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Chthamalus montagui as biomonitor of metal contamination in the northwest coast of Portugal

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Abstract The concentrations of seven metals (Cd, Cr, Cu, Fe, Mn, Ni and Zn) were determined in coastal seawaters and soft and hard tissues of the barnacle *Chthamalus montagui* from the northwest coast of Portugal to assess the potential use of *C. montagui* as biomonitor of metal contamination. The results of this study showed that *C. montagui* soft tissues can be used for monitoring metal bioavailabilities in these coastal seawaters: (1) there were significant correlations ($p<0.05$) between the metal concentrations in soft tissues and their concentrations in seawaters and (2) barnacle soft tissues were sensitive to spatial variation of metal bioavailabilities, accumulating different amounts of metals in different locations. The range of concentrations in tissues were: 0.59–1.7 mg Cd kg^{-1}, 0.5–3.2 mg Cr kg^{-1}, 0.72–3.0 mg Ni kg^{-1}, 1.2–6.7 mg Cu kg^{-1}, 9–26 mg Mn kg^{-1}, 214–785 mg Fe kg^{-1} and 178–956 mg Zn kg^{-1}; (3) mean logarithmic bioaccumulation factors (log BAF) of Fe, Cr and Cd were higher, 5.49, 4.93 and 4.46, respectively, than mean log BAFs of Mn, Zn, Cu and Ni, 4.03, 3.97, 3.74 and 3.61, respectively. In contrary, *C. montagui* shell plates were not a good matrix to monitor metal bioavailability in these coastal seawaters, with no significant correlations ($p<0.05$) between metal concentrations in the shell and in seawater. Regarding the high Zn concentrations obtained in the coastal seawaters and *C. montagui* soft tissues, all seawaters from northwest coast of Portugal should be classified as “moderately/remarkably polluted”.

Keywords Barnacle · *Chthamalus montagui* · Biomonitor · Metals · Coastal waters · Portugal

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Introduction

A biomonitor of anthropogenic contamination should be a species: (1) sessile or with restricted mobility, (2) ubiquitous and sufficiently abundant in the sampling area, (3) available in all seasons, (4) easy of sampling, (5) with predisposition for a consistent uptake of contaminants, (6) with high capacity to accumulate contaminants above environment levels and (7) with predisposition to retain contaminants for a sufficient time after reduction in the environment (Barbaro et al.

1978). On other hand, biomonitor species should be capable of accumulating trace contaminants in their tissues, responding to the contaminant bioavailable fractions (dissolved and particulate phases; Blackmore et al. 1998). Macroalgae, oysters, mussels, clams and barnacles are examples of organisms that have these characteristics and have been used as biomonitors of contaminant bioavailabilities in coastal waters (Ruelas-lnzunza and Páez-Osuna 1998; Philips and Rainbow 1988, 1990; Rainbow and Smith 1992; Fialkowski and Newman 1998; Morillo et al. 2008 and Morillo and Usero 2008).

Different barnacle species have been used around the world to estimate metal levels in marine waters (Alexander and Rowland 1966; Stenner and Nickless 1975; Barbaro et al. 1978; Anil and Wagh 1988; Philips and Rainbow 1988, 1990 and Rainbow and Smith 1992). These works monitored several metals (Ag, Cd, Co, Cr, Cu, Ni, Pb and Zn) and showed that all barnacle species responded to the metal levels in the waters, although different species accumulated different amounts of metals. Metal levels accumulated in barnacle tissues also showed significant spatial and temporal variations, influenced by metal sources of each region at each season (Blackmore and Chan 1997; Blackmore et al. 1998; Blackmore 1999). The extensive biomonitoring programme of metal contamination in Hong Kong coastal waters using different barnacle species, between 1986 and 1998, represented a benchmark for similar works elsewhere in the world (Rainbow and Blackmore 2001). The cosmopolitan barnacle *Amphibalanus amphitrite* is considered a good organism to monitor metals in different parts of the world since it showed significant correlations between metal concentrations in its tissues and in environmental coastal waters, particularly for Cu, Mn, Ni and Zn (Fialkowski and Newman 1998; Morillo et al. 2008; Morillo and Usero 2008). Acorn barnacles *Semibalanus balanoides*, *Elminius modestus* and *Lepas anatifera* from coastal waters of North Wales (UK) accumulated high concentrations of Zn in their soft tissues, mainly in granules of the midgut, which directly reflected environmental concentrations (Walker et al. 1975). Other studies also concluded that different species of barnacles can be successfully used as biomonitors of metal availabilities in coastal waters and had good spatial discrimination (Rainbow et al. 2000, 2002, 2004a; Turkmen et al. 2005; Silva et al. 2006).

