Asian Fisheries Society, Manila, Philippines
Available online at www.asianfisheriessociety.org

Monitoring of Cadmium Accumulation in Cephalopods Processed in Gujarat Coast

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Abstract

Heavy metal accumulation in cephalopods has been one of the intricate issues in seafood export scenario in India. In recent years, many export consignments of cephalopods processed in Gujarat region were rejected on account of high levels of cadmium. To elucidate this problem, cephalopods landed at different landing centres and processed by various processing units were monitored during the period 2006-07. Among commercial samples of cephalopods, cuttlefish tentacles (0.52±0.08 ppm), squid tentacles (0.28±0.02 ppm) and frozen octopus (0.59±0.1 ppm) recorded highest accumulation of Cadmium. Organ-specific analysis revealed highest accumulation of Cd in the gut of *Sepiella inermis* (3.34±0.43 ppm) and Squid *Uroteuthis (Photololigo) duvauceli* (5.31±0.43 ppm). Similarly, in the Purpleback flying squid *Sthenoteuthis oualaniensis*, which is now a days landed by multiday fishing trawlers, highest residue level of Cd was observed in the liver (568.5 ±15 ppm), followed by gills, eyes and tentacles. Among different species of Octopus, highest accumulation was recorded in *Octopus macropus* (2.28± 1.11 ppm), followed by *O. membranaceus* (1.86 ±0.35 ppm) and *O. defilippi* (1.72 ±0.82 ppm). In order to find out the source of Cd, simulation studies were carried out by soaking squid muscle in ink, signifying the fact that poor post harvest handing and consequent rupture of ink sac, followed by soaking in ink-mixed water could be a reason behind high levels of Cd accumulation in cephalopods landed in Gujarat coast.

Introduction

Gujarat with about 20% of the country's coastline, 33% of the continental shelf area (1,64,000 sq. km) and over 2,00,000 sq. km of EEZ ranks second among the maritime states in marine fish production (CMFRI 2005). The annual marine fishery potential of the state is estimated at 0.57 million tons (CMFRI 1997), which is about 17% of the all-India potential. The width of the Indian continental shelf is greatest off Gujarat offering scope for exploitation of several types of finfish and shellfish resources

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by both traditional and mechanized fishing. The cephalopod landings in Gujarat increased by more than two folds during the five-year period from 0.234 lakh tons in 2002 to 0.49 lakh tons in 2006, with a concomitant increase in catch rate from 5.05 $kg.h^{-1}$ in 2003 to 14.74 kg h^{-1} in 2006. Total no. of fish processing units in Gujarat is 63, out of which 27 are EU approved units. Cephalopod landings in 2006 constituted 9.9% of marine landings and 23% of marine fish export of Gujarat. The cephalopod resource is primarily constituted mainly by *Uroteuthis* (*Photololigo) duvauceli*, *Sepia pharaonis, S. aculeata S. brevimana S. prashadi, Sepiella inermis and Octopus vulagris, O. membranaceus* and *O. defilippi*. These cephalopods were processed for various value added products like rings, fillets, skewer, fruity mix and exported to Italy, Spain and other EU and non-EU countries. Cephalopods have a key role in many marine eco systems (Amaratunga 1983; Rodhouse 1989). They represent an essential link in marine trophic chains and are found from polar to tropical ecosystems and both in shallow and deep oceanic environments. One of the peculiar features of cephalopods is that they concentrate several trace elements such as Ag, Cd, Cu and Zn at sometimes very high concentrations. Elevated concentrations of such heavy metals, especially cadmium, not only results in biomagnification, as they constitute a major food source for top predator species (Klages 1996), but also pose a health hazard to the human population that consume the species.

Recently, import regulations have increased on heavy-metal residues in fish. These include arsenic, tin, cadmium, lead, chromium, mercury and nickel. The EU limits levels of heavy metals and other environmental contaminants that can be present in fish and fishery products. In general, levels are disallowed if dietary intake would likely exceed acceptable daily or weekly intake for humans (Directive 91/493/EC). Member States are required to implement a monitoring system to check the level of contamination of fish and fishery products, produced both domestically and imported. Specifically, the EU has published cadmium and 1mg/kg in cephalopods (Regulation 2001/466/EC). Since 2004, heavy-metal residue has replaced antibiotic and microbiological contamination as the biggest concern of the future. In the first half of 2004, more than one-third of total Chinese seafood rejections in the U.S. were on account of high levels of cadmium, mercury and other heavy metals. Heavy-metal concentrations are often determined by variables such as water contamination, mining activity and effluent treatment activities in the fishing region. (Kulkarni 2005). In 2004-05, there were four rejections of export consignments sent from India to European Union countries on account of high level of cadmium in cephalopods.

