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Elemental composition of cephalopods from Portuguese continental waters

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ABSTRACT

Essential and contaminant elements concentrations were determined in the muscle tissue of octopus (*Octopus vulgaris*), squid (*Loligo vulgaris*) and cuttlefish (*Sepia officinalis*), caught off the Portuguese coast in 2004–2005. As expected, the largest concentrations found correspond to Cl, S, K, Na, P and Mg (average values between 629 mg (100 g)$^{-1}$, for Cl, and 435 mg kg^{-1}, for Mg, in octopus and squid, respectively). Above average concentrations of Zn, Cu, Fe and Sr were also found. The highest total Hg concentration was found in cuttlefish (0.36 mg kg^{-1}); however, this value did not exceed the recommended limit proposed by EU (0.5 mg kg^{-1}). Lead levels observed in all samples were always significantly lower than the EU limit (1.0 mg kg^{-1}). Regarding Cd, the 1.0 mg kg^{-1} limit was only exceeded in two octopus samples. It may be concluded that the cephalopods studied do not constitute cause for concern, in terms of toxic elements, and could be safely used for daily intake of essential elements. Nevertheless, the squid contribution for elemental DI is minor in comparison to the other two species.

1. Introduction

Fish and shellfish are considered some of the most interesting dietary products, and their nutritional benefits are well-known. Seafood is rich in protein, with a balanced amino acid composition and a high proportion of polyunsaturated fatty acids (Belitz, Grosch, & Schieberle, 2004; Oehlenschläger, 1997). These species also contain most of the 90 natural elements (Causeret, 1962; Lall, 1995). The largest concentrations correspond to carbon, hydrogen, nitrogen, oxygen and sulphur (structural elements) followed by chlorine, potassium, phosphorus, sodium, magnesium and calcium (Lall, 1995; Oehlenschläger, 1997). Other elements are present at lower levels, being described as trace or ultratrace elements. These elements are classed as essential, when their biological roles are well-known, such as occurs with iron, copper, zinc, iodine, manganese, selenium or fluorine; non-essential, when their physiological functions have not been clearly demonstrated, such as occurs with nickel, vanadium and arsenic; and toxic, such as mercury, lead and cadmium (Lall, 1995).

Cephalopods are an excellent source of some essential elements (Oehlenschläger, 1997); however, given the morphological and biological characteristics associated to their habitat, some contaminant metals may accumulate in their tissues (Bustamante, Caurant, Fowler, & Miramand, 1998; Bustamante, Grigioni, Boucher-Rodoni, Caurant, & Miramand, 2000; Soldevilla, 1987). Among seafood species, cephalopods represent one of the most important groups captured in Europe (Fernández-Rueda & García-Flórez, 2007). In Portugal, cephalopods represented, in 2005, only approximately 8% of wholesale market sales; however, the corresponding values in terms of auction transaction were approximately three times higher than those registered for fish species (Fonseca, Campos, & Garcia, 2002).

Although, numerous studies on the elemental composition of cephalopod species exist (Napoleão, Pinheiro, & Sousa Reis, 2005; Raimundo, Caetano, & Vale, 2004; Seixas, Bustamante, & Pierce, 2005a, 2005b; Villanueva & Bustamante, 2006), their objectives were mostly related to environmental contamination and its use in biological monitoring. Most of these studies focus on amounts present in several organs such as digestive glands, branchial hearts and gills (Bustamante, Lahaye, Durnez, Churlaud, & Caurant, 2006; Miramand & Bentley, 1992; Miramand, Bustamante, Bentley, & Kouéta, 2006).

The primary aim of this work was to quantify the levels of a high number of essential and toxic elements in the muscle tissue of three cephalopods species much enjoyed by Portuguese consumers, using various analytical techniques. From a public health perspective, this study can provide consumers with better knowledge of nutritional characteristics and contamination problems associated to these species. Additionally, possible relationships

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between concentrations and specimen age were also researched, for all species.

2. Materials and methods

2.1. Sample collection and preparation

Octopus (*Octopus vulgaris*), squid (*Loligo vulgaris*) and cuttlefish (*Sepia officinalis*), were purchased at the Peniche auction, in central Portugal (Atlantic North-eastern European coast), from May to September in 2004–2005. Collected specimens were immediately stored in plastic bags, which were placed in ice and taken to the laboratory. Upon arrival, mantle length, total weight, sex and maturity stages were recorded (Table 1). Edible parts (mantle and arms) were removed and homogenised in a food blender. Homogenised samples were vacuum-sealed in individual plastic bags, coded for easy identification, and stored at −21 °C until required for analysis. Specimen age was estimated by growth models (Bettencourt, 2000; Moreno, Azevedo, Pereira, & Pierce, 2007; Raya, 2001); the corresponding results are presented in Table 1.

