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

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Abstract: Concentrations of cadmium (Cd), copper (Cu), mercury (Hg), and zinc (Zn) were determined by atomic absorption spectrophotometry in the muscle tissues (arms and mantle) of 3 commercial cephalopods (*Loligo vulgaris, Octopus vulgaris*, and *Sepia officinalis*) caught in 3 different Tunisian coastal regions. The highest concentrations found correspond to the essential elements Cu and Zn. Octopuses and cuttlefish showed the highest levels of those elements whereas squid presented with significantly higher values of Hg in both muscular tissues. This may be related to different feeding behavior and detoxification processes among benthic and pelagic cephalopods. Variation of element concentrations between seasons was different between species and seemed to be mostly dependent on the sampling site. From a public health standpoint, average concentrations of Cd, Cu, Hg, and Zn measured in edible tissues of cephalopods from this study did not reveal, in general, any risk for consumers. The estimated target hazard quotients for Cd and Hg for consumers of the selected species were below 1 and within the safety range for human health. Moreover, their consumption could provide in an important contribution to the daily dietary intake of Cu for the Tunisian population, especially regarding the consumption of octopus and cuttlefish muscles.

Keywords: cephalopods, DRI, metals, PTWI, Tunisian coast, target hazard quotient

Introduction

Marine resources constitute a mandatory part of the diet of many populations around the world. Seafood consumption is recommended for its nutritional qualities and benefits. The benefits of seafood derive mainly from its content of high-quality proteins and its high content of omega3 polyunsaturated fatty acids—that is, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—and other nutrients (Castro-González 2002; Domingo and others 2007). Omega3 fatty acids have demonstrated protective effects in preventing coronary heart disease, reducing arrhythmias and thrombosis (Oomen and others 2000; Kris-Etherton and others 2002), lowering plasma triglyceride levels (Harris 1997; Ismail 2005), and reducing blood clotting tendency (Din and others 2004; Ismail 2005). Among the commercial marine resources, cephalopods represent one of the most important groups of species in the world to fisheries, comprising about 4% of total catches according to the FAO (2013). During the last decades, cephalopods have been recognized as an appropriate alternative to traditional marine resources and their consumption is likely to continue to increase in the future (Caddy and Rodhouse 1998; Piatkowski and others 2001). They are generally very rich in n-3 fatty acids (Ozogul and others 2008), as well as being excellent sources of proteins and of a large number of essential elements (Oehlenschlager 1997; Storelli 2009). In parallel to the nutritional benefits of consuming seafood, there is a non-negligible risk of contamination given the bioaccumulation/biomagnification capacities occurring in marine organisms. This is true certainly of cephalopods; they can be considered as "potentially" hazardous for consumers, given their ability to accumulate high levels of Cd, even in environments of low metal contamination (Bustamante and others 1998a; Dorneles and others 2007; Kojadinovic and others 2011).

To settle this specific controversy, several studies have been published examining various aspects of the risks and benefits of cephalopod consumption (Storelli and Marcotrigiano 1999; Lourenço and others 2009; Storelli 2009; Storelli and others 2010). However, there is scarce or no data available on the nutritional and health risks associated with the consumption of cephalopods from Tunisian waters. Health organizations worldwide have compiled the following procedures. The Joint FAO/WHO Expert Committee on Food Additives (JEFCA) has released guideline values and advisories to help consumers to manage risks and maximize benefits. JECFA has also established a provisional tolerable weekly intake (PTWI) of 7 and 4 μg/kg body weight per week for Cd and Hg, respectively (JECFA 2006, 2010) and the Institute of Medicine has set the Dietary Reference Intakes (DRIs, IOM 2001) and recommendations for the intake of micronutrients to prevent nutritional inadequacy, which could lead to nutrient deficiencies and consequent impairment of health.

In this context, this study was undertaken to: (1) quantify the levels of non-essential (Cd and Hg) and essential (Cu and Zn) metals in the edible parts (arms and mantle) of the European squid *Loligo vulgaris*, the common octopus *Octopus vulgaris*, and the

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common cuttlefish *Sepia officinalis* caught from 3 different Tunisian coastal regions during different seasons; (2) to estimate the sanitary and nutritional qualities of these fishing products. The selected species are commercially relevant because of their abundance and high consumption in Tunisia. The country is ranked among the top countries with high per capita supply of cephalopods (0.3 kg/capita/year in 2009; FAOSTAT 2013).

Materials and Methods

Sample collection and preparation

Common octopus (*Octopus vulgaris*), European squid (*Loligo vulgaris*), and common cuttlefish (*Sepia officinalis*) were collected seasonally (spring, summer, autumn, and winter) over a 1-y period (December 2009 to November 2010) from 3 major commercial harbors (Bizerte, Monastir, and Sfax) spread evenly across the Tunisian coast (north, east, and southeast, respectively; see Figure 1). The collected samples were immediately stored in hermetically closed plastic bags, placed on ice, stored in thermo-insulated containers and transported to the laboratory at the Univ. of Tunis. On arrival at the laboratory, dorsal mantle length and total weight of samples were determined (Table 1). Samples of arm muscle (A) and mantle muscle (M) were collected and vacuum sealed in individual plastic bags, coded for easy identification, stored at −25 °C and kept for elemental analysis.

Sample analysis. Separated tissue samples were either dried to a constant weight for several days at 60 °C or freeze-dried and then homogenized in a mortar and porcelain pestle. An aliquot of approximately 300 mg of each dried material was digested with 5 mL of 65% ultrapure HNO_3 (Merck®) at 80 °C on a hotplate until the solution was clear. Next, the acid was evaporated and the residue was dissolved in 0.3 M ultrapure nitric acid. Blanks were carried through the procedure in the same way as the samples. Cu and Zn were determined by flame atomic absorption spectrophotometry (AAS), whereas Cd was analyzed by graphite furnace AAS using a Hitachi spectrophotometer Z5000 with Zeeman correction.

The total Hg concentrations were determined by analyzing Hg directly in the powder obtained from the tissues 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 (Bustamante and others 2006a). Hg determination involved evaporation of the metal by progressive heating until 800 °C 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.

