Proc. Internatioal Symp. on Nutrients and Bioactive Substances in Aquatic Organisms, Cochin 16 & 17 Sept 1999:254 (1994)

Reprinted from, "NUTRIENTS AND BIOACTIVE SUBSTANCES IN AQUATIC ORGANISMS". Publ. Society of Fisheries Technologists (India), Cochin, 1994: pp. 254-265.

Trace Metals in Cephalopod Molluscs - A Unique Phenomenon in Metal Accumulation

P.T. Lakshmanan and Jose Stephen

Central Institute of Fisheries Technology
Cochin - 682 029. India

Concentrations of trace metals, viz. mercury(Hg), cadmium(Cd), lead(Pb), copper(Cu), zinc(Zn), arsenic(As) and selenium(Se) were monitored in frozen squid (*Loligo duvacelii*) and cuttlefish (*Sepia pharaonis*) products. The whole soft parts of cuttlefish and squid often contained high levels of Cd(>2ppm) and As (>1ppm). A high concentration of Cd was always associated with high levels of Cu and Zn in both the molluscs. Hg content was very low and ranged between 50-100 ppb. Pb was present in low concentrations. Liver was found to be the major site of accumulation for Cd, Cu and Zn, in these species. The order of accumulation of the metals, in general was liver> gonad > gills > muscle. Se content often exceeded 3 ppm in the whole soft parts. However a corresponding build up was not found in liver or other organs.

Cephalopod molluscs, particularly cuttlefish and squid form an important component in the marine products export of India. During 1992-93, 49300 tonnes of frozen squid and cuttlefish were exported to different countries. The principal species exported from India are the Indian squid (*Loligo duvacelii*) and cuttlefish, *Sepia pharaonis* and *S. prashadi*. Since molluscs in general, concentrate in their bodies certain trace metals from the hydrosphere it becomes necessary to monitor the levels of these metals in seafoods. From the view point of food safety, Hg, Cd, Pb, As, Se, Cu, Zn, Fe and Mn are of great significance. Stringent standards are being enforced by the importing countries. Compulsory inspection for Cd and Hg content of squid and cuttlefish has been introduced recently.

Trace metals in molluscs have been reported from various parts of the world (Brookes & Rumsby, 1965; Eustace, 1974; Topping, 1973; Ratkowsky *et al.*, 1974; Tanaka *et al.*, 1974; Bryan *et al.*, 1977; Talbot

& Chegwidden, 1982; Eisenberg & Topping, 1984; Sadig & Alam, 1989). Concentrations of Hg and other trace metals were reported in various tissues of the squid, *Loligo opalescens* (Falandysz, 1990; 1991; Dehlenschlager, 1991). Cantoni *et al.* (1986) studied the Zn/Cd ratio in squids and cuttlefish imported into Italy from four different countries and showed that about 50% of the samples had Cd content in excess of the tolerance limit. Tonasavic *et al.* (1988) determined toxic and essential elements in samples of Yogoslav -produced and imported marine products and found that 50% of squid samples exceeded the tolerance limit for Cd (1mg kg^{-1}). However, such studies on cephalopod products are scanty from India. (Ramamurthy, 1979; Lakshmanan, 1988, 1989; Patel & Chandy, 1988; Dious & Kasinathan, 1992).

In the present study, the levels of Hg, Cd, Pb, Cu, Zn, Fe, Mn, As and Se in frozen squid and cuttlefish products in different forms from the industry are reported.

Materials and Methods

Frozen squid, *Loligo duvacelii* and cuttlefish of the *Sepidae* family, namely *Sepia pharaonis* and *S. prashadi* in different forms viz. cuttlefish whole (CFW), cuttlefish whole cleaned (CFWC), fillets, squid whole (SQW), squid whole cleaned (SQWC), tubes and tentacles were collected from seafood processing factories at Cochin and Quilon over a period of 5 years (1986-92). A total of 280 samples were analysed for the study. The whole body weight of the samples were in the range of 50-1000 g for cuttlefish and 50-500 g for squid.

