Iranian Journal of Fisheries Sciences 17(2) 267-287 2018
DOI: 10.22092/IJFS.2018.115794

The relationships between heavy metals (As, Cd, Cu, Zn, Pb, Hg, Ni) levels and the size of pharaoh cuttlefish (*Sepia pharaonis*) from Persian Gulf

Roomiani L.[1*]; Jamili Sh.[2]; Askary Sary A.[1] ; Ahmadi S.[1]

Received: March 2016 Accepted: August 2016

Abstract

Concentrations of both essential (Zn and Cu) and non- essential (As, Cd, Pb, Hg, Ni) metals were measured in the organs (mantle, branchial hearts, gill and digestive gland) of *Sepia pharaonis* captured in the coastal waters of Bushehr Province in the Persian Gulf (Iran). The relationships between *S. pharaonis* size (mantle length) and metal concentrations in tissues were investigated by linear regression analysis. Metal concentrations (As µg g^{-1} d.w.) was highest in the digestive gland and lowest in the mantle of *S. pharaonis*, except for Ni. The concentrations of metals were found to follow the order: Zn> Cu>Pb> Ni>Hg> Cd> As. The mean Zn concentrations ranged from 37.72± 5.32 to 9.32± 3.73 µg g^{-1} d.w, whereas the mean As concentrations ranged from 0.01± 0.007 to 0.06± 0.05 µg g^{-1} d.w, respectively. Results of linear regression analysis showed that significant relationships between metal concentrations and fish size were positive, except for cadmium concentrations in the gills. The digestive gland of *S. pharaonis* would seem to constitute a good potential indicator of heavy metal concentrations in the marine environmental. Results from this study revealed that except for Pb the concentration of metals in *S. pharaonis* samples were below the threshold values As recommended by the FAO and EC guidelines.

Keywords: *Sepia pharaonis*, Digestive gland, Heavy metals, Persian Gulf

1-Department of Fisheries, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.
2-Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization, Tehran, Iran.
*Corresponding author's E-mail: L.roomiani@iauahvaz.ac.ir

Introduction

Mollusk cephalopods are known for their importance in trophic marine ecosystems both As predators and prey. The economic importance of their fisheries has recently grown worldwide to compensate for finfish stock depletion (Le Pabica *et al*., 2015).The catch of cephalopods showed an increase of up to 14.1 % in the year 2012, giving a specific total landing of 5.9% for each group (Department of Fisheries, 2012). Cephalopods not only consume various organisms that are well known for their bioaccumulation capacity for both essential and non-essential metals, but they also constitute a major food source for several fish and cetacean species, therefore, they may be important vectors for metal transfer along aquatic food chains (Pereira *et al*., 2009).

The pharaoh cuttlefish *Sepia pharaonis* lives offshore during the winter season and makes long reproductive migrations in spring to mate and to spawn in coastal waters. The eggs are laid in shallow water areas and are therefore potentially subjected to chronic and or acute contamination (Reid *et al*., 2005). During their 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 (Duysak *et al*., 2013). Despite such a short life cycle, the strong capability of cuttlefish to concentrate a large number of metals in their tissues as well as the major role of the digestive gland in the bioaccumulation processes have been previously shown (Danis *et al*., 2005; Miramand *et al*., 2006; Lacoue-Labarthe *et al*., 2009).This capability seems to be shared by many other species of cephalopods, including Octopodide, Teuthoidea and Nautilidae. High accumulation of trace elements associated with the short life span led several authors to consider cephalopod species As potential indicators of environmental contamination. Several studies have reported high metals concentrations in tissues of cephalopod species (Bustamante *et al*., 2004a; Seixas *et al*., 2005 a,b; Raimundo *et al*., 2008; Raimundo *et al*., 2014; Le Pabica *et al*., 2015).

Heavy metals are considered a major anthropogenic contaminant in coastal and marine environments worldwide. They pose a serious threat to human health, living organisms and natural ecosystems because of their toxicity, persistence and bioaccumulation characteristics. Heavy metals can contribute to degradation of marine ecosystems by reducing species diversity and abundance and through accumulation of metals in living organisms and food chains. Anthropogenically, heavy metals can be introduced to coastal and marine environments through a variety of sources, including industries, wastewaters and domestic effluents (Fu and Wang, 2011).

Cuttlefish, *S. pharaonis*, of the Persian Gulf have not been examined to our knowledge on the relationships

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

between tissue metal concentrations and cuttlefish size. Thus, the aim of this study is to determine heavy metal (Cd, As, Ni, Zn, Cu, Hg, Pb) levels in mantle, gill, digestive gland and branchial hearts tissues of *S. pharaonis* from the north-west Persian Gulf, to evaluate the metal distribution into various internal and external organs and to investigate the relationships between fish size (length) and metal concentrations in the tissues.

Materials and methods

Sample collection and pretreatment

Sediment samples were collected in coastal water of Bushehr Province, south of Iran. All sediment samples were stored in air- tight plastic bags immediately after sampling, and were kept at 4 ºC in an ice box before reaching the laboratory for further treatments.

In the laboratory, sediment were weighted (wet weight) using a top-loading balance, wrapped in aluminum foil and frozen at -20 ºC overnight before freeze-drying. Freeze-dried sediments were weighted again (dry weight), sieved (63µm) and wrapped in aluminum foil and stored in a desiccator before determination of heavy metals. Approximately 0.25 g of freeze-dried and homogenized sediment sample was weighed and pre- digested overnight with a mixture of acids [9ml: 3mL: 1mL of concentrated nitric acid (69%), hydrofluoric acid (48%) and hydrochloric acid (37%)]. For digestion, triplicates of blank (pure solvents), and standard reference materials (National Institute of Standards and Technology: Standard Reference Materials 1944- New York Jersey waterway sediment for sediment sample) were used for checking the veracity of data (Kwok *et al*., 2014).

40 specimens of *S. pharaonic* were collected in July 2015 from commercial catches landed in the coastal waters of Bushehr. All samples were kept frozen by storing them in an ice box, and transported to the laboratory as soon as possible. In the laboratory (after measuring the weight and length of each species), branchial hearts, gill, digestive gland and mantle were totally removed under partially defrost condition. The selection of key organs in the present study was carried out on the basis of their structural and functional properties in uptake, distribution, biotransformation, storage and elimination. The samples were then rinsed with purified water to remove foreign particles and patted dry with paper towels.

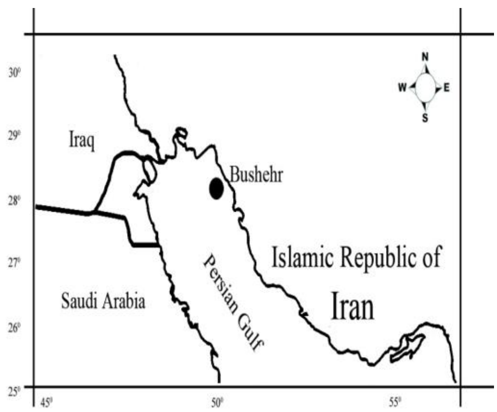

Figure 1: Location of the sampling site of *Sepia pharaonis* in the Persian Gulf.

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

Acid digestion and metal quantifications

After separation, individual tissue samples were freeze-dried, ground and homogenized for the analysis of heavy metals. The procedure for the extraction of heavy metals was based on standard methods 3052 (Microwave-assisted total heavy metal digestion) (USEPA, 1996). TORT1 (Lobster hepatopancreas reference material for trace metals) (National Research Conuncil, Canada) was used As Standard Reference Material for the digestion of cuttlefish, to verify the accuracy of metal determination. The recovery rates 90%± 10% (Table 1).

