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Interannual patterns of variation in concentrations of trace elements in arms of
Octopus vulgaris

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Abstract: Concentrations of essential (copper, iron, manganese, selenium, and zinc) and non-essential (arsenic, cadmium, mercury, and lead) trace elements were measured in arms of *Octopus vulgaris*. The cephalopods were sampled from commercial fishery landings at two sites on the Portuguese coast in spring 2002 and 2003. Mercury was determined using an Advanced Mercury Analyser Spectrophotometer (AMAS) and other trace elements were measured using inductively coupled plasma-atomic emission spectrometry (ICP-AES). Since high levels of arsenic were detected, identification of the forms present was carried out using high performance liquid chromatography (HPLC) followed by ICP-MS. Mean concentrations of trace elements analysed were in the following order: As > Zn > Fe > Cu Cd > Pb > Se > MnHg. The results of speciation of arsenic demonstrate that virtually all arsenic was in the arsenobetaine form, which is the less toxic form. The concentrations of several trace elements were generally high in samples from Viana in 2002. Cadmium concentrations were above the legal limit for human consumption in samples from Viana in 2002 and two of these animals also had lead concentrations that exceeded legal limits. Mercury appeared in all samples but levels were within legally defined safe limits. No relationship was detected between trace element concentrations and size or maturity of octopus.

Keywords: Cephalopods; Metals; Bioaccumulation; Contamination; Human exposure

1. Introduction

Like several other cephalopod species, the common octopus *Octopus vulgaris* is part of the traditional diet of coastal communities in southern Europe. In Portugal, *O. vulgaris* is an important target species in fisheries and has a high economic, social and cultural value. Therefore, contaminant levels in cephalopods are also of direct concern to public health. Several studies have revealed that cephalopods have the capacity to accumulate trace elements at high levels in their tissues (e.g. Miramand and Bentley, 1992; Bustamante, 1998; Bustamante et al., 1998a,b, 2000, 2002a,b; Storelli and Marcotrigiano, 1999). Among tissues, the digestive gland and branchial hearts appeared to play a major role in bioaccumulation processes of toxic elements such as cadmium and silver, which are detoxified and sequestered, sometimes over a very long time scale (Bustamante et al., 2002a, 2004; Beuerlein et al., 2002). In general, feeding is considered to be the primary pathway for trace element bioaccumulation in cephalopods and second seawater. Although *O. vulgaris* is benthic, living in direct contact with the substratum, which represents another possible pathway for trace element accumulation.

O. vulgaris can therefore constitute a significant source of essential elements for man but also represents a source of exposure to toxic elements. Thus, there is a need to assess the trace element concentrations in their tissues. Although the highest concentrations of metals may be found in digestive glands of cephalopods (e.g. Miramand and Bentley, 1992; Bustamante et al., 2000), muscular parts, i.e. arms and mantle, are the most commonly eaten by humans and the total amount of toxic elements in these tissues may still be significant.

Only a few studies investigating elements concentrations in octopus from Portugal have been published to date (Seixas et al., 2002, in press; Raimundo et al., 2004). In the former article, the technique used for lead analyses (i.e. particle-induced X-ray emission), did not allow the quantification of this metal in octopus arms (Seixas et al., 2002). Secondly, the others studies were limited to a single period (i.e. 2003, Raimundo et al., 2004) or to a low number of elements (only Hg, Seixas et al., in press). Finally, the bioaccumulation and distribution between tissues of various trace elements in octopus from Portuguese waters have been also studied (Seixas and Pierce, in press, submitted). Therefore, there is a need to provide more data on trace element concentrations in edible tissue of octopus to better evaluate the intake of human populations feeding on this cephalopod. Thus, the present study brings together data on nine different essential and non-essential trace elements in edible tissues (the muscular arms) of octopus *O. vulgaris* collected over two years (2002 and 2003) from two sites on the Portuguese coast, in more and less polluted areas. In addition to examining geographical and interannual differences in concentrations, relationships between levels of different elements, and variation in levels in relation to sex and maturity, were examined.

2. Material and methods

2.1. Sampling and sample preparation

Octopus were sampled from commercial fishery landings in Cascais, which situated mid-way down the coast of Portugal, with a strong influence of the Tagus River (the largest river in Portugal), and in Viana do Castelo, situated in the north of the Portugal and influenced by freshwater flows into the Rias Gallegas (NW Spain) and the rivers closer to the area (Lima river and Minho river) (Fig. 1).

Octopuses were sampled in spring of 2002 and of 2003, 3 females and 3 males from each area in each year. Results for mercury in arm samples from 2002 were previously used in a comparison of levels in different tissues (digestive gland, branchial hearts, gills, mantle, arms and gonads; Seixas et al., in press).

Fresh animals were taken back to the laboratory and dissected. Total length, mantle length, total weight, sex and maturation state were determined in each animal. The maturation state was evaluated by direct observation of colours of reproductive structures (Gonçalves, 1993). The maturity index used was from Guerra (1975) and is based on microscopic analyses and measurements of ovules and spermatophores. Gonad weights and digestive gland weights were expressed as percentages of total body weight, i.e. as gonadosomatic (GSI) and digestive gland (DGI; see Silva et al., 2002) indices, respectively.