The various trace metals were determined in the edible and inedible parts of the squid and cuttlefish and also in whole (bone/pen free) and whole cleaned samples. The samples were homogenised and sub-samples were wet digested according to standard methods (AOAC, 1975). Samples for the determination of Hg was wet digested in a Bethge's apparatus using con. HNO_3 and con. H_2SO_4 (4:1 V/V). To study the distribution pattern of metals in different parts of the body, fresh samples of squid and cuttlefish or frozen specimens after partial thawing were dissected into various body components like liver, gills, gonad, viscera and skin and digested as described above. Cd, Pb, Cu, Zn, Fe

and Mn were determined using a GBC 902 atomic absoprtion spectrophotometer (AAS). As and Se were determined in an Inductively coupled Plasma atomic emission spectrometer (ICP). Mercury content in the digested samples was determined using a Mercury Analyser (ECIL Model MA 5800).

Results and Discussion

The results of analysis of various tissues and whole soft parts of squid and cuttlefish for the trace elements are presented in Tables 1 & 1a. The distribution pattern of metals in the body components are summarised for cuttlefish in Tables 2 & 2a and for squid in Table 3.

Table 1. Mercury, cadmium, lead, arsenic and selenium contents of squid (*Loligo* spp) and cuttle fish (*Sepia* spp) products (mg kg $^{-1}$ wet wt.) mean, standard deviation and range.

Product	n	Mercury*	Cadmium	Lead	Arsenic	Selenium
Cuttle fish whole (CFW)	52-76	66.3±46.1 6.6-179.0	3.89±3.23 0.07-10.96	0.66±1.16 0.0-8.10	11.74±1.62 9.46-13.86	4.56±0.38 4.23-4.98
Cuttle fish whole cleaned (CFWC)	36-44	39.1±21.0 8.1-87.5	1.38±2.05 0.06-7.56	1.55±0.59 0-2.05	10.64±3.32 7.60-14.67	5.01±1.06 4.06-6.34
Cuttle fish fillet	40-51	32.9±16.5 8.1-58.9	0.40±0.25 0.10-0.90	0.71±1.15 0-6.30	10.64±3.32 7.60-14.67	5.01±1.06 4.06-6.34
Tentacles	11-21	28.6±18.9	0.37±0.19	0.90±0.75	ND	ND
Squid whole (SQW)	25-47	54.6±21.9 10.1-87.6	1.63±2.27 0.08-8.008	0.87±0.77 0-1.57	3.35±1.16 2.26-4.52	3.87±0.41 3.45-4.30
Squid whole cleaned (SQWC)	12-28	45.9±25.0 8.0-76.4	0.23±0.17 0.07-0.68	1.13±1.15 0.28-4.85	2.01±4.52 1.86-2.15	3.90±0.55 3.32-4.43
Tentacles	8-14	39.4±15.7 13.6-60.6	0.49±0.52 0.07-2.18	1.41±1.02 0.-3.14	ND	ND

* Expressed in µg kg^{-1}. ND - Not determined. +For arsenic and selenium sample size is between 6-8.

n Number of samples

The levels of total Hg in the different tissues of both cuttlefish and squid are very low. The edible muscle of these two species had Hg concentration below 100 µg kg^{-1}, the mean values are <50 µg kg^{-1}. Among the 68 samples of CFW examined, 14 samples (21.5%) showed

Hg above 100 µg kg^{-1} the highest value being 179 µg kg^{-1}. The maximum value observed for Hg in SQW was 87.6 µg kg^{-1}. These values for Hg are far below the limit of 1 mg kg^{-1} permitted for seafoods (FAO, 1983). The lower range of concentration often fall below 10 µg kg^{-1}. The values for Hg obtained in the present study are quite comparable with the levels reported by Ramamurthy (1979) in cuttlefish (6-40 ppb) and Patel & Chandy (1988) in cephalopods (50-200 ng/g by wt) caught from Indian waters. Falandysz (1990) also reported low values for Hg in the squid, *Loligo opalescens*. The concentrations of Hg in the different body components of the two species were also low. Gonad recorded the highest values (85 µg kg^{-1} in cuttlefish and 87 µg kg^{-1} in squid) followed by liver. The distribution pattern being gonad > liver > gills > viscera > muscle/ skin in cuttlefish and gonad > liver > mantle (tube) > skin in squid. The results indicated that cephalopod molluscs did not exhibit unusual tendency for mercury accumulation.