2.2. Analytical methods

Determination of K, Na, Mg, Ca, Zn, Fe, Cu, Mn, Ni, Cd and Pb levels was performed by flame atomic absorption spectrometry, according to the procedures described by Jorhem (2000). All analyses were carried out at least in duplicate; an external calibration method was used for quantitative analysis. Edible portion samples (5–10 g wet weight) were dry-ashed at 500 °C under a gradual temperature increase. Ash was dissolved in concentrated nitric acid and the solution obtained was evaporated to dryness. The final residue was redissolved with 12 or 5 ml nitric acid 15% (v/v) and transferred to 25 or 10 ml volumetric flasks (10 ml for Pb, Cd and Ni, 25 ml for other elements); final volumes were adjusted with ultrapure water. Quantification of these elements was performed using a Spectr AA-20 spectrophotometer with deuterium background correction (Varian). Detection limits (DL, mg kg^{-1}, wet weight) were 0.01 (K), 0.09 (Na), 0.02 (Mg), 0.08 (Ca), 0.06 (Zn), 0.32 (Fe), 0.02 (Cu), 0.01 (Mn), 0.02 (Ni), 0.01 (Cd) and 0.02 (Pb).

Total Hg was determined by cold vapour atomic absorption spectrometry (CVAAS) according to the procedure developed by Hatch and Ott (1968) and described by Joiris, Holsbeek, Bouquegneau, and Bossicart (1991). For each specimen, 1 g of homogenised edible portion sample was digested with concentrated sulphuric acid. Sample mercury (Hg^0 and Hg_2^{2+}) was subsequently oxidised to Hg^{2+}, using potassium permanganate. Following Hg^{2+} reduction to Hg^0 with stannous chloride, volatile Hg^0 was bubbled into the Bacharach Coleman MAS-50D Mercury Analyser closed system (λ = 253.7 nm). Samples were analysed twice. Concentrations were calculated by interpolation using a linear calibration curve obtained by measuring the absorbance of standard solutions. The detection limit was 0.01 mg kg^{-1}, wet weight.

Phosphorus was determined spectrophotometrically according to ISO Standard 13730 (1996). Samples (5 g wet weight) were dry-ashed at 500 °C, followed by acid digestion and colorimetric measurement of a yellow compound resulting from the reaction between phosphorus and an ammonium vanadate and ammonium molybdate mixture, at 430 nm. The detection limit was 0.01 mg kg^{-1}, wet weight.

In order to analyse Cl, S, Br, Sr, Rb and Se, homogenised samples were freeze-dried for 48 h, at −45 °C and low pressure (approximately 10^{-1} atm). Samples were powdered and immediately vacuum-sealed, in individual coded plastic bags, which were subsequently stored at −21 °C, until further analysis. Concentrations of these six elements were determined using an EDXRF spectrometer, according to Carvalho, Santiago, and Nunes (2005). Powdered samples were pressed into 2.0 cm diameter pellets. Pellets were glued onto Mylar film, on a sample holder, and directly placed in the path of an X-ray beam, for quantification. Two pellets were prepared for each tissue sample. The detection limits (mg kg^{-1}, dry weight) for these elements were 10 (Cl), 10 (S), 0.8 (Br), 0.5 (Sr), 1.1 (Rb) and 0.6 (Se).

Blanks were always tested in the same conditions as the samples. All laboratory ware was cleaned with HNO_3 (10%) or HCl (20%) for 24–48 h and rinsed with ultrapure water (18.2 MΩ cm), to avoid contamination. Chemical reagents were pro analysis or superior. Commercial standard solutions (Merck, 1000 mg l^{-1}) were used for some elements.

Analytical data for elements are reported in mg $(100\,g)^{-1}$ or mg kg^{-1}, depending on element, on a wet weight basis. Mean moisture content in each species was 78% in cuttlefish and squid and 83% in octopus. These values were used for the conversion of results to dry weight basis for comparison with other studies.

Five certified reference materials were tested in the same conditions as the samples, in order to assess analytical method accuracy: LUTS-1 (non defatted lobster hepatopancreas), TORT-2 (Lobster hepatopancreas), DORM-2 (Dogfish muscle) from National Research Council of Canada, SMRD-2000 (Canned matrix meat) from Swedish Meats R&D and Scan Foods/National Food Administration, Sweden and MA-A-2 (Fish flesh) from International Atomic Energy Agency. Results obtained in this study were in good agreement with certified values (Table 2).

2.3. Statistical analysis

All data were analysed using the STATISTICA 6.1 software (Stat soft, Inc., Tulsa, OK74104, USA). Pearson's correlation between elements was performed. The Student *t*-test was used to evaluate the influence of sex on the concentration of various elements, for each species. Single factor ANOVA analysis was used to confirm the existence of significant differences between age groups, within the same species. Due to the absence of normality and variance homogeneity (Lilliefors-test and Levene-test, respectively), the Kruskal–Wallis non-parametric test was used to evaluate differences in element concentrations between studied cephalopods (Zar, 1999). Differences were considered statistically significant when $p < 0.05$.