Samples of arm, a piece of around 10 g were taken from each animal. Prior to the determination of the concentrations of trace elements, all these samples were stored frozen (between -20 °C to -40 °C) in individual plastic bags. Samples were then freeze-dried, and reduced to a powder using a porcelain mortar and pestle.

2.2. Analytical procedure to determine mercury

For mercury analysis, two aliquots ranging from 10 to 20 mg of dried material were analysed directly in an AMA (Advanced Mercury Analyser Spectrophotometer), Altec AMA 254. Hg determination involved evaporation of mercury by progressive heating to 800 °C in an oxygen atmosphere for 3 min and subsequent amalgamation on a gold-net. The net was then heated to liberate the collected mercury, which was measured by UV atomic absorption spectrophotometry. Quality assurance was assessed using lobster hepatopancreas TORT-2 (NRCC) and dogfish liver and muscle, DOLT-2 and DORM-2 (NRCC) respectively, as reference materials. These standards were treated and analysed under the same conditions as the octopus samples, and recoveries of mercury ranged from 99% to 101%. The detection limit, calculated as 3 SD of the mean of eight blanks, was 0.005 for Hg (mg kg⁻¹ dry wt). Mercury concentrations in octopus arm are also reported in mg kg⁻¹ dry wt.

2.3. Analytical procedure to determine other elements

Arsenic, cadmium, copper, iron, manganese, lead, selenium and zinc concentrations were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) after acid digestion with 5 ml of 65% HNO₃ in a microwave then completed to 50 ml with Milli-Q quality water. The same procedure was applied to blanks and reference materials, TORT-2, DOLT-2 and DORM-2. The results for standard reference materials displayed recoveries of the elements ranging from 88% to 110%. The detection limits were (mg kg⁻¹ dry wt): 8.5 (As), 0.8 (Cd), 0.8 (Cu), 1.7 (Fe), 0.8 (Mn), 0.8 (Pb), 0.8 (Se) and 1.7 (Zn). Trace element concentrations in octopus arm are also reported in mg kg⁻¹ dry wt.

2.4. Analytical procedure for speciation of arsenic

The speciation of arsenic was determined in samples from Viana and Cascais collected in 2003. A part of each sample was freeze-dried, then powdered with a porcelain mortar and pestle. The other part of each sample was homogenised and analysed fresh. The homogenate or powder was then digested with 0.1 g of trypsin and 10 ml 0.1 M ammonium bicarbonate for 12 h in a water bath at 37 °C.

Arsenic speciation was carried out by high performance liquid chromatography (HPLC) followed by inductively coupled plasma-mass spectrometry (ICP-MS). HPLC analysis was carried out on a Varian ProStar, using a Hamilton PRP1 column. For the mobile phase, we used tetrabutylammonium dihydrogenophosphate 0.5 mM and sodium phosphate 4 mM. For each run, 20 µl of sample was injected, and total time for elution of all species was 15 min.

ICP-MS analysis was carried out on a Varian Ultra Mass 700, with the following settings: plasma flow 15.0 l/min, auxiliary flow 1.05 l/min, nebulizer flow 0.90 l/min, sampling depth 7 mm, power 1.30 kW and pump rate 28 rpm. The ultramass ion optical specifications were: extraction lens -600 V, first lens -260 V, second lens -11.80 V, third lens 0 V, fourth lens -60 V and photon stop -11.20 V. The ultramass scan conditions were: points per peak 1, reading spacing 0.100 AMU, and time scan acquisition 10 ms/point.

2.5. Statistical procedures

Statistical analysis was carried out using STATISTICA (StatSoft, Inc., 1995). ANOVA was used to test the influence of sex, year and location on element concentrations in arms. When the level was below the detection limit it was assumed that the concentration was half the detection limit: this was the case for manganese (1 value), selenium (4 values) and cadmium (17 values). Correlations between state of maturation (an ordinal variable) and concentrations in tissues were analysed with the Spearman rank order correlations. To quantify relationships between other parameters (e.g. total length and total weight) and concentrations of elements, the Pearson coefficient of correlation was used. The

Ward's Method of tree clustering, which is based on an analysis of variance approach to evaluate the distance between clusters, was used to provide an indication of the overall similarity of different samples. The significance level for statistical analyses was always set at $\alpha = 0.05$.

3. Results

3.1. Trace element concentrations in relation to body size and maturation

There were no significant correlations between concentrations of trace elements in arms of octopus sampled and body length or state of maturation (Table 1). Only in the case of iron was a significant (negative) relationship seen between concentration and body weight (Table 1). Given that Table 1 reports 27 correlation coefficients, at least one would be expected to be significant ($p < 0.05$) by chance alone. Thus, while noting the small sample size, the general conclusion is that trace element concentrations are not related to body size or maturation state.

3.2. Essential elements (copper, iron, manganese, selenium, zinc)

The average concentrations in octopus arms ranged between 1 and 2 mg kg⁻¹ for selenium, 1.5 and 2.5 mg kg⁻¹ for manganese, 40 and 50 mg kg⁻¹ for iron, 4 and 203 mg kg⁻¹ for copper, and 50 and 300 mg kg⁻¹ for zinc (Fig. 2).

