

## Heavy Metal Concentrations in Tissues of Short-Finned Squid *Illex coindetii* (Cephalopoda: Ommastrephidae) (Vérany, 1839) in Iskenderun Bay, North-Eastern Mediterranean

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**Abstract.** Metal levels (Cd, Cr, Cu, Fe, Mn, Pb and Zn) in the gill, digestive gland, arm and mantle of female and male short-finned squid (*Illex coindetii*) in Iskenderun Bay were investigated. All elements differed in accumulation in tissues and sex significantly ( $p < 0.05$ ). Concentrations of Cd in the tissues of male and female short-finned squid samples ranged between 0.68-9.51  $\mu\text{g/g}$ . Cu concentrations in all tissues were found and ranged between 5.97 and 324.1  $\mu\text{g/g}$ . Fe concentrations in the mantle of female and male samples were found 9.13 and 9.83  $\mu\text{g/g}$ , respectively. Pb concentrations in the mantles of female and male samples were found between 1.49-2.37  $\mu\text{g/g}$ . The highest Zn concentrations in hepatopancreas of male and female samples were found 85.29 and 66.8  $\mu\text{g/g}$  respectively ( $P < 0.05$ ). In the mantle tissue of *I. coindetii*, metal levels independent of sex were in the following order:  $\text{Zn} > \text{Cu} > \text{Fe} > \text{Pb} > \text{Mn} > \text{Cd} > \text{Cr}$ . In accordance with limit values, the present study found out that the mantle tissue of cuttlefish, *I. coindetii*, was overlimited with Cd and Pb.

**Key words:** Squid, *Illex coindetii*, sex, heavy metals, risk evaluation, Iskenderun Bay.

### INTRODUCTION

Many marine organisms have the potential to bio-concentrate high levels of metals from their environment. Cephalopods are regarded as key species in many marine ecosystems (Amaratunga, 1983; Rodhouse, 1989). They represent an essential link in marine trophic chains and are consumed by a number of marine top predators including fish, sea birds and mammals (Croxall and Prince, 1996; Smale, 1996; Klages, 1996). Over 80% of odontocete species and two baleen whale species regularly include cephalopods in their diets (Clarke, 1996).

The cephalopod accounts for a considerable component of the world's fisheries (Boyle and Rodhouse, 2006; FAO, 2007). They are consumed throughout the world, both as food and feed supplement and hence have great commercial value (Navarro and Villanueva 2000; Koueta and Boucaud-Camou, 2001). These squids are popular for protein source and their gustative quality. Cephalopods are served not only fresh, but also dried, frozen and chilled products (Paredi and

Crupkin, 1997; Ueng and Chow, 1998; Hurtado *et al.*, 2001).

In general, many metals occur naturally in marine environments. Some of them are classified as pollutants only when added by man in sufficient amounts to produce deleterious effects on some features of the ecological system (Freedman, 1989; Yousafzai and Shakoori, 2008). Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches substantially high levels. Accumulation levels vary considerably among metals and species. Heavy metals mainly accumulate in metabolically active tissues. The liver tissue is highly active in the uptake and storage of heavy metals. It is well known that large amount of metallothionein induction occurs in liver tissue of aquatic organisms. It is also a good indicator of chronic exposure to heavy metals because it is the site of metal metabolism (Miller *et al.*, 1992). However, high levels of metals accumulate in the gill tissues by absorption and adsorption. Many studies have shown presence of different heavy metals in the form of different cephalopods species (Bryan, 1968; Miramand and Bentley, 1992; Bustamante *et al.*, 2002a,b).

The short-finned squid, *Illex coindetii* (Verany, 1839) (Cephalopoda: Ommastrephidae) is an Atlanto-Mediterranean species inhabiting various soft substratum, mostly muddy bottoms with a

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broad vertical distribution from shallow waters up to 1000 m depth (Sanchez *et al.*, 1998; Vafidis *et al.*, 2008). Owing to its commercial interest and also wide range of spread, there are many studies about the species biology, fisheries and stock management (Vafidis *et al.*, 2008).