200 mg of the dry tissue was digested with a mixture of 4mL of HNO_3 (65% v/v) and 2 mL of H_2O_2 (30% v/v) at 60 ºC for 12 h (5mL acid mix/1g dry weight tissue), at 80 ºC for 1 h, and at 100 ºC for 1 h. All lab ware was cleaned with HNO_3 and HCl and rinsed with Milli- Q water. Concentrations of Zn, Cu, Cd, Ni, As and Pb were determined either by flame atomic absorption spectrometry (Perkin Elmer A Analyst 100) (Table 2) or electrothermal atomic absorption (Perkin Elmer Zeeman 4110ZL) depending on the metal concentration.

The total Hg concentration was determined by analyzing Hg directly in the powder obtained from the tissue with an Advanced Mercury Analyzer (ALTEC AMA 254) on aliquots ranging from approximately 10 mg of dry sample weighed to the nearest 0.01 mg. Hg determination involved evaporation of the metal by progressive heating until 800 ºC was reached, the sample was then held under oxygen atmosphere for 3 min, and subsequently amalgamated in a gold-net. Afterward, the net was heated to liberate the collected Hg, which was then measured by atomic absorption spectrophotometry (Raimundo *et al*., 2014).

The accuracy of these analytical methods was assessed by the analysis of international certificate standards. Measured and certified values did not differ significantly (p<0.05) (Table 1).

All concentrations are given as ranges and medians expressed as microgram per gram of dry weight of tissue. Bioaccumulation factor (BAF) was calculated according to Kwok *et al*. (2014). BAFs higher than 100 percent indicate bioaccumulation of the contaminant in the sample.

Table 1: Observed and certified values of elemental concentrations as micrograms per gram dry weight in Standard reference materials DORM-2 from the Nationals Research Council, Canada.

Element	Certified value (μg g^{-1})	Obtained value (μg g^{-1})	Recovery %
As	6.70± 0.66	6.99± 0.092	101
Cu	2.34± 0.16	2.25± 0.18	96
Zn	25.60± 2.30	24.80± 2.20	97
Cd	0.043± 0.008	0.041± 0.006	102
Ni	1.40± 0.30	1.30± 0.20	97
Pb	0.065±0.008	0.069± 0.007	105
Hg	4.60± 0.26	4.20± 0.20	100

Table 2: The operating parameters for working elements for flame absorption spectrometer.

Element	Wave length (nm)	Slit width	Lamp current (mA)
Zn	324.8	0.7	15
Cu	324.8	0.7	15
Pb	283.3	0.7	15
Ni	232.0	0.2	30
Cd	228.8	0.7	8
As	240.7	0.2	30

Statistical analysis

Statistical analysis of data was carried out using SPSS statistical package programs. Data used here were plotted on graph to see their distributions. Data showed mostly normal distribution or close to normal distribution and therefore, no transformation was done for statistical analyses. The linear regression analyses were applied to data to compare the relationships between size and heavy metal concentrations in the tissues. Correlations between elemental concentrations in tissue were calculated using Spearman correlation. Residual values were evaluated for independence by means of the Durbin-Watson test, and the significance used for statistical analyses was $p<0.05$.

Results

The mean heavy metals in the sediments of Bushehr Province were: Zn (50.23±2.42), Cu (13.11±0.66), Cd (0.78±0.05), Pb (2.02±0.21), Ni (50.26±1.88), Hg (1.35±0.11) and As (0.20± 0.08) µg g^{-1} d.w.

The weight and total length of cuttlefish ranged from 130- 3350 g and 95- 365 cm, respectively. Table 3 shows mean metal concentrations and their standard deviation in the tissues of *S. pharaonis*.

Mean concentrations of metals varied as follows: **As**: 0.06 µg g^{-1} d.w.in gill, 0.02 µg g^{-1} d.w. in branchial hearts, 0.08 µg g^{-1} d.w. in digestive gland and 0.01µg g^{-1} d.w. in mantle; **Cd**:0.08 µg/d.w. in gill, 0.06 µg g^{-1} d.w. in branchial hearts; 0.98 µg g^{-1} d.w. in digestive gland and 0.02 µg g^{-1} d.w. in mantle; **Cu**: 6.95 µg g^{-1} d.w. in gill, 2.16 µg g^{-1} d.w. in branchial hearts, 9.01 µg g^{-1} d.w. in digestive gland and 1.34 µg g^{-1} d.w. in mantle; **Zn**: 28.86 µg g^{-1} d.w. in gill, 14.21 µg g^{-1} d.w. in branchial hearts, 37.72 µg g^{-1} d.w. in digestive gland and 9.32 µg g^{-1} d.w. in mantle, **Pb**: 1.10 µg g^{-1} d.w. in gill, 0.64 µg/g d.w. in branchial hearts, 3.67 µg g^{-1} d.w. in digestive gland and 0.52 µg/ g d.w. in mantle; **Hg**: 0.25 µgg^{-1} d.w. in gill, 0.10 µg g^{-1} d.w. in branchial hearts, 0.33 µg g^{-1} d.w. in digestive gland and 0.08 µg g^{-1} d.w. in mantle; **Ni**: 0.24 µg g^{-1} d.w. in gill, 0.29 µg g^{-1} d.w. in branchial hearts, 0.29 µg g^{-1} d.w. in digestive gland and 0.27 µg g^{-1} d.w. in mantle.

Regardless of being essential or non-essential metals, their concentrations in the digestive gland showed significant variations. The highest metal concentrations were found in the digestive gland, while the lowest concentrations were found in the mantle of *S. pharaonis*. Zinc concentrations in

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

the organs were much higher than the concentrations of other metals, whereas arsenic concentrations in tissues were lower than other metals. The mean Zn concentrations ranged from 37.72± 5.32 to 9.32±3.73 µg g^{-1} d.w, whereas the mean As concentrations ranged from 0.01±0.007 to 0.06±0.05 µg g^{-1} d.w, respectively (Table 3).

Results showed significantly higher concentrations in the digestive gland, gill and branchial hearts compared to the mantle. All differences were statistically valid. Availability of metals was established as the following ranking: Zn> Cu>Pb> Ni> Hg> Cd>As in the tissues of cuttlefish. In general, metal distribution in cuttlefish tissues followed the pattern: Digestive gland>gill>branchial hearts> mantle, except for Ni (digestive gland>branchial hearts>mantle>gill).

The relationships between metal concentrations and cuttlefish size are shown in Table 3. In the *S. pharaonis*, mean concentrations of metals in gill, branchial hearts, digestive gland and mantle tissues ranged from (0.06 to 28.86 µg g^{-1} d.w.), (0.10 to 14.21 µg g^{-1} d.w.), (0.08 to 37.72 µg g^{-1} d.w.) and (0.01 to 9.32 µg g^{-1} d.w.), respectively (Table 3).

Significant positive relationships were found between length and metal levels in the tissues of cuttlefish. A strong positive linear effect of size on Pb (R=94.55%) and Ni (R=94.46%) concentrations was detected in the branchial hearts. A linear positive correlation was detected between Zn levels and the body size in the branchial hearts (R=94.29%) and digestive gland (R=94.24%), respectively. Significant differences were seen between length and Cu levels in tissues of cuttlefish (p<0.05). Results did not show any significant relationship between length and cadmium levels in the branchial hearts (R=7.57%), but there were significant differences between metal concentrations in the digestive gland with other organs (p<0.001). Nickel was accumulated at the same concentrations with no significant differences between the gills and branchial hearts (p>0.001) and the mantle and digestive gland (p>0.01). Significant correlations were found between As in all tissues resulting in R values ranging from 70.99% (p<0.001) to 85.02% (p<0.001). Results showed a strong positive relationship between length and Hg levels in the mantle (R=94.53% and p<0.001).

