Vanadium, rubidium and potassium in *Octopus vulgaris* (Mollusca: Cephalopoda)*

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SUMMARY: The levels of vanadium, rubidium and potassium were determined in *Octopus vulgaris* caught during commercial fishing activities at three locations (Cascais, Santa Luzia and Viana do Castelo) in Portugal in autumn and spring. We determined the concentration of these elements in digestive gland, branchial heart, gills, mantle and arms in males and females. At least five males and five females were assessed for each season/location combination. Elemental concentrations were determined by Particle Induced X-ray Emission (PIXE). Vanadium was detectable only in digestive gland and branchial heart samples. Its concentration was not correlated with total weight, total length or mantle length. There were no differences in concentrations of V, Rb and K between sexes. There were significant differences in vanadium concentrations in branchial hearts in autumn between samples from Viana do Castelo and those from the other two sites. We found a significant positive relationship between the concentration of vanadium and those of potassium and rubidium in branchial hearts. Branchial hearts appear to play an important role in decontamination of V.

Keywords: vanadium, rubidium, potassium, octopus, metals, pollution.

RESUMEN: VANADIO, RUBIDIO Y POTASIO EN *OCTOPUS VULGARIS* (MOLLUSCA: CEPHALOPODA). – Se determinaron los niveles de vanadio, rubidio y potasio en *Octopus vulgaris* capturado durante las actividades pesqueras en tres localidades de Portugal (Cascais, Santa Luzia y Viana do Castelo). Las concentraciones de estos elementos se determinaron en la glándula digestiva, corazón branquial, branquias, manto y en los brazos de machos y hembras en otoño y primavera. Al menos cinco machos y cinco hembras se muestrearon para cada combinación de temporada/localidad. Las concentraciones de estos elementos fueron determinadas por el método de Particle Induced X-ray Emission (PIXE). El vanadio fue solamente detectado en glándula digestiva y en los corazones branquiales y su concentración no tuvo correlación con el peso total, longitud total o longitud del manto. No se detectaron diferencias significativas en la concentración de V, Rb y K, entre machos y hembras aunque sí en las cantidades de vanadio acumulado en la glándula digestiva en otoño y primavera en muestras de Cascais y Santa-Luzia. En el caso del V en los corazones branquiales, se encontraron diferencias significativas en el otoño entre concentraciones en muestras de Viana do Castelo y las de las otras dos localidades. Se encontró una relación significativa positiva entre la concentración de vanadio y las de potasio y rubidio en los corazones branquiales. Concentraciones de Rb y K están correlacionadas en todos los tejidos excepto los musculares. Los corazones branquiales parecen jugar un papel importante en la descontaminación del V.

Palabras clave: vanadio, rubidio, potasio, pulpo, metales, polución.

INTRODUCTION

Vanadium is an element that, in recent years, has attracted considerable environmental and scientific interest because of its wide industrial applications, large releases into the environment and complex chemistry (Nriagu, 1998). The concentration of dissolved vanadium in seawater is typically 0.001-0.003 mg/l (WHO, 1988, 2001) and both marine animals and plants play an important role in its transfer. Vanadium does not react chemically with seawater but is continuously removed by biochemical processes and

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by absorption. Invertebrates accumulate vanadium principally through their diet (Miramand and Fowler, 1998), and have high levels of vanadium when compared with vertebrates—in which the concentrations are so low that detection is difficult.

Ascidians are known to accumulate vanadium in their blood cells (vanadocytes) in concentrations of 10^6 to 10^7 times those found in seawater (Kanda *et al.*, 1997; Michibata and Kanamori, 1998) and they have proteins, vanabins, which bind vanadium ions (Ueki *et al.*, 2003). The reason for this accumulation is not known (Nielsen and Uthus, 1994). However, vanadium concentrations in benthic invertebrates such as annelids, crustaceans, echinoderms and molluscs are generally low, with levels varying from 1 to 4 mg/kg dry weight (Miramand and Fowler, 1998).

