bheemunipatnam region), and 0.01 to 0.059 ppm (Kakinada region). The highest value observed for Hg in the whole cuttlefish was 0.098 ppm and a reduction of around 30% of Hg content was seen in the edible muscle. The edible muscle in cuttlefish along the east coast had Hg in the range of 0.010 to 0.071 ppm. The highest mean values of Hg in edible muscle were noted in Visakhapatnam 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 0.186± 0.0419, 0.071±0.01179 and 0.081±0.028 ppm from Visakhapatnam, Bheemunipatnam, and Kakinada regions respectively. The liver of cuttlefish caught off the Visakhapatnam region recorded the highest values, *viz* 0.291 ppm (Table 1). 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, 0.01 to 0.062, and 0.01 to 0.082 ppm in cuttlefish from Visakhapatnam, Bheemunipatnam, and Kakinada regions respectively. The highest body burden of Hg in gills was noted in Kakinada region samples compared to levels in other regions. The distribution pattern of Hg in the body components analysed was of the order: liver > gills > muscle at all three regions. The geographic trends of Hg in the whole soft tissues and muscle of cuttlefish were Visakhapatnam>Kakinada>Bheemunipatnam and Visakhapatnam > Bheemunipatnam >Kakinada respectively.

Cadmium (Cd) is a toxic metal from the viewpoint of public health, whole cuttlefish (*Sepiella inermis*) had a higher level of Cd than in the edible muscle the highest value observed being 6.08 ppm from the Visakhapatnam region. Concentrations of Cd in whole soft tissues of cuttlefish at the three regions were 3.247 ± 1.553 ppm (Visakhapatnam region), 1.196 ± 0.442 ppm (Bheemunipatnam region), and 3.22 ± 1.466 ppm (Kakinada region). Around 20% of the whole cuttlefish had Cd content > 3 ppm, the tolerance limit. However, the Cd content in the muscle of cuttlefish was < 3 ppm in all three regions. The mean Cd content in the edible muscle at the three regions was 0.481 ppm (Visakhapatnam region), 0.374 ppm (Bheemunipatnam region), and 0.912 ppm (Kakinada region). The liver was the major site of Cd accumulation in cuttlefish from all the regions 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, while the Cd content in liver samples from Bheemunipatnam and Kakinada regions were in the range of 0.84 to 32.8 ppm and 4.5 to 92.0 ppm respectively. Around 62% 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 cuttlefish exhibited concentrations of 2.093 ± 0.928, 1.825 ± 0.381 and 3.425±2.717 ppm at Visakhapatnam Bheemunipatnam, and Kakinada regions respectively. All the liver and gill samples from Bheemunipatnam region had Cd content above 1.8 ppm. The lowest levels were noted in gill samples of Kakinada region (0.51ppm). However, 25% of the gill samples from the Visakhapatnam region showed Cd content above 2 ppm. The increasing order of abundance of Cd in the body components of cuttlefish were liver > gills >muscle. This was true for samples from all three regions. A similar geographic trend as that of Hg was noted in the distribution of Cd in the whole soft tissues of cuttlefish. However, in the muscle tissue collected from the different regions, the increasing order of Cd was as follows: Kakinada > Visakhapatnam. > Bheemunipatnam.

In the whole soft parts of cuttlefish (*Sepiella inermis*), Pb levels varied from 0.46 to 1.48 ppm at Visakhapatnam, 0.24 to 0.82 ppm at Bheemunipatnam, 0.155 to 0.88 ppm at Kakinada. Of 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 in the samples from the three regions the range of values being 0.1 to 1.14 ppm at Visakhapatnam, 0 to 1.42 at Bheemunipatnam, and 0.1 to 0.85 ppm at Kakinada. The lower range of Pb, in the various body components analysed in general was zero. The liver concentration of Pb ranged from 0 to 4.31 ppm covering all the stations. The mean Pb content was highest in gill samples of cuttlefish from Visakhapatnam (1.210 ppm) and the lowest average levels were noted in Bheemunipatnam region samples. The distribution pattern in general being gills> liver > muscle. The mean Pb content in cuttlefish in the whole soft parts varied from region to region and was of the order: Visakhapatnam > Bheemunipatnam> Kakinada.