Comparisons between localities of essential trace elements demonstrated significant differences only for selenium (Table 2), although it should be noted that sample sizes were relatively small for all comparisons ($N = 24$ animals overall). In 2002 there were higher levels of selenium in the Viana samples ($t = 2.44$, $p = 0.04$) but this difference was not apparent in 2003 ($t = -2.04$, $p = 0.07$).

Of the essential elements, only copper and selenium showed significant interannual variation (Table 2). In Viana samples, concentrations were higher in 2002 (Cu: $t = 2.63$, $p = 0.03$; Se: $t = 3.95$, $p < 0.01$). In Cascais samples, the between-year differences for these elements were not significant (Cu: $t = 0.83$, $p = 0.43$; Se: $t = 1.19$, $p = 0.27$).

Iron concentrations were higher in females than in males (Table 2, Fig. 2) but concentrations of the other essential elements were similar in both sexes.

3.3. Non-essential elements (arsenic, cadmium, mercury, lead)

Although toxic in all but trace amounts, arsenic is normally considered to be an essential element (Anke et al., 1997). However, in cephalopods there is no published information on its role, so it is considered here along with the other non-essential (and toxic) elements.

Cadmium concentrations were higher in 2002 than in 2003. Viana samples from 2002 had an average cadmium concentration of around 20 mg kg⁻¹ while values for the 2003 samples were all below the detection limit (Fig. 2). Cadmium was detected in five out of six animals from Viana in 2002 and in two of the Cascais animals from 2002.

The average concentrations of arsenic, mercury and lead in octopus arms were 40–130 mg kg⁻¹, 0.15–0.45 mg kg⁻¹ and 3–4 mg kg⁻¹, respectively (Fig. 2).

Comparisons of non-essential trace elements between localities demonstrated significant differences only for arsenic and mercury. Differences between localities for arsenic were significant in 2003 ($t = -4.03$, $p < 0.01$), with higher levels in Cascais samples, but difference was not significant in 2002 ($t = -1.54$, $p = 0.16$). Concentrations of mercury were significantly higher in the Cascais samples in both years ($t = -3.46$, $p = 0.01$; $t = -10.57$, $p < 0.01$, respectively).

There were also significant interannual differences in concentrations of arsenic and mercury. In Viana samples, concentrations were higher in 2002 (As: $t = 4.63$, $p < 0.01$; Hg: $t = 2.39$, $p = 0.04$), although in Cascais there was no significant difference between years (As: $t = 1.19$, $p = 0.26$; Hg: $t = 0.97$, $p = 0.35$).

To determine the species of arsenic present, we used both dried and fresh samples; both gave identical results. The results were also the same for both localities, Viana and Cascais. The chemical form of arsenic was almost 100% arsenobetaine, while As III (arsenite) and As V (arsenate) were below the detection limit of 5 µg l⁻¹ in all samples.

3.4. Correlations between different trace elements

Concentrations of arsenic and mercury were positively correlated, as were those of zinc, cadmium and copper (Table 3). Selenium concentrations were correlated with those of copper, manganese, zinc and cadmium. Iron concentrations were correlated with those of manganese and lead.

Most of these relationships also emerge from the Ward's diagram (Fig. 3), notably the close relationship between cadmium, copper and zinc (see also Fig. 4), although lead and iron are in different halves of the classification.

4. Discussion

4.1. Essential elements

The concentrations of the essential elements—copper, iron, manganese, selenium and zinc—varied over two orders of magnitude, with the lowest average concentrations for manganese and selenium below 2.5 mg kg⁻¹ and the highest for zinc reaching up to 70 mg kg⁻¹, and the following overall pattern of

relative concentrations: $Se \approx Mn < Cu < Fe < Zn$. These essential elements are thought to be regulated by homeostatic processes.

Manganese is involved in protein, lipid and carbohydrate metabolism (NAS, 2002), is a cofactor for enzymes (particularly those involved in phosphorylation), and is involved in glycolysis and the synthesis of cholesterol, DNA, RNA and fatty acids (Bowen, 1979; Goyer, 1995; Barceloux, 1999a; Roth, 2003). It is also essential to the activity and development of nervous tissues (Ledig et al., 1991). Iron is a component of a number of proteins such as ferritin (Nardi et al., 1971; NAS, 2000, 2002). Copper is an important component of respiratory pigment hemocyanin (Barceloux, 1999b). Indeed, 85% of the total copper in *O. vulgaris* is bound to this respiratory pigment (D'Aniello et al., 1986) and hemocyanin represents 98% of the blood proteins in cephalopods (Ghiretti, 1966). Zinc acts as a cofactor in a variety of cellular processes, including DNA synthesis (Barceloux, 1999c). Selenium is a component of glutathione peroxidase, which is an important enzyme for processes that protect lipids in polyunsaturated membranes from oxidative degradation (Barceloux, 1999d).

However, the concentrations of trace elements could also vary in relation to several endogenous (biological) and exogenous (environmental) factors. Among them, the sex of the animal is likely to be one of the most important. However, in our study, the concentrations of essential elements did not vary significantly with gender, except for iron. Indeed, female octopuses exhibited higher iron concentrations independently of the year or the area. This result is difficult to explain but might be link to different metabolic needs in relation to egg production.