Vanadium is an essential trace element for nitrogen-fixing bacteria (WHO, 2001; Zaslavsky *et al.*, 1999) but there is no consensus on its role in animals. Some authors state that it has an unknown role; other authors say that it is a non-essential metal. Vanadium is toxic to vertebrates even at low concentrations (Owusu-Yaw *et al.,* 1990) and it is also known to be toxic to invertebrates (e.g. larvae of *Crassostrea gigas*; Fichet and Miramand, 1998) and fish (Chakraborty *et al.,* 1998). In a study on gobies, Miramand *et al.* (1992) suggested that vanadium does not bioaccumulate in the food chain.

Rubidium is widely distributed in nature. It has no known biological role but is said to stimulate the metabolism. The only study to date on a mollusc showed it to be toxic to oysters (Salaun and Truchet, 1996). In vertebrates, the metabolism of rubidium is closely related to that potassium. Indeed, it has been suggested that rubidium represents a nutritional substitute for potassium (Bruce and Duff, 1968). However, in oysters the rubidium metabolism clearly differs from that of potassium (Salaun and Truchet, 1996). Potassium is an essential element for all organisms. Absorption by animals from the diet is passive and does not require any specific mechanism. In vertebrates the kidneys are the main regulators of body potassium.

In several species, including humans, vanadium in the form of vanadate (the +5 oxidation state), is a powerful inhibitor of Na,K-ATPase (Cantley and Aisen, 1979). In rats, the inhibition of Na,K-ATPase caused by vanadate is dependent on the concentration of potassium (K) in muscle (Searle *et al.,* 1983). There is evidence that, under appropriate conditions, K+ or its congeners such as Rb+ become bound to Na,K-ATPase in a way that slows down its release. This type of binding is called occlusion (Kaufman *et al.* 1999). Na,K-ATPase has the same apparent affinity for potassium and rubidium (Rb) ions because of its hydrolytic activity (Cheval and Doucet, 1990). Vanadate optimises the occlusion of ATPase by rubidium (Rabon *et al.*, 1993) because rubidium binds much better to the protonated pump (form E2) than the unprotonated pump (Blostein, 1985; Milanic and Arnett, 2002). The intrinsic affinity of this enzyme for vanadate—and the interaction between this element and both rubidium and potassium—was demonstrated in an assay in which the enzyme was incubated with various concentrations of vanadate in the presence of K+ or Rb+ (Toustrup-Jensen and Vilsen, 2002).

The octopus (*Octopus vulgaris*) is a cephalopod mollusc with a high growth rate and a short life span. It is benthic and its diet is essentially composed of bivalves, crustaceans, other cephalopods and fishes (Lee, 1994). *O. vulgaris* has a high economic and cultural value in Portugal, and is an important fishery resource throughout southern Europe. Cephalopods, such as octopus, are known to bioaccumulate high levels of certain metals, notably cadmium, and represent an important route of transfer to top marine predators, including humans (Bustamante *et al.,* 1998). The present study examines levels of two poorly documented metals, vanadium and rubidium, both potentially toxic, in *O. vulgaris* from the Portuguese coast. Given the known links between vanadium, rubidium and potassium metabolism in animals, potassium concentrations are also recorded.

To provide information on variation in concentrations around Portugal, octopus were collected at three sites on different parts of the Portuguese coast, all important fishing areas. In view of the short lifespan and sex-related differences in growth and maturation (Mangold, 1989), seasonal and sex-related differences are also investigated. Previous work on lead levels in this species indicated differences between the sexes (Seixas *et al.*, 2002). Lastly, we analyse the relationships between concentrations of the three elements and discuss the possible implications of the observed levels and interactions for the health of the octopus and its predators.

MATERIAL AND METHODS

Sampling and sample preparation

Animals landed by commercial fisheries were sampled at three locations along the Portuguese coast: (a) Viana do Castelo, situated in the north of the country, (b) Cascais in the centre, with a strong influence of the Tagus River (the largest river in Portugal), and (c) Santa-Luzia, situated in the Algarve region on the south coast of Portugal. Animals in these zones were sampled over two seasons of the year, autumn (November, 1999) and spring (May, 2000). We sampled 60 octopuses: 10 animals (5 males and 5 females) in each season and each zone.