Table 1a. Copper, zinc, iron and manganese contents of squid and cuttle fish products (mg kg^{-1} wet wt.) Mean, ± SD and range

Product	n	Copper	Zinc	Iron	Manganese
Cuttle fish whole (CFW)	31-76	9.34±6.37 1.20-35.08	12.76±4.58 10.11-34.32	7.65±6.27 1.66-26.91	0.59±1.50 0-7.91
Cuttle fish whole cleaned(CFWC)	36-44	3.74±2.98 0.73-12.33	14.83±3.59 7.64-23.32	6.04±2.30 1.85-11.18	0.29±0.34 0.10-1.07
Cuttle fish fillet	40-51	1.62±1.02 0.37-4.69	10.66±3.54 6.34-22.24	4.01±3.81 0.17-13.40	0.20±0.15 0-0.67
Tentacles	11-21	2.70±1.03 1.72-4.54	15.87±1.92 12.19-18.20	6.83±3.44 3.82-10.63	0.45±0.42 0.14-1.27
Squid whole	25-47	3.51±3.00 0.35-13.89	12.23±4.81 6.38-19.88	8.93±6.06 2.84-20.26	0.32±0.24 0.04-0.69
Squid whole cleaned/tube	12-28	2.83±2.25 0.50-12.09	12.11±6.68 4.57-30.47	5.06±3.39 0.78-16.30	0.38±0.20 0.03-0.72
Tentacles	8-14	2.87±5.64 0.44-5.05	15.49±5.64 9.16-25.69	5.20±4.55 0.88-12.89	0.45±0.19 0.19-0.59

n Number of samples

The cadmium content of whole cuttlefish and occasionally whole squid was very high (Table 1). Of the 75 samples of CFW, 58.7%

exceeded the tolerance limit (2 mg kg^{-1}). Relatively higher levels of cadmium were also detected in whole cleaned cuttlefish. 22% samples exceeded the limit. However, all the fillet and tentacle samples had relatively low values for Cd and was far below the tolerance limit. The elevated levels of Cd in some of the CFWC samples appear to come from liver. Whole, *L. duvacelii* squid contained on an average 1.63 mg Cd kg^{-1} and ranged between 0.08 - 8.08 mg kg^{-1}. Of the 47 samples of SQW 7 samples (about 15%) exceeded the permissible limit for Cd. Other edible parts of the squid (mantle, tentacles and SQWC) contained Cd at much lower level (Table 1). Thus, both in cuttlefish and squid, the edible parts contain cadmium at lower levels with average values 0.40 and 0.23 mg kg^{-1} respectively. It has been observed that fillets or tube prepared from whole animals having high cadmium content did not show elevated levels of the metal. Similar observation was made by Dehlenschlager (1991) in the squid *Loligo opalescens*.

Table 2. Distribution pattern of mercury, cadmium, lead, arsenic and selenium in the body parts of cuttlefish (mg kg^{-1}). Mean ±S.D and range.

Body part	n	Mercury *	Cadmium	Lead	Arsenic	Selenium
Muscle (fillet)	41-51	32.9±16.5 8.1-58.9	0.40±0.25 0.10-0.93	0.71±1.15 0-6.30	10.64±3.32 7.64-14.67	5.01±1.07 4.06-6.34
Liver	16-29	93.5±48.9 23.1-167.0	155.83±130.64 10.79-448.20	0.00 0-0	836.03±289.64 488.76-1202.0	6.39±3.04 3.41-9.78
Gills	10-10	63.9±46.1 10.5-161.8	6.48±3.97 0.69-13.46	1.84±1.36 0-3.65	88.58±34.08 65.00-139.12	4.25±2.90 2.97-8.58
Gonad	4-12	85.5±37.6 20.3-126.0	7.07±4.77 0.87-12.40	3.64±4.16 0-12.50	107.72±27.60 79.54-133.43	3.32±0.35 2.94-3.79
Viscera	6-10	44.5±20.4 19.0-74.0	5.04±1.01 4.14-6.01	1.62±1.47 0.37-3.67	ND	ND
Skin	8-10	25.4±12.1 10.3-46.0	0.61±0.38 0.11-1.04	1.84±2.16 0.21-5.47	ND	ND