3. Results and discussion

3.1. General

Data relative to 16 elements in common octopus, squid and common cuttlefish are presented in Table 3. Mean and standard deviation histograms are shown in Figs. 1 and 2, in order to com-

Table 1
Characteristics of studied cephalopods

Common name (specie)	*N*	Mantle length range (mm)	Weight (g)	Sex	Sex maturation state	Estimated age month (number in each class)
Common octopus (*O. vulgaris*)	10	115–245	427–4900	8 ♂,2 ♀	II, III	3 (2); 5 (2); 6 (3); 7 (3)
Squid (*L. vulgaris*)	10	130–420	65–1244	5 ♂,5 ♀	II, IV, V	7–8 (3); 9–10 (4); 11–13 (3)
Common cuttlefish (*S. officinalis*)	12	165–315	538–3100	6 ♂,6 ♀	III	6–8 (5); 9–12 (3); 13–15 (2); 24 (2)

Table 2
Results of analysis ($n = 4$) for some certified reference materials (mean ± sd, in mg kg^{-1})

		K	Na	P	Mg	Ca	Zn	Fe	Cu	Sr	Mn	Se	Ni	Hg	Pb	Cd
LUTS-1	Certified	948 ± 72	–	–	89.5 ± 4.1	203 ± 33	–	11.6 ± 0.9	15.9 ± 1.2	–	1.20 ± 0.13	–	0.200 ± 0.034	–	–	–
	Obtained	955 ± 23			90.8 ± 2.2	197 ± 16		12.1 ± 0.4	15.4 ± 0.2		1.28 ± 0.03		0.195 ± 0.009			
SMRD-2000	Certified	1859 ± 85	8533 ± 281	1075 ± 47	–	70.3 ± 8.3	–	6.33 ± 1.66	–	–	–	–	–	–	–	–
	Obtained	1938 ± 38	8346 ± 280	1108 ± 11		66.1 ± 7.4		4.94 ± 0.09								
TORT-2	Certified	–	–	–	–	–	180 ± 6	–	–	45.2 ± 1.9	13.6 ± 1.2	–	2.50 ± 0.19	0.27 ± 0.06	0.35 ± 0.13	26.7 ± 0.6
	Obtained						175 ± 1			43.4 ± 3.2	13.1 ± 0.1		2.37 ± 0.08	0.28 ± 0.00	0.35 ± 0.06	26.8 ± 0.1
DORM-2	Certified	–	–	–	–	–	–	142 ± 10	–	–	3.66 ± 0.34	–	–	4.64 ± 0.26	–	–
	Obtained							141 ± 4			3.62 ± 0.18			4.48 ± 0.12		
MA-A-2	Certified	–	–	–	–	–	–	–	–	–	–	1.7 ± 0.3	–	–	–	–
	Obtained											1.5 ± 04				

pare electrolyte, structural and nutrient element concentrations in the three cephalopod species. Total concentrations of Hg, Pb and Cd, metals considered toxic and regulated by the EU Commission (2006), are summarised in Table 4. Elements with related physiological roles were grouped together. Similar patterns were observed for all elements for the three cephalopod species; in general terms, the highest concentrations were found in octopus and the lowest in squid. The main elements were S, Cl, K, Na, P, Mg and Ca, followed by Br, Zn, Fe, Cu, Sr, Rb, Mn and Ni.

3.2. Electrolytes

The most abundant elements were the electrolytes K, Cl and Na, as illustrated in Fig. 1. Potassium contributes to the intracellular ion balance, as a monovalent cation; the two other elements constitute the main extracellular anions and cations, respectively, assuming an important role in acid–base balance (Lall, 1995). The chloride concentration was approximately 630 mg $(100\ g)^{-1}$ in octopus; this value was significantly lower in squid ($p = 0.000$), of approximately 270 mg $(100\ g)^{-1}$. Chloride data in seafood is scarce. In a study of fish species performed by Oehlenschläger (1997), lower values than those found in the present work were described. Nevertheless, in fish from Indian coastal areas, Cl concentrations are the highest, probably due to pollutant organochlorine compounds (Garg & Ramakrishna, 2006). Average Na levels displayed an identical pattern to Cl. As usually stated for fish, Na concentration in these cephalopod species is also equal to the molar amount of Cl. In general, Na contents are considerably higher in shellfish than in finfish (Vlieg, Lee, & Grace, 1991). The highest concentration of this element was found in octopus (572 mg $(100\ g)^{-1}$), followed by cuttlefish (266 mg $(100\ g)^{-1}$) and squid (157 mg $(100\ g)^{-1}$). Similar contents were found in other studies involving molluscs (Lall, 1995; Segar, Collins, & Riley, 1971; Villanueva & Bustamante, 2006; Vlieg et al., 1991). Octopus was the cephalopod species showing the lowest K concentration, as opposed to other electrolytes (Fig. 1). Average K concentrations were between 223 mg $(100\ g)^{-1}$, in octopus, and 289 mg $(100\ g)^{-1}$, in cuttlefish. The average K concentration in octopus was significantly lower than in cuttlefish ($p = 0.01$); values for squid were not significantly different from the average values observed for the other two species. The range found in this study agrees with those obtained by several authors for cephalopods (Carvalho et al., 2005; Karakoltsidis, Zotos, & Constantinides, 1995; Lall, 1995; Villanueva & Bustamante, 2006).