Total length, mantle length, total weight, sex and maturation state were determined for each animal. The maturation state was determined by direct observation of colours of reproductive structures (Gonçalves, 1993). The maturity index follows Guerra (1975), based on microscopic analyses and measurements of ovules and spermatophores.

The tissues collected for analysis were digestive glands, branchial hearts, gills, mantles and arms. The tissue samples were stored frozen between -20 ^{o}C and -40 ^{o}C prior to analysis. We could not analyse the samples of branchial hearts, gills, mantles and arms from Viana do Castelo in spring due to problems that occurred during the storage of the samples.

Analytical procedure

The concentrations of vanadium, potassium and rubidium were determined using PIXE (Particle Induced X - ray Emission). First we freeze-dried the samples. This was followed by microwave acid digestion with 9:1 v/v HNO_3 and H_2O_2, 4 minutes at 300 W. We used yttrium (Y) as the internal standard. After that, three aliquots of 10 μl of sample from each animal and tissue were analysed. The technique of PIXE was available in a Van der Graff accelerator at the Technological and Nuclear Institute of Portugal (ITN). Irradiation of the sample was made by a sheaf of protons of 2.2 MeV with 5 mm diameter and 6.5 nA mm^{-2} intensity in a vacuum. A Mylar™ of 350 μm thickness was used to eliminate the contribution of X-rays of low energy (lower than potassium). The crystal where the emission of X-ray was detected was made of Si(Li). For each X-ray detected one electrical signal was produced, which was processed in a multi-channel system (MCA). The spectra were analysed with the computer programs AXIL and DATTPIXE (International Atomic Energy Agency), which calculated the concentration of elements.

Detection limits were for V: 0.4 mg/kg, Rb: 1.28 mg/kg, K< 0.0 mg/kg.

The results for each tissue are given relative to dry weight of tissue (mg kg^{-1} dry weight - dw).

Statistical procedures

Statistical analysis of the data was carried out using STATISTICA (StatSoft, Inc., 1995). We used 3-way ANOVA (factors: sex, season and location) for elements in digestive glands and 2-way ANOVA (factors: sex and season; data missing for one location) for elements in branchial hearts. When significant variation was detected using ANOVA, Student's t-tests were used to identify where those differences occurred. To analyse the correlations between state of maturation (an ordinal variable) and concentration of elements, we used Spearman rank correlations. For quantifying relationships between other parameters, such as total length, mantle length and total weight, and concentrations of elements, we used the Pearson coefficient of correlation.

RESULTS

The averages and standard deviations of weights and total lengths of the animals from each sampling location and occasion are given in Table 1.

The body weights of the sampled animals differed significantly between locations, being largest

TABLE 1. – The average (± 1 standard deviation) of weights, total and mantle lengths and state of maturation of octopus for each sex, location and season.

Season	Parameters	Viana do Castelo		Cascais		SantaLuzia	
		Females	Males	Females	Males	Females	Males
Autumn	Weight (g)	1249 ± 231	1249 ± 304	1266 ± 660	1524 ± 628	3788 ±1480	3560 ± 676
	Total Length (mm)	870 ± 69	878 ± 140	872 ± 123	892 ± 120	1085 ± 185	1070 ± 125
	Mantle Length (mm)	154 ± 8	155 ± 11	154 ± 26	166 ± 29	224 ± 38	232 ± 18
	Maturation	2.3 ± 1.6	3.0 ± 0.0	2.1 ± 0.7	2.4 ± 0.9	2.0 ± 0.7	2.4 ± 0.9
Spring	Weight (g)	939 ± 199	906 ± 249	2183 ± 424	1052 ± 314	2246 ± 807	3157 ± 2215
	Total Length (mm)	768 ± 65	758 ± 50	914 ± 61	746 ± 91	1020 ± 139	1073 ± 208
	Mantle length (mm)	134 ± 10	134 ± 11	192 ± 16	146 ± 17	189 ± 22	188 ± 36
	Maturation	2.1 ± 1.5	1.4 ± 0.5	2.8 ± 0.8	3.0 ± 0.0	2.0 ± 0.0	2.7 ± 0.6

