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Metal Concentrations in Different Tissues of Cuttlefish (*Sepia officinalis*) in İskenderun Bay, Northeastern Mediterranean

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Abstract

Metal levels (Cd, Cr, Cu, Fe, Mn, Pb and Zn) in the gill, hepatopancreas, ovary, testis and mantle of male and female cuttlefish *Sepia officinalis* in İskenderun Bay were investigated. All elements differ in accumulation in tissues and sex significantly ($p<0.05$). Concentrations of Cd in the tissues of male and female cuttlefish samples ranged between 0.12-34.7 mg/kg. Cr levels in tissues of males and females ranged between 0.59-1.26 mg/kg. Cu concentrations in all tissues were found and ranged between 8.83 and 1296 mg/kg. Fe concentrations in the mantle of female and male samples were found 0.35 and 0.36 mg/kg, respectively. Pb concentrations in the mantles of female and male samples were found between 1.74-1.79 mg/kg. The highest Zn concentrations in hepatopancreas of male and female samples were found 181 mg/kg and 272 mg/kg respectively ($P<0.05$). In the mantle tissue of *S. officinalis*, metal levels independent of sex were in the following order: Cu>Zn>Cd>Pb>Cr>Mn>Fe. In accordance with limit values, the present study found out that the mantle tissue of cuttlefish, *S. officinalis,* was overlimited with Cd and Pb

Keywords: Cuttlefish, *Sepia officinalis*, sex, heavy metals, risk evaluation

İskenderun Körfezi'nden Avlanan Mürekkep Balığı (*Sepia officinalis*)'nın Farklı Dokularındaki Metal Konsantrasyonları

Özet

İskenderun Körfezi'nden avlanan dişi ve erkek mürekkep balıklarının (*S. officinalis*) solungaç, hepatopankreas, ovaryum, testis ve mantosundaki metal seviyeleri (Cd, Cr, Cu, Fe, Mn, Pb ve Zn) araştırılmıştır. İncelenen metaller, dokular ve cinsiyetler arasında önemli ölçüde farklılık göstermiştir ($p<0.05$). Dişi ve erkek örneklerin dokularındaki Cd içerikleri 0.59-1.26 mg/kg arasında değişmiştir. Cr seviyeleri ise 0.59-1.26 mg/kg arasında değişim göstermiştir. Erkek ve dişi bireylerin dokulardaki Cu konsantrasyonları 8.83 ve 1296 mg/kg arasında bulunmuştur. Dişi ve erkek bireylerin Fe konsantrasyonları sırasıyla 0.35 ve 0.36 mg/kg olarak bulunmuştur. Dişi ve erkek örneklerdeki Pb konsantrasyonları 1.74-1.79 mg/kg bulunmuştur. Dişi ve erkek bireylerin hepatopankreasındaki en yüksek Zn konsantrasyonları sırasıyla 181 mg/kg ve 272 mg/kg ($P<0.05$). *S. officinalis* bireylerinin manto dokusunda birikim gösteren ağır metal seviyeleri sırasıyla Cu>Zn>Cd>Pb>Cr>Mn>Fe olarak tespit edilmiştir. Mürekkep balığının manto dokusunda birikim gösteren Cd ve Pb değerleri limit değerlerinin üzerinde bulunmuştur.

Anahtar Kelimeler: Mürekkep balığı, *Sepia officinalis*, cinsiyet, ağır metal, risk değerlendirmesi.

Introduction

The cephalopods represent important economic seafood for human consumption and contribute to 14% of the world fisheries, according to FAO (2004). Cuttlefish, which has recently been captured in various amounts ranging from 11.000 to 15.000 tons annually throughout the world, occupies an important place among cephalopoda (FGIS, 2004). Highly preferred by consumers, common cuttlefish *Sepia officinalis* is distributed from the Baltic and northern sea in the east Atlantic to Africa, and the Mediterranean (Roper *et al*., 1984).

In the Turkish coasts of Mediterranean Sea, common cuttlefish is the most largely caught species of the cephalopods. According to Anonymous (2010) report, 1,502 and 1,258 tons of cuttlefish were caught in 2008 and 2009, respectively, in Turkey.