İskenderun Bay, located in the north-eastern part of Mediterranean Sea, is potentially threatened by anthropogenic activities such as fishing, shipping, and industrial activities. Yet, there are no studies on heavy metal contamination in squid *I. coindetii* in İskenderun Bay except for cuttlefish in İskenderun Bay (Duysak *et al.*, 2013) and in Mersin Bay (Ayas and Ozogul, 2011).

The aim of this study was to determine the concentration of Cd, Cr, Cu, Fe, Mn, Pb and Zn in gill, digestive gland, arm and mantle tissues of male and female short-finned squid and to assess the health risks from exposure of metals to consumers through consumption of the local squid *I. coindetii* captured from İskenderun Bay.

## MATERIALS AND METHODS

A total of 60 (30 male and 30 female) short-finned squid collected by fishermen in November 2011 from İskenderun Bay, were immediately transported to the laboratory, where male and female squids was classified according to sex and size. Squid mantle lengths were measured and their bodies were weighed, before dissection. Approximately 1 g of the gill, arm, mantle and digestive gland tissues from each *I. coindetii* were dissected, washed with distilled water, packed in polyethylene bags and kept at -21°C until analysed.

Sample preparation and analysis were carried out according to the procedure described by UNEP/FAO/IOC/IAEA (1984). Tissues were digested with concentrated nitric acid and perchloric acid (2:1 v/v) at 60°C for 3 days. After dilution, metal contents of tissue measured on a inductively coupled plasma atomic emission spectrometry (ICP-AES) (Varian model, Liberty Series II; Palo Alto, USA) and metal concentration in the tissue was presented as µg/g. For calibration ICP-AES was used as a High Purity Multi Standard.

The quality of data was checked against the analysis of standard reference material DORM-2

(National Research Council of Canada; dogfish muscle and liver MA-A-2/TM Fish Flesh) (Table I).

**Table I.- Observed and certified values of elemental concentrations as micrograms per gram wet weight in Standard reference materials DORM-2 from the Nationals Research Council, Canada ( $n = 2$ ).**

DORM-2	Certified values (µg/g)	Measured values	Recovery (%)
Cd	0.04±0.008	0.05±0.009	104
Cr	0.20±0.01	0.2±0.009	99
Cu	2.34±0.16	2.26±0.17	96
Fe	142±10	137±11	96
Mn	0.05±0.006	0.05±0.007	97
Pb	0.06±0.007	0.07±0.008	106
Zn	25.6±2.3	24.9±2.4	97

Differences between male and female short-finned squid tissues for any metal concentration were checked by “Data analyses” built in SPSS statistical package programs. Two-way analysis of variance tests with significance levels of 5% were conducted on each metal to test for significant differences between sexes. T test was used to compare data among tissues. Data showed mostly normal distribution or close to normal distribution and therefore, no transformation were done for statistical analyses. Data shown in different letters are significant at the 0.05 level.

## RESULTS

The mean elemental concentrations (µg/g) in the gill, digestive gland, arm and mantle of female and male *I. coindetii* samples collected from İskenderun Bay are given in Table II (male: 15.07±0.36 cm and 109.9±6.8 g; female: 18.26±0.42 cm and 142.29±4.84 g). Replicate analyses of reference materials showed good accuracy with recovery rates for metals between 96% and 106%. The results showed good agreement between the certified and the analytical values.