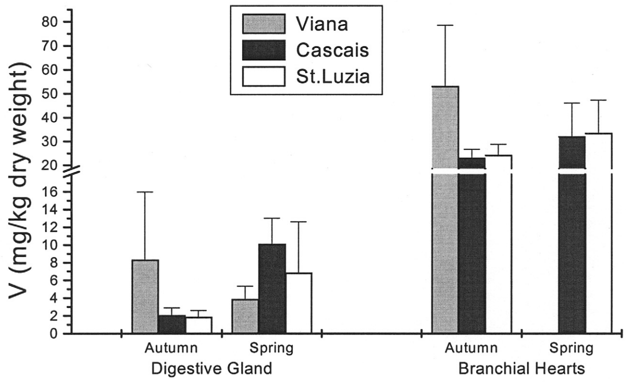

FIG. 1. – Concentration of vanadium in digestive glands and branchial hearts.

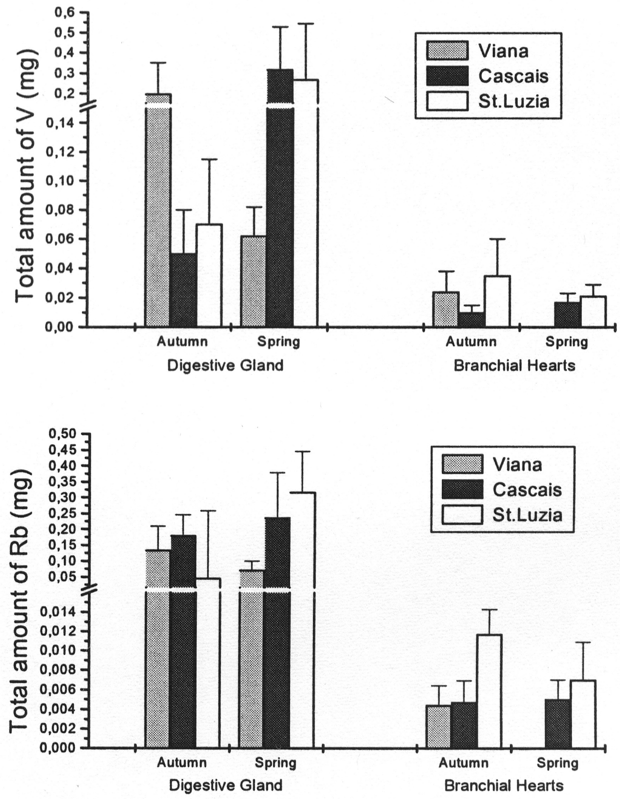

FIG. 2. – Total amount of vanadium and rubidium in digestive glands and branchial hearts.

at Santa Luzia and smallest at Viana do Castelo (Viana/Cascais: t=-2.53, N=40, p=0.015; Viana/St. Luzia: t=-6.56, N=40, p=0.00; Cascais/St. Luzia: t=-4.97, N=40, p=0.00).

Vanadium was detected only in digestive glands and branchial hearts. In gills, mantles and arms the levels were lower than the detection limit (< 0.4 mg/kg). Rubidium and potassium were detected in all tissues analysed.

There were no significant differences between sexes in concentrations of any of the three elements measured in the tissues analysed (Table 2). Hence the data for both genders were treated together in subsequent analyses.

Vanadium

The concentrations and total amounts of vanadium in digestive glands and branchial hearts for samples from each location-season combination are shown in Figure 1 and 2 respectively. The concentration of vanadium in branchial hearts was markedly higher than in digestive glands (F=9.05, p=0.00), although estimated total amounts present were higher in the latter organ. Concentrations in the two tissues were positively correlated with each other (r=0.38, N=50, p=0.04).