* Expressed in µg kg^{-1}. ND - Not determined. + For As and Se sample size is between 4-8.
n Number of samples

Among the body components studied, liver contained the highest level of Cd in both the species. The cuttlefish liver, particularly of large

specimens had the metal to the extent of 448 mg Cd kg^{-1} and the mean value was 155.83 mg kg^{-1} (Table 2). Low levels were found in smaller animals. In squid liver the highest value was 65.38 mg Cd kg^{-1} (mean, 38.43 mg kg^{-1}). While liver constitutes about 3% of the bone-free body weight of cuttlefish, it was only 0.5% in squid. Further, the gonad which is also an important site of Cd accumulation may also contribute to the total body burden of Cd at the time of gonad maturity. Gills and viscera also contain significantly high levels of Cd in both the molluscs (Table 2 & 3). Thus, the viscera of cuttlefish and squid, including liver, are inedible and should be removed without contaminating edible parts. The pattern of accumulation in different body parts of cuttlefish and squid are liver > gonad > gills > viscera > skin > muscle in cuttlefish and liver > gills > viscera > gonad > skin > muscle in squid.

Table 2a. Distribution pattern of copper, zinc, iron and manganese in the body parts of cuttlefish (mg kg^{-1} wet wt). Mean ± S.D. and range.

Body part	n	Copper	Zinc	Iron	Manganese
Muscle (fillet)	41-51	1.62±1.02 0.37-4.69	10.66±3.54 6.34-22.24	4.01±3.81 0.17-13.40	0.20±0.15 0-0.67
Liver	16-29	56.16±45.67 9.68-191.04	80.40±42.91 21.85-167.71	40.50±18.14 10.04-74.93	0.63±0.42 0.14-1.27
Gills	10-10	19.86±6.51 9.60-26.54	13.80±6.65 7.54-31.70	21.67±26.32 3.22-65.40	0.70±0.29 0.32-1.14
Gonad	4-12	5.07±1.11 3.41-6.28	17.82±7.19 11.78-31.03	14.20±3.04 11.57-17.34	0.87±0.45 0.36-1.56
Viscera	6-10	9.50±3.49 6.32-15.00	19.95±6.82 11.08-28.85	22.71±4.49 18.40-27.96	0.52±0.10 0.411-0.603
Skin	8-10	7.81±1.80 5.86-10.05	22.83±8.84 8.91-29.58	5.62±3.83 2.36-10.53	0.37±0.19 0-0.60

n Number of samples

High levels of cadmium in cephalopod molluscs have been reported from different parts of the world. Falandysz (1989) found high levels of cadmium (2.9 - 10 mg kg^{-1} wet wt) in the edible parts of canned squid *Loligo patagonic*. Raw whole squid contained on an average 4.0 mg Cd

Table 3. Distribution pattern of trace metals (mercury, cadmium, lead, copper, zinc, manganese and iron) in the squid body parts (mg kg^{-1}). Mean ± S.D. and range.

Body part	n	Mercury*	Cadmium	Lead	Copper	Zinc	Manganese	Iron
Mantle	28	38.5±19.5	0.23±0.17	1.13±1.15	2.83±2.25	12.04±6.68	0.38±0.20	5.06±3.39
(tube)		8.08-73.5	0.06-0.49	0.26-2.90	0.50-12.04	4.55-30.47	0.03-0.72	0.79-16.28
Liver	6-10	65.3±23.7	38.43±21.4	0.89±0.98	77.22±79.52	29.22±22.0	-	20.36±16.76
		14.3-75.4	14.32-65.38	0-2.22	12.79-178.94	6.71-29.87		5.81-36.59
Gills	7-9	NA	5.59±4.51	2.32±1.59	21.69±12.07	19.90±12.26	ND	9.11±1.50
			1.32-13.46	0.68-3.72	10.50-32.31	6.86-41.70		
Gonad	4-6	87.0±42.8	2.45±1.79	11.13±2.68	11.48±0.98	19.10±7.09	0.25±0.10	40.17
		4.80-126.0	0.88-5.14	8.04-12.86	10.74-12.00	11.56-25.88	0.14-0.32	
Viscera	4-6	NA	3.04±0.31	3.17±1.62	24.50±7.75	8.12±0.65	ND	52.27±8.55
		2.68-3.25	1.16-4.54	17.56-31.34	7.54-10.60	42.40-57.40		
Skin	5-6	30.4±17.1	0.68±0.17	0.66±0.91	10.03±1.84	22.19±5.92	0.74±0.27	8.03±7.42
		12.8-45.5	0.44-0.91	0-1.70	8.20-11.89	18.17-30.91	0.48-1.03	1.03-15.9