This investigations showed that different sexes contained different metal levels (Table III). Concentrations of Cd in the gill, digestive gland, arm and mantle tissues of male and female

**Table II.- Mean elemental composition ( $\pm$ SD) ( $\mu\text{g/g}$  wet weight) in gill, hepatopancreas, arm and mantle of female and male *I. coindetii* individuals.**

Metal	Sex	Gill	Arm	Mantle	Digestive gland
Cd	Female	1.23 $\pm$ 0.11 <sup>a,x</sup>	0.68 $\pm$ 0.06 <sup>a,x</sup>	1.35 $\pm$ 0.59 <sup>a,x</sup>	7.40 $\pm$ 1.05 <sup>b,x</sup>
	Male	1.38 $\pm$ 0.10 <sup>a,x</sup>	1.16 $\pm$ 0.48 <sup>a,y</sup>	0.90 $\pm$ 0.29 <sup>a,x</sup>	9.51 $\pm$ 1.02 <sup>b,y</sup>
Cr	Female	7.97 $\pm$ 1.22 <sup>a,x</sup>	0.62 $\pm$ 0.07 <sup>b,x</sup>	0.53 $\pm$ 0.06 <sup>b,x</sup>	0.48 $\pm$ 0.07 <sup>b,x</sup>
	Male	8.75 $\pm$ 2.03 <sup>a,x</sup>	0.61 $\pm$ 0.09 <sup>b,x</sup>	0.62 $\pm$ 0.06 <sup>b,x</sup>	0.72 $\pm$ 0.08 <sup>b,x</sup>
Cu	Female	83.25 $\pm$ 5.87 <sup>a,x</sup>	6.75 $\pm$ 0.51 <sup>b,x</sup>	13.18 $\pm$ 2.04 <sup>b,x</sup>	205.8 $\pm$ 35.5 <sup>c,x</sup>
	Male	81.28 $\pm$ 9.26 <sup>a,x</sup>	5.97 $\pm$ 0.52 <sup>b,x</sup>	13.14 $\pm$ 1.91 <sup>b,x</sup>	324.1 $\pm$ 43.2 <sup>c,y</sup>
Fe	Female	131.35 $\pm$ 11.20 <sup>a,x</sup>	7.23 $\pm$ 1.49 <sup>b,x</sup>	9.13 $\pm$ 1.35 <sup>b,x</sup>	67.7 $\pm$ 1.66 <sup>b,x</sup>
	Male	127.36 $\pm$ 11.42 <sup>a,y</sup>	7.56 $\pm$ 1.71 <sup>b,x</sup>	9.83 $\pm$ 1.89 <sup>b,x</sup>	86.6 $\pm$ 1.58 <sup>b,y</sup>
Mn	Female	45.28 $\pm$ 5.29 <sup>a,x</sup>	0.52 $\pm$ 0.09 <sup>b,x</sup>	1.33 $\pm$ 0.18 <sup>b,x</sup>	1.32 $\pm$ 0.22 <sup>b,x</sup>
	Male	52.46 $\pm$ 8.73 <sup>a,y</sup>	0.50 $\pm$ 0.05 <sup>b,x</sup>	1.74 $\pm$ 0.43 <sup>b,x</sup>	1.97 $\pm$ 0.45 <sup>b,y</sup>
Pb	Female	2.27 $\pm$ 0.30 <sup>a,x</sup>	1.88 $\pm$ 0.12 <sup>b,x</sup>	1.49 $\pm$ 0.07 <sup>b,x</sup>	1.77 $\pm$ 0.12 <sup>b,x</sup>
	Male	3.10 $\pm$ 0.22 <sup>a,y</sup>	1.82 $\pm$ 0.14 <sup>b,x</sup>	2.37 $\pm$ 0.58 <sup>b,y</sup>	1.68 $\pm$ 0.10 <sup>b,x</sup>
Zn	Female	21.70 $\pm$ 1.66 <sup>a,x</sup>	9.13 $\pm$ 0.50 <sup>b,x</sup>	14.28 $\pm$ 3.16 <sup>b,x</sup>	66.28 $\pm$ 7.42 <sup>c,x</sup>
	Male	20.12 $\pm$ 1.77 <sup>a,x</sup>	9.38 $\pm$ 0.46 <sup>b,x</sup>	11.52 $\pm$ 0.58 <sup>b,y</sup>	85.29 $\pm$ 7.30 <sup>c,y</sup>

Letters x and y show differences among sex; a, b and c among tissues.