Cuttlefish have a short life span of around 2 years. The spawning season is from early spring to

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mid-summer followed by mass adult mortality, and hatching follows from mid-summer to autumn (Boletzky, 1983; Dunn, 1999). Previous studies of the spatial and temporal patterns of cuttlefish abundance show that there is an annual migration in this area, and large inter-annual fluctuations in landings (Boucaud-Camou and Boismery, 1991; Dunn, 1999; Denis and Robin, 2001). Given the short life span, large inter-annual fluctuations in landings, and the regular annual migration cycle, it is expected that marine environment conditions have an important impact on cuttlefish recruitment and distribution (Boletzky, 1983). During short life span, the growth rate of cuttlefish is very high. This exceptional growth rate can be explained in terms of their active metabolism owing to their carnivorous diet (Mangold, 1989).

Cephalopods are regarded as key species in many marine ecosystems. They represent an essential link in marine trophic chains and are eaten by many marine top predators, fish, birds and mammals (Clarke, 1996; Croxall and Prince, 1996; Klages, 1996; Smale, 1996, Bustamante *et al*., 2002).

The metal levels of cuttlefish (*S. officinalis*) in İskenderun Bay, Northeastern Mediterranean Sea has not been studied and there is currently no such literature available. However, there have been some studies on the elemental composition of cephalopod species in other Seas (Raimundo *et al*., 2004; Napoleão *et al*., 2005; Miramand *et al*., 2006; Miramand and Bentley, 1992; Miramand *et al*., 2006; Lourenço *et al*., 2009; Pereira *et al*., 2009; Ayas and Ozogul, 2011; Chouvelon *et al*., 2011). Their objectives were mostly related to environmental contamination and its use in biological monitoring. Most of these studies focus on amounts present in several organs such as digestive glands, branchial hearts and gills. These studies reported the major role of the digestive gland in the bioaccumulation mechanisms, this organ suspected to have a key function in the metabolism of many metals in cephalopods (Miramand and Bentley, 1992, Bustamante *et al*., 2002, 2004). Thus, the digestive gland of cephalopods constitutes the main storage organ for essential (Co, Cu, Fe, and Zn) and non essential elements (Ag, Cd, Pb, and V) independently of the considered species and of its area of origin (Miramand and Guary, 1980; Smith *et a*l., 1984; Finger and Smith, 1987; Miramand and Bentley, 1992, Bustamante *et al*., 1998, 2002, 2004, Miramand *et al*., 2006). However, the common cuttlefish *Sepia officinalis* makes long reproductive migrations in spring to mate and to spawn in coastal waters (Boucaud-Camou and Boismery, 1991). The eggs are laid in shallow water areas and are therefore potentially subjected to chronic and/or acute contaminations. In addition to the direct contamination from the environment, the cuttlefish egg could also possibly suffer from a second contamination pathway, viz. the transfer of metals from the gravid female during the prespawning period. Indeed, the somatic tissues of the gravid cuttlefish female are partly used to produce the eggs (Guerra and Castro, 1994) and a fraction of their contaminant burden may therefore be transferred to the eggs and then to offspring. Several studies have shown that essential metals (Peake *et al*., 2004) but also toxic elements (Sellin and Kolok, 2006) are maternally transferred to the eggs (Lacoue-Labarthe, 2008).

In this context, the aim of this study was to investigate the concentration of metals (Cd, Cr, Cu, Fe, Mn, Pb and Zn) in gill, mantle, hepatopancreas, ovary and testis of male and female cuttlefish captured from İskenderun Bay.

Materials and Methods

Sample Preperation

The samples were caught by fishermen on March 2011 from Iskenderun Bay, Eastern Mediterranean Sea. Specimens collected during the sampling period were immediately transported to the laboratory. In the laboratory, male and female cuttlefish was grouped in sex and size. Total cuttlefish length and weight were measured to the centimeter and gram before dissection (male: 13.84±0.16 cm and 165.24±7.86 g; female: 13.41±0.24 cm and 188.29±5.78 g). For analysis, whole ovary and testis, hepatopancreas, gills and mantle of each cuttlefish were dissected. We took advantage of this to choose 16 male and 14 female cuttlefish.