The independence of residuals was confirmed by the Durbin- Watson test.

Figs. 2 to 8 shows the median, the percentiles 25th and 75th, minimum and maximum concentrations of As, Cd, Cu, Zn, Ni, Hg and Pb in the digestive gland, gill, branchial gland and mantle of *S. pharaonis* captured in the Persian Gulf. Outliers and extreme points were not plotted to better visualize differences among tissues within smaller scales. Enhanced levels were observed in digestive gland in comparison to mantle, gill and branchial hearts. Zinc and copper were the most abundant elements. Although

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

being considered as essential elements to organisms, concentration medians in the digestive gland ranged in order of magnitude for Zn (28- 45 µg g^{-1}) and two for Cu (6-16µg g^{-1}). Narrower intervals were observed in branchial hearts (12-18 µg g^{-1} for Zn and 1.20-3.5 µg g^{-1}). Partitioning among the four tissues varied with the element, medians decreasing from:0.01µg g^{-1} (As) to 5.00 µg g^{-1} (Pb) in digestive gland, 0.01µg g^{-1} (As) to 2.00µg g^{-1} (Pb) in gill, 0.01 µg g^{-1} (As) to 0.90µg g^{-1} (Pb) in branchial hearts and 0.01µg g^{-1} (As) to 1.80µg g^{-1} (Pb) in mantle. BAFs of metals are shown in Table 4. The BAFs for cuttlefish ranged between 0.47 and 181.68. BAF_S of Cd and Pb (> 100 %) were observed in the digestive gland of *S. pharaonis* (125.64 and 181.68%). BAFs of metals in tissues of *S. pharaoins* follow this pattern: digestive gland>gill>branchial hearts > mantle, except for Ni: digestive gland= branchial hearts > mantle>gill.

Table 3: The relationships between heavy metal concentrations and fish length in the tissues of the cuttlefish (*Sepia pharaonis*).

Metal	T[1]	Mean± SD	Equation[2]	df	R-sq	P[3]	DW[4]	G[5]
As	G	0.0617±0.052	Y= -0.0683 + 0.000603 (X)	38	70.99%	*	0.48	a
	Bh	0.0233±0.013	Y = -0.01592 + 0.000174 (X)	35	75.15%	*	1.10	b
	Dg	0.0841±0.053	Y = -0.0614 + 0.000675(X)	38	85.02%	*	1.42	a
	Ma	0.0181±0.007	Y = -0.01923 + 0.000147 (X)	26	74.34%	*	1.41	b
Cd	G	0.0864± 0.066	Y = -0.0795 + 0.000770 (X)	38	71.29%	*	0.42	b
	Bh	0.0626± 0.084	Y = -0.0055 + 0.000316 (X)	38	7.57%	NS	2.06	b
	Dg	0.9859± 0.357	Y = -0.0039 + 0.004591 (X)	38	88.92%	*	0.43	a
	Ma	0.02± 0.007	Y = -0.01315 + 0.000132 (X)	28	78.64%	*	1.29	b
Cu	G	6.951± 2.440	Y = 0.511 + 0.02987 (X)	38	80.82%	*	0.62	b
	Bh	2.162± 0.597	Y = 0.555 + 0.007455 (X)	38	84.12%	*	0.65	c
	Dg	9.013± 2.469	Y = 2.139 + 0.03188 (X)	38	89.95%	*	0.61	a
	Ma	1.345± 0.512	Y = -0.0835 + 0.006627 (X)	38	90.31%	*	0.71	c
Zn	G	28.86± 6.570	Y = 10.90 + 0.08328 (X)	38	86.79%	*	0.49	b
	Bh	14.215± 1.101	Y = 11.078 + 0.014553 (X)	38	94.29%	*	1.32	c
	Dg	37.728± 5.321	Y = 22.567 + 0.07032 (X)	36	94.24%	*	0.62	a
	Ma	9.323± 3.739	Y = -0.166 + 0.04401 (X)	38	74.75%	**	0.17	d
Pb	G	1.1021± 0.590	Y = -0.551 + 0.007665 (X)	38	88.08%	*	1.98	b
	Bh	0.645± 0.151	Y = 0.2114 + 0.002009 (X)	38	94.55%	*	0.89	c
	Dg	3.672± 1.012	Y = 0.861 + 0.013038 (X)	38	89.50%	*	0.59	a
	Ma	0.52± 0.489	Y = -0.536 + 0.004898 (X)	38	54.03%	*	1.87	c
Hg	G	0.2597± 0.112	Y = -0.0493 + 0.001433 (X)	38	87.52%	*	1.08	b
	Bh	0.10974± 0.048	Y = -0.02136 + 0.000608 (X)	38	85.84%	*	0.44	c
	Dg	0.3308± 0.123	Y = -0.0061 + 0.001563 (X)	38	86.30%	*	0.63	a
	Ma	0.08333± 0.049	Y = -0.05730 + 0.000652 (X)	38	94.53%	*	0.77	c
Ni	G	0.2469± 0.125	Y = -0.0930 + 0.001576 (X)	38	85.60%	*	1.85	a
	Bh	0.2936± 0.135	Y = -0.0929 + 0.001792 (X)	38	94.46%	*	0.50	a
	Dg	0.2955± 0.124	Y = -0.0154 + 0.001425 (X)	37	68.62%	**	0.97	a
	Ma	0.2759± 0.229	Y = -0.3070 + 0.002703 (X)	38	74.66%	**	0.15	a

1- Tissue: G=Gill, Bh=Branchial hearts, Dg=Digestive gland, Ma=Mantle. 2- In the equations, Y is metal concentration (µg/ g d.w) and X is total length (mm). 3- *p*-value= * p<0.001, ** p<0.01, NS: Not Significant p>0.05. 4- Durbin-Watson test statistic. 5- Grouping Information Using the Tukey Method and 95% Confidence.

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

Table 4: Bioaccumulation factors heavy metals in the different tissues of *Sepia pharaonis*.

BAF_S	As	Cd	Cu	Zn	Pb	Hg	Ni
Gill	30.00	10.25	53.01	57.45	54.45	18.51	0.47
Branchial hearts	10.00	7.69	16.47	28.28	31.68	7.40	0.57
Digestive gland	40.00	125.64	68.72	75.09	181.68	24.44	0.57
Mantle	5.00	2.56	10.22	18.55	25.74	5.92	0.53

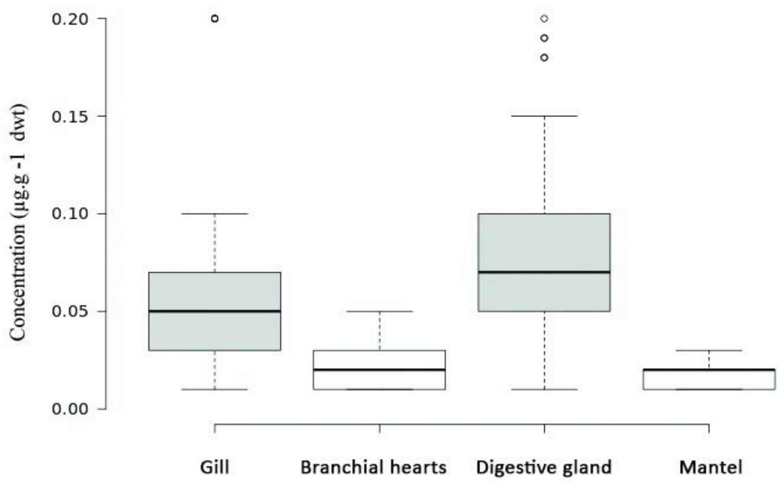

Figure 2: Median, 25% and 75% percentile, minimum and maximum of As concentrations (µg g^{-1} d.w.) in the tissues of *Sepia pharaonis*.