The concentration of vanadium in digestive gland was not significantly correlated with total length, mantle length or wet weight of the animals.

TABLE 2. – Results of ANOVA for effects of location, season and gender on concentrations of metals in digestive glands branchial hearts, gills, mantle and arms. The table shows the F values, followed by the associated number of samples (N) and probability (p) in parentheses. Significant effects are shown in bold face.

Tissue	Metal	Gender	Season	Location
Digestive glands	V	0.00 (60, 0.98)	**4.17 (60, 0.048)**	**3.90 (60, 0.04)**
	Rb	0.39 (60, 0.54)	2.40 (60, 0.13)	**3.49 (60, 0.04)**
	K	0.15 (60, 0.70)	**12.49 (60, 0.00)**	2.63 (60, 0.08)

Tissue	Metal	Gender	Season	Location (autumn)	Location (spring)
Branchial	V	3.21 (50, 0.08)	0.08 (50, 0.78)	**9.10 (50, 0.00)**	0.02 (50, 0.89)
Hearts	Rb	3.61 (50, 0.07)	1.18 (50, 0.28)	0.31 (50, 0.74)	0.08 (50, 0.78)
	K	1.13 (50, 0.29)	0.36 (50, 0.55)	**13.90 (50, 0.00)**	0.15 (50, 0.71)
Gills	Rb	0.00 (50, 0.98)	0.00 (50, 0.96)	0.75 (50, 0.50)	2.44 (50, 0.13)
	K	0.05 (50, 0.83)	2.95 (50, 0.09)	1.55 (50, 0.24)	**5.51 (50, 0.02)**
Mantle	Rb	1.13 (50, 0.30)	1.87 (50, 0.19)	0.13 (50, 0.88)	**7.5 (50, 0.00)**
	K	2.11 (50, 0.15)	3.61 (50, 0.07)	0.01 (50, 0.99)	0.87 (50, 0.38)
Arms	Rb	0.73 (50, 0.40)	1.34 (50, 0.26)	2.76 (50, 0.10)	**6.84 (50, 0.03)**
	K	0.01 (50, 0.93)	1.17 (50, 0.29)	**7.22 (50, 0.01)**	3.70 (50, 0.08)

TABLE 3. – Student's t-test results for comparisons between the three locations (above) and the two seasons (autumn and spring) (bottom). The table shows the t values and associated probability (p) in parentheses. Significant differences are shown in bold face. These tests are used to indicate which differences were significant if ANOVA showed that there was significant variation.

Tissue	Element	Season	Viana/Cascais	Viana/St. Luzia	Cascais/St. Luzia
Digestive gland	V	Autumn	**2.30 (0.04)**	**2.37 (0.03)**	0.36 (0.73)
Digestive gland	V	Spring	**-3.48 (0.01)**	-0.85 (0.42)	1.53 (0.15)
Digestive gland	Rb	-	**-2.07 (0.048)**	**-2.49 (0.02)**	-0.42 (0.69)
Branchial heart	V	Autumn	**3.72 (0.00)**	**2.20 (0.049)**	-0.52 (0.61)
Branchial heart	K	Autumn	**4.64 (0.00)**	**4.08 (0.00)**	1.33 (0.20)
Arms	K	Autumn	1.10 (0.29)	**3.01 (0.02)**	**4.68 (0.00)**
Tissue	**Element**	**Viana**	**Cascais**	**St. Luzia**	
Digestive gland	V	0.96 (0.36)	**-7.50 (0.00)**	**-2.44 (0.03)**	
Digestive gland	K	-0.20 (0.85)	**-3.03 (0.01)**	-1.37 (0.20)	

There was a significant positive correlation between the maturation state and the concentration of vanadium in digestive gland (R=0.35, N=60, p=0.03). However, vanadium concentration in branchial hearts was not correlated with maturation state and it should be noted that, since 24 different correlation coefficients were computed (3 metals in 2 tissues in relation to 4 biological variables), at least one of them would be expected to be significant (P<0.05) by chance alone.