3.3. Structural elements

Phosphorus and sulphur were the main structural elements found in seafood; in general terms, equal amounts of these two elements were found in marine species (Oehlenschläger, 1997). In this study, concentrations ranged between 107 and 285 mg $(100\ g)^{-1}$ and 197 and 444 mg $(100\ g)^{-1}$, respectively (Table 3). Squid showed the highest average value for P (260 mg $(100\ g)^{-1}$), clearly above the average 200 mg $(100\ g)^{-1}$ value found in the majority of seafood. Significant minimum P concentrations were observed in octopus samples, when compared to squid ($p = 0.000$) and cuttlefish ($p = 0.001$). Identical amounts were described by Lall (1995), in octopus, and wild juvenile cephalopods (Villanueva & Bustamante, 2006). Other authors reported similar concentrations for bivalve and fish species (Oehlenschläger, 1997; Segar et al., 1971; Teeny, Gauglitz, Hall, & Houle, 1984). Significantly larger concentrations of S were found in cuttlefish, when compared to squid ($p = 0.001$) and octopus ($p = 0.029$); the average value was 338 mg $(100\ g)^{-1}$. The lowest average concentration was found in squid (229 mg $(100\ g)^{-1}$). Vlieg et al. (1991) reported identical results for some squid species. The highest S concentrations in

Table 3
Elemental contents (wet basis) in edible part of the three studied cephalopods (mean ± standard deviation, median and range)

Elements	Common octopus (n = 10)		Squid (n = 10)		Common cuttlefish (n = 10)	
	Mean ± sd (median)	Range	Mean ± sd (median)	Range	Mean ± sd (median)	Range
Br (mg kg^{-1})	34.0 ± 3.7^a (34.1)	26.8–40.2	13.3 ± 1.4^b (13.7)	11.3–15.3	21.5 ± 2.9^c (21.4)	17.6–24.9
Ca (mg kg^{-1})	213 ± 108^a (177)	76–405	136 ± 43^a (128)	89–211	134 ± 26^a (135)	89–179
Cl (mg $(100\ g)^{-1}$)	629 ± 97^a (652)	460–786	267 ± 36^b (266)	202–328	439 ± 75^a (436)	326–527
Cu (mg kg^{-1})	3.8 ± 1.6^a (3.4)	2.6–8.1	1.5 ± 0.2^b (1.5)	1.3–1.8	4.5 ± 2.5^a (4.3)	1.7–10.3
Fe (mg kg^{-1})	4.2 ± 1.7^a (3.6)	1.9–6.8	1.7 ± 1.2^b (1.2)	0.7–4.7	1.4 ± 0.7^b (1.2)	0.6–2.5
K (mg $(100\ g)^{-1}$)	223 ± 38^a (227)	154–295	261 ± 55^{ab} (242)	193–343	289 ± 46^b (306)	210–359
Mg (mg kg^{-1})	938 ± 262^a (823)	651–1473	435 ± 108^b (433)	283–610	567 ± 99^b (506)	467–705
Mn (mg kg^{-1})	0.31 ± 0.07^a (0.30)	0.22–0.44	0.16 ± 0.03^b (0.17)	0.11–0.22	0.11 ± 0.04^b (0.09)	0.06–0.20
Na (mg $(100\ g)^{-1}$)	572 ± 143^a (519)	399–793	157 ± 33^b (158)	90–194	266 ± 60^b (255)	186–352
Ni (mg kg^{-1})	0.02 ± 0.01^a (0.02)	<0.02–0.04	0.02 ± 0.01^a (0.02)	<0.02–0.04	0.05 ± 0.02^b (0.03)	0.02–0.09
P (mg $(100\ g)^{-1}$)	147 ± 39^a (140)	107–195	260 ± 20^b (262)	230–285	249 ± 23^b (256)	208–280
Rb (mg kg^{-1})	0.44 ± 0.16^a (0.41)	0.22–0.73	0.68 ± 0.12^b (0.66)	0.51–0.95	0.77 ± 0.12^b (0.78)	0.54–0.91
S (mg $(100\ g)^{-1}$)	257 ± 54^a (243)	207–383	229 ± 24^a (226)	197–270	338 ± 55^b (340)	235–444
Se* (mg kg^{-1})	<QL*	–	<QL	–	<QL	–
Sr (mg kg^{-1})	3.8 ± 0.5^a (3.8)	3.0–4.7	1.8 ± 0.2^b (1.7)	1.6–2.3	2.3 ± 0.3^b (2.3)	1.9–2.7
Zn (mg kg^{-1})	17.7 ± 2.2^a (16.9)	15.6–23.0	12.6 ± 1.3^b (12.3)	10.8–15.2	17.7 ± 2.3^a (17.2)	14.0–22.5

Mean ± sd with equal superscript letters for same element, indicates no statistical differences within species ($p > 0.05$).
* Selenium range always between DL and QL (quantification limit), error percentage too large to quantify concentration.

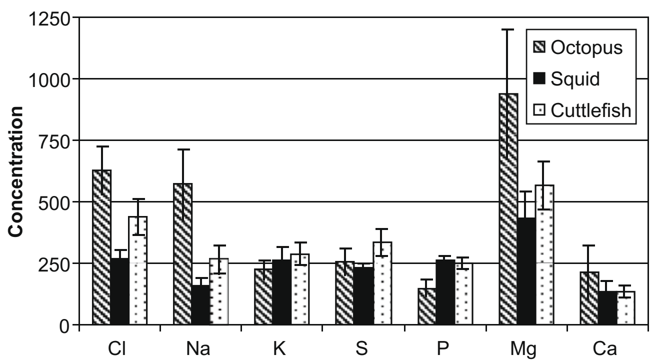

Fig. 1. Mean + sd comparison of macro nutrient elements in cephalopods (Cl, Na, K, S, P in mg $(100\ g)^{-1}$, Mg and Ca in mg kg^{-1}).