Levels of Chromium and Nickel:

Chromium (Cr) is considered one of the least toxic of trace elements. Chromium (iii) as a part of the human tolerance factor was identified as an essential micronutrient for mammals. The mean values of Cr and the concentrations of Cr from the three regions are presented in (Tables 1 to 3) Cr content in whole cuttlefish (*Sepiella inermis*) varied from region to region and was in the range 0.05 to 6.43 ppm at Visakhapatnam, 0.52 to 3.51 Bheemunipatnam, and 0.06 to 7.54 ppm at Kakinada. Around 11% of the whole cuttlefish showed Cr content above 12 ppm. However, Cr content in the muscle of cuttlefish was below the tolerance limit at Visakhapatnam, Bheemunipatnam and Kakinada regions (Table 4). The mean values being 0.662, 0.55 and 0.822 ppm, respectively. The lower range of Cr in the edible muscle in general, was zero except in Kakinada region samples. Cr content in the liver also showed variation with region. The mean values being 2.317 ± 2.312 at Visakhapatnam, 2.717±1.022 ppm at Bheemunipatnam and 7.5±4.406 ppm at Kakinada. As in the case of Cr content in muscle, in liver samples also the lower range, in general, was zero except in cuttlefish from Bheemunipatnam and

Kakinada region. The mean Cr value in liver was < 8 ppm in all the regions. Mean values of Cr in gills of cuttlefish from Visakhapatnam, Bheemunipatnam and Kakinada regions were 1.12, 0.845, and 0.661 ppm respectively. The concentrations of Cr in the different organs of cuttlefish varied among stations and followed the order: liver > gills > muscle. The geographic trend in the distribution of Cr in the whole soft tissues were in the order; Kakinada >Visakhapatnam > Bheemunipatnam (Tables 1 to 3).

Nickel (Ni) in excess is toxic to aquatic organisms and persistent in the aquatic environment. Nickel content in whole cuttlefish varied from region to region and was in the range 0.19 to 5.92 ppm at Visakhapatnam, 0.04 to 2.08 ppm at Bheemunipatnam, and 0.53 to 2.56 ppm at Kakinada region. However, Ni content in the muscle of cuttlefish was below the tolerance limit at all the regions. The mean values being 0.297, 0.362, and 0.821 ppm, respectively. Slightly elevated levels of Ni were found in the liver of cuttlefish from Kakinada region (3.82 ppm) (Tables 3). Ni levels ranged from 0 to 5.32 ppm in gills along the east coast of India and the highest value observed was in Kakinada region (5.32 ppm). The distribution pattern of Ni in the body components from the three regions was of the order: liver > gills > muscle (Tables 1 to 3). Whole cleaned cuttlefish (*Sepiella inermis*) samples analysed showed lower mean values of all the five metals analysed. Toxic metals like Hg, Cd and Ni showed a significant reduction in metal levels. Around 30% of reduction in Hg content was found in whole cleaned samples. The proper evisceration of the visceral parts was found to bring down Cd and Cr content by around 70% and 35% respectively. Significant reductions of Cu, Zn and Ni levels were also noted.