Comparison of essential trace element concentrations in octopus from different localities showed significant differences only for selenium. Octopus from Viana in 2002 were characterised by relatively high and variable concentrations of selenium compared to those from Cascais. However, our values for the other samples were quite similar to values recorded in squid from the English Channel (see Table 4).

The values for selenium obtained in the present study were broadly in line with results from samples of *O. vulgaris* from three localities on the Portuguese coast in 1999–2000 (Seixas and Pierce, in press). Published data for other cephalopods refer to concentrations of elements per unit wet weight. For octopus arms the normal ratio of wet weight to dry weight is approximately 5:1 (S. Seixas, unpublished data), so our values are rather high compared to those recorded in other cephalopods (see Table 4).

In relation to manganese and iron, again levels were similar to those found in 1999/2000 (Seixas and Pierce, submitted) and all were high compared to published results for cephalopods (Table 4). Our values for copper were somewhat higher than recorded in other cephalopods, although a value equivalent to almost 40 mg kg^{-1} dry wt has been recorded in squid (Falandysz, 1989). It may be seen that zinc concentrations calculated in the present study were similar to the values found in the same area in 1999/2000 and generally comparable with values recorded for other cephalopods.

4.2. Non-essential elements

Levels of arsenic observed in 2002 did not differ between localities but in 2003 there were differences between Viana and Cascais. 2003 was a year with important floods that could have moved a large amount of sediment from the Tagus estuary to the adjacent coast (S. Seixas, personal observation). In fact, one of the major inputs of arsenic to the marine environment is river runoff (WHO, 2001).

The toxicity of arsenic depends on the chemical form. In order of decreasing toxicity, the common forms are: arsenite (+3), arsenate (+5), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA, MSMA), arsenobetaine, arsenocholine and trimethylarsine oxide (Hamasaki et al., 1995). The chemical form of arsenic recorded in the present study was almost 100% arsenobetaine, while As III (arsenite) and As V (arsenate) were below the detection limit of $5 \mu\text{g l}^{-1}$ in all samples. In most marine animals, arsenobetaine ($(\text{CH}_3)_3\text{As} + \text{CH}_2\text{-COO}^-$) is the sole or major arsenic compound, with other organo-arsenic compounds and inorganic arsenic generally occurring as minor constituents (Francesconi and Edmonds, 1997). Arsenobetaine plays an important role in accumulation of arsenic in the trophic chain (Kubota et al., 2003). The transformation by animals of other forms of arsenic into arsenobetaine can be a mechanism of detoxification, because arsenobetaine is not found in seawater. The mechanism of this transformation is, however, not clear (Bettencourt, 1990). The dominant form of arsenic in oxygenated marine and brackish waters is arsenate, which is very toxic (Neff, 1996).

The other non-essential elements measured in this study are toxic to the organisms. In fact cadmium (Cd^{2+}) is known to be able to substitute for Zn^{2+} (WHO, 1992). In living animals, cadmium is transported to the liver or equivalent structure and induces the synthesis of metallothionein (e.g. George and Viarengo, 1985; Cosson et al., 1991). Continuous synthesis of metallothionein is necessary for the continued sequestration of the cadmium ions (Adams et al., 1993). This association with metallothionein can be considered as a mechanism of cadmium detoxification of the organism (Viarengo and Nott, 1993; Roesijadi, 1996). Mercury is considered to be one of the most problematic metals in marine ecosystems because of its bioaccumulation and biomagnification in marine foodwebs (EPA, 2001). The predominant form of mercury in seafood is methyl mercury (NAS, 1991). This is the form that is most toxic to organisms, which it provokes deleterious effects in the nervous system and interferes with the process of cell division (Horvat, 2001; Pinho et al., 2002). Lead is a classic chronic or cumulative poison, which produces a continuum of deleterious effects on animals and humans (WHO, 2000). Aquatic organisms take up and accumulate lead from water, sediment or/and food. Lead in water is fixed by lysosomes when water crosses the branchial epithelium of bivalves. When lead in food arrives in the digestive tract, there is immobilisation by interstitial cells and capture by amoebocytes (Amiard, 1988; Galdies and Axiak, 1992). In animals, lead affects a great number of enzymes and physiological systems (WHO, 2000).

The absence of cadmium in detectable amounts in 2003 suggests that cadmium contamination may be localised in space or time; clearly further sampling is needed to fully understand the extent of

contamination in the fished octopus population, particularly in the Viana area. Studies carried out by the National Water Institute (INAG) in the same area on mussels, between May and June of 1999, verify that levels were high especially at Viana. In fact the values for cadmium concentration in octopus arms obtained in this study are very high when compared with values in the literature for other cephalopods (Table 4), adding weight to the idea that a local source of contamination is responsible for the general increase of Cd concentrations in molluscs from this area.

Mercury concentrations for this species reported in the literature (Table 4) range from values considerably lower than recorded in the present study (Plessi et al., 2001) to around an order of magnitude higher (once corrected to refer to dry weight; Renzoni et al., 1973). There were few references in literature about levels of lead in arms of cephalopods, although results from squid (probably *Loligo* spp.) from the English Channel indicate a concentration at least an order of magnitude lower than that recorded here (Table 4).