Accuracy and reproducibility of the preparation were tested by preparing analytical blanks and replicates of Lobster Hepatopancreas (TORT-2), Dogfish liver (DOLT-3), and Dogfish Muscle (DORM-2) reference standards (Natl. Research Council, Canada)

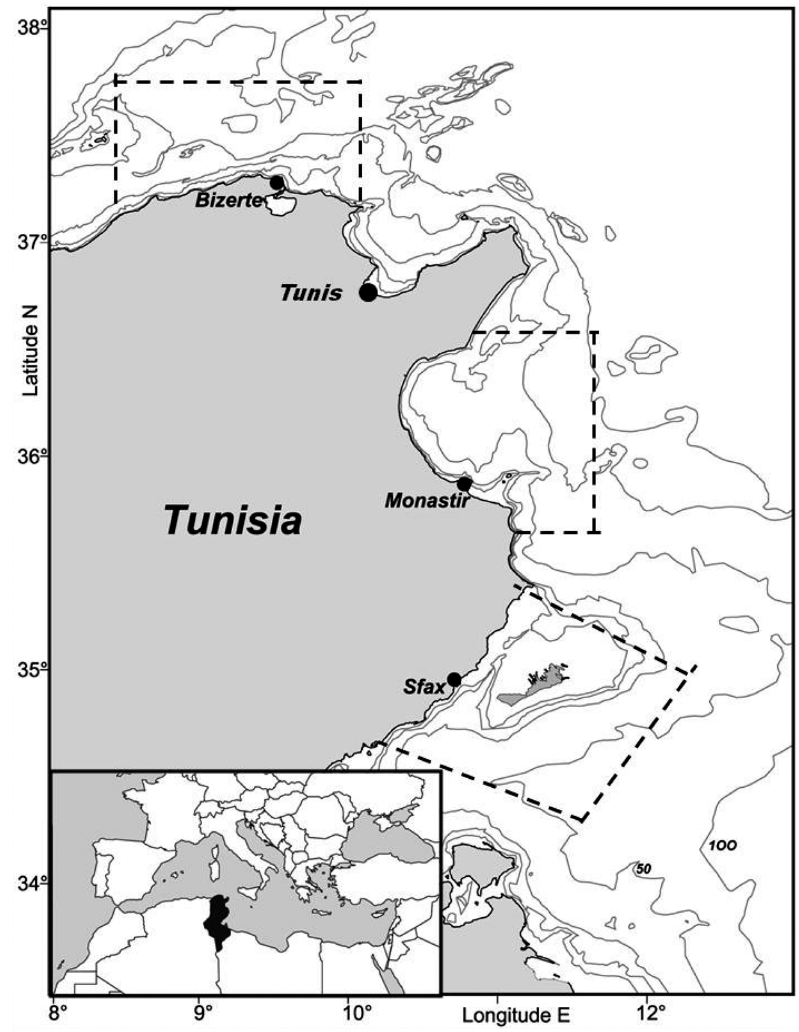

Figure 1–Sampling locations (Bizerte, Monastir, and Sfax) along the Tunisian coast.

Table 1–Mean ± SD and ranges of dorsal mantle length (DML, mm) and weight (g) of cephalopod species from the sampling locations along the Tunisian coast.

	Bizerte (Northern coast)			Monastir (Eastern coast)			Sfax (Southern coast)		
Species	***n***	**DML**	**Weight**	***n***	**DML**	**Weight**	***n***	**DML**	**Weight**
European squid	21	154 ± 28	118 ± 64	37	171 ± 29	128 ± 47	37	182 ± 32	156 ± 66
Loligo vulgaris		122–227	58–318		120–225	47–207		120–256	59–297
Common octopus	20	112 ± 18	506 ± 228	27	104 ± 21	476 ± 208	25	117 ± 22	655 ± 256
Octopus vulgaris		85–150	286–1267		72–160	114–983		72–155	194–1033
Common cuttlefish	35	110 ± 22	171 ± 90	38	108 ± 16	155 ± 55	38	108 ± 18	163 ± 99
Sepia officinalis		70–145	40–396		80–139	74–258		65–170	41–682

n, number of individuals.

along with each set of samples. Recovery rates were respectively equal to 92 ± 7% for Cd, 94 ± 8% for Cu, 100 ± 3% for Hg, and 101 ± 12% for Zn. Detection limits (DL, μg/g, dry weight [dw]) were 0.034 (Cd), 0.5 (Cu), 0.006 (Hg), and 3 (Zn).

All data were computed on a μg/g wet weight (ww) basis. Concentrations of elements in μg/g ww were obtained from data in dw using the dry/wet weight ratios measured in all samples. These ratios indicated a mean moisture content of 78% in cuttlefish and squid and 83% in octopuses, which matched well with those previously reported for the same species (for example, Ozogul and others 2008; Lourenço and others 2009).

Statistical analyses

Levels of elements in the edible parts of the studied species were represented by median rather than average values because of their non-normal distribution. Differences in median levels between species, as well as between seasons, were performed by Kruskal–Wallis (KW) tests followed by Wilcoxon tests for independent samples using Bonferroni's *P*-value correction. Levels of significance of the null hypotheses associated with these tests will be divided into classes of *P*-values represented by the following codes: NS ≥ 0.05; $^{*} < 0.05$; $^{**} < 0.01$; $^{***} < 0.001$. All statistical analyses were performed using the GNU R statistical system (The R Development Core Team 2008).

Assessment of risk and nutritional benefits for human consumers

The risk to health was assessed by the calculation of the estimated weekly intake (EWI) and the target hazard quotient (THQ) for muscle tissues, considering the average Cd and Hg concentrations in both arms and mantle of selected cephalopod species caught along the Tunisian coast. In this calculation, metal sources supplied by other meals or by drinking water on the same day or week were not taken into account, that is, only metal intake coming from the seafood has been considered.

The EWIs of non-essential elements (Cd and Hg) were calculated by multiplying the respective concentrations in each cephalopod species by the average weight of that species consumed (33 g/person/day; FAO 2009), and reporting to the average body weight for Tunisian people (60 kg). The obtained values were compared to the PTWI defined by the JECFA for these elements (that is, 7 μg Cd/kg body weight [JECFA 2006] and 4 μg Hg/kg body weight [JECFA 2010]).

Moreover, the non-carcinogenic effects through cephalopod consumption by the local inhabitants were assessed based on the THQ (USEPA 1989). The THQ is an estimate of the risk level (non-carcinogenic) due to pollutant exposure. Thus, a THQ below 1 means that the exposed human population is unlikely to experience obvious adverse effects. The methodology for estimating THQ was available in the U.S. EPA region III Risk-based Concentration Table (USEPA 2013) and is described by the following equation:

$$\mathrm{THQ} = \left(\frac{\mathrm{EF} \times \mathrm{ED} \times \mathrm{FIR} \times \mathrm{C}}{\mathrm{RFD} \times \mathrm{WAB} \times \mathrm{TA}}\right) \times 10^{-3}$$

where EF is the exposure frequency (365 days/year); ED is the exposure duration (70 y), equivalent to the average lifetime; FIR is the food ingestion rate (33 g/person/day; FAO 2009); *C* is the metal concentration in cephalopods (μg/g ww; RFD is the U.S. EPA's reference dose [$\mathrm{Cd} = 1 \times 10^{-3}$ μg/g/d, $\mathrm{Hg} = 5 \times 10^{-4}$ μg/g/d]; USEPA 2013); WAB is the average body weight (60 kg), and TA is the average exposure time for non-carcinogens (365 days/year × ED).