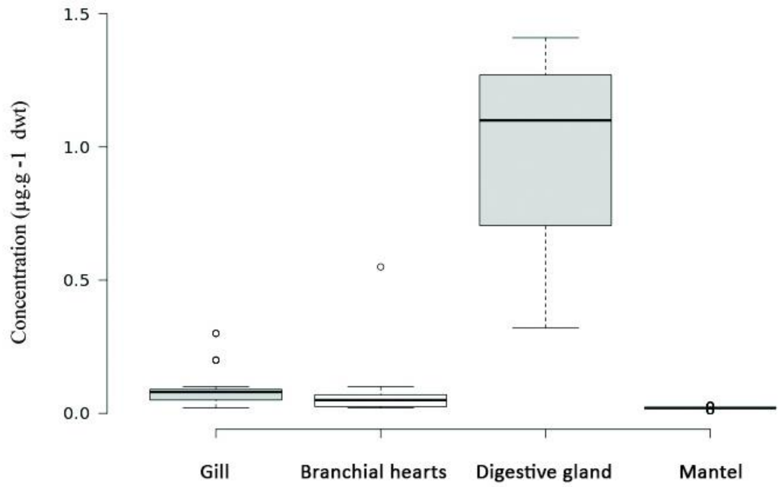

Figure 3: Median, 25% and 75% percentile, minimum and maximum of Cd concentrations (µg g^{-1} d.w.) in the tissues of *Sepia pharaonis*.

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

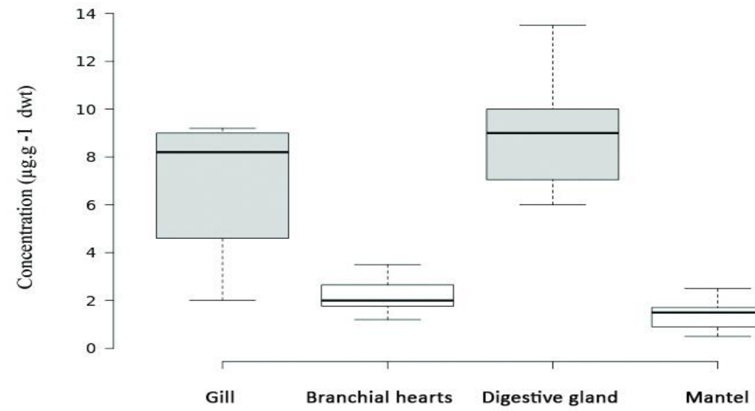

Figure 4: Median, 25% and 75% percentile, minimum and maximum of Cu concentrations (µg g^{-1} d.w.) in the tissues of *Sepia pharaonis*.

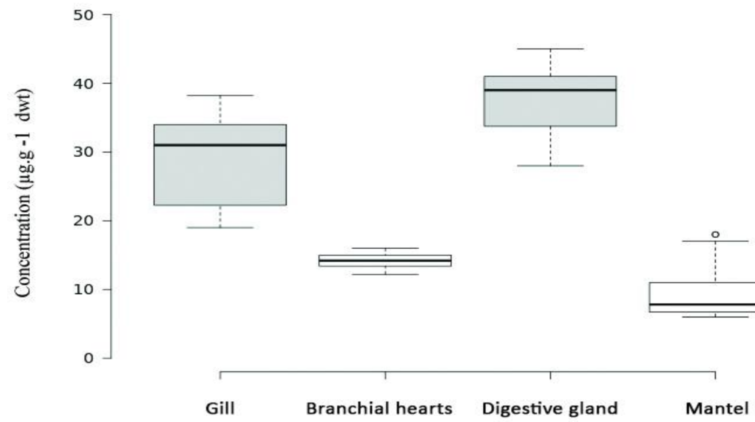

Figure 5: Median, 25% and 75% percentile, minimum and maximum of Zn concentrations (µg g^{-1} d.w.) in the tissues of *Sepia pharaonis*.

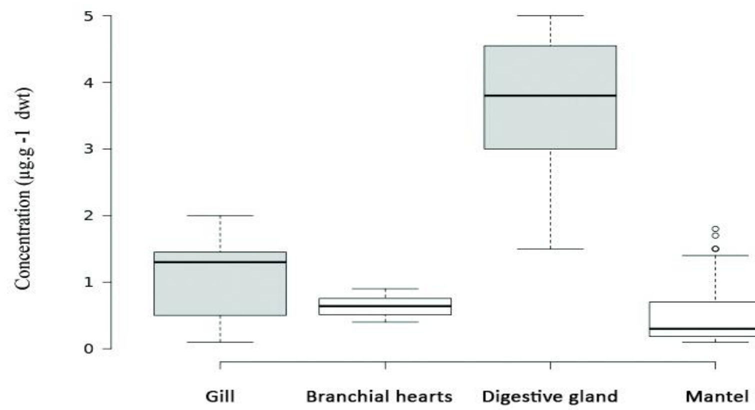

Figure 6: Median, 25% and 75% percentile, minimum and maximum of Pb concentrations (µg g^{-1} d.w.) in the tissues of *Sepia pharaonis*.

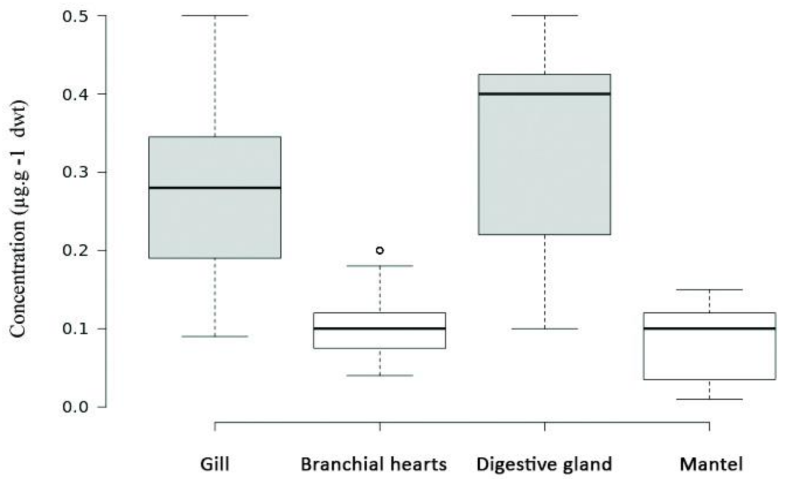

Figure 7: Median, 25% and 75% percentile, minimum and maximum of Hg concentrations (µg g^{-1} d.w.) in the tissues of *Sepia pharaonis*.