4.3. Correlations between concentrations of different trace elements

The association between manganese and iron in the octopus was evident from the Ward's diagram (Fig. 3). Manganese and iron have synergistic effects: the absorption of iron depends on manganese although the concentration of manganese is frequently lower than iron (Roth, 2003). There were no significant differences between localities and years in concentrations of either manganese or iron.

Concentrations of cadmium, copper and zinc were all positively correlated with each other (Table 3). Such correlations between these elements have been noted by various including in other studies on cephalopods (Bustamante, 1998; Bustamante et al., 2002a,b). These correlations can be explained by the high affinity that copper demonstrates for proteins responsible for the regulation of zinc, the metallothioneins (Viarengo, 1989). Cadmium also demonstrates a great affinity to metallothioneins, which provides a mechanism for detoxification of the animals (Cosson et al., 1991). Consequently, cadmium behaves more like essential element than like a toxic element (Fig. 3).

There was a positive correlation between concentrations of selenium and cadmium. Selenium has been reported to be involved in the detoxification processes of several metals, e.g. cadmium, copper, mercury and silver, allowing the formation of insoluble complexes of metal-selenide (Goyer, 1995). A correlation between concentrations of selenium and cadmium in mantle tissue of the octopus *Eledone cirrhosa* (Barghigiani et al., 1993) was interpreted for the presence of protection mechanism based on the formation of a Cd-Se complex and subsequent release from the body. Thus selenium may also have a role in detoxification of cadmium in *O. vulgaris*.

4.4. Concern for public health

Based on the present results, arsenic is not a concern to humans who consume octopus from Portuguese coastal waters, because arsenobetaine is known to be rapidly excreted into urine in humans and is both metabolically inert and non-toxic (Fodor, 2001; Urieta et al., 2001).

Concentrations of cadmium in octopus arm samples from Viana were higher than the maximum levels of this metal allowed for human consumption, namely 1.0 mg kg^{-1} wet weight (EC rule no. 466/2001), which corresponds (in octopus arm tissue) to 5.0 mg kg^{-1} dry wt. This level or higher occurred in 4 out of 5 animals in which cadmium was detected in 2002. Further studies are needed to assess the risk of octopus consumption by Portuguese people, particularly considering the chemical form of cadmium in octopus arms.

Maximum levels of mercury allowed for human consumption are 0.5 mg kg^{-1} fresh weight (EC, rule no. 466/2001), which corresponds (in arm tissue) to 2.5 mg kg^{-1} dry wt. Portugal signed the Paris convention, under which it was established that the maximum level of mercury in animals for human consumption is 0.3 mg kg^{-1} fresh weight, which corresponds to approximately 1.5 mg kg^{-1} dry wt. The levels of mercury in our study were rather high but below the permitted limit. The highest value in this study was 0.56 mg kg^{-1} dry wt. The fact that this species can accumulate mercury to concentrations well above safe limits has been shown previously by Renzoni et al. (1973) for *O. vulgaris* from the Tyrrhenian Sea, who recorded an average concentration in muscle of 1.65 mg kg^{-1} wet weight, roughly equivalent to 8 mg kg^{-1} dry wt.

Maximum levels of lead allowed in food for human consumption are 1.0 mg kg^{-1} wet weight (EC rule no. 466/2001), which corresponds (in arm tissue) to 5.0 mg kg^{-1} dry wt. Among the concentrations of lead determined in this study, two values were above this limit: 5 mg kg^{-1} dry wt was recorded in an octopus from Cascais in 2002 and 6.0 mg kg^{-1} dry wt was recorded in an animal from Cascais in 2003. Again there seems to be the potential for harmful effects, although focused on Cascais rather than Viana, and further study is needed.

4.5. Conclusion

Our study provided new data on element trace concentrations in edible arm tissue of the commonly eaten *O. vulgaris* from Portugal and clearly demonstrated the need to take into account the chemical form of the elements when aiming to evaluate the risk for human consumption. Indeed, despite relatively high levels of arsenic, this element was mainly present under a nontoxic form, i.e. arsenobetaine. In the same way, further analyses concerning the speciation of cadmium and lead are needed to determine their bioavailability to consumers as some specimens could exhibit concentrations exceeding the limit values fixed by the European Community for those metals.

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Caption to figures

Fig. 1. Map showing locations of the sampling ports.

Fig. 2. Concentrations of trace elements (mg kg^{-1} dry wt) in octopus arms sampled in 2002 and 2003 from Viana and Cascais.

Fig. 3. Ward's method tree diagram for the trace elements analysed in arms of octopus from Portugal.

Fig. 4. Regression relationship between copper and zinc concentrations (mg kg^{-1} dry wt) in arms of *Octopus vulgaris* from Portugal.