In the case of essential trace metals (Cu and Zn), a nutritional evaluation was carried out by comparing the estimated dietary intakes from the consumption of edible tissues of the studied cephalopod species with the RDI values, that is, the recommended dietary allowance (RDA) recommended by the Institute of Medicine (IOM 2001) for these elements.

Results and Discussion

Metal levels in edible tissues

Considering the results of all combined sites and for all 4 seasons, the median concentrations of Cd, Cu, Hg, and Zn in the edible tissues (arms and mantle) of European squid, common octopuses, and common cuttlefish, expressed in wet weight basis are presented in Table 2. The following sequence of metal concentration was observed in all muscular samples for all studied species: $\mathrm{Zn} > \mathrm{Cu} > \mathrm{Hg} \approx \mathrm{Cd}$. Metals such as Cu and Zn are essential for cephalopod metabolism because they play an important role in biological functions, whereas non-essential metals such as Cd and Hg have no known biological functions and can be toxic, especially in the most sensitive stages (Bryan 1976). In this study, Zn levels vary over an order of magnitude ranging from 2.6 to 45.3 μg/g ww and 4.0 to 33.6 μg/g ww in the arms and the mantle, respectively. The highest median concentration (KW, $P < 0.001$) was observed in octopuses (arms: 14.2 μg/g; mantle: 11.7 μg/g) and cuttlefish (arms: 15.0 μg/g; mantle: 11.1 μg/g) followed by squid (arms: 12.7 μg/g; mantle: 9.8 μg/g; Table 2). These results fall within the same range of data obtained for the same species from the European coasts (Miramand and Bentley 1992; Raimundo and others 2004; Napoleão and others 2005; Seixas and others 2005; Lourenço and others 2009). For Cu, the levels found ranged from 0.50 to 10.30 μg/g in the arms and from 0.59 to 21.13 μg/g in the mantle in cephalopods from Tunisia. The lowest concentrations were detected in squid for both muscle tissues with median

Table 2–Concentrations of Cd, Cu, Hg, and Zn (μg/g ww) in cephalopods muscle tissues from the Tunisian coasts for 4 seasons (winter 2009 and, spring to autumn 2010). Interspecies comparison results are given in the last columns.

Metal	Muscle tissue	European squid $n = 95$ Median (range)		Common octopus $n = 72$ Median (range)		Common cuttlefish $n = 111$ Median (range)		Hypotheses tests results
Cd	Arms	0.041 (0.014–0.17)	a	0.026 (0.001–0.20)	b	0.026 (0.006–0.10)	b	KW: <0.001
	Mantle	0.045 (0.009–0.16)	a	0.043 (0.006–0.27)	a	0.041 (0.007–0.18)	a	KW: NS
Cu	Arms	1.94 (0.50–7.22)	c	3.29 (1.49–10.62)	b	5.33 (2.31–10.30)	a	KW: <0.001
	Mantle	1.22 (0.59–6.13)	c	4.90 (1.67–13.93)	a	3.63 (0.80–21.13)	b	KW: <0.001
Hg	Arms	0.062 (0.030–0.68)	a	0.027 (0.010–0.11)	c	0.049 (0.020–0.15)	b	KW: <0.001
	Mantle	0.072 (0.030–0.95)	a	0.031 (0.010–0.10)	c	0.057 (0.019–0.22)	b	KW: <0.001
Zn	Arms	12.7 (2.8–19.7)	b	14.2 (6.4–45.3)	a	15.0 (2.6–39.4)	a	KW: <0.001
	Mantle	9.8 (4.0–18.7)	b	11.7 (5.3–32.3)	a	11.1 (6.2–33.6)	a	KW: <0.001

The significances of the differences among species are indicated by letters.
KW, Kruskal–Wallis; NS, not significant.

concentrations of 1.94 and 1.22 μg/g in the arms and mantle, respectively. Cuttlefish revealed significantly higher concentrations than octopuses in the arms but were lower in the mantle (Table 2). This situation is consistent with the results of Lourenço and others (2009) on cephalopods from Portugal (that is, 2.6–8.1 μg/g ww and 1.7–10.3 μg/g ww for octopus and cuttlefish, respectively). Furthermore, the concentrations of Cu in cuttlefish and octopus tissues are in agreement with results for mantle muscle of *Sepia officinalis* from Turkish waters (that is, 7.31–8.02 μg/g ww; Ayas and Ozogul 2011) and for *Octopus vulgaris* from Portuguese coasts (that is, 7–16 μg/g dw, Carvalho and others 2005; 5.5–14 μg/g dw, Raimundo and others 2005). The high levels of such essential metals found in benthic species (octopus and cuttlefish) compared to those found in the pelagic species (squid) confirms the results found for the same species by Lourenço and others (2009).

Cephalopods are carnivorous, active predators and have very high feeding rates (Rodhouse and Nigmatullin 1996). Thus, their diet represents a major pathway of exposure for many elements including Cu and Zn (Bustamante and others 2002, 2004, 2006b). This should explain the high values of the essential elements reported in this study. The octopus, with the highest concentration of these elements in its tissues, consumes a wider range of prey than the 2 other species studied: in addition to polychaetes and mollusks, octopuses mainly consume crustaceans (Smith 2003) such as crabs that contain high levels of Cu and Zn, which may lead subsequently to a higher intake of these elements (Rjeibi and others 2014). However, an uptake of waterborne elements also occurs from exposure to seawater (Bustamante and others 2002). To the best of our knowledge, no data have been published on the respective proportions in cephalopods of these elements incorporated from food and seawater. Among the 3 cephalopod species, squid show the lowest concentrations of essential elements. According to some authors (Pierce and others 1994; Coelho and others 1997), fish represents the main component of the squid diet, comprising about 90% of the prey found in their stomachs. This may explain the lower values of essential elements in this species, because fish contain fewer minerals than crustaceans, the preferred prey of octopuses.

