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: $\Sigma$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 $\Sigma$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 *Apoponichthys* 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 $\Sigma$Hg was found to be <50 $\mu$g/kg in the edible muscle in around 90% of the samples and $\Sigma$Hg 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 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.