Analysis of Variance (ANOVA) of the data followed by the ‘t test’ was employed to compare the means among the three regions, *viz.*, Visakhapatnam, Bheemunipatnam and Kakinada regions. The results of ANOVA of some of the important metals in the muscle and whole soft tissues of cuttlefish are presented in Tables 5 and 6. The results showed that the variance among the three groups was highly significant ($p<0.01$) with respect to Cd content in the muscle of cuttlefish. However, a significant difference was noticed among the three regions with respect to Hg, Cd, Cr, and Ni concentrations ($p<0.05$) in the whole soft tissue of cuttlefish. Hg content in the whole soft tissues of cuttlefish from the Visakhapatnam region was significantly higher ($p<0.01$) than Kakinada and Bheemunipatnam region samples. Kakinada cuttlefishes showed significantly higher ($p<0.05$) levels of Hg than Bheemunipatnam samples. Cd content in cuttlefish from the Visakhapatnam region was significantly higher ($p<0.01$) than Bheemunipatnam and Kakinada region Cuttlefish, while, Kakinada samples exhibited significantly higher ($p<0.05$) levels than Bheemunipatnam samples. Statistically, no significant difference was noted in Hg and Cd content between Visakhapatnam and Kakinada region samples. Pb content in whole soft parts of cuttlefish from Bheemunipatnam and Kakinada regions was significantly higher ($p<0.05$) than in Visakhapatnam samples. In the muscle tissue of *Sepiella inermis*, Hg content in the Visakhapatnam region was significantly higher ($p<0.01$) than Bheemunipatnam and Kakinada regions. Cd content in the muscle tissue of *Sepiella inermis* from the Kakinada region was significantly higher ($p<0.01$) than Bheemunipatnam and Visakhapatnam regions. Statistically, no significant difference was noted in the Cr and Ni levels in *Sepiella inermis* muscle from the three regions.

Concentrations of Hg in Cuttlefish (*Sepiella inermis*) were far below the limit of 1mg/kg permitted for seafood 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; Prafulla *et al.*, 2001; Saleh Al Farraj *et al.*, 2011; Nurjanah *et al.*, 2012; Onder *et al.*, 2013 and Jamil *et al.*, 2014) The concentration of Hg in the different body components obtained in the present study were also low, and the distribution pattern being in the order liver > gills > muscle in Visakhapatnam, Bheemilipatnam and Kakinada region. The results indicated that cuttlefish did not exhibit an unusual tendency for Hg accumulation.

High levels of Cd (>3ppm) have been observed in around 20% of the whole cuttlefish. Higher levels of Cd in whole *Loligo* spp. have 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 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), Prafulla *et al*., (2001) found that Cd content in the muscle of squid *L duvauceli* and *Doryteuthis sibogae* collected for the west coast of India was < 2ppm and he stated that in the squid, mantle tissues accumulated relatively low values for Cd and was far below the tolerance limit. 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. Dorneles *et al*., (2007) reported very high Cd concentrations; reach up 1000μg/g wet wt, in the digestive gland of the shortlived species *Illex argentinus.* They stated that this value represents the highest cadmium level ever reported for a cephalopod. 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 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.

In one of the studies carried out by Tewari *et al*., (2001) along the Alang coast, which is the biggest ship-breaking yard in the world, the Cd levels in the offshore waters were 177.16% more than in the control site that ranged from 3.26±1.1 ppm to 24.03±2.1 ppm. Similarly, in another study carried out along Brazialian coast, it was observed that anthropogenic action like high industrial waste discharge, upwelling and cannibalism of Argentine short-finned squid were the possible reasons for remarkably high cadmium concentrations (Dorneles *et al*., 2007). The potential to accumulate trace minerals like cadmium is positively correlated with trophic levels due to biomagnifications through the food web (Young 2001). It was also found that cephalopods act as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean Cephalopods constitute an important source of Cd for cephalopod predators, and this results in bioaccumulation in higher predators (Bustamante *et al*., 1998). In the present study, liver formed the major site of Cd accumulation in cuttlefish from all the regions. However, all the mantle and tentacles samples had relatively low values for cadmium and were far below the tolerance limit. Cephalopods have been considered a significant source of Cd to their predators (Muirhead and Furness, 1988; Law *et al*., 1997; and Bustamante *et al*., 1998 a). High levels of Cd have been found in striped dolphins (Honda and Tatsukawa, 1983), Arctic marine mammals (Dietz *et al*., 1998), Juan Fernandez fur seals (Ochoa- Acuna and Francis, 1995) and it has been attributed to high cephalopod consumption in the diet. The environmental factors and feeding habits of cuttlefish, wherein a variety of fish, shellfish and crustaceans are consumed, probably contribute to high levels of the metals in cuttlefish (Bryan *et al*., 1983). Being a voracious fish eater, and carnivorous in nature, cuttlefish receive and accumulate heavy metals from their food fishes and also from cephalopods. Cannibalism that has been noticed in cuttlefish (Jinadasa, 2014) might have also contributed to high Cd levels.