Digestion Procedures

This approach was modified from Tüzen (2003). A homogenized 1-2 g sample was placed in a 20 ml digestion tube, and 5 ml of high purity nitric acid (Merck) were added then the samples were heated to dissolve at 60˚C for 7 days. After digestion the samples were filtered through Whatman-Quantitative (No: 42, 110 mm £) filter paper. The digested portion was then diluted to a final volume of 20 ml. A blank digest was carried out in the same way. All metals were determined against aqueous standards. Digested samples were analyzed three replicates for each metal.

Analytical procedures

Determination of all metal concentrations was carried out by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Varian model, Liberty Series II; Palo Alto, USA) equipment located at Mustafa Kemal University. For the calibration of the ICP-AES as a High Purity Multi Standard was used. The used absorption lines are given in Table 1.

Metal concentrations were calculated mg kg^{-1} wet weight. The quality of data was checked by the analysis of standard reference material DORM-2

Table 1. Absorption line and detection limit of metals

Elements	Absorbsiyon line (nm)	Detection limit (ppm)
Cd	226.502	0.015
Cr	267.716	0.040
Cu	324.754	0.020
Fe	259.940	0.015
Mn	257.610	0.003
Pb	220.353	0.14
Zn	213.856	0.009

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

Statistical Analysis

To test the differences between the element concentrations in seasons, one way ANOVA was performed. The posthoc test (Tukey) was applied to determine statistically significant differences following ANOVA.

Results

The mean elemental concentrations (mg/kg wet weight) in the gill, testis, hepatopancreas, mantle and ovary of female and male *S. officinalis* are given in Table 3.

Concentrations of Cd in the gill, hepatopancreas, testis, ovary and mantle tissues of male and female cuttlefish samples ranged between 0.12-34.7 mg/kg. The lower concentration (0.12 mg/kg) of this metal was noted in the testis of male samples. Cd concentrations did not change among tissues in male and female samples significantly. Changes in Cd concentrations between hepatopancreas tissues were higher than that of the other tissues (Table 3).

Cr concentrations were given in the tissues of *S. officinalis* (Table 3). Cr concentrations in gill, hepatopancreas and mantle tissues were not changed between sexes significantly. Cr levels in tissues of males and females ranged between 0.59-1.26 mg/kg. The highest level of Cr was detected in mantle and gill tissues. The concentration of Cr in the mantle tissues of males and females were found 1.13-1.17 mg/kg, respectively.

Variations in Cu concentrations occurred in tissues of *S. Officinalis*. It observed that significant variations in all tissues of Cu concentrations were found and ranged between 8.83 and 1296 mg/kg. The highest value was found in the hepatopancreas of males, while the lowest values were detected in the mantle and testis of male samples (Table 3). Although Cu levels between mantle and testis of male were not different ($P>0.05$), hepatopancreas of male samples differ significantly. Gender difference was not affected the Cu content of mantle and gill. Cu contents among sexes were not changed in tissues of mantle and gills.

Higher concentrations of Fe were detected in the gill of male and female samples compared to other tissues, whereas lower concentrations of this metal were noted in the mantle of samples (Table 3). Fe concentrations in the mantle of female and male samples were found 0.35 and 0.36 mg/kg, respectively. Fe concentrations were not changed significantly in all tissues.

Some variations in Mn concentrations occurred in tissues of *S. officinalis* ($P<0.05$). The highest values were found as 3.56 and 4.88 mg/kg in the gills of male and females, respectively, while the lowest values were in the mantle of all samples (Table 3). Concentrations of Mn in mantle of male and female samples were found same as 0.97 mg/kg. Gender difference was not affected the Mn concentrations of mantle tissue except testis and ovary.