Considering the alarming state of cephalopod processing in Gujarat and frequent rejections because of high cadmium level, the present study was carried out to monitor the level of Cd in various commercial samples of cephalopods for the period 2006-07. The present study also attempted organ-wise accumulation of cadmium in various species of cephalopods and studied whether soaking of cephalopods in chilled water for weight gain (as practised by suppliers for economic benefits) had any effect on further escalation in cadmium levels.

Materials and Methods

Cadmium levels in commercial samples of cephalopods (squid, octopus and cuttlefish) were monitored during the period 2006-07. In case of squid (*Uroteuthis Photololigo duvauceli*), commercial samples obtained from various fish processing units located at Veraval, Gujarat in the forms of fresh raw material, frozen raw material, whole cleaned, frozen tubes, frozen rings, skewer and frozen tentacles were analysed. Similarly, for the cuttlefish the commercial products were in the form of fresh raw material, frozen whole cleaned, cuttlefish products and frozen tentacles were selected. In Octopus, the commercial samples were in the form of fresh raw material, frozen raw material and frozen whole cleaned. Samples were analyzed in triplicates, following the method of AOAC official method 999.10 (AOAC 2000).

Samples were digested in Teflon containers using a microwave digester (Ethos plus High Performance Microwave Labstation, Milestone, USA). Tissues were homogenized, 3.0 g of wet tissue was weighed into 100 mL Teflon vials and digested overnight with 7 mL of pure nitric acid (AR grade, specific gravity: 1.38, Qualigens, India) and 3.0 mL of hydrogen peroxide. The microwave parameters were 700W power for 1 hour, with 40 minute heating time and 20 minute ventilation time. The digested contents were transferred to acid washed polypropylene bottles and made up to 25 mL with double distilled water and subjected to Cd content analysis by Atomic Absorption Spectrophotometer (GBC 932AA, GBC Scientific Instruments, Australia).

In order to determine, in which tissue Cd level gets accumulated in higher level, organ-wise analysis of Cd level in different species of cephalopods such as Cuttlefish *Sepia pharaonis,* Indian Squid *Uroteuthis (Photololigo) duvauceli)* and Purpleback flying squid *Sthenoteuthis oualaniensis* was carried out. Freshly landed samples were obtained from landing centre and Cd levels were analysed in different tissues like meat, tentacles, skin, ctenidia (gills) and liver/viscera as per the procedure described above. In different species of Octopus (*Octopus vulgaris, Octopus defilippi, Octopus dolfusi, Octopus macropus* and *Octopus membranaceus*), the edible parts were analyzed for accumulation of cadmium following the above described procedure.

In almost all landing centres of Gujarat, cephalopods are purchased by some middlemen (i.e. suppliers), who in turn, sell them to fish processing units. But in the process, they soak the species in chilled water for around 5-15 hours, so that cephalopods adsorb water and thereby the weight of individual species increases and in the process the suppliers get higher price for Kg body weight. During soaking and also due to improper handling and transport (by open transport vehicles commonly called as “Chakda”), the pre-processing quality of the products deteriorates. There is a possibility of rupture of ink sac and internal organs like liver, which could attribute to higher Cd levels in cephalopods. In order to elucidate this phenomenon a simulated soaking experiment was carried out in the laboratory for *Sepia prashadi*, *Sepia brevimana* and *Uroteuthis (Photololigo) duvauceli*. The weight gain was measured at 0, 5, 10 and 15h intervals and finally the Cd level was determined in meat and tentacles after 15 hours of soaking.

For determining any significant difference among commercial products of cephalopods, organ-wise Cd content and Cd levels in after 15 h of soaking, statistical analysis was performed by one way ANOVA procedure of SPSS 12.0.1. The graphs were plotted by using Sigma Plot 10.0. Mann-Whitney U Statistic was followed to determine any significant difference in Cd accumulation level in different species of Octopus.

Results

Cadmium levels in commercial samples of cephalopods

As shown in Fig.1, in 578 commercial samples of squid, highest mean (±S.E.) Cd accumulation value of 0.286± 0.02 ppm was observed in tentacles followed by frozen raw material (FZRM) 0.229± 0.012 ppm, skewer 0.221±.029 ppm, fresh raw material (FRM) 0.207±.026 ppm, whole cleaned (WC) 0.205±0.013 ppm, squid rings 0.157±0.013 ppm and frozen tubes (0.147± 0.02) ppm.