* Expressed in μg kg^{-1}. ND - Not determined.
n Number of samples.

inermis in Portonovo samples. In cuttlefish, the concentration of copper was the highest in the liver, followed by gills, viscera, skin and gonad (Table 2). The copper content in the liver varied widely. In squid also liver contained the highest level of copper. Copper content was in the following order: liver> viscera> gills> gonad> skin> mantle. Squid and cuttle fish muscle showed low levels of copper (Table 3). Falandysz (1991) and Martin & Flegal (1975) also reported that in *L. opalescens*, the highest concentration of copper was in the liver.

The level of zinc was comparable in the various products from cuttlefish and squid. In both species, tentacles had higher mean values for Zn (15.87 and 15.49 mg kg^{-1}) than the muscle (Table 1a). The range of values in whole *Sepia* spp. (10.11 - 34.32 mg kg^{-1}) and *L. duvacelii* (6.38 - 19.88 mg kg^{-1}) in the present study are comparable to the level of Zn found in whole raw *L. pantagonica* squid (16-18 mg kg^{-1}) (Falandysz, 1989) and the mean values reported by Hall *et al.* (1978) for *L. opalescens* (12±4 and 21±11 mg kg^{-1}). The distribution pattern of Zn among the body parts (Tables 2a & 3) clearly indicated that liver in both species form the major site of Zn accumulation. The concentration of Zn in different organs is in the order liver> skin> viscera> gonad> gills> muscle in cuttlefish and liver> skin> gills and gonad> muscle> viscera in squid. In general, the edible muscle show relatively low levels of Zn.

Generally, low levels of iron are found in the muscle of the two species (Table 1a). Whole animals had slightly higher levels than mantle and tentacle. Liver of cuttlefish is richest in iron content (40.5±18.14 mg kg^{-1}) and in squid viscera has the highest level (52.27 mg kg^{-1}). Falandysz (1991) also observed higher content of Fe in the liver of *L. opalescens* (16 - 20 mg kg^{-1}). The levels of iron found in the mantle and tentacles in the present study (Tables 2a & 3) are comparable to the iron content in the mantle, arms, crown and fins of *L. patagonica* (1.8 - 3.4 mg kg^{-1}) (Falandysz, 1989).

The mean values of Mn in CFW and SQW are 0.59 and 0.32 mg kg^{-1} respectively. In cuttlefish, the order of abundance is gonad followed by gills and liver. In other body parts, the levels are almost similar (Table 2a). Mn could not be determined in the various body parts of

squid. The level of Mn in *L. duvacelii* and *Sepia*. spp observed in the present study compares well with the values for whole *L.opalescens* (0.59±0.25mg kg^{-1}) reported by Falandysz (1991) and 0.31±0.15 reported by Hall *et al.* (1978).

Arsenic and selenium are the other two elements in cephalopod molluscs that cause concern. All the 24 samples of cuttlefish and squid (including mantle) analysed had high levels of As and Se. The As and Se content in *Sepia* spp. were significantly higher than that in *L. duvacelii* (Table 1) with mean values 11.74 and 4.56 mg kg^{-1} respectively. Analysis of body parts in *Sepia* sp. showed that liver had the highest concentration of As (836±289 mg kg^{-1}) followed by gonad (107.7 mg kg^{-1}) and gills (88.58 mg kg^{-1}). However a corresponding build up of Se was not observed in the liver or other organs. This indicates that in cuttlefish, liver is the storage site for As and there is selective accumulation of the element. Independent of environmental pollution, many marine organisms contain appreciable amounts of As in their tissue (Shiomi *et al.*, 1984). These researchers reported that carnivorous gastropods are characterised by high levels of As in tissues especiallly in muscle. The As content in the muscle of carnivorous gastropods ranged from 16.8 ppm (in *Neptunea arthritica)* to 67.9 ppm (in *Reishia bronni*). They also observed high As content (340.1 µg g^{-1} wet wt) in the mid gut gland of *charonia sauliae*. The health implications of the high level of Cd and As in the cephalopod molluscs is yet to be studied.