Non-essential Cd and Hg do not have a known biological function in cephalopods' metabolism and are therefore not regulated by homeostatic processes. The amount of Cd and Hg in cephalopod organisms would therefore 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 and 0.006 to 0.27 μg/g in the arms and mantle, respectively. Regardless of the geographical origin, squid revealed significantly higher values in the arms with a median concentration of 0.041 μg/g, followed by octopuses and cuttlefish (median concentration: 0.026 μg/g). Although there were no significant differences between species detected in the mantle, squid showed the highest levels (0.045 μg/g; Table 2). In cephalopods, Cd preferentially accumulates in internal organs like the digestive gland, but is poorly concentrated in the muscle tissues, where the concentrations are usually very low (Bustamante and others 1998b; Raimundo and others 2004, 2005; Miramand and others 2006). Although the biological half-life of Cd is relatively long in whole cephalopods (that is, >257 d; Bustamante and others 2002), this parameter remains to be quantified for muscle tissues specifically. The median Cd content for the octopuses and cuttlefish in this study was lower than that found in studies conducted in other locations, like the Portuguese waters (0.33 and 0.23 μg/g; Lourenço and others 2009) and the Adriatic sea (0.87 and 0.27 μg/g; Storelli and others 2006), but concerning squid, it was in the same range of that from Spain and the British Isles (Villanueva and Bustamante 2006; Pierce and others 2008).

Concerning Hg, levels varied from 0.010 to 0.68 μg g^{-1} in the arms and from 0.010 to 0.95 μg g^{-1} in the mantle. The same trend was detected for both muscle tissues in the 3 analyzed species: significantly higher concentrations (KW, $P < 0.001$) were found in the arms and mantle of squid (0.062 and 0.072 μg g^{-1}), followed by cuttlefish (0.049 and 0.057 μg/g) and octopuses (0.027 and 0.031 μg/g; Table 2). This different Hg distribution among the studied species is in accordance with the process of bioaccumulation of this metal in cephalopods, which is dominated by the diet (Lacoue-Labarthe and others 2009). The highest concentration of Hg found in squid reflets their behavior of feeding mainly on fish and other cephalopod species (Pierce and others 1994; Coelho and others 1997). Such a pelagic diet would lead to higher Hg exposure and hence to higher Hg bioaccumulation in pelagic cephalopods, because their prey contains higher organic Hg loads than those of benthic species (Cossa and others 1990; Chouvelon and others 2012). The concentration of Hg in the edible parts of the cephalopod species under investigation were generally slightly lower than the levels reported by authors in other areas of the world (Bustamante and others 2006a; Storelli and others 2006; Villanueva and Bustamante 2006; Lourenço and others 2009). In a previous study, we showed that these differences were not due to important differences either in size or in weight distribution, and thus they reflect actual geographic variations (Rjeibi and others 2014). Within the Tunisian zone, the eastern coast may be subjected to potential natural inputs from nearby Hg ferrous belts and previous mining areas (Joiris and others 1999), whereas the southern coast is especially contaminated by Cd from the phosphate extraction waste (Hamza-Chaffai

Table 3–According to seasons, concentrations of trace metals (μg/g ww) in arms and mantle of European squid, common octopus, and common cuttlefish collected from Bizerte, Monastir, and Sfax.