The ANOVA results on variation in concentration of vanadium in relation to effects of location and season are shown in Table 2 and, where significant variation was identified, the differences are summarised in Table 3.

Significantly higher concentrations of vanadium were accumulated in digestive gland in spring than in autumn, for both Cascais and Santa-Luzia samples (Fig. 1). However, in the Viana do Castelo samples, there were no differences between seasons. The concentrations in autumn and spring varied in relation to sample location. In autumn, levels of vanadium in digestive gland for Viana do Castelo were higher than at the other two locations, while in spring levels were higher at Cascais than at Viana do Castelo.

For the autumn branchial heart samples, there were differences between the concentration of vanadium in Viana and the other two places, the level being higher in Viana.

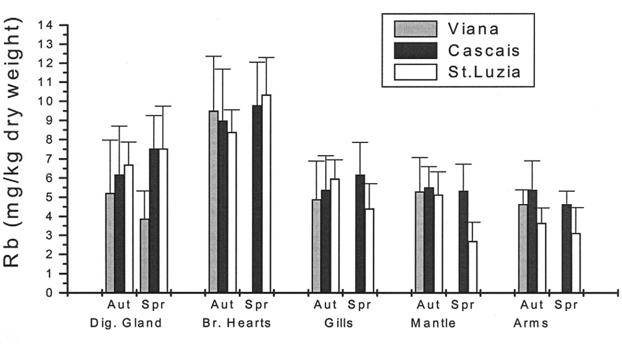

FIG. 3. – Concentration of rubidium in tissues analysed.

Rubidium

Concentrations of rubidium were slightly (but not significantly) higher in branchial hearts than in all other tissues (Fig. 3), although (as for vanadium) the total amount present in the digestive gland was greater than in the branchial hearts (Fig. 2). The concentrations of Rb in digestive glands, branchial hearts, gills, mantles and arms were not correlated with each other.

Concentrations of rubidium in digestive glands, branchial hearts, gills, mantles and arms were not correlated with total length, mantle length, wet weight or maturation of the animals.

ANOVA results on variation in concentration of rubidium in relation to locations and seasons are shown in Table 2 and, where significant variation was identified, the differences are summarised in Table 3.

The levels of rubidium showed no differences between seasons or locations for digestive glands, branchial hearts and gills; in mantles and arms there were differences between locations in spring.

Potassium

In general, the concentrations of potassium were fairly similar in all studied tissues (Fig. 4).

Concentrations of K were correlated with biological parameters of the animals only in gills and arms: with total length (r=-0.40, N=50, p=0.01 and r=-0.39, N=50, p=0.03 respectively), mantle length (r=-0.33, N=50, p=0.04 and r=-0.43, N=50, p=0.01 respectively) and wet weight (r=-0.39, N=50,

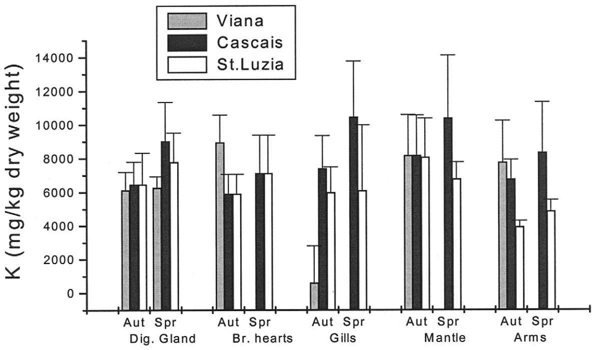

FIG. 4. – Concentration of potassium in tissues analysed.

p=0.01 and r=-0.44, N=50, p=0.01 respectively). Concentrations of K were not correlated with maturation in any of the tissues analysed.

Concentrations of K in digestive glands were correlated with concentrations of K in branchial hearts, gills, mantles and arms (r=0.35, p=0.04; r=0.33, p=0.04; r=0.37, p=0.03; r=0.46, p=0.01 respectively; N=50 in all cases). Concentrations of K in arms were also correlated with those in branchial hearts, gills and mantles (r=0.62, p=0.00; r=0.50, p=0.01; r=0.64, p=0.00 respectively; N=50 in all cases).