Data shown with different letters are statistically significant at the differences  $p < 0.05$  level.

squid samples ranged between 0.68-9.51  $\mu\text{g/g}$ . The lower concentration of this metal was noted in the arm of female sample. Cd concentrations varied among arm and digestive gland tissues in male and female samples significantly ( $p < 0.05$ ). Changes in Cd concentrations between digestive gland tissues were higher than that of the other tissues (Tables II, III).

**Table III.- Heavy metal accumulation in gill, hepatopancreas, arm and mantle of female and male *I. coindetii* individuals.**

Metals	Female	Male
Cd	Digestive gland > Mantle $\geq$ Gill $\geq$ Arm	Digestive gland > Arm $\geq$ Gill $\geq$ Mantle
Cr	Gill > Arm $\geq$ Mantle $\geq$ Digestive gland	Gill > Digestive gland $\geq$ Mantle $\geq$ Arm
Cu	Digestive gland > Gill > Mantle $\geq$ Arm	Digestive gland > Gill > Mantle $\geq$ Arm
Fe	Gill > Digestive gland $\geq$ Mantle $\geq$ Arm	Gill > Digestive gland $\geq$ Mantle $\geq$ Arm
Mn	Gill > Mantle $\geq$ Digestive gland $\geq$ Arm	Gill > Digestive gland $\geq$ Mantle $\geq$ Arm
Pb	Gill > Arm $\geq$ Digestive gland $\geq$ Mantle	Gill > Mantle $\geq$ Arm $\geq$ Digestive gland
Zn	Digestive gland > Gill > Mantle $\geq$ Arm	Digestive gland > Gill > Mantle $\geq$ Arm

Cr concentrations in mantle tissues of males and females ranged between 0.48-8.75  $\mu\text{g/g}$ . The highest levels of Cr were detected in gill tissues. Cr

concentrations in tissues did not change between sexes significantly ( $p > 0.05$ ). The lowest Cr concentration was found in female digestive gland tissue (Tables II, III).

The highest value of Cu was found in digestive gland tissue (324.1  $\mu\text{g/g}$ ), while the lowest values were detected in the arm tissue (5.97  $\mu\text{g/g}$ ) of male samples (Table II). Significant variations of Cu concentrations were found in all tissues ( $p < 0.05$ ). Cu contents between sexes did not change in tissues of mantle, arm and gills, except in digestive gland tissue.

Some variations in Fe concentration occurred in gill tissues of *I. coindetii* ( $p < 0.05$ ) in both sexes. The highest values were found as 131.35 and 127.36  $\mu\text{g/g}$  in the gills of female and male, respectively, while the lowest values were in the arm and mantle tissues of all samples (Tables II, III). Fe concentrations changed in gill and digestive gland tissues significantly between sexes ( $p < 0.05$ ).

Significantly higher concentrations of Mn were detected in the gill of male and female samples compared to other tissues, whereas lower concentrations of this metal were noted in the arms of samples (Table III). Mn concentrations in the mantle of female and male samples were found 1.33 and 1.74  $\mu\text{g/g}$ , respectively (Table II).

There were no considerable differences in Pb

levels of arm and digestive gland tissues in the samples between sexes ( $p > 0.05$ ). The highest Pb concentrations in gill tissues of male samples were found  $3.10 \mu\text{g/g}$ , while the lowest  $1.49 \mu\text{g/g}$  found in mantle tissue of female *I. coindetii*. In mantle tissues these values varied between  $1.49$  and  $2.37 \mu\text{g/g}$  for female and male short-finned squid, respectively (Table II).