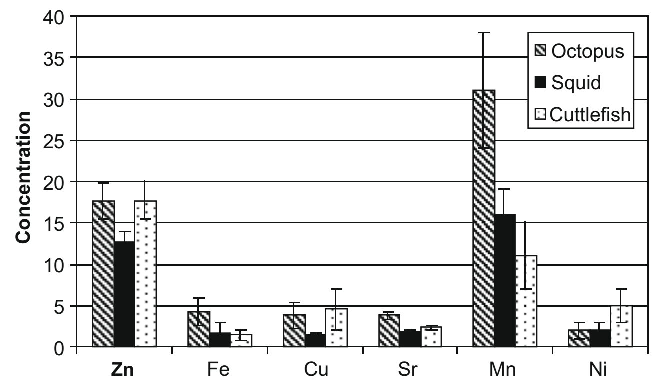

Fig. 2. Mean + sd comparison of micro nutrient elements in cephalopods (Zn, Fe, Cu and Sr in mg kg^{-1}, Mn and Ni in µg $(100\ g)^{-1}$).

juveniles of these three species were reported by Villanueva and Bustamante (2006).

3.4. Nutrient elements

Regarding macro elements, the lowest levels observed corresponded to Mg and Ca. Fishery products are considered poor sources of Mg and Ca (Lall, 1995). Nevertheless, Mg concentrations are always higher than Ca concentrations (Oehlenschläger, 1997). This fact was also observed this study (Fig. 1). The magnesium:calcium ratio (mg/mg) in cephalopods was approximately 3 or 4. The average Mg concentration observed in octopus (938 mg kg^{-1}) was significantly higher than in the other two cephalopod species ($p < 0.05$), ranging between 651 and 1473 mg kg^{-1}. Squid samples showed the lowest levels, ranging between 283 and 610 mg kg^{-1} (Table 3). No significant differences were observed within species in what concerns average Ca levels, although the highest concentration (405 mg kg^{-1}) was found in octopus and the lowest in squid and cuttlefish (89 mg kg^{-1}). These levels are within the ranges observed by other researchers for some cephalopods, bivalve and gastropoda species (Carvalho et al., 2005; Oehlenschläger, 1997; Segar et al., 1971; Vlieg et al., 1991), although lower concentrations were observed in wild juvenile cephalopods in a study performed by Villanueva and Bustamante (2006). A previous study (Karakoltsidis et al., 1995) also reported some differences, namely in squid.

Iron, Cu, Zn and Mn are important elements for health. These elements play the role of functional elements in various metalloenzymes, which have particular catalytic function in living organisms. As frequently observed, concentrations of these elements in cephalopods are high, when compared to other seafood species (Bustamante et al., 2000; Causeret, 1962; Lall, 1995; Raimundo

Table 4
Cadmium, lead and total mercury (mg kg^{-1} wet basis) contents in edible part of the three studied cephalopods (mean ± standard deviation, median and range)

Toxic elements	Commom octopus (N = 10)		Squid (N = 10)		Common cuttlefish (N = 12)	
	Mean ± sd (median)	Range	Mean ± sd (median)	Range	Mean ± sd (median)	Range
Cd	0.38 ± 0.39^a (0.33)	0.03–1.3	0.04 ± 0.03^b (0.04)	0.01–0.10	0.31 ± 0.28^a (0.23)	0.03–1.0
Pb	0.02 ± 0.01^a (0.02)	<0.02–0.04	0.10 ± 0.03^b (0.09)	0.07–0.14	0.04 ± 0.01^a (0.04)	0.03–0.05
Total Hg	0.13 ± 0.06^a (0.12)	0.07–0.21	0.05 ± 0.02^b (0.05)	0.02–0.08	0.15 ± 0.10^a (0.11)	0.08–0.37

Mean ± sd with equal superscript letters for same element, indicates no statistical differences within species ($p > 0.05$).