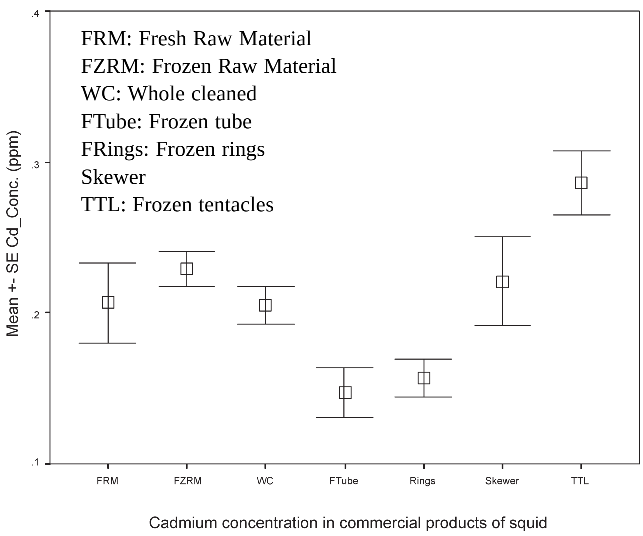

Figure 1. Cadmium concentrations in commercial products of squid

In FZRM, maximum content of 1.14 ppm was observed, which was beyond threshold limit of 1 ppm as per EU norms. One way ANOVA showed significant difference between Cd levels in different products of squids (P<0.05). Posthoc analysis b using S-N-K test revealed Cd level in tentacles to be significantly different from other commercial products of squid.

For 469 commercial samples of cuttlefish, as shown Fig. 2, highest mean (±S.E.) Cd accumulation value of 0.524±0.078 ppm was observed in tentacles (TTL) followed by frozen whole cleaned (FWC) 0.323±.014 ppm, frozen raw material (FZRM) 0.284±0.020 ppm, fresh raw material (FRM) 0..279 ± .021ppm and assorted products 0.276 ± 0.030 ppm.

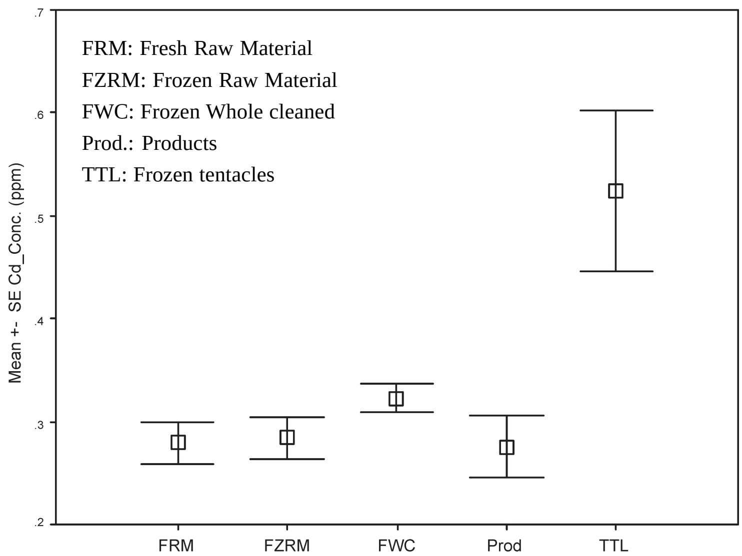

Figure 2. Cadmium concentrations in commercial products of cuttlefish

In some of the FWC and TTL products of cuttlefish samples the Cd levels were above the acceptable limit of 1 ppm. One way ANOVA showed significant difference between Cd levels in different products of cuttlefish (P<0.05). Posthoc analysis b using S-N-K test revealed Cd level in tentacles to be significantly different from other commercial products of cuttlefish.

As shown in Fig. 3, for 43 commercial samples of octopus, highest mean (±S.E.) Cd accumulation value of 0.587 ± .09 ppm was observed in frozen raw material (FZRM), followed by fresh raw material (FRM) 0.5 ± 0.94 ppm and whole cleaned (WC) 0.455 ± 0.09 ppm.