Lead constitutes a serious health hazard to both children and adults. The adverse toxic effect caused by Pb on humans was documented by Subramanian (1988). The high blood lead level can cause kidney dysfunction, brain damage, anemia and can inhibits the normal functioning of many enzymes (Forstner and Wittmann 1983; USFDA 1993). In the present study concentrations of Pb in muscle of cuttlefish (*sepiella inermis*) were less than the permissible limit (1.5 mg/kg) 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 of 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, 11% of whole samples had Pb content above 1.5 ppm, the tolerance limit.

Cr and Ni levels in the edible muscle of cuttlefish *sepiella inermis* were several folds lower than levels reported by Prafulla *et al*., (2001). Schumacher *et al*., (1992) found that the mean concentrations of chromium in the flesh of cuttlefishes 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 squid species *Loligo duvauceli* and *Doryteuthis sibogae* was < 1.0 ppm. Ahdy *et al.*, (2007) concluded that the average concentration of Cr was 2.2 μg/g in cuttlefish. Ichihashi *et al.*, (2001) studied Cr concentrations were higher in juveniles than in adults, in the squid *Sthenoteuthis oualaniensis*. Saleh Al Faraj (2011) found that the mean average concentration of Cr in *Sepia Pharaonis* mantles was 0.22 mg/kg. Bustamante *et al.*, (1998a); Miramand and Bentley (1992) reported Ni levels of 16.3±7.8 and 1.3+0.4 ng/dry wt in cephalopods, *Nautilus macromphalus* and *Sepia officinalis* respectively. 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. On the other hand, Craig and Overnell, (2003) observed that adults of the species *Loligo forbesi* have an average Ni concentration of 1.1 mg/kg wet wt. In the present study the Cr and Ni contents are in permissible limits in all the samples from all the three regions.

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 removed). As cuttlefishes 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 *Sepiella Inermis* 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 results in higher levels in the edible mantle. In order to reduce Cd and other trace metals in the edible muscle of cuttlefish, 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.

CONCLUSION

Monitoring programmes and research on bioaccumulation of heavy metals in aquatic environments samples have become widely important due to concerns over accumulation and toxic effects in aquatic organisms and to humans through the food chain. The relationship between trace metal content in biota with the surrounding environment such as water, sediment, and food are environmental factors correlated with metal accumulation in the internal organs of marine animals. The present result has shown that variation in heavy metal concentrations exists between the cuttlefish (*sepiella inermis*) collected from different regions. Environment differences in conjunction with age, nature and availability of food are the probable factors responsible for the wide range of metal concentrations found in *sepiella inermis* from the three different regions. Therefore, the baseline data of the study will be useful for regular ecological monitoring considering the impact of pollution in the aquatic environment.

Table 1: 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	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)	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)	0.662 ± 0.479 (0 -2.11)	0.297±0.243 (0-0.92)
LIVER	28	0.186± 0.041 (0.16-0.29)	52.291 ± 36.025 (4.21-114.01)	0.533±0.330 (0.1-1.18)	2.317 ±2.312 (0-7.11)	1.854 ±1.478 (0.21-5.86)
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)	1.12 ±1.360 (0-5.74)	0.920 ±0.692 (0.2-2.54)

* number of samples analyzed

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 Bheemunipatnam Region

WHOLE/ BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	CHROMIUM	NICKEL
WHOLE	22	0.029 ± 0.012 (0.02-0.06)	1.196 ±0.442 (1.09-3.22)	0.482 ±0.205 (0.24-0.82)	1.722 ± 1.182 (0.5-3.51)	0.828± 0.720 (0.04-2.08)
MUSCLE	27	0.0192± 0.008 (0.01 - 0.03)	0.374±0.314 (0-0.89)	0.341 ±0.450 (0-1.42)	0.55± 0.374 (0-0.91)	0.362±0.299 (0-0.84)
LIVER	28	0.07± 0.017 (0.04-0.09)	16.839±10.352(0.84-32.8)	0.860 ±0.378 (0-1.52)	2.717 ±1.022 (0.45-3.94)	1.493 ±0.945 (0.26- 2.89)
GILLS	24	0.0412 ±0.015 (0.01-0.06)	1.825 ±0.381 (0.81 -2.21)	0.587 ±0.480 (0-1.21)	0.845 ±0.798 (0.21-3.22)	1.025 ± 0.917 (0-2.51)