Pb concentrations in gill, hepatopancreas and mantle did not vary significantly between the sexes. Pb concentrations in the mantles of female and male specimen were found between 1.74-1.79 mg/kg (Table 3). Pb concentrations in tissues of female species did not vary significantly except in gills. Higher concentrations of Pb were observed in hepatopancreas and mantles of male samples, while lower values were detected tissues in testis and gill between 1.11-1.25 mg/kg, respectively.

There were no considerable differences in the Zn levels of mantle and gill in the samples of both sexes. However, Zn values in hepatopancreas were changed depending on the gender. Zn concentrations were not changed between tissues of male and female samples except hepatopancreas (Table 3). The highest Zn concentrations in hepatopancreas of male and female samples were found 181 mg/kg and 272 mg/kg respectively ($P<0.05$). Different samples ranged from 7.60 to 272 mg/kg for all tissues, while the same in mantle tissues were between 7.60-8.18 mg/kg (Table 3).

Discussion

The accumulation of all metal concentrations changed among tissues and sexes significantly. The relationship between metal accumulation and tissues found in this study may be due to the difference in metabolic activities between tissues. The various

Table 2. 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	0.043 ± 0.008	0.045 ± 0.009	104
Cr	0.200 ± 0.01	0.199 ± 0.009	99
Cu	2.34 ± 0.16	2.26 ± 0.17	96
Fe	142 ± 10	137 ± 11	96
Mn	0.050 ± 0.006	0.0485 ± 0.007	97
Pb	0.065 ± 0.007	0.069 ± 0.008	106
Zn	25.6 ± 2.3	24.9 ± 2.4	97

Table 3. Mean elemental composition (±standard deviation) (mg/kg wet weight) in gill, testis, hepatopancreas, mantle and ovary of male and female *S. officinalis* individuals

Metal	Sex	Gill	Testis	Hepatopancreas	Mantle	Ovary
Cd	Female	2.17±1.09^{a,x}	-	34.7±67^{b,x}	2.39±0.37^{a,x}	3.69±0.92^{a}
	Male	0.99±0.57^{a,y}	0.12±0.12^{a}	14.4±4.94^{b,x}	1.73±0.57^{a,y}	-
Cr	Female	0.78±0.48^{a,x}	-	0.59±0.29^{a,x}	1.17±0.19^{b,x}	1.13±0.25^{b}
	Male	1.26±1.88^{b,x}	0.37±0.25^{a}	0.72±0.24^{ab,x}	1.13±0.24^{b,x}	-
Cu	Female	349±383^{b,x}	-	999±270^{c,x}	11.4±19^{a,x}	34.5±25^{a}
	Male	237±225^{b,x}	8.83±3.53^{a}	1296±433^{c,y}	12.3±18^{a,x}	-
Fe	Female	155±144^{c,x}	-	79.5±18^{b,x}	0.35±1.53^{a,x}	3.56±2.71^{a}
	Male	150±201^{b,x}	3.27±4.08^{a}	70±24^{a,x}	0.36±1.40^{a,x}	-
Mn	Female	3.56±2.71^{b,x}	-	1.97±0.70^{a,x}	0.97±0.12^{a,x}	1.19±0.19^{a}
	Male	4.88±5.11^{b,x}	1.20±3.04^{a}	2.95±3.76^{ab,x}	0.97±0.13^{a,x}	-
Pb	Female	1.24±0.37^{a,x}	-	1.541±0.43^{b,x}	1.74±0.36^{b,x}	1.64±0.42^{b}
	Male	1.25±0.49^{a,x}	1.11±0.37^{a}	1.68±0.50^{b,x}	1.79±0.25^{b,x}	-
Zn	Female	9.29±2.03^{a,x}	-	181±55^{b,x}	7.60±0.79^{a,x}	14.0±2.70^{a}
	Male	8.47±1.92^{a,x}	17.7±2.66^{a}	272±98^{b,y}	8.18±1.05^{a,x}	-

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

factors such as season, length and weight, physical and chemical status of water can play a role in the tissue accumulation of metals. Seasonal changes of metal concentrations may result from factors such as growth cycle, reproductive cycle and changes in water temperature.

In the present study, the weight (0.12 mg/kg) of testes were much higher the weight (3.69 mg/kg) of ovaries. We think that is the mean reason for Cd concentrations which were higher in the ovaries of females when compared to male gonads.