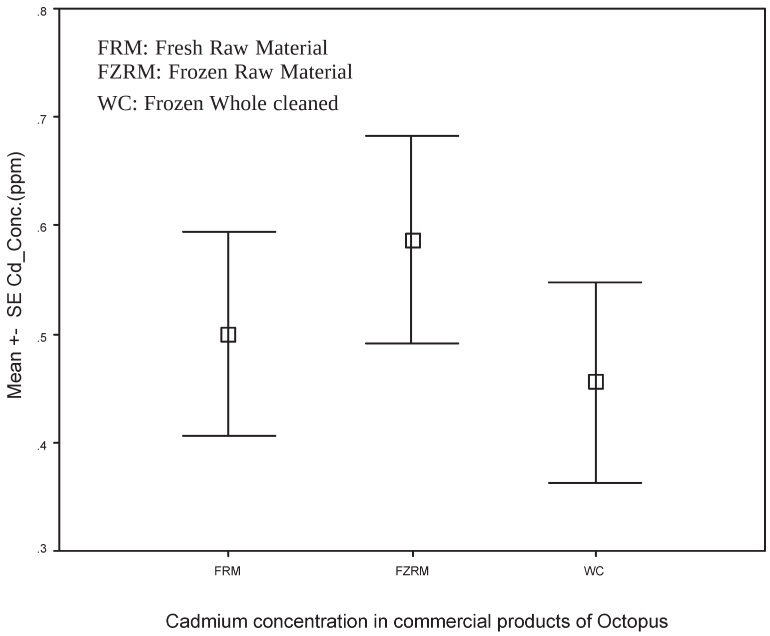

Figure 3. Cadmium concentrations in commercial products of Octopus

In some samples FZRM and WC, the Cd level was found to be higher than the acceptable limit of 1 ppm. One way ANOVA showed no significant difference between Cd levels in different products of octopus (P>0.05).

Organ-wise analysis of Cadmium accumulation

Cadmium levels were analysed in different organs of freshly landed specimens of *Sepia pharaonis, Uroteuthis (Photololigo) duvauceli)* and *Sthenoteuthis oualaniensis*. As shown in Table 1, highest mean (±S.E.) In *Sepia pharoanis* cadmium level of 27.114± 8.76 ppm was observed in liver/viscera, followed by gills 2.904± 0.45 ppm, skin 1.444± 0.29 ppm, tentacles 0.477± 0.07 ppm and meat 0.384± 0.04 ppm.

Table 1. Mean Cadmium content in different organs of cephalopods

Organs	Cuttlefish *Sepia pharaonis*	Squid *Uroteuthis (Photololigo) duvauceli*)	Purpleback flying squid *Sthenoteuthis oualaniensis*
Meat	0.384± 0.04 [a]	0.349 ± 0.07 [a]	0.47±0.21[a]
Tentacles	0.477± 0.07 [a]	0.421± 0.04 [a]	2.135±0.22[b]
Skin	1.444± 0.29 [a]	3.358 ± 0.97 [b]	1.784±0.04[b]

Ctenidia (Gills)	2.904± 0.45 [a]	1.366 ± 0.2 [a, b]	10.414±0.3[c]
Liver/Viscera	27.114± 8.76 [b]	2.659 ± 0.38 [b]	568.5±15[d]

Mean values in the column with different superscripts are significantly different ($P<0.05$)

In *Uroteuthis (Photololigo) duvauceli*), highest mean (±S.E.) Cd level was observed in skin 3.358 ± 0.97 ppm, followed by liver/viscera 2.659 ± 0.38 ppm, ctenidia/ gills 1.366 ± 0.2 ppm, tentacles 0.421± 0.04 ppm and meat 0.349 ± 0.07 ppm. In Purpleback flying squid *Sthenoteuthis oualaniensis* highest mean (±S.E.) Cd level of 568.5±15 ppm was observed in liver/viscera, followed by ctenidia (gills) 10.414±0.3 ppm, tentacles 2.135±0.22 ppm and meat 0.47±0.21 ppm. In all the species of cephalopods, significant difference was observed in organ-wise accumulation ($P<0.05$). Post-hoc analysis using S-N-K test revealed accumulation of cadmium in liver/viscera of *Uroteuthis (Photololigo) duvauceli*) *Sthenoteuthis oualaniensis* and was significantly different from other organs.