ANOVA results on variation in concentration of potassium in relation to locations and seasons are shown in Table 2 and, where significant variation was identified, the differences are summarised in Table 3. The levels of potassium in digestive glands showed statistically significant differences only between seasons: in samples from Cascais, the levels were higher in spring.

In branchial hearts and arms, the concentrations of potassium differed between locations in autumn. In branchial hearts levels were similar for Cascais and St. Luzia, but the concentrations for both places were significantly lower than at Viana. In arms the levels were similar at Viana and Cascais but these two locations had higher values than St. Luzia.

Correlations between elements

For branchial hearts, there were positive correlations between the concentrations of vanadium and rubidium (r=0.32, N=60, p=0.02) and those of vanadium and potassium (r=0.41, N=60, p=0.01). On the other hand, both graphs indicate a wide scatter of data and, as indicated by the r^2 values, only around 13 and 17% respectively of variation in rubidium and potassium concentrations is explained by a linear relationship with vanadium concentration.

Concentrations of Rb and K were positive correlated in digestive glands, branchial hearts and gills (r=0.70, N=60, p=0.00; r=0.60, N=50, p=0.00; r=0.58, N=50, p=0.00 respectively). In mantles and arms there was no correlation between Rb and K.

DISCUSSION

In *Octopus vulgaris*, we found that branchial hearts had higher concentrations of vanadium than digestive glands. This is similar to the results of Miramand and Guary (1980), who studied the Mediterranean octopus. Miramand and Fowler (1998) found that branchial hearts in cephalopods constitute only 0.2% of the whole animal weight but contain 1-6% of the total vanadium body burden. Higher concentrations of rubidium were also higher in branchial hearts than in other tissues.

We found higher total quantities of vanadium and rubidium in the digestive gland (as might be expected given the relative size of these organs). In *Sepia officinalis*, vanadium in digestive glands represents 40% of the total found in the animals (Miramand and Fowler, 1998).

In contrast to results for vanadium and rubidium, there were no consistent differences in potassium concentration between different organs.

In branchial hearts, the highest concentration of vanadium was found in the samples from Viana in autumn; in other samples the levels varied between 22 and 33 mg/kg. It can be suggested that the Viana area is more contaminated with vanadium than other locations studied and, if so, that the contamination source is probably oil. During the summer, precipitation and riverine inputs are lower, so the accumulation in coastal areas of pollutants such as oils (an important source of vanadium contamination in the ocean, Crans *et al.*, 1998) would be higher, and this would be reflected in animals captured in autumn. In winter, levels of precipitation and riverine inputs are higher and strongly influence coastal conditions.

Values of V and K in digestive glands obtained in this study are broadly similar to data from the literature (Table 4). However, the highest concentration of vanadium in digestive glands recorded in the present study, in the samples from Cascais in spring (10.1±2.92 mg kg^{-1} dw), is somewhat higher than the values for other cephalopod species in the literature. Levels of Rb in digestive glands were a little higher than values found for other cephalopods.

TABLE 4. – Concentration of V, Rb and K (mg/kg dw) in digestive glands and branchial hearts as found in the present study and the studies on cephalopods. [*Values that refer to wet weight.] (In the present study, ratios of wet weight to dry weight were 2.3 in digestive gland and 4.4 in branchial hearts).