				Arms								Mantle							
Sampling location	Cephalopod species	Seasons	*n*	Cd Mean ± SD		Cu Mean ± SD		Hg Mean ± SD		Zn Mean ± SD		Cd Mean ± SD		Cu Mean ± SD		Hg Mean ± SD		Zn Mean ± SD	
Bizerte	European squid	Spring	4	0.054 ± 0.025	a	5.81 ± 1.58	a	0.588 ± 0.105	a	12.35 ± 1.57	a	0.034 ± 0.012	a	3.93 ± 1.47	a	0.706 ± 0.280	a	10.66 ± 3.20	a
		Summer	8	0.045 ± 0.020	a	1.69 ± 1.16	b	0.039 ± 0.011	b	16.29 ± 2.95	a	0.034 ± 0.011	a	1.07 ± 0.37	b	0.048 ± 0.019	b	9.33 ± 0.72	a
		Autumn	9	0.040 ± 0.016	a	2.04 ± 0.45	ab	0.051 ± 0.011	ab	14.67 ± 2.15	a	0.035 ± 0.010	a	1.75 ± 0.79	ab	0.062 ± 0.014	ab	9.91 ± 0.40	a
	Common octopus	Spring	7	0.016 ± 0.008	a	3.64 ± 1.15	a	0.060 ± 0.035	a	12.13 ± 0.85	b	0.031 ± 0.012	a	5.68 ± 1.26	a	0.045 ± 0.021	a	8.68 ± 0.68	ab
		Summer	6	0.023 ± 0.014	a	4.27 ± 2.42	a	0.031 ± 0.014	ab	11.60 ± 2.76	ab	0.033 ± 0.015	a	8.64 ± 4.03	a	0.043 ± 0.028	ab	12.07 ± 1.05	a
		Autumn	7	0.021 ± 0.005	a	3.00 ± 0.78	a	0.020 ± 0.009	b	14.46 ± 0.59	a	0.028 ± 0.012	a	6.49 ± 2.06	a	0.023 ± 0.010	b	6.82 ± 1.47	b
	Common cuttlefish	Winter	11	0.051 ± 0.025	a	6.63 ± 2.36	ab	na		10.78 ± 5.64	c	0.10 ± 0.037	a	9.59 ± 4.95	a	na		18.34 ± 6.20	a
		Spring	7	0.020 ± 0.007	b	7.21 ± 1.71	a	0.036 ± 0.011	a	17.80 ± 3.16	ab	0.024 ± 0.015	b	3.39 ± 2.14	ab	0.035 ± 0.014	a	12.01 ± 4.07	ab
		Summer	8	0.023 ± 0.015	b	5.46 ± 1.40	ab	0.033 ± 0.007	a	21.77 ± 2.76	a	0.027 ± 0.014	b	4.27 ± 1.60	ab	0.045 ± 0.012	a	14.93 ± 2.53	ab
		Autumn	9	0.022 ± 0.015	b	4.36 ± 1.55	b	0.051 ± 0.064	a	12.65 ± 2.39	bc	0.025 ± 0.014	b	2.12 ± 1.20	b	0.037 ± 0.006	a	10.60 ± 2.13	b
Monastir	European squid	Winter	9	0.071 ± 0.048	a	2.17 ± 0.58	ab	na		10.93 ± 4.42	ab	0.049 ± 0.029	a	0.94 ± 0.25	b	na		12.03 ± 5.13	ab
		Spring	10	0.064 ± 0.038	a	2.65 ± 1.09	a	0.096 ± 0.017	a	12.45 ± 0.91	b	0.050 ± 0.009	a	1.18 ± 0.45	ab	0.104 ± 0.026	a	7.99 ± 2.38	b
		Summer	10	0.036 ± 0.011	a	1.68 ± 0.37	b	0.055 ± 0.012	b	13.71 ± 3.91	ab	0.050 ± 0.015	a	1.80 ± 0.64	a	0.075 ± 0.022	b	9.83 ± 1.29	ab
		Autumn	8	0.054 ± 0.015	a	1.59 ± 0.22	b	0.064 ± 0.010	b	14.94 ± 1.741	a	0.049 ± 0.010	a	1.23 ± 0.23	ab	0.081 ± 0.011	ab	11.40 ± 0.86	a
	Common octopus	Winter	6	0.008 ± 0.004	b	2.09 ± 0.72	b	na		17.27 ± 5.21	a	0.018 ± 0.011	b	3.01 ± 1.71	a	na		13.88 ± 6.07	a
		Spring	5	0.004 ± 0.002	b	4.17 ± 0.93	ab	0.024 ± 0.004	a	13.30 ± 1.79	a	0.022 ± 0.010	b	3.59 ± 1.71	a	0.029 ± 0.005	a	11.00 ± 3.12	a
		Summer	9	0.020 ± 0.011	ab	3.21 ± 1.00	ab	0.033 ± 0.011	a	14.93 ± 3.52	a	0.036 ± 0.015	ab	4.88 ± 1.73	a	0.039 ± 0.014	a	10.48 ± 2.46	a
		Autumn	7	0.040 ± 0.012	a	6.02 ± 2.89	a	0.028 ± 0.009	a	13.99 ± 1.41	a	0.050 ± 0.013	a	5.18 ± 1.39	a	0.030 ± 0.011	a	11.70 ± 1.23	a
	Common cuttlefish	Winter	10	0.053 ± 0.019	a	5.62 ± 2.20	a	na		12.04 ± 10.48	b	0.124 ± 0.036	a	6.40 ± 2.58	a	na		11.70 ± 1.37	a
		Spring	10	0.018 ± 0.006	b	6.86 ± 2.41	a	0.083 ± 0.029	a	14.09 ± 0.75	b	0.045 ± 0.022	b	4.12 ± 1.56	ab	0.085 ± 0.029	a	9.22 ± 0.98	b
		Summer	9	0.029 ± 0.017	ab	5.47 ± 0.84	a	0.051 ± 0.011	b	18.98 ± 2.34	a	0.031 ± 0.017	b	4.21 ± 1.86	ab	0.058 ± 0.011	b	11.23 ± 1.00	ab
		Autumn	9	0.017 ± 0.006	b	4.84 ± 1.54	a	0.055 ± 0.015	ab	15.98 ± 0.63	ab	0.038 ± 0.021	b	2.70 ± 1.41	b	0.068 ± 0.018	ab	9.25 ± 1.72	b
Sfax	European squid	Winter	10	0.040 ± 0.007	a	2.42 ± 0.88	a	na		5.85 ± 2.64	b	0.020 ± 0.009	b	1.26 ± 0.41	a	na		8.54 ± 3.13	b
		Spring	10	0.034 ± 0.013	a	1.88 ± 0.34	a	0.066 ± 0.012	a	11.38 ± 0.73	a	0.055 ± 0.039	a	1.75 ± 0.59	a	0.070 ± 0.026	a	8.14 ± 3.26	b
		Summer	9	0.049 ± 0.026	a	1.93 ± 0.37	a	0.067 ± 0.031	a	11.97 ± 2.98	a	0.074 ± 0.029	a	1.23 ± 0.57	a	0.094 ± 0.074	a	9.45 ± 0.36	ab
		Autumn	8	0.041 ± 0.015	a	2.48 ± 0.63	a	0.068 ± 0.019	a	14.13 ± 2.29	a	0.045 ± 0.018	ab	1.72 ± 0.85	a	0.090 ± 0.026	a	11.19 ± 0.86	a
	Common octopus	Winter	7	0.071 ± 0.036	ab	4.14 ± 2.11	a	na		27.19 ± 12.42	a	0.145 ± 0.054	ab	6.08 ± 2.59	a	na		20.41 ± 8.04	a
		Spring	8	0.047 ± 0.019	b	2.58 ± 0.67	a	0.022 ± 0.003	b	14.36 ± 1.62	bc	0.062 ± 0.024	b	4.30 ± 1.92	a	0.025 ± 0.003	ab	15.40 ± 6.27	a
		Summer	5	0.086 ± 0.032	ab	4.36 ± 1.32	a	0.039 ± 0.012	a	19.53 ± 2.67	ab	0.173 ± 0.031	a	6.51 ± 1.17	a	0.051 ± 0.006	a	23.98 ± 9.87	a
		Autumn	5	0.154 ± 0.032	a	3.50 ± 0.67	a	0.028 ± 0.006	ab	12.83 ± 1.40	c	0.244 ± 0.043	a	4.40 ± 1.89	a	0.035 ± 0.006	b	13.38 ± 0.68	a
	Common cuttlefish	Winter	10	0.052 ± 0.008	a	5.36 ± 1.39	ab	na		11.90 ± 9.23	b	0.062 ± 0.016	a	3.24 ± 0.57	b	na		18.55 ± 6.80	a
		Spring	10	0.024 ± 0.010	b	4.14 ± 1.03	b	0.064 ± 0.023	a	13.92 ± 1.01	b	0.052 ± 0.017	a	2.93 ± 0.65	b	0.079 ± 0.030	a	9.35 ± 0.74	c
		Summer	10	0.018 ± 0.007	b	4.72 ± 1.36	ab	0.076 ± 0.010	a	18.55 ± 4.15	a	0.023 ± 0.012	b	6.16 ± 1.92	a	0.091 ± 0.014	a	13.38 ± 2.71	ab
		Autumn	8	0.030 ± 0.006	ab	5.83 ± 1.12	a	0.035 ± 0.011	b	15.45 ± 1.06	ab	0.038 ± 0.012	ab	3.13 ± 0.92	b	0.041 ± 0.016	b	9.97 ± 1.53	bc

To assess whether the differences between seasons were significant, Kruskal–Wallis test followed by multiple comparison test with Bonferroni adjustment method were performed. The significances of the differences among seasons are indicated by letters.
na, not analyzed.

and others 2003; Smaoui-Damak and others 2003; Banni and others 2005, 2007).

Seasonal variations of heavy metal concentrations in the edible tissues of the 3 cephalopod species are shown in Table 3. In general, a similar seasonal profile was observed for the different metals in both muscular tissues. In most cases, the variation among seasons was different between species and seemed to be dependent on the sampling site. Considering the essential elements, the maximum concentration of Cu was obtained in spring for squid and cuttlefish from Bizerte and Monastir, and in autumn for octopuses from Monastir and cuttlefish from Sfax. In the case of Zn, higher levels were found in summer for the arms tissue of cuttlefish in all sampling sites, whereas maximum values were obtained in winter for the mantle. Squid caught from the eastern and southeastern coast and octopuses from the northern coast showed higher Zn levels in autumn (Table 3).