et al., 2004). Of these four elements, zinc concentrations were the most relevant, corroborating the hypothesis that zinc is always present in seafood, but concentrations found in molluscs are generally higher (Celik & Oehlenschläger, 2004; Lall, 1995). In the present study, average levels reached 17.7 mg kg^{-1}, in octopus and cuttlefish samples (Fig. 2 and Table 3), while squid showed the lowest significant average value, of 12.6 mg kg^{-1} ($p = 0.000$). Similar values had also been found in previous studies (Miramand & Bentley, 1992; Napoleão et al., 2005; Seixas et al., 2005b; Soldevilla, 1987; Vlieg et al., 1991). The highest average Cu concentrations, approximately 4.0 mg kg^{-1}, were found in octopus and cuttlefish samples (no significant differences were observed), with some samples reaching 10 mg kg^{-1}. The maximum value found for squid samples was 1.8 mg kg^{-1} (Table 3). Soldevilla (1987), Seixas et al. (2005b) and Napoleão et al. (2005) found identical amounts for the same octopus species. Nevertheless, some results found in literature for similar species do not agree with the former (Carvalho et al., 2005; Miramand & Bentley, 1992; Miramand et al., 2006; Segar et al., 1971; Villanueva & Bustamante, 2006). In a different study, high concentrations were found in squid; however the species is not specified (Lall, 1995). Iron values found in octopus and squid samples were identical to Cu values (Fig. 2). The highest significant average Fe concentration, approximately 4.0 mg kg^{-1}, was found in octopus ($p = 0.00$); the concentration of this element was only approximately 1.5 mg kg^{-1} in squid and cuttlefish. Some authors observed higher levels (Karakoltsidis et al., 1995; Lall, 1995; Miramand et al., 2006; Seixas et al., 2005b; Villanueva & Bustamante, 2006) in identical species caught in other areas; however, similar concentrations were found in octopus specimens from the Portuguese coast (Napoleão et al., 2005), the Saharan Fishing Bank (Soldevilla, 1987) and the French Atlantic coast (Miramand & Bentley, 1992). Vlieg et al. (1991) also reported higher values than those observed in this study for arrow squid (*Nototodarus* sp.) and broad squid (*Sepiotheutis bilineata*). These different Fe concentrations may be explained by the different maturity stages of the specimens in question, sampling period and different habitats (Carvalho et al., 2005). Published data indicate that Mn amounts are generally low and identical in most seafood species (Astorga España, Rodríguez Rodríguez, & Díaz Romero, 2007; Bustamante et al., 2000; Oehlenschläger, 1997), although higher concentrations are found in some lamellibranchia and gastropoda (Segar et al., 1971). Manganese concentrations in this study were low, ranging between 0.06 and 0.44 mg kg^{-1}, in cuttlefish and octopus, respectively. The Mn profile was similar to that displayed for Fe (Fig. 2). The highest average concentration was found in octopus (0.31 mg kg^{-1}), followed by squid (0.16 mg kg^{-1}) and cuttlefish (0.11 mg kg^{-1}) (Table 3). These values correspond to the usual concentrations found in cephalopods (Miramand & Bentley, 1992; Napoleão et al., 2005; Seixas et al., 2005b; Soldevilla, 1987). Levels were higher in cuttlefish juveniles, reaching approximately 1.4 mg kg^{-1} (Villanueva & Bustamante, 2006). Significant differences in average Fe, Cu, Zn and Mn concentrations were always found between squid and octopus ($p = 0.003$, $p = 0.002$, $p = 0.000$ and $p = 0.01$, respectively).

The concentrations of the last four elements found in this study suggest that cephalopods may contribute significantly to the daily intake (DI) needs, especially in what concerns Cu and Zn.

Little is known about the function of Sr and Rb in organisms. Moreover, the number of studies on the concentrations of these two elements in fishery products is small; nevertheless, Varo (1992) found that Sr levels in seafood could be 20 times higher than in meat. The results of this study indicate that cephalopods may constitute a good source of Sr. Concentrations found were between 1.6 mg kg^{-1} (squid) and 4.7 mg kg^{-1} (octopus), The Sr profile was identical to that observed for some elements (Figs. 1 and 2); the average concentration in octopus (3.8 mg kg^{-1}) was significantly higher than those found in cuttlefish (2.3 mg kg^{-1}) and squid (1.8 mg kg^{-1}). Vlieg et al. (1991) found identical concentrations in squid species. Lamellibranchia and gastropoda species (Segar et al., 1971) also showed similar levels. Concentrations measured by Villanueva and Bustamante (2006) in the same species were higher, particularly in cuttlefish, possibly because this study focused on juveniles. Lowest concentrations were observed in studies performed in fish species (Garg & Ramakrishna, 2006; Teeny et al., 1984). Rubidium concentrations ranged between 0.22 and 0.95 mg kg^{-1}, respectively, in octopus and squid. These concentrations were lower than Sr concentrations and agree with the existing literature (Carvalho et al., 2005; Villanueva & Bustamante, 2006). In a study of fish species from different regions of India, performed by Garg and Ramakrishna (2006), a large dispersion of Rb concentrations was observed. No significant differences were found between squid and cuttlefish, for both elements. A similar concentration pattern was observed for Rb and K in the three species (octopus < squid < cuttlefish), which can be explained by the electrochemical affinity of these elements.

The last essential trace element analysed was Ni. A few years ago, the physiological functions of this element were not clear (Lall, 1995). Nowadays it is thought that Ni plays a role in hormone, lipid and cell membrane metabolism, as well as activating enzymes associated with the glucose breakdown and use (Acu-Cell, 2007). The nickel concentration profile was different from profiles observed for other microelements (Fig. 2). The average levels observed in octopus and squid, of 0.02 mg kg^{-1} (no significant differences were found), were lower than the average level observed in cuttlefish, of 0.05 mg kg^{-1}. These concentrations are considered low, possibly because the specimens collected originate from an unpolluted ocean area. These results agree with those published by other authors (Carvalho et al., 2005; Miramand & Bentley, 1992; Varo, 1992). Nevertheless, Napoleão et al. (2005) and Villanueva and Bustamante (2006) observed higher concentrations of Ni in the same octopus species.