However, acorn barnacle *Chthamalus montagui* has never been used as biomonitor of metal contamination in coastal waters, and it is the dominant species in the northwest (NW) Atlantic coast of Portugal. Thus, the main objectives of this study are:

1. To evaluate the potential use of *C. montagui* as biomonitor of metal contamination in NW Atlantic coast of Portugal;
2. To increase the information on metal concentrations in coastal seawaters and tissues of *C. montagui* of NW coast of Portugal. This data collection will allow to construct a database for comparison purposes in future works;
3. To assess spatial variations of metal bioavailabilities along NW coast of Portugal;
4. To establish relationships between metals in coastal seawaters and *C. montagui* tissues from NW coast of Portugal;
5. To calculate bioaccumulation factors of metals in soft tissues of *C. montagui* from NW coast of Portugal.

Materials and methods

Study area

Twenty-two sampling sites were chosen along NW coast of Portugal, between the cities of Esmoriz (location 0) and Moledo (location 21; Fig. 1). They were set in places that can be influenced by anthropogenic activity such as beaches, marinas, river mouths, industrial and domestic effluents. As examples:

1. "Paramos" (location 1) is located near a wastewater treatment plant, which was under-dimensioned and cannot treat all the receiving load;
2. "Valadares" (location 2) and "Canidelo" (location 3), located southwards of Douro estuary, are under influence of potential contamination from Oporto southwards water currents promoted by northwesterly winds (Martins et al. 2002);
3. "Foz" (location 4), located in downstream of Douro estuary, is surrounded by the second largest urban area of the country, Oporto, which

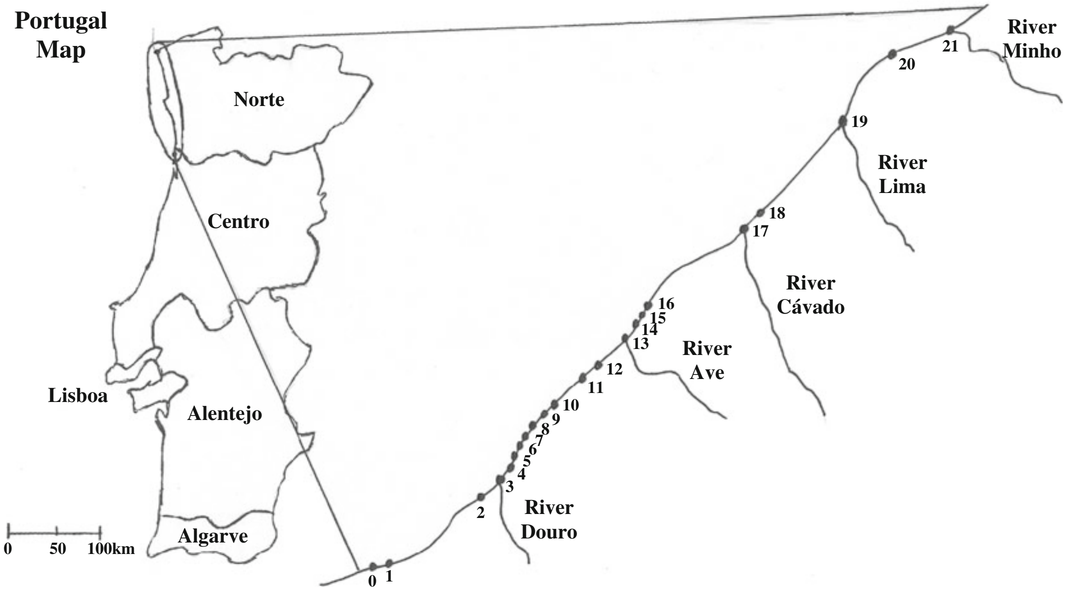

Fig. 1 Geographical distribution of the 22 sampling locations along the NW coast of Portugal

shows high anthropogenic contamination (Martins et al. 2002);

4. "Leça da Palmeira" (location 8) is near to an oil refinery plant, where organic and inorganic contamination may be expected;
5. "Vila do Conde" (locations 14 and 15) urban area involves a coastal region already considered contaminated by the Portuguese Water Institute (INAG), which is directly affected by untreated industrial and domestic effluents (Salvado 2009).
6. "Póvoa de Varzim" (location 16) and "Viana do Castelo" (location 19) are areas potentially contaminated by industrial and domestic effluents and by naval shipyard influences, which can contribute with discharges of wastes from tanker washings and ballast.
7. "Moledo" (location 21) is a coastal area located southwards of Minho estuary, which can be influenced by anthropogenic metal contamination.

All these coastal waters (location 0 to location 21) were classified by the INAG as "good/acceptable" quality, except locations 2 ("Valadares"), 13 ("Árvore"), 14 ("Vila do Conde") and 15 ("Vila do Conde–Norte"), which were classified as "bad/interdicted" for recreation use due to high concentrations of pathogenic microorganisms (Salvado 2009; Table 1).