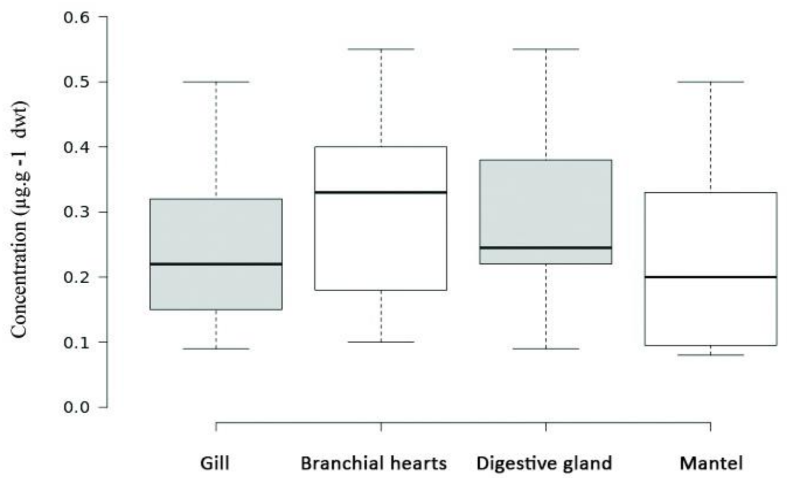

Figure 8: Median, 25% and 75% percentile, minimum and maximum of Ni concentrations (µg g^{-1} d.w.) in the tissues of *Sepia pharaonis*.

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

Discussion

Among pollutants that can accumulate in aquatic organisms, metals are of great interest since they can induce oxidative stress due to their redox potential and persistence in the environment (Semedo *et al*., 2012). Despite the limited number of specimens, to the best of our knowledge, this is the first attempt to assess the effect of biological parameters on trace element bioaccumulation in *S. pharaonis* of the Persian Gulf. Therefore, comparison to previous works can only be done with other species.

Many studies highlight that in cephalopods, the digestive gland has a central role in the bioaccumulation of trace elements particularly for toxic metals such as Ag and Cd (Ichihashi *et al*., 2001; Bustamante *et al*., 2004a; Rjeibi *et al*., 2014). The partly similar element composition observed for males and females is in line with our findings for the squids *Todarodes filippovae* and *Illexar gentinus* (Gerpe *et al*., 2000; Kojadinovica *et al*., 2011). The digestive gland was the preferential organ of the cuttlefish for the accumulation of As, Cd, Cu, Zn, Pb, Hg and Ni, reported for *Architeuthis dux* and *T. filippovae* (Bustamante *et al*., 2008; Kojadinovica *et al*., 2011). High retention of potentially toxic elements is admittedly associated with detoxification mechanisms existing in the digestive gland, like metallothioneins and high molecular weight proteins. Our study revealed higher concentrations of metals in the digestive gland than other organs of *S. pharaonis*. Mean concentrations of essential and non-essential metals in the gill, digestive gland, mantle and branchial hearts of cuttlefish showed great variations. Statistical comparisons revealed that metal concentrations were significantly different in each tissue of cuttlefish. Previous data from the different studies also showed that cephalopod species contained strikingly different metal levels in their tissues (Jinadasa, 2014; Duysak and Dural, 2015; Rjeibi *et al*., 2015). Rjeibi *et al*. (2015) showed that the amount of Cd and Hg in cephalopod organisms would reflect the environmental levels of these metals in the ocean. The Cd content for all studied species was in the range of 0.001 to 0.20 µg g^{-1} and 0.006 to 0.27 µg g^{-1} in the arms and mantle, respectively. The median Cd content for the cuttlefish in this study was higher than that found in a study by Rjeibi *et al*. (2015). This may be related to the differences in ecological needs, swimming behaviors and the metabolic activities among different cephalopod species.

In cephalopods, the digestive gland consists of 6 to 10% of the total body of the total body weight, and has a major physiological function in the digestive process: it supplies most of the digestive enzymes, plays an important role in digestive gland absorption, is a storage site for essential nutrients, and finally excretes part of the digestive residues (Bustamante *et al*., 2008). In our study, in contrast to the digestive gland, the concentrations measured in the mantle of cuttlefish are low. The mantle is not exposed to metal absorption phenomena by direct contact with seawater, unlike other mollusks. The digestive gland is well- known for its role in storage and detoxification processes (Bustamante *et al*., 2002; Storelli *et al*., 2006).

The branchial hearts are considered as excretion tissues, allowing the depuration and/ or the storage of various elements (Kojadinovica *et al*., 2011). Consequently, in most cephalopod species, these two tissues (digestive gland and branchial hearts) contain the highest concentrations of many trace elements (Miramand and Fowler, 1998). The tendency of metals

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

concentrations in digestive gland with length indicates that cuttlefish had not successfully detoxified the non-essential metals. The enhanced levels of metals in the digestive gland may be related to various factors because this is the most operative function containing cell membrane, intact cells, nuclei, granules and other cellular components of unknown function (Villanueva and Bustamante, 2006). Molluscs are known to have a number of sub-cellular systems for accumulation, regulation and immobilizing of metals during phases of excess. The partition of metals in these sub- cellular fractions is related to the fact that storage takes place in compartments that are particularly rich in, or capable of synthesizing relative large quantities of metal- binding ligands, although the metal partitioning between soluble and insoluble fractions of the digestive gland varies with the cephalopod species (Storelli *et al*., 2010). Zn and Cu are the essential elements, and Zn was the most abundant heavy metal followed by Cu in tissues of cuttlefish according to the results in the present study. Pb, Hg, Cd, As and Ni as the non- essential elements had relatively lower concentrations than the essential ones.

Zn and Cu are cofactors in many enzyme systems present in the digestive gland and their involvement in many metabolic processes can explain the higher concentrations measured of these metals in comparison to non- essential elements Ni, Pb, Cd, Hg and As (Rjeibi *et al*., 2014). The involvement of these elements in a number of metabolic functions, such as in metal- dependent enzymes, may explain their high concentrations. However, enrichment at certain levels of the food web may result in broader concentration intervals in the digestive gland of cephalopods. An element of interest of cephalopods Cu, is an important element of respiratory pigments and hemocyanin represents 98% of the blood proteins in cephalopods. Therefore copper is required in large concentrations (Jinadasa, 2014). It was considered that the accumulation of heavy metals in the body of marine organisms mostly depended on feeding behavior (Zhang *et al*., 2015). Table 5 compares the metal concentrations registered in the whole digestive gland of *S. pharaonis* from Persian Gulf with values reported in the literature for cephalopod species (no data in Iran).

Table 5: Comparison of metal levels (µg g^{-1} w.d.) in the digestive gland of *S. pharaonis* from Iran with cephalopod data from the literature.