	Locations	V	Rb	K	Authors
Digestive Gland					
Octopus vulgaris	Viana do Castelo	7.2 ± 6.9	5.0 ± 2.5	6173 ± 942	Present study
	Cascais	6.5 ± 4.7	6.8 ± 2.2	7747 ± 2257	Present study
	Santa Luzia	5.0 ± 5.1	7.1 ± 1.8	7125 ± 1861	Present study
	Monaco	4.5 ±1.0			Miramand and Guary (1980)
Eledone cirrhosa	English Channel	3.3 ± 0.5			Miramand and Bentley (1992)
Todarodes pacificus	Pacific coast	5.5*	1.5*	4900*	Ichihashi *et al*. (2001b)
	Sea of Japan	0.22*	1.1*	3600*	Ichihashi *et al*. (2001b)
	Nemuro Strait	0.66*	0.91*	2700*	Ichihashi *et al*. (2001b)
Stenotheuthis oualaniensis	Japan	0.74	0.54	4450	Ichihashi *et al*. (2001a)
Sepia officinalis	English Channel	5.0 ± 1.3			Miramand and Bentley (1992)
Nautilus macromphalus	New Caledonia	8.8 ± 2.0			Bustamante *et al*. (2000)
Branchial hearts					
Octopus vulgaris	Viana do Castelo	53.0 ± 25.4	9.5 ± 2.9	8946 ± 1627	Present study
	Cascais	27.5 ± 11.0	9.4 ± 2.5	6508 ± 1855	Present study
	Santa Luzia	28.1 ± 9.9	9.1 ± 1.7	5876 ± 2793	Present study
	Monaco	25 ± 2			Miramand and Guary (1980)
Eledone cirrhosa	English Channel	6.0 ± 1.0			Miramand and Bentley (1992)
Sepia officinalis	English Channel	7.1± 0.2			Miramand and Bentley (1992)

In branchial hearts, the level of V recorded at Viana was very high compared with the values in the literature. However, our results for other locations were similar to values obtained in Monaco for the same species (Table 4). In general, levels recorded in *Octopus vulgaris* are higher than those in other cephalopods. There are no reports in the literature on Rb and K levels in branchial hearts.

Our data are consistent with the hypothesis of Miramand and Fowler (1998) that branchial hearts play an important role in accumulation and excretion of V. Vanadium is known to affect the functioning of the enzyme Na,K-ATPase: it converts this enzyme to the protonated state. Our results indicate relationships between the concentrations of vanadium and potassium and between those of vanadium and rubidium in branchial hearts. It should be noted that, in cephalopods, Na,K-ATPase is responsible for the excretion of NH_4^+ (Boucher-Rodoni and Mangold, 1994). Thus, if high vanadium levels adversely affect enzyme activity, the excretion of ammonium may be affected. However, at present it is not known whether the concentration of vanadium found in branchial hearts is sufficient to induce alterations in concentrations of potassium and rubidium or to have deleterious effects on octopus health.

In octopus we do not yet know the effects of vanadium. In human beings, problems with the enzyme Na,K-ATPase, associated with high levels of potassium and vanadium, can cause heart diseases and hypertension (IPCS, 1990). The cardiac cycle and the pressure volume loops of the systemic heart of *Octopus vulgaris* are functionally not dissimilar to those described in mammals (Berne and Levy, 1992 in Agnisola and Houlihan, 1994).

The correlations between concentrations of Rb and K in all tissues with the exception of muscle (mantles and arms) could indicate a relationship between them. However, further evidence is needed.

Another aspect that may be considered is that *Octopus* may be one of the species responsible for the bioaccumulation of vanadium in higher trophic levels. Cephalopods are regarded as being an important link in marine food chains (Piatkowski *et al.,* 2001).

Studies on bioaccumulation of vanadium in pinnipeds (northern fur seal *Callorhinus ursinus*; Steller's sea lion *Eumetopias jubatus*; harbour seal *Phoca vitulina;* and ribbon seal *Phoca fasciata*) caught in the northern Pacific show high concentrations in liver, hair and bone (Saeki *et al*., 1999). High levels of V were reported in liver of marine mammals captured in Alaska after they were subjected to oil contamination over a long period of time (Mackey *et al*., 1996), although these levels remained lower than those measured in the tissues of cephalopods, especially branchial hearts.

Given increasing scientific interest in vanadium and considering that cephalopods have a high economic value and represent an important path for transfer of contaminants to top predators in marine food webs, further studies on vanadium levels are needed. The relationships between vanadium, rubidium and potassium also deserve further attention.

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