Figure 1

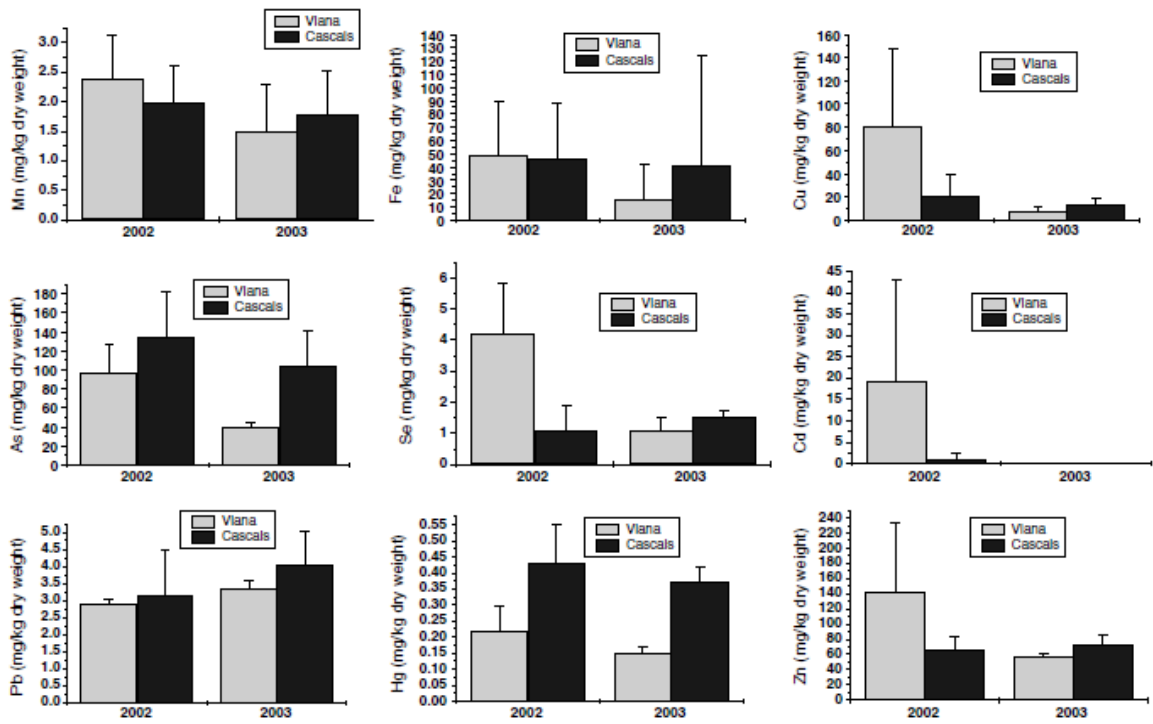


Figure 2

Tree Diagram for 9 Variables
Ward's method
1-Pearson r

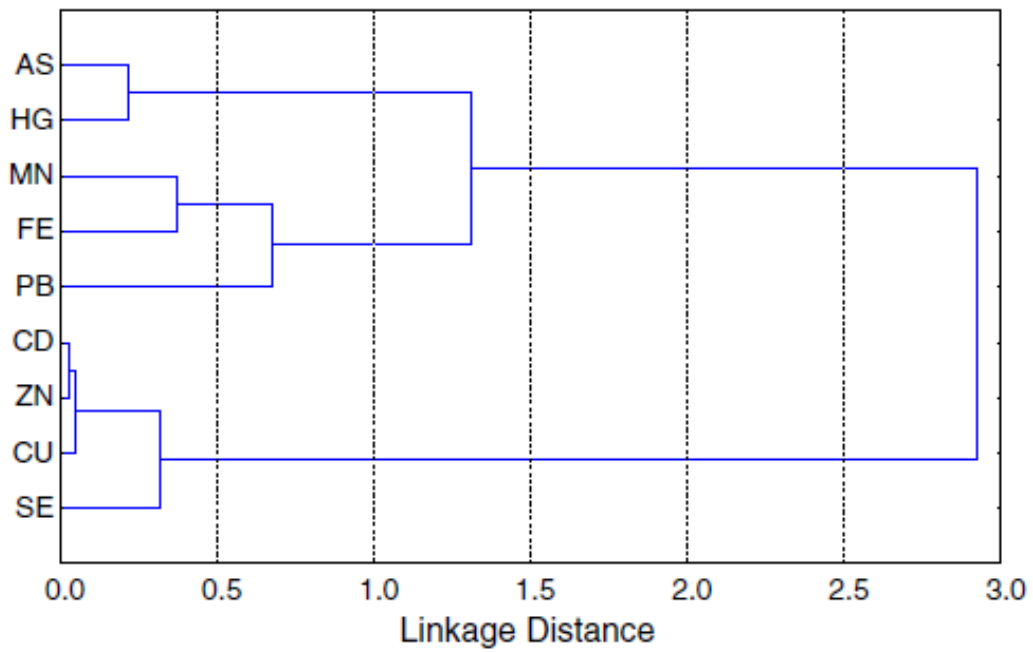


Figure 3

Cu vs. Zn
 $Zn = 47.424 + 1.2099 \times Cu$
Correlation: $r = 0.95783$

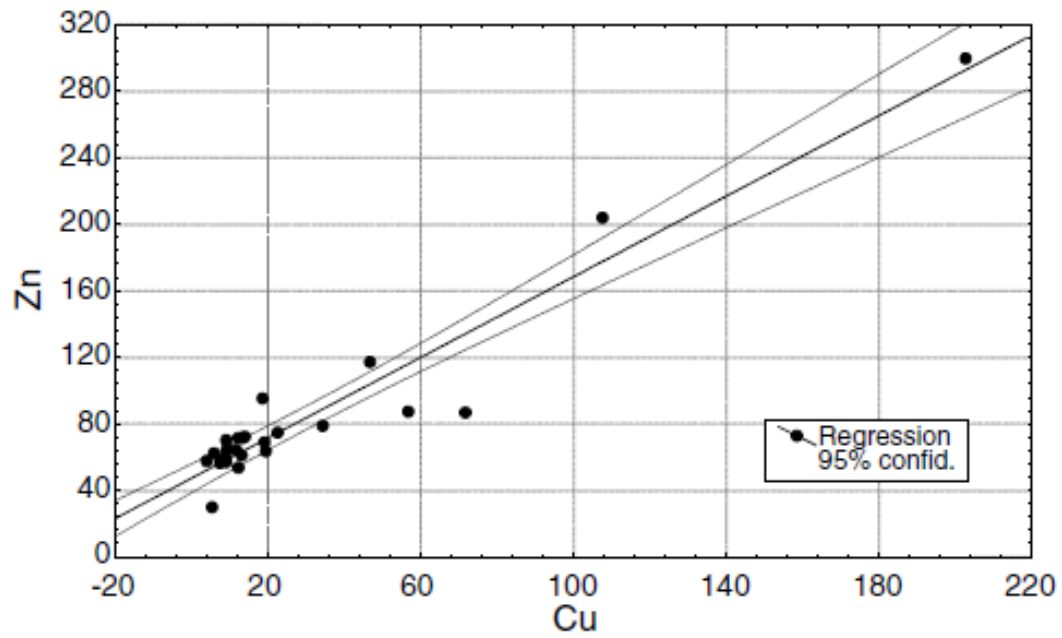


Figure 4

Table 1. Pearson's correlations (R), between concentrations of the elements and (a) weight, (b) length; Spearman Rank order correlations (Rs), between concentrations of the elements and state of maturation

Element	Weight	Length	Maturation
<i>Essential elements</i>			
Cu	-0.15 (0.50)	0.01 (0.98)	-0.11 (0.61)
Fe	-0.41 (0.04)	-0.12 (0.58)	-0.19 (0.38)
Mn	-0.24 (0.26)	-0.15 (0.50)	0.07 (0.74)
Se	-0.10 (0.66)	-0.01 (0.95)	-0.19 (0.38)
Zn	-0.10 (0.64)	0.08 (0.72)	-0.12 (0.59)
<i>Non-essential elements</i>			
As	0.02 (0.92)	0.10 (0.66)	0.02 (0.92)
Cd	-0.08 (0.70)	0.02 (0.93)	-0.18 (0.40)
Hg	0.04 (0.87)	0.16 (0.47)	-0.12 (0.57)
Pb	-0.21 (0.33)	0.11 (0.60)	0.11 (0.60)

Probability values (p) appear in parentheses. Sample size was 24 in all cases. Significant correlations are indicated in bold face.