Species	Zn	Cu	Cd	Pb	As	Ni	Hg	Authors
Sepia officinalis	-	-	-	-	-	-	0.53-2.4	Raimundo *et al.* (2014)
S. officinalis	220-5678	68-5054	10-557	-	-	-	-	Raimundo *et al.* (2005)
S. officinalis	-	-	-	-	-	-	1.3-2.7	Storelli *et al.* (2006)
Todarodesfilippovae	1.2-307	5-865	34-883	0.02-1.5	6-41	0.2-13	-	Pierce *et al.* (2008)
S. officinalis	1435	1289	108	802	-	-	0.27-0.95	Pereira *et al.* (2009)
S. officinalis	571±47	315±3	13±0.35	1.10±0.06	-	1.5±0.3	-	Miramand and Bentley (1992)
S. officinalis	-	-	-	-	-	-	0.2	Alcobia (1995)
Eledonecirrhosa	646±86	456±11	24±1.75	1.17±0.09	-	2.5±0.1	-	Miramand and Bentley (1992)
Ommastrephes bartrami	163±55	195±212	287±202	-	-	-	-	Martin and Flegal (1975)
Dosidicusgigas	22-333	6.8-560	57-509	0.063-0.7	8.1-20	1.1-7.4	-	Raimundo *et al.* (2014)
*Loligo*sp.	3.99-16.3	0.65-10.7	0.03-0.47	0.07-0.76	-	0.06-0.94		Sivaperumal *et al.* (2007)
Octopus vulgaris	410-2873	639-1597	10-252	1.5-7.2	-	-	-	Raimundo *et al.* (2008)
O. vulgaris	200-14721	139-3140	19-761	0.037-44	-	-	-	Raimundo *et al.* (2004)
O. vulgaris	198-14721	137-1465	20-269	-	-	-	-	Raimundo *et al.* (2005)
O. vulgaris	1463±726	1768±1010	-	6.9±3.2	-	-	-	Napoleao *et al.* (2005)
O. vulgaris	-	-	-	-	-	-	0.58-3.43	Seixas *et al.* (2005b)
Sepia esculenta	-	-	-	-	-	-	0.23-0.48	Ahmad *et al.* (2015)
S.pharaonis	-	-	-	-	-	-	0.30-0.36	Ahmad *et al.* (2015)
Todarodesfilippovae	88.5-94.3	137-218	98.5-246	0.07-0.52	11.5-17.1	0.55-3.54	0.33-0.14	Kojadinovic *et al.* (2011)
S.pharaonis	5.74-9.44	2.19	0.04	0.25-0.45	-	0.21-0.40	-	Al Farraj *et al.* (2011)
S.pharaonis	37.72±5.32	9.01±2.46	0.98±0.35	3.67±1.01	0.08±0.05	0.29±0.12	0.33±0.12	Present study

a: mg kg^{-1} in the mantle.

As observed for various cephalopod species, median concentrations of Cd in the pharaoh cuttlefish were elevated (0.32-1.40 µg g^{-1} w.d.). The comparison with the literature (Table 5) shows that those registered values were one order of magnitude above the ones obtained for *S. officinalis*, *T. filippovae*, *D. gigas*, *O.vulgaris*, comparable to *O. bartrami*, and lower than in *Loligo sp.*,.

Broad intervals of concentrations, like those observed for other species, is in line with the hypothesis of accumulation being linked to detoxification mechanisms preventing the toxicity effect of such high Cd accumulation. Similar mechanisms are considered in the digestive gland of the various cephalopod species (Raimundo *et al.*, 2010). To our knowledge mantle of cephalopods reflecting

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

environmental availability of Cd has been rarely emphasized and it is line with our study.

Concentration of Zn, Cu, Pb, Hg and Ni were compared to values found in other species (Table 5). Narrow intervals suggest that accumulated values primarily reflect the ingested food composition rather than retention mechanisms in response to toxicity.

Contrary to all the determined elements in this work, As concentrations in gills were similar to the values registered in the digestive gland. A similar distribution pattern was observed for as in *A. dux* captured in Spanish waters (Bustamante *et al*., 2008) and in *T. filippovae* from Tasmania and southern Indian Ocean (Kojadinovica *et al*., 2011). This partitioning has been attributed to different mobility of As chemical form (Francesconi, 2010).

The mantle of *S. pharaonis* presented low element concentrations, as found in other cephalopods (Miramand and Bentley, 1992; Raimundo *et al*., 2004). The contrasting difference of Cd concentrations between mantle and digestive gland is remarkable in the cuttlefish, as well as in other cephalopods. Presumably the detoxification mechanism existing in the digestive gland prevents the partitioning of this potential toxic element for other tissues, namely mantle (Raimundo *et al*., 2010). The mantle tissue of *S. pharaonis* was characterized by the lowest concentrations of most trace elements. The muscle tissue of *T. filippovae* was characterized by the lowest concentrations of trace elements (Kojadinovica *et al*., 2011). These results are consistent with data reported for other cephalopod species (Storelli and Marcotrigiano, 1999; Bustamante *et al*., 2008).

The results obtained in the current work pointed to relations between metal concentrations in tissues and the biological parameters of cuttlefish. It is generally accepted that trace element accumulation in living organisms controlled by specific uptake, detoxification and elimination mechanisms, depends significantly also on the size-specific metabolic rate of organisms (Farkas *et al*., 2003). Although heavy metal accumulation in fish generally increase with age (Raimundo *et al*., 2014), the literature reflects a lack of consensus on this type of relation in cephalopods. Whereas some studies showed similar concentrations in small and large individuals (Seixas *et al*., 2005; Raimundo *et al*., 2009), others indicated correlation between accumulated levels and size of cephalopods (Rossi *et al*., 1993; Pereira *et al*., 2009). Squid size was positively correlated to Ag, As, Cd, Hg and Zn concentrations in Tasmanian squid and negatively correlated to all but Hg and Zn concentrations in Amsterdam squid (Kojadinovic *et al*., 2011). The discrepancy among these observations may result from the effect of other factors on the metal accumulation in cephalopods, such as quality and quantity of food and variation of growth rates with the temperature (Raimundo *et al*., 2014). Lifestyle also influences the metal accumulation, with cephalopods benthic species displaying higher total heavy metals than pelagic ones (Bustamante *et al*., 2006).

Results showed that there were positive relationships between cuttlefish sizes and metal levels in most cases. Relationship between metal concentrations and size of cuttlefish could not be compared to other studies in cephalopods in Iran (Persian Gulf), due to lack of data.

Al- Farraj *et al*. (2011) showed statistically significant positive correlations were detected between the concentrations of Ni, Pb, Cd and Cr and the mantle length of *S. pharaonis*, while Zn displayed a significant negative correlation. This study concluded that the levels of the investigated heavy metals in the cuttlefish were generally low and/ or well within the maximum permitted concentrations imposed by different organizations and authorities, and consequently within in the safe limits for human consumption. Seixas *et al*. (2005a) found no significant correlations between the concentration of mercury in tissues and any of the measures of size, condition, and reproductive status of *Octopus vulgaris*. A study of *E. cirrhosa* in the Tyrrhenian Sea (Rossi *et al*., 1993) showed that the concentration of mercury was correlated with length. Among different organs analyzed, the digestive gland displayed the highest Hg concentrations, which strongly suggests that food is a major pathway for Hg accumulation in cephalopods, that line with our results. Our results for concentrations of mercury in the digestive gland were consistent with results for *S. pharaonis* (Ahmad *et al*., 2015). On the other hand, in the study of Rjeibi *et al*. (2014), Hg concentrations were positively correlated with size in all tissues of *S. officinalis*, but significant negative relationships were seen for Pb and Cu in all tissues.

The positive relationship between heavy metal levels and cuttlefish size was attributed to the variations in feeding habitat as different stages of cuttlefish life. The discrepancy of these observations resulted probably from the more prominent effect of different factors, such as food availability (i.e., quality and quantity of food) and growth rates (which may be affected by temperature) (Merciai *et al*., 2014), on the metal accumulation in cephalopods.

Significant, positive and linear relations were obtained for the digestive gland, kidneys, gills and mantle analyzed of *S. officinalis*, with the mantle being the tissue that presented the strongest relation (r^2=0.99), followed by the digestive gland (r^2=0.86), branchial hearts (r^2=0.86), kidney (r^2=0.72) and gill (r^2=0.61) (Raimundo *et al*., 2014). In our study, the strongest relation were obtained for As and Cd in digestive gland (r^2 = 0.85; r^2=0.88), Zn, Ni and Pb in branchial

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

hearts (r^2=0.942; r^2=0.944; r^2=0.945), Cu and Hg in the mantle (r^2=0.90; r^2=0.94), that line with our results for Hg. The digestive gland plays a major role in the metabolism of all metals in cuttlefish, and especially Cd. Because of the metal dilution with somatic growth, it was necessary to consider the tissues separately and particularly the digestive gland as a storage organ of metals.