In the edible parts of different species of Octopus such as *Octopus vulgaris*, *Octopus defilippi*, *Octopus dolfusi*, *Octopus macropus* and *Octopus membranaceus*, highest (mean ± S.E.) accumulation of Cd was observed in *Octopus macropus* (2.28± 1.11 ppm), followed by *O. membranaceus* (1.86 ±0.35 ppm), *O. defilippi* (1.72 ±0.82 ppm), *Octopus vulgaris* (0.59 ± 0.03 ppm) and *Octopus dolfusi* (0.18± 0.01 ppm). Mann-Whitney U statistic revealed accumulation of Cd in *Octopus dolfusi* was significantly different from other species of Octopus (Table 2).

Table 2. Cadmium accumulation in the edible parts of different species of Octopus

Species	Mean ± S.E.
Octopus vulgaris	0.59 ± 0.03[a]
Octopus defilippi	1.72± 0.82[a]
Octopus dolfusi	0.18± 0.01[b]
Octopus macropus	2.28± 1.11[a]
Octopus membranaceus	1.86± 0.35[a]

Mean values in the column with different superscripts are significantly different ($P<0.05$).

Soaking of cephalopods for varying duration

As shown in Fig 4, the mean (±S.E.) weight in *Sepia prashadi* increased from 70.77 ±5.23 g to 94.07 ±6.98g after 10h of soaking and reduced to 92.34± 6.93 g after 15h.

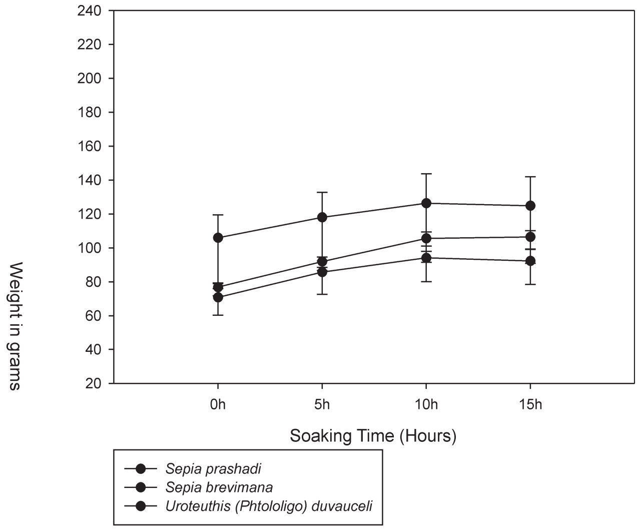

Figure 4. Weight gain in different species of cephalopods in response to varying soaking duration

In *Sepia brevimana* the mean (±S.E.) weight increased from 76.79±2.55g to 106.48±3.69 g after 15h. In *Uroteuthis (Photololigo) duvauceli* the mean weight increased from 105.96± 13.49 g to 126.33±17.37g after 10 h and slightly decreased to 124.91±17 g after 15h. Using Univariate analysis of GLM, it was revealed that there was significant difference (P<0.05) among species and hour-wise soaking in uptaking of moisture by different species of cephalopods, whereas the interactive effect of species vs hour had no significant difference (P>0.05).

As depicted in Table 3, after 15 hours of soaking highest mean (±S.E.) cadmium level in meat was found in *Sepia prashadi* 0.835 ± 0.17 ppm, followed by *Sepia brevimana* 0.806±0.08 ppm and *Uroteuthis (Photololigo) duvauceli* in 0.438 ± 0.1 ppm.

Table 3. Cadmium accumulation in various species of cephalopods after 15 h of soaking

Species	Mean (± S.E.) Cadmium accumulation	
	Meat	Tentacle
Sepia prashadi	0.8350 ±.17350 [a]	1.3010 ±.46803
Sepia brevimana	0.8060 ±.08100 [a]	0.9480 ±.15007
Uroteuthis (Photololigo) duvauceli	0.4380 ±.10048 [b]	0.3800 ± .03774

*Mean values in the column with different superscripts are significantly different (P<0.05)

Similarly, in tentacles, highest mean (±S.E.) cadmium level was found to be 1.3 ±0.47 ppm in *Sepia prashadi*, followed by 0.948 ±0.15 ppm *in Sepia brevimana* and 0.38 ± .038 in *Uroteuthis (Photololigo) duvauceli*. The Cd accumulation was significantly different ($P<0.05$) in meat of different species whereas no significant difference ($P>0.05$) was observed in that of tentacles.