Table 2. Results of ANOVA for effects of locality, year and gender on element concentrations

Element	Locality	Year	Gender
<i>Essential elements</i>			
Cu	4.10 (0.06)	7.72 (0.01)	0.39 (0.54)
Fe	0.02 (0.89)	1.242 (0.28)	5.56 (0.03)
Mn	0.13 (0.72)	2.11 (0.17)	1.79 (0.20)
Se	10.17 (0.01)	8.61 (0.01)	0.79 (0.39)
Zn	2.81 (0.11)	4.12 (0.06)	0.77 (0.39)
<i>Non-essential elements</i>			
As	11.88 (<0.00)	11.48 (<0.00)	0.72 (0.41)
Cd	4.35 (0.05)	4.99 (0.04)	0.75 (0.40)
Hg	44.788 (<0.00)	5.67 (0.034)	2.52 (0.13)
Pb	1.64 (0.22)	3.59 (0.08)	1.76 (0.20)

F values are given, with probability (p) in parentheses. Significant variation is indicated by use of bold face.

Table 3. Significant (Pearson) correlations between concentrations of different elements

Elements	Correlation
As	+Hg ^{***}
Cd	+Cu ^{***} , +Zn ^{***} , +Se ^{***}
Cu	+Cd ^{***} , +Se ^{***} , +Zn ^{***}
Fe	+Mn ^{***} , +Pb ^{**}
Hg	+As ^{***}
Mn	+Fe ^{***} , +Se [*]
Pb	+Fe ^{**}
Se	+Cu ^{***} , +Mn [*] , +Zn ^{***} , +Cd ^{***}
Zn	+Cd ^{***} , +Cu ^{***} , +Se ^{***}