There is some legislation regulating the maximum concentrations of trace metals. The guideline value adopted by the World Health Organization for As in cephalopods is 2 µg g^{-1} w.w. The maximum permitted level of Hg allowed for human consumption is 0.5 mg kg^{-1} fresh weight (EC, rule n8 466/ 2001) in cephalopods. The guideline value adopted by the European Commission for Pb in cephalopods is 1 µg g^{-1} w.w. The maximum Cd and Pb levels were reported as 1.0 and 0.5µg kg^{-1} by FAO, although FAO limits for Zn and Cu were 30 µg kg^{-1}. Results from this study revealed that except for Pb the metal levels in *S. pharaonis* samples were below the threshold values as recommended by the FAO and EC guidelines.

Acknowledgment

The authors wish to thanks the Ahvaz Branch, Islamic Azad University for the financial support. The authors would like to thanks all the editors and reviewers for their comments in the development and improvement of this paper.

References

Ahmad, N.I., Noh, M.F.M., Mahiyuddin, W.R.W., Jaafar, H., Ishak, I., Azmi, W.N.F.W., Veloo, Y. and Mokhtar, F.A., 2015. The mercury levels in crustaceans and cephalopods from Peninsular Malaysia. *Environmental Science and Pollution Research*, 22, 12960-12974.

Alcobia, H., 1995. Biologia e acumulacao de mercurio no choco *Sepia officinallis* (Linnaeus, 1758) do estuario do Sado. Relatorio de estagio de licenciatura. IPIMAR and FCUL; Lisboa. 46P.

Al-Farraj, S., El-Gendy, A., Alyahya, H. and El-Hedeny, M., 2011. Heavy metals accumulation in the mantle of the common cuttlefish *Sepia pharaonis* from the Persian Gulf. *Australian Journal of Basic and Applied Sciences*, 5, 897–905.

Bustamante, P., Cosson, R.P., Gallien, I., Caurant, F. and Miramand, P., 2002. Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations. *Marine Environmental Research*, 53, 227–41.

Bustamante, P., Teyssié, J.L., Danis, B., Fowler, S.W., Miramand, P., Cotret, O. and Warnau, M., 2004. Uptake, transfer and distribution of silver and cobalt in tissues of the common cuttlefish *Sepia officinalis* at different stages of its life cycle. *Marine Ecology Progress Series*, 269, 185–195.

Bustamante, P., Morales, C.F., Mikkelsen, B., Dam, M. and Caurant, F., 2004a. Trace element bioaccumulation in grey seals *Halichoerus grypus* from the Faroe Islands. *Marine Ecology Progress Series*, 267, 291–301.

Bustamante, P., Lahaye, V., Durnez, C., Churlaud, C. and Caurant, F. 2006. Total and organic Hg concentrations in cephalopods of the North Eastern Atlantic waters: influence of geographical origin and feeding ecology. *Science of the Total Environment*, 368, 585–96.

Bustamante, P., Gonzalez, A., Rocha, F., Miramand, P. and Guerra, A., 2008. Metal and metalloid concentrations in the giant squid *Architeuthis dux* from Iberian waters. *Marine Environmental Research*, 66, 278–287.

Danis, B., Bustamante, P., Cotret, O., Teyssie, J.L., Fowler, S.W. and Warnau, M., 2005. Bioaccumulation of PCBs in the cuttlefish *Sepia officinalis* from seawater, sediment and food pathways. *Environmental Pollution*, 134, 113–122.

Department of Fisheries. Annual Fisheries Statistics., 2012. prefix. http:// www.dof.gov.my/ en/ perangkaan- tanhunan- 2012. Accessed 6 January 2014.

Duysak, O., Ersoy, B. and Dural, M., 2013. Metal concentrations in different tissues of cuttlefish (*Sepia officinalis*) in Iskenderun Bay, Northeastren Mediterranean. *Turkish Journal of Fisheries and Aquatic Science*, 13, 205-210.

Duysak, O. and Dural, M., 2015. Heavy metal concentrations in tissues of short-finned squid *Illex coindetii* (Cephalopoda: Ommastrephidae) (Vérany, 1839) in Iskenderun Bay, North-Eastern Mediterranean. *Pakistan Journal Zoology*, 47(**2**), 447-453.

EC–European Communities 2001. Commission regulation (EC) No. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuff. Official Journal of the European Union, 1. 77/1.

FAO (Food and Agriculture Organization), 1983. Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fishery Circular No. 464, Rome, Italy, 764P.

FAO/WHO, (2006). Evaluation of certain food additives and contaminants. Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives.WHO technical report series. No. 940.

Farkas, A., Salanki, J. and Specziar, A., 2003. Age- and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. *Water Research*, 37, 959- 964.

Francesconi, K.A., 2010. Arsenic species in seafood: origin and human health implications. *Pure and Applied Chemistry*, 82, 373-381.

Fu, F. and Wang, Q., 2011. Removal of heavy metal ions from wastewaters: a review. *Journal of Environmental Management*, 92, 407–418.

Gerpe, M., deMoreno, J. and Patat, M., 2000. Cadmium, zinc and copper accumulation in the squid *Illex argentinus* from the southwest Atlantic Ocean. *Marine Biology*, 136, 1039–1044.

Ichihashi, H., Nakamura, Y., Kannan, K., Tsumura, A. and Yamasaki, S.I., 2001. Multi-elemental concentrations in tissues of Japanese common Squid (*Todaro despacificus*). *Archives of Environmental Contamination and Toxicology*, 41,483–490.

Jinadasa, B.K.K.K., 2014. Concentration of trace metals in the squids (*Loligo duvauceli*, *Sepio teuthislessoniana*) and cuttlefish (*Sepia latimanus*) from the north-western coast of Sri Lanka. *Journal of Aquatic Science*, 2, 5-10.

Kojadinovica, J., Jackson, C.H., Cherel, Y., Jackson, G.D., Bustamante, P., 2011. Multi-elemental concentrations in the tissues of the oceanic squid *Todaro desfilippovae* from Tasmania and the southern Indian Ocean. *Ecotoxicology and Environmental Safety*, 74, 1238–1249.

Kwok, C.K., Liang, Y., Wang, H., Dong, Y.H., Leung, S.Y. and Wong, M.H., 2014. Bioaccu-mulation of heavy metals in fish and Ardeid at Pearl River Estuary, China. *Ecotoxicology and Environmental Safety*, 106, 62–67.

Lacoue-Labarthe, T., Warnau, M., Metian, M., Oberhänsli, F., Rouleau, C. and Bustamante, P., 2009. Biokinetics of Hg and Pb accumulation in the encapsulated egg of the common cuttlefish *Sepia officinalis*: radiotracer experiments. *Science of the Total Environment*, 407, 6188–6195.

Le Pabica, Ch., Caplata, Ch., Lehodeya, J.P., Milinkovitchd, T., Kouetaa, N., Philippe Cossone, R.Ph. and Bustamante, P., 2015. Trace metal concentrations in post-hatching cuttlefish *Sepia officinalis* and consequences of dissolved zinc exposure. *Aquatic Toxicology*, 159, 23–35.