Considering the non-essential metals, Cd levels have been reported higher in winter for cuttlefish samples from Bizerte (KW, $P < 0.01$) and Monastir, and in autumn for octopuses from Monastir (Table 3). For Hg, higher concentrations were detected in squid and octopus samples from the northern coast and in cuttlefish from the eastern coast in spring, whereas they tended to increase in summer for all selected species from the southern coast (Table 3).

For safety purposes, authorities and environmental agencies around the world have set standards for metal concentrations in food. The Ministry of Agriculture, Fisheries and Food in the United Kingdom (MAFF 1995) limits the levels of Cu and Zn in the muscle at 20 and 50 μg/g ww, respectively, whereas the Western Australian Food and Drug Regulations List has established a Zn maximum limit in fish of 40 μg/g ww. In Tunisia, there is no legislation regarding Cu and Zn levels in food or in products of animal or plant origin. Using the above-mentioned values as a guideline, average concentrations of Cu and Zn found in this study were below the proposed limits, with the exception of one cuttlefish caught in winter on the northern coast for Cu in the mantle, and 2 octopuses from the southeastern coast for Zn in the arms.

For non-essential metals, the Tunisian authority (Directorate General for Veterinary Services; DGSV 2006) as well as the European legislation (Official Journal of the European Communities 2001) stipulates that the maximum concentrations for Cd and Hg in cephalopods should be 1 and 0.5 μg/g ww, respectively. None of the muscle samples reached this limit for Cd. For Hg, only 3 squid caught in spring from the northern coast (Bizerte) exceeded the limit proposed by the European Community. Despite this fact, the average levels did not exceed the indicative value.

Assessment of contamination risk and nutritional benefits for human consumers

In this study, the Cd and Hg weekly intakes for cephalopods from Tunisia, based on average concentrations, were calculated by considering a weekly average consumption of 231 g (7 days × 33 g) for the Tunisian population (FAO 2009). As shown in Table 4, the average EWIs represented very low percentages of the reference values (ranging from 1.37% to 15.76% for a person weighing 60 kg). The highest dietary intake of Cd and Hg assessed in this study was associated with the consumption of octopuses from Sfax (maximum of 26.18 μg/wk) and of squid from Bizerte (maximum of 37.81 μg/wk), respectively (Table 4), which are still far under the limits (420 μg/wk for Cd and 240 μg/wk for Hg). It is therefore unlikely that Cd and Hg intakes through cephalopod

Table 4–Metal concentrations (μg/g ww), estimated Cd and Hg weekly intakes (EWIs; μg/kg bw), estimated dietary intake for Cu and Zn (mg/day), and target hazard quotients (THQs) for Cd and Hg following the consumption[a] of edible tissue (mantle and arms muscles) of cephalopods caught off 3 different Tunisian regions.

	Cephalopod	Average concentrations		Estimated weekly intake[a]		Percent of PTWI[b]		Target hazard quotients		Average concentrations		Estimated daily intake[a]		Percent of DRIs[c]	
	species	Cd	Hg	Cd	Hg	Cd	Hg	Cd	Hg	Cu	Zn	Cu	Zn	Cu	Zn
Bizerte	European squid	0.039	0.164	0.15	0.63	2.17	15.76	0.02	0.18	2.26	12.34	0.07	0.41	8.31	5.09–3.70
	Common octopus	0.025	0.037	0.10	0.14	1.37	3.58	0.01	0.04	5.23	10.92	0.17	0.36	19.18	4.50–3.28
	Common cuttlefish	0.040	0.040	0.15	0.15	2.18	3.83	0.02	0.04	5.55	14.74	0.18	0.49	20.36	6.08–4.42
Monastir	European squid	0.053	0.079	0.20	0.31	2.92	7.66	0.03	0.09	1.67	11.58	0.06	0.38	6.13	4.78–3.47
	Common octopus	0.026	0.031	0.10	0.12	1.44	3.04	0.01	0.03	4.08	13.28	0.13	0.44	14.98	5.48– 3.98
	Common cuttlefish	0.045	0.067	0.17	0.26	2.48	6.47	0.02	0.07	5.07	12.76	0.17	0.42	18.57	5.26–3.83
Sfax	European squid	0.044	0.076	0.17	0.29	2.43	7.28	0.02	0.08	1.83	9.93	0.06	0.33	6.70	4.09–2.98
	Common octopus	0.113	0.032	0.44	0.12	6.23	3.06	0.06	0.03	4.41	18.40	0.15	0.61	16.17	7.59–5.52
	Common cuttlefish	0.038	0.066	0.14	0.25	2.06	6.37	0.02	0.07	4.44	13.95	0.15	0.46	16.26	5.75–4.18

[a] Based on a daily consumption of 33 g of cephalopod (FAO 2009) for a person weighing 60 kg.

[b] PTWI: Provisional Tolerable Weekly Intake (Cd: 7 μg/kg bw; Hg: 4 μg/kg bw; JECFA 2006, 2010).

[c] DRIs: Dietary Reference Intake (Cu: 0.9 mg/day; Zn: 8 (women); 11 (men) mg/day; IOM 2001).

consumption would cause any risk for average consumers. Indeed, it would be necessary to eat more than 7 times the daily average consumption in the specific case of the squid and octopus that presented the highest concentration in this study (381 and 962 g, respectively). Assuming that most of the Hg found in cephalopod muscles is in methyl-Hg form (ranging from 67% to 83%; Bustamante and others 2006a) and that the standard PTWI value of 1.6 $\mu g\ kg^{-1}$ body weight (JECFA 2010) equals 96 μg MeHg per week for a 60 kg person, we have also estimated the related risk. With the worst hypothesis (83% of MeHg), the average EWI accounted for MeHg was in the range of 15.11% to 32.7%, 6.31% to 7.43%, and 7.94% to 13.43% of the PTWI due to the consumption of squid, cuttlefish, and octopuses, respectively. However, when calculating the maximum safe consumption using the highest MeHg concentrations, the PTWI was reached with only 183.5 g of squid from the northern coast (Bizerte). Taking this result into consideration, it seems that the Hg content in squid from Bizerte may present a health hazard if consumed excessively.

According to the report of USEPA (2013), the dose calculations are made using the standard assumptions for an integrated USEPA risk analysis, including the exposure over an entire 70-y lifetime and for a 60 kg body weight for an average Tunisian adult. In addition, it was assumed in accordance with the USEPA (1989) that the ingested dose is equal to the absorbed contaminant dose and that cooking has no effect on the contaminants (Cooper and others 1991). Table 4 shows the results of estimated THQs for Cd and Hg, caused by consuming muscle tissues of the selected species from different sampling locations. The obtained values were all below the acceptable safe value of 1. Therefore, our results infer that consumption of these cephalopod species at the present rate may not be hazardous to the human population.