3.5. Non-metals

Selenium and bromine are non-metals, classed as essential or toxic, depending on their concentration (Lall, 1995). Bromine data in seafood is relatively scarce; however, this element is known to have anti-seizure properties, as potassium bromide or sodium bromide, appearing to be effective trace elements regarding the prevention of hyperthyroid conditions (Acu-Cell, 2007). In this study, Br concentrations found in octopus ranged between 26.8 and 40.2 mg kg^{-1}, whereas average Br values found in squid and cuttlefish samples were lower than those found in octopus (13.3 and 21.5 mg kg^{-1}, respectively). Significant differences were observed between the three species ($p = 0.000$ for squid/octopus and $p = 0.03$ for squid/cuttlefish and octopus/cuttlefish). Garg and Ramakrishna (2006) found similar average Br concentrations, between 43 and 93 mg kg^{-1} (dry weight basis), in fish from different regions of India, having considered this element as a pollutant.

Selenium in low concentrations, in addition to its role as an essential micronutrient for normal growth and reproduction, has a protective effect against toxic elements in organisms (Barghigiani, Pellegrini, ĎUlivo, & De Ranieri, 1991; Feroci, Badiello, & Fini, 2005). Although Se was found in all samples analysed, the concentrations observed ranged between the detection limit (DL) and the quantification limit (QL). Concentrations found in squid and cuttlefish samples were close to the QL (1.0 mg kg^{-1}, dry weight; approximately 0.2 mg kg^{-1}, wet weight) whereas concentrations found in octopus were closed to the DL (0.6 mg kg^{-1}, dry weight; approximately 0.1 mg kg^{-1}, wet weight). Similar values were found in studies performed in molluscs (Astorga España et al., 2007; Napoleão et al., 2005; Plessi, Bertelli, & Monzani, 2001; Seixas et al., 2005b). Seafood species usually showed values between 0.10 and 0.60 mg kg^{-1}, wet weight basis (Carvalho et al., 2005; Lall, 1995; Oehlenschläger, 1997; Plessi et al., 2001; Varo, 1992).

3.6. Toxic elements

Non-essential functions are known for Hg, Cd and Pb, which are considered harmful (Ruiter, 1995). Concentrations in marine organisms reflect environmental pollution (Belitz et al., 2004; Carvalho et al., 2005; Ruiter, 1995); bioaccumulation and biomagnification are observed in some organisms. Lead is considered a chronic or accumulative poison (Seixas et al., 2005b). In the species studied, Pb concentrations were the lowest, among toxic elements. The profile observed for this element is different from the profiles observed for the remaining two. Of all samples analysed, squid reached the highest concentration, 0.14 mg kg^{-1}, representing almost one tenth of the limit proposed by the EU for cephalopods (1.0 mg kg^{-1}), whereas average concentrations were low in cuttlefish and octopus samples, of 0.04 and 0.02 mg kg^{-1}, respectively. These values agree with the results published by some authors (Martí-Cid, Bocio, Llobet, & Domingo, 2007; Miramand & Bentley, 1992) despite being lower than results published by others (Raimundo et al., 2004; Seixas et al., 2005; Villanueva & Bustamante, 2006).

Mercury is one of the most toxic elements, with seafood representing one of its major sources in the human food chain (Plessi et al., 2001). Total Hg levels detected in cephalopods were always lower than the limit set by the EU (0.5 mg kg^{-1}), in all samples studied. Average Hg concentrations were similar in octopus and cuttlefish, of approximately 0.14 mg kg^{-1}; however, maximum concentrations were found in cuttlefish (0.37 mg kg^{-1}) whereas the lowest levels, of approximately 0.02 mg kg^{-1}, were found in squid. These values agree with results reported by other authors, for the same species (Bustamante et al., 2006; Pierce, Stowasser, Hastie, & Bustamante, 2007; Villanueva & Bustamante, 2006). In general terms, it may be said that total Hg concentrations in cephalopods are low (Plessi et al., 2001; Raimundo et al., 2004; Seixas et al., 2005a, 2005b; Villanueva & Bustamante, 2006) and do not represent a risk for human consumption.

Molluscs very often accumulate cadmium in digestive glands (Bustamante et al., 1998); however, minor amounts are also found in the mantle and arms (Miramand & Bentley, 1992). In most organisms, this element may compete with Fe, Cu and Zn, which turns its presence into a serious hazard (Carvalho et al., 2005). Regarding the cephalopods studied, modest average amounts were found in squid (0.04 mg kg^{-1}), when compared to octopus and cuttlefish, in which average concentrations of 0.38 and 0.31 mg kg^{-1} were found, respectively (Table 4). In squid, data obtained agrees with data obtained by other authors (Martí-Cid et al., 2007; Pierce et al., 2007; Villanueva & Bustamante, 2006). The limit proposed by the EU for cadmium (1.0 mg kg^{-1}) was reached in one cuttlefish sample and exceeded in two octopus samples, despite this fact the average levels did not exceed the indicative value. Bustamante et al. (1998), Raimundo et al. (2004) and Miramand et al. (2006) found identical concentrations for similar species. Nevertheless, lower concentrations were reported in cuttlefish, in different studies (Martí-Cid et al., 2007; Miramand & Bentley, 1992; Villanueva & Bustamante, 2006). In octopus, some authors also observed higher levels (Seixas et al., 2005b; Soldevilla, 1987), possibly due to the different environments.