Significant variations of Zn concentrations were found in all tissues ( $p < 0.05$ ). Zn concentrations in the mantles of female and male specimens were found between  $14.28$ - $11.52 \mu\text{g/g}$  (Table II). Higher concentrations of Zn were observed in digestive gland and in the gill of samples, while lower values were detected in both arm and mantle tissues (Table III).

## DISCUSSION

Cephalopods are predominant in the tropic system and they are eaten by many oceanic animals, such as marine mammals and sea birds. Some studies on elemental bio-accumulation show that cephalopods accumulate high levels of trace elements, particularly cadmium (Cd) and copper (Cu) in different parts of the body *viz.*, muscle tissue, liver, digestive gland etc. (Miramand and Guary 1980; Smith *et al.*, 1984). Thus cephalopods represent important species for studying the transfer of heavy metals into marine food webs. Oehlenschlaeger (1989) frequently observed high concentrations of toxic heavy metals, especially cadmium and lead in squids (*Loligo* sp.) and squid products from India and Thailand. Hadj *et al.* (1986) have observed the total mercury content of fish, bivalves, shrimp and cuttlefish, which are very low and varying from  $29 \pm 14 \mu\text{g/g}$  to  $198 \pm 18 \mu\text{g/g}$  when compared to the tolerated concentrations fixed by FAO for ( $5000$ - $1000 \mu\text{g/g}$ ) mercury and is not dangerous for human health. Barska *et al.* (1988) have studied the most toxic lead, cadmium, zinc, copper, mercury concentrations in two species of squid: *Loligo patagonica* and *Illex argentinus*.

The present study showed that generally metal levels are higher in digestive gland and gill, while it is lower in arm and mantle for both sexes in short-finned squid *I. coindetii*. Our study suggested that *I. coindetii*, caught from İskenderun Bay, was

contaminated with Pb and Cd when this study was carried out. When studies already available were checked, it was observed that the highest accumulation of heavy metal occurred in digestive gland in squids as well as in all cephalopods (Bustamante *et al.* 2002a, 2006). Consequently, this organ appears to have a key function in the metabolism of cadmium in cephalopods (Miramand and Guary, 1980; Smith *et al.*, 1984; Finger and Smith, 1987; Miramand and Bentley, 1992; Bustamante *et al.*, 1998, 2002a). Very high levels of cadmium in the tissues of cephalopods would be expected to be toxic to the organisms unless efficient storage and detoxification mechanisms were available (Simkiss and Taylor, 1982; Phillips and Rainbow, 1989; Bustamante *et al.*, 2002b).

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). Bustamante *et al.* (2002) suggests that the major part of cadmium is associated with lysosomes and cytosolic proteins, which play a function key in the storage and detoxication of this element. Also the heterogeneity of cadmium concentrations among the different species of cephalopod molluscs (curled octopus,  $0.25 \mu\text{g/g}$ ; horned octopus,  $0.32 \mu\text{g/g}$ ; spider octopus,  $0.59 \mu\text{g/g}$ ; European squid,  $0.29 \mu\text{g/g}$ ; broadtail squid,  $0.18 \mu\text{g/g}$ ) is not an unexpected finding but reflects, not only their great taxonomic diversity, but also ecologic difference (Bustamante *et al.*, 1998; Storelli and Marcotrigiano, 1999; Craig and Overnell, 2003; Raimundo *et al.*, 2004; Storelli *et al.*, 2006).

This study showed that the levels of all metal concentrations found in males were generally higher than in female *I. coindetii*. It was observed that female short-finned squid were in their last maturity stage at the time of the study. It is known that female squids do not feed themselves during spawning period. Thus, it is natural that they include less heavy metal concentrations than males.

The metal concentrations in mantle tissue of *I. coindetii* from different parts of Mediterranean Sea and other sea were determined (Storelli and Marcotrigiano, 1999). These studies were carried out with only edible organs and with certain heavy metals. On the other hand it is not practical to

compare the studies carried out in different areas loaded with different heavy metal contaminants.