Martin, J. and Flegal, A., 1975. High copper concentrations in squid livers in association with elevated levels of silver, cadmium, and zinc. *Marine Biology*, 30, 51–55.

Merciai, R., Guasch, H., Kumar, A. and Sabater, S. 2014. Trace metal concentration and fish size: Variation among fish species in a Mediterranean river. *Ecotoxicology and Environmental Safety*, 107, 154- 161.

Miramand, P. and Bentley, D. 1992. Concentration and distribution of heavy metals in tissues of two cephalopods, *Eledo necirrhosa* and *Sepia officinalis*, from the French coast of the English Channel. *Marine Biology*, 114, 407–14.

Miramand, P. and Fowler, S.W., 1998. Bioaccumulation and transfer of vanadium in marine organisms. In: Nriagu JO, editor. Vanadium in the environment part 1: chemistry and biochemistry. Wiley Series in Advances in Environmental Sciences and Technology, New York: John Wiley & Sons; pp. 167–97.

Miramand, P., Bustamante, P., Bentley, D. and Koueta, N., 2006. Variation of heavy metal concentrations (Ag, Cd, Co, Cu, Fe, Pb, V, and Zn) during the life cycle of the common cuttlefish *Sepia officinalis*. *Science of the Total Environment*, 361, 132–143.

Napoleão, P., Pinheiro, T. and Sousa Reis, C., **2005**. Elemental characterization of tissues of *Octopus vulgaris* along the Portuguese coast. *Science of the Total Environment*, 345, 41–9.

Pereira, P., Raimundo, J., Vale, C. and Kadar, E., 2009. Metal concentrations in digestive gland and mantle of *Sepia officinalis* from two coastal lagoons of Portugal. *Science of the Total Environment*, 407, 1080–1088.

Pierce, G., Stowasser, G., Hastie, L., Bustamante, P., 2008. Geographic, seasonal and on to genetic variation in cadmium and mercury concentration sinsquid (Cephalopoda: Teuthoidea) from UK waters. *Ecotoxicology and Environmental*, 70, 422–432.

Raimundo, J., Caetano, M. and Vale, C., 2004. Geographical variation and partition of metals in tissues of *Octopus vulgaris* along the Portuguese coast. *Science of the Total Environment*, 325, 71–81.

Raimundo, J., Pereira, P., Vale, C., Caetano, M., 2005. Fe, Zn, Cu and Cd in the digestive gland and muscle tissues of *Octopus vulgaris* and *Sepia officinalis* from coastal areas in Portugal. *Ciencias Marinas*, 31, 243-251.

Raimundo, J., Vale, C., Duarte, R. and Moura, I., 2008. Sub-cellular partition in of Zn, Cu, Cd and Pb in the digestive gland of native *Octopus vulgaris* exposed to different metal concentrations (Portugal). *Science of the Total Environment*, 390, 410–416.

Raimundo, J., Vale, C., Caetano, M., Cesario, R. and Moura, I., 2009. Lead isotope signature in digestive gland of *Octopus vulgaris* and sediments from the Portuguese coast. *Aquatic Biology,* 6, 25–30.

Raimundo, J., Costa, P. M., Vale, C., Costa, M.H. and Moura, I., 2010. Metallothioneins and trace elements in digestive gland, gills, kidney and gonads of *Octopus vulgaris*. *Comparative*

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

Biochemistry and Physiology. 152, 139–146.

Raimundo, J., Vale, C. and Rosa, R., 2014. Trace element concentrations in the top predator jumbo squid (*Dosidicus gigas*) from the Gulf of California. *Ecotoxicology and Environmental Safety*, 102, 179-186.

Raimundo, J., Pereira, P., Vale, C., Canario, J. and Gaspar, M., 2014. Relations between total mercury, methylmercury and selenium in five tissues of *Sepia officinalis* captured in the south Portuguese coast. *Chemosphere*, 108, 190–196.

Reid, A., Jereb, P. and Roper, C.F.E., 2005. Family Sepiidae. P. Jereb and C.F.E Roper, eds. Cephalopods of the word. An annotated and illustrate catalogue of species known to date. Volume 1. Chambered nautiluses and sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae). FAO Species Catalogue for Fishery Purposes. Rome, FAO, 4, 57- 152

Rjeibi, M., Metian, M., Hajji, T., Guyot, T., Chaouacha-Chékir, R.B. and Bustamante, P., 2014. Interspecific and geographical variations of trace metal concentrations in cephalopods from Tunisian waters. *Environmental Monitoring* and *Assessment*, 186, 3767–3783.

Rjeibi, M., Metian, M., Hajji, T., Guyot, T., Chaouacha-Chekir, R.B. and Bustamante, P., 2015. Seasonal survey of contaminants (Cd and Hg) and micronutrients (Cu and Zn) in edible tissues of cephalopods from Tunisia: assessment of risk and nutritional benefits. *Journal of Food Science*, 80, 199-206.

Rossi, A., Pellegrini, D., Belcari, P. and Barghigiani, C., 1993. Mercury in *Eledone cirrhosa* from the Northern Tyrrhenian Sea: contents and relations with life cycle. *Marine Pollution Bulletin*, 26, 683–686.

Seixas, S., Bustamante, P. and Pierce, G.J., 2005a. Interannual patterns of variation in concentrations of trace elements in arms of *Octopus vulgaris*. *Chemosphere*, 59, 1113–24.

Seixas, S., Bustamante, P. and Pierce, G. 2005b. Accumulation of mercury in the tissues of the common octopus *Octopus vulgaris* (L.) in two localities on the Portuguese coast. *Science of the Total Environment*, 340, 113–22.

Semedo, M., Reis-Henriques, M.A., Rey-Salgueiro, L., Oliveira, M., Delerue-Matos, C., Morais, S. and Ferreira, M., 2012. Metal accumulation and oxidative stress biomarkers in octopus (*Octopus vulgaris*) from Northwest Atlantic. *Science of the Total Environment*, 433, 230-237.

Sivaperumal, P., Sankar, T.V. and Viswanathan Nair, P.G., 2007. Heavy metal concentrations in fish,

[DOR: 20.1001.1.15622916.2018.17.2.2.2]

shellfish and fish products from internal markets of India vis-s-vis international standards. *Food Chemistry*, 102, 612–620.

Storelli, M.M. and Marcotrigiano, G.O. 1999. Cadmium and total mercury in some cephalopods from the South Adriatic Sea (Italy). *Food Additives and Contaminants*, 16, 261–265.

Storelli, M.M., Giacominelli-Stuffler, R., Storelli, A. and Marcotrigiano, G.O., 2006. Cadmium and mercury in cephalopod molluscs: estimated weekly intake. *Food Additives and Contaminants*, 23, 25–30.

Storelli, M.M., Garofalo, R., Giungato, D. and Giacominelli-Stuffler, R., 2010. Intake of essential and non-essential elements from consumption of octopus, cuttlefish and squid. *Food Additives and Contaminants,* Part B, 3, 14–8.

USEPA, 1996. Method 3052: Microwave assisted acid digestion of siliceous and organically based matrices.

Villanueva, R. and Bustamante, P., 2006. Composition in essential and non-essential elements of early stages of cephalopods and dietary effects on the elemental profiles of *Octopus vulgaris* para larvae. *Aquaculture*, 261, 225–40.

Zhang, L., Shi, Z., Jiang, Z., Zhang, J., Wang, F. and Huang, X., 2015. Distribution and bioaccumulation of heavy metals in marine organisms in east and west Guangdong coastal regions, South China. *Marine Pollution Bulletin*, 10, 1-8.