Present study shows that concentrations of various trace metals in *Sepia* spp. and *L. duvacelii*, in general, are sufficiently low in the edible parts (fillets and tubes). Concentrations of total mercury is less than 50 µg kg^{-1} in 95% of the samples. However, the whole soft parts particularly of cuttlefish often contained higher levels of As and Se. The highest concentrations of the trace metals (except Pb and Mn) were found in the liver of cuttlefish. In squid also, liver was found to be the major site of accumulation for Cd, Cu and Zn and As. The distribution of Se is rather uniform in the various tissues of these species and often exceeded the limit of 3ppm. Concentrations of zinc in the tissues were less variable than those of other metals possibly due to zinc regulation. The results obtained clearly emphasise the need for the removal of liver and viscera before processing. The abnormally high levels of As and

Cd in cephalopods are unique phenomenon and requires further investigations.

Acknowledgement

The authors are grateful to Dr. K. Gopakumar, Director, Central Institute of Fisheries Technology, Cochin for according permission to publish this paper.

References

AOAC (1975) *Official Methods of Analysis.* 12th edn. Association of Official Analytical Chemists, Mc Graw-Hill, New York

Brookes, R.R. & Rumsby, M.G. (1965) *Limnol Oceanogr.* **10**, 521

Bryan, G.W., Potts, G.W & Forster, G.R. (1977) *J. Mar. Biol. Ass. U.K.*, **57**, 75

Cantoni, C., Caltaneo, P, & Balgaretti,C. (1986) *Industrie Alimentari*, **25**, 637

Clark, R.B (1986) in *Marine Pollution.* Oxford University Press, New York, p.94

Dehlenschlager, J. (1991) *Information fur die Fischwirtschaft* **38**, 62

Dious, S.R.J. & Kasinathan,R. (1992) *Indian J. Mar. Sci,* **21** 224

Eisenberg, M. & Topping, J.J. (1984) *J. Environ. Sci. Health.* **198**, 649

Eustace, IJ (1974) *Aust. J. Mar. Freshwat. Res.*, **25**, 209

FAO (1983) *Compilation of legal limits for hazardous substances in fish and fishery products* P. 102

Falandysz, J. (1989) *Food Add. contam.* **6**, 483

Falandysz, J.(1990) *Food. Chem,* **38**, 171

Falandysz, J. (1991) *J. Sci Food Agric.*, **54**, 79

Hall, R.A., Zook, E.G, & Meaburn, G.M. (1978) NOAA Techn Rep. NMFS SSRF-721

Lakshmanan, P.T.(1988) *Fish. Technol.*, **25**, 142

Lakshmanan, P.T. (1989) *Fishing Chimes,* **9**, 16

Martin, J.H. & Flegal, A.R.(1975) *Mar. Biol.* **30**, 51

Patel, B, & Chandy, J.P. (1988) *Indian J. Mar. Sci.* **17,** 55

Ramamurthy, V.D. (1979) *Bull. Jap. Soc. Sci. Fish.,* **45,** 1405

Ratkowsky, D.A, Thrower, S.J., Eustace, I.J. & June, O. (1974) *J. Fish Res Bd Can* **31,** 1165

Sadiq, M. & Alam, I. (1989) *Bull. Environ. Cont. Toxicol.* **42,** 111

Shiomi, K, Shinagawa. A, Igarashi, T., Hirota, K., Yamanaka, H. & Kikuchi, T. (1984) *Bull. Jap. Soc. Sci. Fish,* **50**, 293

Talbot, V. & Chegwidden, A. (1982) *Aust. J. Mar. Freshwat. Res.* **33,** 779

Tanaka, Y., Ikebe, K., Tanaka, R. & Kunita, N. (1974) *J.Food Hyg. Soc. Japan.* **15,** 390

Tonsavic, Z., Darmati, D., Vlajkovic,M. & Darmati, S. (1988) *Hrana Lshrang,* **29**, 155

Topping,G. (1973) *Aquaculture,* **1**, 379