Therefore, our study was the first attempt investigating the metal concentrations in gill, digestive gland, arm and mantle of short-finned squid in İskenderun Bay and other parts of Turkish coasts. In addition, it would not be advisable to compare cephalopods in heavy metal accumulation ratio with other aquatic organisms having different metabolism rates and life cycle. Hence, though not done on the same species, a comparative study with cuttlefish already included in the same class as cephalopods having a similar rate of metabolism, life cycle and body form would be the best choice.

Duysak *et al.* (2013) reported the same metals accumulation levels in mantle tissue of *S. officinalis* caught from İskenderun Bay as: 1.73 to 2.39 (µg/g) Cd, 1.13 to 1.17 (µg/g) Cr, 11.4 to 12.3 (µg/g) Cu, 0.35 to 0.36 (µg/g) Fe, 0.97 (µg/g) Mn, 1.74 to 1.79 (µg/g) Pb, 7.60 to 8.18 (µg/g) Zn. Whole metal levels were similar to those obtained in our study except Fe. Fe concentrations were 30 times lower compared with our findings. This situation may have resulted from having the similar feeding pattern of both species. This difference in Fe concentration might have been caused by different factors such as different species of cephalopods and regional differences, sexual maturation stages, size of specimens and feeding habits.

In our study, metal levels in the mantle tissue of *I. coindetii* independent of sex were in the following order: Zn>Cu>Fe>Pb>Mn>Cd>Cr (Table III). Ayas and Ozogul (2011) reported this order as: Zn>Fe>Cu>Cd>Cr>Pb in the metal levels of *S. officinalis* in Mersin Bay, another part of the Northeastern Mediterranean. Ayas and Ozogul (2011) reported Cd, Cr, Pb, Cu, Zn and Fe levels of *S. officinalis* mantle tissue caught from Mersin Bay as with 2.34 to 3.89 (µg/g) Cd, 0.30 to 0.63 (µg/g) Cr, 0.15 to 0.54 (µg/g) Pb, 2.35 to 14.90 (µg/g) Cu, 23.22 to 51.88 (µg/g) Zn, 5.12 to 10.65 (µg/g) Fe. Cd and Zn levels reported by these researchers were found higher than in our study. Ayas and Ozogul (2011) mentioned that the mantle tissue of common cuttlefish, *S. officinalis*, caught from Mersin Bay, was contaminated with Cd and Zn in the seasons when that the study was carried out. Similarly, our

study showed that short-finned squid mantle tissue had the highest Pb concentration in İskenderun Bay during November 2011. These differences may also be related to the study area and different specimens and habitats.

Cephalopods are potentially an essential source of increased load of cadmium in human tissues. As a result these 'high-risk' consumer groups should be taken in to consideration when evaluating the implications of heavy metal contamination of cephalopod tissue for public health (Pierce *et al.*, 2008).

Anonymous (2005) regulates food contamination levels for the mantle tissue of cephalopods as 1 (µg/g) Cd, 1 (µg/g) Pb. The hygienic-sanitary aspect may be considered acceptable since total cadmium and lead content of these squid meat is higher than the limit prescribed by the rules in force and does not allow, on the basis of the consumption, the tolerable intakes recommended by the Joint FAO/WHO and Turkish Food Codex to be exceeded. In accordance with these limit values, the present study found out that short-finned squid mantle tissue had higher Pb and Cd concentrations in İskenderun Bay during November 2011. This study suggested that cephalopod species in İskenderun Bay are not recommended for human consumption. This situation may show that Mediterranean has been considerably affected from the sources of pollution. According to these results, detected heavy metal pollution threat these areas should be taking into consideration to protect the biodiversity and human health in this ecosystem.

This study also showed that, like the other cephalopod species, *I. coindetii* inhabiting the İskenderun Bay, could be used as a good indicator organism, for Cd and Pb accumulation studies, *I. coindetii* because of high metabolic activity speed and short life span.

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