In parallel to the negligible risk related to the metal contamination of this seafood, results of essential elements (Cu and Zn) concentrations were analyzed from a nutritional point of view. Given the DRIs published by the IOM (2001), the nutritional contribution that the edible part of the species offers in terms of essential elements was calculated (in DRI percent; see Table 4). The DRI relates to a set of the reference values (RDA) given in concentration level per day (Table 4). The estimated daily intake of each element was calculated using the average content obtained in each muscle tissues for the selected species and considering a meal of 33 g of seafood. Results from Table 4 show that the contribution to daily intake through the consumption of muscle tissues of the selected species was lower than the required DRIs. The contribution of octopuses and cuttlefish from all sampling sites are more important than that of squid, especially for Cu.

Conclusions

In summary, levels of the metals analyzed (Cd, Cu, Hg, and Zn) in the edible parts of 3 cephalopods species (*L. vulgaris*, *O. vulgaris*, *S. officinalis*) from various Tunisian coastal regions were broadly comparable to those reported in similar international studies for the same species. We showed that the average concentrations for these metals were below the limits provided by international authorities and environmental agencies. In addition, the consumption of cephalopods from Tunisian waters did not exceed the PTWIs for the non-essential elements investigated, indicating that average consumers should not be at risk. In fact, the studied cephalopods specie would provide a reasonable percentage of the DRIs for the essential elements (Cu and Zn) in the average human diet.

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Author Contributions

Conceived and designed the experiments: P. Bustamante, R. Ben Chaouacha-Chekir, T. Hajji Performed the experiments: M. Rjeibi and T. Guyot Analyzed the data: M. Rjeibi and M. Metian Wrote the paper: M. Rjeibi, P. Bustamante, M. Metian

References

Ayas D, Ozogul Y. 2011. The effects of season and sex in the metal levels of mature common cuttlefish (*Sepia officinalis*) in mersin bay, northeastern Mediterranean. J Food Sci 76:121–4.

Banni M, Jebali J, Daubeze M, Clerandau C, Guerbej H, Narbonne JF, Boussetta H. 2005. Monitoring pollution in Tunisian coasts: application of a classification scale based on biochemical markers. Biomarkers 10:105–16.

Banni M, Dondero F, Jebali J, Guerbej H, Boussetta H, Viarengo A. 2007. Assessment of heavy metal contamination using real-time PCR analysis of mussel metallothionein mt10 and mt20 expression: a validation along the Tunisian coast. Biomarkers 12:369–83.

Bryan GW. 1976. Some aspects of heavy metal tolerance in aquatic organisms. In: Lockwood A.P.M., editor. Effects of pollutants on aquatic organisms. London, UK Cambridge University Press. p 7–34.

Bustamante P, Caurant F, Fowler SW, Miramand P. 1998a. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. Sci Total Environ 220:71–80.

Bustamante P, Cherel Y, Caurant F, Miramand P. 1998b. Cadmium, copper and zinc in octopuses from Kerguelen Islands, Southern Indian Ocean. Polar Biol 19:264–71.

Bustamante P, Teyssie J, Fowler S, Cotret O, Danis B, Miramand P, Warnau M. 2002. Biokinetics of zinc and cadmium accumulation and depuration at different stages in the life cycle of the cuttlefish *Sepia officinalis*. Mar Ecol Prog Ser 231:167–77.

Bustamante P, Teyssie J, Danis B, Fowler S, Miramand P, Cotret O, 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. Mar Ecol Prog Ser 269:185–95.

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

Bustamante P, Teyssie J, Fowler S, Wamau M. 2006b. Assessment of the exposure pathway in the uptake and distribution of americium and cesium in cuttlefish (*Sepia officinalis*) at different stages of its life cycle. J Exp Mar Biol Ecol 331:198–207.

Caddy JF, Rodhouse PG. 1998. Cephalopod and groundfish landings: evidence for ecological change in global fisheries? Rev Fish Biol Fish 8:431–44.

Carvalho M, Santiago S, Nunes ML. 2005. Assessment of the essential element and heavy metal content of edible fish muscle. Anal Bioanal Chem 382:426–32.

Castro-González MI. 2002. Ácidos grasos omega 3: beneficios y fuentes. Interciencia 27:128–36.

Chouvelon T, Spitz J, Caurant F, Mèndez-Fernandez P, Autier J, Lassus-Débat A, Chappuis A, Bustamante P. 2012. Enhanced bioaccumulation of mercury in deep-sea fauna from the bay of biscay (north-east Atlantic) in relation to trophic positions identified by analysis of carbon and nitrogen stable isotopes. Deep-Sea Res Part I Oceanogr Res Pap 65:113–24.

Coelho M, Domingues P, Balguerias E, Fernandez M, Andrade JP. 1997. A comparative study of the diet of *Loligo vulgaris* (Lamarck, 1799) (Mollusca: Cephalopoda) from the south coast of Portugal and the Saharan Bank (Central-East Atlantic). Fish Res 29:245–55.

Cooper CB, Doyle ME, Kipp K. 1991. Risks of consumption of contaminated seafood: the quincy bay case study. Environ Health Perspect 90:133–140.

Cossa D, Thibaud Y, Roméo M, Gnassia-Barelli M. 1990. Le mercure en milieu marin. Biogéochimie et écotoxicologie. Rapports scientifiques et techniques de l'IFREMER 19 Brest.

DGSV. 2006. Note de service n° 742/200 du 28 mars 2006 fixant les Limites Maximales de Résidus des médicaments vétérinaires et des contaminants dans les produits avicoles, les poissons d'élevage et le gibier sauvage à plumes.

Din JN, Newby DE, Flapan AD. 2004. Omega 3 fatty acids and cardiovascular disease—fishing for a natural treatment. BMJ 328:30–35.

Domingo JL, Bocio A, Martí-Cid R, Llobet JM. 2007. Benefits and risks of fish consumption: part II. RIBEPEIX, a computer program to optimize the balance between the intake of omega-3 fatty acids and chemical contaminants. Toxicology 230:227–33.

Dorneles PR, Lailson-Brito J, dos Santos RA, Silva da Costa PA, Malm O, Azevedo AF, Machado Torres JP. 2007. Cephalopods and cetaceans as indicators of offshore bioavailability of cadmium off Central South Brazil Bight. Environ Pollut 148:352–9.

FAO. 2009. Food and Agriculture Organization. FAOSTAT. Statistical databases. Available from http://faostat.fao.org. Accessed 2014 Feb 2.