*number of samples analysed

Table 3: Trace metal concentrations (Mean ± S.D, Range, ppm wet wt.) in the whole soft parts and body components of Cuttlefish *Sepiella inermis* collected off Kakinada Region

WHOLE/ BODY COMPONENT	n*	MERCURY	CADMIUM	LEAD	CHROMIUM	NICKEL
WHOLE	14	0.05±0.012 (0.01 - 0.06)	3.22 ±1.466 (1.04-5.3)	0.68 ±-0.268 (0.15-0.88)	4.323 ±2.548 (0.06-7.54)	1.264 ±0.771 (0.53-2.56)
MUSCLE	22	0.022±0.012 (0.01-0.05)	0.912±0.271 (0.05-1.0)	0.625±0.237 (0.1-0.85)	0.822±0.534 (0.52-2.25)	0.821 ±0.749 (0-2.52)
LIVER	18	0.08±0.028 (0.03-0.11)	54.24 ±32.915 (4.53-92.01)	0.54±0.212 (0-0.68)	7.5±4.406 (1.56-12.64)	3.82±2.338 (0-7.36)
GILLS	17	0.041±0.024 (0.01-0.08)	3.425 ±2.717 (0.511-8.51)	0.811 ±0.529 (0-1.5)	0.661±0.469 (0-1.42)	1.547 ± 1.752 (0-5.32)

*****number of samples analyzed

Table 4. Environment contaminants, tolerances, action levels and guidance levels as per FDA and EU regulations

Toxic Elements	Limits as per US FDA	Food Commodity	Limits as per EU norms (for all marine products) mg/kg
Cadmium	3 4	Crustacean Molluscan bivalves	0.5-3.0*
Chromium	12 13	Crustacean Molluscan bivalves	-
Lead	1.5 1.6	Crustacean Molluscan bivalves	0.5-10.0*

Nickel	70 80	Crustacean Molluscan bivalves	-
Mercury	1.0	All fish	1.0

Table 5: Analysis of variance (ANOVA) of heavy metals in muscle of *Sepiella inermis* (Regional variation)

Mercury

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.0087	2	0.0044	21.1186	0.0000
Within Groups	0.0184	89	0.0002		
Total	0.0271	91			

Cadmium

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	4.2450	2	2.1225	37.9741	0.0000
Within Groups	4.9746	89	0.0559		
Total	9.2196	91			

Lead

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	1.0499	2	0.5249	4.4537	0.0143
Within Groups	10.4898	89	0.1179		
Total	11.5397	91			

Chromium

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.9039	2	0.4520	2.0144	0.1394
Within Groups	19.9683	89	0.2244		
Total	20.8722	91			

Nickel

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	4.2383	2	2.1192	10.8880	0.0001
Within Groups	17.3223	89	0.1946		
Total	21.5606	91			

Table 6: Analysis of variance (ANOVA) of heavy metals in the whole of *Sepiella inermis* (Regional variation)

Mercury

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.0403	2	0.0202	110.1835	0.0000
Within Groups	0.0143	78	0.0002		
Total	0.0546	80			

Cadmium

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	67.0112	2	33.5056	18.2642	0.0000
Within Groups	143.0903	78	1.8345		
Total	210.1015	80			

Lead

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	3.0128	2	1.5064	21.5725	0.0000
Within Groups	5.4467	78	0.0698		
Total	8.4595	80			

Chromium

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	73.4769	2	36.7385	10.5613	0.0001
Within Groups	271.3303	78	3.4786		
Total	344.8072	80			

Nickel

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	102.9508	2	51.4754	36.9246	0.0000
Within Groups	108.7374	78	1.3941		
Total	211.6882	80			

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