* p < 0.05; ** p < 0.01; *** p < 0.005

Table 4. Concentration of trace elements (mg kg⁻¹) in arms of cephalopods

Species	Locality	Year	Sample size	Essential elements					Non-essential elements				Authors
				Cu	Fe	Mn	Se	Zn	As	Cd	Hg	Pb	
<i>Octopus vulgaris</i>	Viana do Castelo	2002	6	81 ± 68	49 ± 41	2.39 ± 0.72	4.2 ± 1.6	142 ± 92	97 ± 30	19 ± 24	0.22 ± 0.08	2.9 ± 0.2	Present study
<i>Octopus vulgaris</i>	Viana do Castelo	2003	6	7.9 ± 2.9	15 ± 27	1.5 ± 0.8	1.1 ± 0.4	58 ± 3	40 ± 5	0.4 ± 0.0	0.15 ± 0.02	3.3 ± 0.3	Present study
<i>Octopus vulgaris</i>	Cascais	2002	6	20 ± 18	47 ± 41	1.97 ± 0.64	1.1 ± 0.8	65 ± 19	133 ± 49	1.2 ± 1.4	0.43 ± 0.13	3.1 ± 1.4	Present study
<i>Octopus vulgaris</i>	Cascais	2003	6	14 ± 5.5	41 ± 83	1.76 ± 0.77	1.5 ± 0.20	74 ± 12	103 ± 38	0.4 ± 0.0	0.37 ± 0.04	4.0 ± 1.0	Present study
<i>Octopus vulgaris</i>	Viana do Castelo	1999/2000	10	27 ± 15	15 ± 4	1.44 ± 0.60	1.3 ± 0.4	78 ± 24	34 ± 14				Seixas and Pierce (in press, submitted)
<i>Octopus vulgaris</i>	Cascais	1999/2000	20–40	33 ± 19	28 ± 19	2.15 ± 0.81	1.2 ± 0.6	69 ± 21	62 ± 28				Seixas and Pierce (in press, submitted)
<i>Octopus vulgaris</i>	Santa Luzia	1999/2000	20	59 ± 77	30 ± 44	1.85 ± 1.99	0.9 ± 0.3	40 ± 24	15 ± 10				Seixas and Pierce (in press, submitted)
<i>Octopus vulgaris</i>	Tyrrhenian Coast	–	5								1.65 ± 0.64*a		Renzone et al. (1973)
<i>Octopus vulgaris</i>	Kastela Bay (Adriatic)	–	NM								0.52a		Buzina et al. (1989)
<i>Octopus vulgaris</i>	Modena (Italy)	–	3								0.04*		Plessi et al. (2001)
<i>Octopus vulgaris</i>	Japan (Pacific Ocean)	1977/1978	19	2.5*b	1.8*b	0.33*b		14*b					Ueda et al. (1979)
<i>Paroctopus dofleini</i>	Japan (Pacific Ocean)	1977/1978	16	4.0*b	1.4*b	0.17*b		13*b					Ueda et al. (1979)
<i>Benthoctopus thielei</i>	Kerguelen	1994/1995	17	3a				138a		0.21a			Bustamante (1998)
<i>Doryteuthis bleekeri</i>	Japan (Pacific Ocean)	1977/1978	20	1.3*b	2.8*b	0.12*b		8*b					Ueda et al. (1979)
<i>Sthenoteuthis bartrami</i>	Japan (Pacific Ocean)	1977/1978	64	2.6*b	1.8*b	0.13*b		14*b					Ueda et al. (1979)
<i>Loligo natanonica</i>	Argentina	1986		7.8*	2.3*	0.45*		12*		0.32*			Falandysz (1989)

Concentration of trace elements (mg kg⁻¹) in arms in our study and found for other authors in cephalopods in dry wt

Table 4 (continued)

<i>Loligo forbesi</i>	British Channel	1993	9	4 ± 1a				59 ± 10a		0.011a		0.20 ± 0.06a	Bustamante (1998)
<i>Loligo opalescens</i>	Monterey (California)		16	4.3 ± 1.0*	2.0 ± 0.4*	0.31 ± 0.08*		25 ± 8*					Falandysz (1991)
<i>Granelone</i> sp.	Kerguelen	1994/1995	17	15a				113a		0.37a			Bustamante (1998)
<i>Todarodes sagittatus</i>	Faroe Islands	1997	20							0.194a			Bustamante (1998)
<i>Todarodes pacificus</i>	Japan (Pacific Ocean)	1977/1978	66	1.2*	1.1*	0.09*		12*					Ueda et al. (1979)
Squid	Brixham	1996	NM	2.6*b	1.7*b	0.2*b	0.28*b	12*b	5.2*b		0.058*b		MAFF (1998)
Squid	Fraserburgh	1996	NM	2.4*b	1.1*b	0.5*b	0.34*b	12*b	4.6*b	0.02*b	0.016*b	0.02*b	MAFF (1998)
Squid	Newlyn	1996	NM	2.6*b	3.8*b	0.4*b	0.27*b	12*b	4.8*b		0.046*b	0.01*b	MAFF (1998)
<i>Nautilus macromphalus</i>	New Caledonia	NM	4							0.191a			Bustamante (1998)

All figures refer to dry weight except where indicated by * (values related to wet weight). a = muscle, b = edible parts, NM = not mentioned. Viana do Castelo, Cascais and Santa Luzia are all sites on the Portuguese coast. The squid studied by MAFF (1998) was probably *Loligo* spp.