The metal concentrations in gill and mantle of *S. officinalis* from Eastern Mediterranean Sea and other sea were determined (Lourenço *et al*., 2009; Ayas and Ozogul, 2011), but these studies were not carried out metal levels in hepatopancreas, ovary and testis of *S. officinalis*. Therefore, our study was the first attempt investigating the metal concentrations in hepatopancreas, ovary and testis of cuttlefish in İskenderun Bay.

In the mantle tissue of *S. officinalis*, metal levels independent of sex were in the following order: Cu>Zn>Cd>Pb>Cr>Mn>Fe (Table 3). 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. Our study was different in terms of the average metal concentrations and also orders. These differences may be related to the study area, Mersin and İskenderun Bay that having more contaminated by heavy metals.

Ayas and Ozogul (2011) noted Cd, Cr, Pb, Cu, Zn and Fe levels of *S. officinalis* mantle tissue caught from Mersin Bay as: 2.34 to 3.89 μg Cd g^{-1}, 0.30 to 0.63 μg Cr g^{-1}, 0.15 to 0.54 μg Pb g^{-1}, 2.35 to 14.90 μg Cu g^{-1}, 23.22 to 51.88 μg Zn g^{-1}, 5.12 to 10.65 μg Fe g^{-1}. Cd levels these reseachers reported were higher than those found in our study. Ayas and Ozogul (2011) reported that the present study found out that the mantle tissue of common cuttlefish, *S. officinalis,* which was caught from Mersin Bay, was contaminated with Cd in the seasons when that the study was carried out. In similarly, the our study found out that cuttlefish mantle tissue had the high Pb and Cd concentrations in İskenderun Bay during March 2011. *S. officinalis,* which was caught from İskenderun Bay, was contaminated with Pb and Cd when that the study was carried out.

Raimundo *et al*. (2005) reported Zn, Fe, Cu, and Cd levels of *S. officinalis* mantle tissue caught from Portuguese coast as: 62.0 to 113.0 μg g^{-1}, 5.4 to 40.0 μg g^{-1}, 3.3 to 19 μg g^{-1}, 0.03 to 0.81 μg g^{-1}. Fe, Cu and Zn levels they reported were higher than those found in our study although Cd levels were lower. Pb and Cr values were lower than that of the our results. This differences might have been caused by the regional differences.

There is some legislation regulating the maximum concentrations of trace metals. The maximum Cr level was reported as 1.0 mg kg^{-1} by FAO, although FAO limits for Cd and Pb were 0.5 mg $kg^{-1,}$ and for Cu and Zn were 30 mg $kg^{-1.}$ According to the Turkish Food Codex, the tolerance levels in seafood are 0.1 mg kg^{-1} for Cd, 1 mg kg^{-1} for Pb, 20 mg kg^{-1} for Cu and 50 mg kg^{-1} for Zn (all expressed as wet mass). There is no information about maximum permissible Mn and Fe concentrations in the Turkish standards and FAO. The FAO/WHO reported that the Provisional Permissible Tolerable Weekly Intake (PTWI) of Cd and Pb was 7 and 25 μg kg^{-1} body weight $week^{-1}$, respectively. In accordance with these limit values, the present study found out that cuttlefish mantle tissue had very high Pb and Cd concentrations in İskenderun Bay during March 2011. In the present study, Cu and Zn levels in mantle were found lower than permissible limits reported by FAO and Turkish Food Codex. Cr level was found 1.0 mg kg^{-1} that permissible limit. However, Cd and Pb values were found higher than permissible limits. From the human health point of view, the Pb and Cd value in the mantle of *S. officinalis* may also be considered as an important problem for human safety.

This study also permitted to initiate a data basis for the *S. officinalis* inhabiting at the İskenderun Bay, in what concerns trace element contents in metabolic organs, reproductive organs and mantle, the tissue that is relevant as a food resource. In Pb and Cd accumulation studies, *S. officinalis* could be used as a good indicator organism due to its short life span and also rapid growth rate.

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