Discussion

It is a proven fact that cephalopods naturally accumulate high levels of number of trace metals including cadmium (Martin and Flegel 1975; Finger and Smith 1987), although it has no biological role in the animal. In the present an effort was made to monitor the cadmium levels in samples of various forms obtained from export consignments of cephalopods. In commercial samples of squid, cuttlefish and octopus, although the mean value of Cd level was always found below the threshold limit of 1 ppm, in some samples of frozen tentacles in squid, frozen whole cleaned and tentacles of cuttlefish and frozen raw material and whole cleaned samples of octopus the values were above the acceptable limit. Although a particular reason can not be ascribed to the occasional high levels of Cd in some of the samples, personal observation of the whole cycle of post harvest handling reveals that ineffective de-skinning of tentacles and rupture of internal organs must be contributing to these high levels. Another reason may be high industrial waste discharge along Gujarat coast especially from the petrochemical, chemical and ship-breaking yards. 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 that this results in bioaccumulation in higher predators (Bustamante et al. 1998).

Liver/viscera in *Sepia pharoanis* and *Sthenoteuthis oualaniensis* accumulated the highest concentration of cadmium. Especially, in *Sthenoteuthis oualaniensis* which is considered as an oceanic squid, the mean Cd concentration was remarkably high i.e. 568.5±15 ppm in liver/viscera. Hence extreme caution should be exercised in utilization of this untapped resource from Indian Ocean. It is known that digestive glands like liver accumulate heavy metals from the environment. Cadmium is known to accumulate in the digestive gland (Bustamante et al. 1998; Finger and Smith 1987; Miramand and Bently 1992) reaching up to 98% of the total body cadmium in some species. Liver serves as a key function in the metabolism of cadmium in cephalopods. In a recent study the highest mean cadmium concentration of 1002.9 ìg/g in wet weight was observed in the digestive gland of sexually mature Argentine short-finned squids (*Illex argentinus*) (Dorneles et al. 2007). While studying the sub-cellular distribution of different heavy metals in *Sepia officinalis,* Bustamante et al. (2006) had reported a different detoxification mechanism for cadmium that leads to their bioaccumulation at relatively high concentration in the digestive gland. In octopus, a predominantly benthic organism, which was not studied in the present experiment, high levels of cadmium were reported in the arms of *Octopus vulgaris* (Sexias et al. 2005). In this case although correlation between Cd level and body weight has not been shown, higher Cd levels were always obtained from small sized cephalopods (personal observation). As per Pierce et al. (2007) in the UK waters concentrations of Cd were generally higher in the in all tissues of *Loligo forbesi* except muscle, which varied in relation to body size due to shift in the diet with growth.

In the edible parts like meat and tentacles the level of cadmium in *Sepia pharaonis* and *Uroteuthis (Photololigo) duvauceli* was found to be below 1 ppm. This finding is in consonance with that of Hussain and Mukundan (2005), who reported that out of 60 samples only in one sample of cuttlefish, the concentration was found to be more than 1 ppm. Earlier while determining Cd level in cephalopods, it was not clear, whether whole body has to be sampled or only the edible parts for analysis. But the present guidelines stipulate drawing samples only from the edible portion of cephalopods (Reddy 2004). A significant difference was observed in Cd levels in different species of Octopus. It must be due to different habitat preference in various species of Octopus as well as difference in size spectrum and feeding niche.

Soaking experiment was carried out in order to elucidate the phenomenon of increase in body weight and simultaneous increase in Cd level. Although weight gain was substantial, increase in Cd level in meat and tentacles beyond EU prescribed acceptable limit was not that pronounced. Only in tentacles of *Sepia prashadi* it increased above 1 ppm. It might be due to accidental rupture of ink sac or digestive gland that accumulates higher level of Cadmium.

Conclusion

Heavy metal accumulation in cephalopods has been a delicate issue for seafood export business. Rejection of consignments having cadmium levels above prescribed levels has brought this issue to limelight. In the present study higher Cd levels were encountered in commercial samples of cephalopods, which must be due to heavy industrial discharge along Gujarat coast. Organ specific analysis points to the fact that the digestive glands must be carefully segregated during post harvest handling and must not be allowed to rupture during pre-processing transport phase. The current practice of soaking of cephalopods in chilled water by the suppliers results in weight gain of individual species; but possesses the potential to elevate cadmium levels due to rupture of internal organs and hence monitoring agencies must stress for abandoning this unusual practice.

Acknowledgement

We would like to thank Dr. K. Devadasan, Director, Central Institute of Fisheries Technology, for his encouragement during the course of study. Special thanks are extended to Mr. K. U. Shekh, Technical Officer for his meticulous technical help and boat crew for their invaluable assistance in collecting specimens during the study.

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Received: 31 December 2007; Accepted: 24 December 2008