FAO. 2013. Yearbook, fishery and aquaculture statistics 2011. Rome: Food and Agriculture Organization of the United Nations.

Hamza-Chaffai A., Pellerin J, Amiard JC. 2003. Health assessment of a marine bivalve *Ruditapes decussatus* from the Gulf of Gabès (Tunisia). Environ Intern 28:609–17.

Harris WS. 1997. n-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nut 65:1645–54.

IOM. 2001. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington DC: The National Academies Press.

Ismail HM. 2005. The role of omega-3 fatty acids in cardiac protection: an overview. Front Biosci 10:1079–1088.

JECFA. 2006. Evaluation of certain food contaminants (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930.

JECFA. 2010. Summary and conclusions of the seventy-second meeting of the joint FAO/WHO expert committee on food additives. Rome, 16–25 February 2010. Geneva: World Health Organisation.

Joiris CR, Holsbeek L, Moatmeri NL. 1999. Total and Methylmercury in Sardines *Sardinella aurita* and *Sardina pilchardus* from Tunisia. Mar Pollut Bull 38:188–92.

Kojadinovic J, Jackson CH, Cherel Y, Jackson GD, Bustamante P. 2011. Multi-elemental concentrations in the tissues of the oceanic squid *Todarodes filippovae* from Tasmania and the southern Indian Ocean. Ecotoxicol Environ Saf 74:1238–49.

Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 113:71–88.

Lacoue-Labarthe T, Warnau M, Oberhänsli F, Teyssié J-L, Bustamante P. 2009. Bioaccumulation of inorganic Hg by the juvenile cuttlefish *Sepia officinalis* exposed to ^{203}Hg radiolabelled seawater and food. Aquatic Biol 6:91–8.

Lourenço HM, Anacleto P, Afonso C, Ferraria V, Martins MF, Carvalho ML, Lino AR, Nunes ML. 2009. Elemental composition of cephalopods from Portuguese continental waters. Food Chem 113:1146–53.

MAFF. 1995. Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of waste at sea, 1993. Aquatique Environment Monitoring Report No. 44. Tech. rep., Directorate of Fisheries Research, Lowestoft, UK.

Miramand P, Bentley D. 1992. Concentration and distribution of heavy metals in tissues of two cephalopods, *Eledone cirrhosa* and *Sepia officinalis*, from the French coast of the English Channel. Mar Biol 114:407–14.

Miramand P, Bustamante P, Bentley D, Kouéta 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*. Sci Total Environ 361:132–43.

Napoleão P, Pinheiro T, Sousa Reis C. 2005. Elemental characterization of tissues of *Octopus vulgaris* along the Portuguese coast. Sci Total Environ 345:41–49.

Oehlenschläger J. 1997. Marine fishnon-a source for essential elements. In: Luten J.B., Børresen T., Oehlenschläger J. editors. Seafood from producer to consumer. Amsterdam, NL: Elsevier. p 641–52.

Official Journal of the European Communities. 2001. Official Journal of the European Communities L 77/1 (16 March 2001), Reg. No. 466/2001.

Oomen CM, Feskens EJ, Räsänen L, Fidanza F, Nissinen AM, Menotti A, Kok FJ, Kromhout D. 2000. Fish consumption and coronary heart disease mortality in Finland, Italy, and The Netherlands. Am J Epidemiol 151:999–1006.

Ozogul Y, Duysak O, Ozogul F, Özkütük AS, Türeli C. 2008. Seasonal effects in the nutritional quality of the body structural tissue of cephalopods. Food Chem 108:847–52.

Piatkowski U, Pierce G, Morais da Cunha M. 2001. Impact of cephalopods in the food chain and their interaction with the environment and fisheries: an overview. Fish Res 52:5–10.

Pierce GJ, Boyle PR, Hastie LC, Santos MB. 1994. Diets of squid *Loligo forbesi* and *Loligo vulgaris* in the northeast Atlantic. Fish Res 21:149–63.

Pierce GJ, Stowasser G, Hastie LC, Bustamante P. 2008. Geographic, seasonal and ontogenetic variation in cadmium and mercury concentrations in squid (Cephalopoda: Teuthoidea) from UK waters. Ecotoxicol Environ Saf 70:422–32.

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

Raimundo J, Pereira P, Vale C, Caetano M. 2005. Fe, Zn, Cu and Cd concentrations in the digestive gland and muscle tissues of *Octopus vulgaris* and *Sepia officinalis* from two coastal areas in Portugal. Cienc Mar 31:243–51.

Rjeibi M, Metian M, Hajji T, Guyot T, Ben Chaouacha-Chékir R, Bustamante P. 2014. Interspecific and geographical variations of trace metal concentrations in cephalopods from Tunisian waters. Environ Monit Assess 186(6): 3767–83.

Rodhouse P, Nigmatullin CM. 1996. Role as consumers. Philos Trans R Soc Lon B Biol Sci 351:1003–22.

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

Smaoui-Damak W, Hamza-Chaffai A, Berthet B, Amiard JC. 2003. Preliminary study of the clam *Ruditapes decussatus* exposed in situ to metal contamination and originating from the Gulf of Gabès, Tunisia. Bull Environ Contam Toxicol 71: 961–70.

Smith CD. 2003. Diet of *Octopus vulgaris* in False Bay, South Africa. Mar Biol 143:1127–33.

Storelli MM. 2009. Intake of essential minerals and metals via consumption of seafood from the Mediterranean sea. J Food Prot 72:1116–20.

Storelli MM, Garofalo R, Giungato D, Giacominelli-Stuffler R. 2010. Intake of essential and non-essential elements from consumption of octopus, cuttlefish and squid. Food Addit Contam Part B 3:14–8.

Storelli MM, Marcotrigiano GO. 1999. Cadmium and total mercury in some cephalopods from the South Adriatic Sea (Italy). Food Addit Contam 16:261–5.

Storelli MM, Giacominelli-Stuffler R, Storelli A, Marcotrigiano GO. 2006. Cadmium and mercury in cephalopod molluscs: Estimated weekly intake. Food Addit Contam 23:25–30.

The R Development Core Team R. 2008. R: a language and environment for statistical computing. Austria: R foundation for statistical computing Vienna.

USEPA. 1989. Risk assessment guidance for superfund. Volume I: human health evaluation manual (Part A), interim final. EPA/540/1–89/002. United States Environmental Protection Agency.

USEPA. 2013. EPA Region III Risk-Based Concentration (RBC) Table 2008 Region III. 1650 Arch Street, Philadelphia, Pennsylvania 19103.

Villanueva R, 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* paralarvae. Aquaculture 261:225–40.