In general terms, mean and median concentrations of these toxic elements are identical (Table 4), which indicates that the cephalopod population in the study habitat may be well-known. Statistical analysis performed for these three toxic metals did not reveal significant differences between octopus and cuttlefish. This fact shows that squid must have a different behaviour from the other two cephalopod species, probably due to its size and diet.

3.7. Influence of age, sex and relationships between element levels

Comparison between age groups was performed for each species. Differences in Hg concentrations were only found in squid. No significant differences were found between age groups in octopus and cuttlefish. Several studies were performed in seafood to test the significance of differences or find correlations between age or size and Hg concentrations; however, the results obtained were inconsistent (Pierce et al., 2007; Raimundo et al., 2004; Seixas et al.,2005a, 2005b). In a similar way to what occurred in the present study, Pierce et al. (2007) found that body size was a significant factor in *Loligo forbesi*, whereas other authors (Raimundo et al., 2004; Seixas et al., 2005b) did not find any significant differences in octopus. Significant differences in element concentrations with sex were only found for Mn and only in squid and cuttlefish ($p = 0.001$ and $p = 0.03$, respectively). Mn concentrations were higher in females of these two species, probably due to reproductive requirements.

Numerous positive and negative correlations were observed among elements for all cephalopod species (data not show). Some of the most significant ($p < 0.005$ and absolute correlation coefficient $r \geqslant 0.8000$) positive correlations found were between K/Mg/Zn, Mn/Fe, Zn/Cu, Fe/Cd, and Cu/Cd. In octopus some negative correlations were also found, such as Sr/K and Sr/P. Seixas et al. (2005b) found similar relationships with Cd. These correlations may be explained by the Cd interaction with the metabolism of these essential metals (Peraza, Ayala-Fierro, Barber, Casarez, & Rael, 1998).

3.8. Daily and provisional tolerable weekly element intakes

Several suggestions have been made regarding daily intake (DI) values relative to elements (Belitz et al., 2004; Lall, 1995; Oehlenschläger, 1997). The values proposed by Belitz et al. (2004) and Acu-cell (2007) are listed in Table 5. Considering the concentrations found in the present work, DI percentages for each element are also shown in Table 5. Analysis of the results included in this table shows that cephalopods may contribute significantly to the DI of S, P, Mg, Zn and Cu, representing 10–38% of the DI. Daily intakes of Ni and Sr are also important. Additionally, Ca, Fe and Mn percentages are small, ranging between 0.3% and 3%. The Na percentage (based on minimum intake for good health) is higher than the K percentage, especially for octopus, which indicates a high Na/K ratio. This fact is important, since some studies suggest an increased risk of developing high blood pressure and cardiovascular disease (Astorga España et al., 2007). The contribution of octopus and cuttlefish is more relevant than that of squid, in general terms. This dif-

Table 5
Daily intake and contribution of each element in octopus, squid and cuttlefish

Elements	Daily intake (DI)[a] (mg)	Percent of DI in a 100 g portion of cephalopods (octopus–squid–cuttlefish)
Ca	1000	2–1–1
Cl	3500	18–8–12
Cu	1–1.5	30–12–36
Fe	15	3–1–1
K	2000–5900	6–7–7
Mg	300–400	27–12–16
Mn	2–5	1–0.5–0.3
Na	<2300	25–7–12
Ni	0.025–0.030	7–7–18
P	800–1200	15–26–25
S	800–1000	28–25–38
Sr[b]	1–5	15–7–9
Zn	10–15	14–10–14

[a] Belitz et al. (2004).
[b] Acu-Cell (2007).

ference could be explained by a different diet. According to the Joint Food and Agriculture Organisation/World Health Organisation (FAO/WHO) (WHO, 1999, 2003) provisional tolerable weekly intakes (PTWI) for Hg, Cd and Pb are 5 $\mu g\ kg^{-1}$ body weight, 7 $\mu g\ kg^{-1}$ body weight and 25 $\mu g\ kg^{-1}$ body weight, respectively. With basis on a weekly average consumption of fishery products in Portugal of 1120 g (160 g/day) (FAO, 2007), an average human body weight of 60 kg and the average Hg, Cd and Pb values found in this study (Table 4), estimated weekly intakes of Hg and Pb are much lower than established PTWIs, for all three species. Nevertheless, the PTWI of 7 $\mu g\ kg^{-1}$ body weight for cadmium is almost reached or slightly exceeded when cuttlefish (6.7 $\mu g\ kg^{-1}$ body weight) and octopus (9.7 $\mu g\ kg^{-1}$ body weight) are consumed. However, it would be very unlikely for a person to consume the aforementioned amounts of these species per week; therefore, the values obtained are overestimated.

4. Conclusion

The elemental profile is quite similar for the three species studied, the major elements being Cl, Na, K, S, P, Mg and Ca, followed by Br, Zn, Fe, Cu, Sr, Rb, Se, Mn and Ni. This indicates that these elements may have the same physiological importance in the three cephalopod species. Results obtained in this study also suggest that cephalopods may constitute a good source of some essential elements, such as P, Mg, Zn and Cu. In general, comparison of element concentrations in octopus, squid and cuttlefish shows that the lowest concentrations of most elements are found in squid; therefore, its contribution for DIs is lower than that of the other two species. Regarding Hg and Pb intakes, consumption does not guide to any concerns, although it should be moderate when considering Cd intake, especially regarding octopus.

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