Reagents and material

All reagents used in this work were at least of pro analysis or equivalent grade, without further purification: nitric acid (Fluka, Suprapure) and acetone (Fluka, HPLC). Metal standard solutions used in the analysis were daily prepared by weight from a stock solution of 1,000 mg L^{-1} (Fluka, pro analysis). Solutions were prepared with high-purity water from a Milli-Q system (conductivity, 0.054 μS cm^{-1} at 25°C).

All materials (sampling and treatment) used in this work were previously decontaminated in nitric acid solution (20%, *v*/*v*), at least for 24 h, washed with deionised water (conductivity <0.066 μS cm^{-1} at 25°C) and dried in the oven (APHA 1998a).

Sampling strategy and analytical methods

Coastal seawaters

At summer of 2009, three independent seawater replicates (n=3) were collected in each site and stored in 500-mL polyethylene bottles which were

Table 1 Classification of the seawater in the 22 locations of the NW coast of Portugal

	Location	Geographical Coordinates	Description	Reference
0	Esmoriz	40° 57′ 28.10″ N 8° 39′ 25.19″ W	Seawall; classification[a]: "good"; south of the Lagoon "Barrinha de Esmoriz"; domestic sewage and industrial effluents	Fernandes et al. 2008; Salvado 2009
1	Paramos	40° 58′ 24.56″ N 8° 39′ 1.76″ W	Seawall; classification[a]: "good"; north of the Lagoon "Barrinha de Esmoriz"; domestic sewage and industrial effluents	Fernandes et al. 2008; Salvado 2009
2	Valadares	41° 6′ 19.69″ N 8° 39′ 50.79″ W	Rock/sand beach; classification[a]: "bad"; domestic sewage	Salvado 2009
3	Canidelo	41° 8′ 13.18″ N 8° 40′ 5.57″ W	Rock/sand beach; classification[a]: "aceptable"; south bank of River Douro mouth; domestic sewage and industrial effluents	Salvado 2009
4	Foz	41° 8′ 57.69″ N 8° 40′ 36.02″ W	Rock/sand beach; classification[a]: "good"; north bank of River Douro mouth; domestic sewage and industrial effluents	Salvado 2009
5	Gondarém	41° 9′ 30.56″ N 8° 41′ 4.29″ W	Rock/sand beach; classification[a]: "acceptable"; urban centre of Oporto city	Salvado 2009
6	Castelo do Queijo	41° 10′ 10.74″ N 8° 41′ 21.21″ W	Rock/sand beach; classification[a]: "acceptable"; urban centre of Oporto city	Salvado 2009
7	Matosinhos	41° 10′ 43.40″ N 8° 41′ 52.33″ W	Seawall; classification[a]: "acceptable"; urban centre of Oporto city	Salvado 2009
8	Leça da Palmeira	41° 11′ 33.81″ N 8° 42′ 28.88″ W	Rock/sand beach; classification[a]: "acceptable"; urban/industrial centre of Oporto metropolitan area; fuel refinery activities	Salvado 2009
9	Aterro	41° 12′ 31.90″ N 8° 42′ 58.45″ W	Rock/sand beach; classification[a]: "good"; industrial centre of Oporto metropolitan area; fuel refinery activities	Salvado 2009
10	Cabo do Mundo	41° 13′ 33.62″ N 8° 43′ 3.01″ W	Rock/sand beach; classification[a]: "good"; urban/industrial centre of Oporto metropolitan area; fuel refinery activities	Salvado 2009
11	Labruge	41° 16′ 14.96″ N 8° 43′ 42.43″ W	Rock/sand beach; classification[a]: "acceptable"; urban/agricultural activities; small piscatorial village	Salvado 2009
12	Vila Chã	41° 17′ 33.93″ N 8° 44′ 5.39″ W	Rock/sand beach; classification[a]: "acceptable"; urban/agricultural activities; small piscatorial village	Salvado 2009
13	Árvore	41° 20′ 19.24″ N 8° 44′ 51.19″ W	Seawall; classification[a]: "interdict"; south bank of River Ave mouth; domestic sewage and industrial effluents	Salvado 2009
14	Vila do Conde (South)	41° 20′ 20.23″ N 8° 44′ 57.15″ W	Seawall; classification[a]: "bad"; north bank of River Ave mouth; domestic sewage and industrial effluents	Salvado 2009
15	Vila do Conde	41° 21′ 4.81″ N 8° 45′ 19.03″ W	Rock/sand beach; classification[a]: "bad"; urban centre; domestic sewage and industrial effluents	Salvado 2009
16	Póvoa de Varzim	41° 21′ 57.80″ N 8° 45′ 46.42″ W	Seawall; classification[a]: "good"; south of urban centre of "Póvoa de Varzim" city; domestic sewage and industrial effluents	Salvado 2009
17	Fão-Ofir	41° 30′ 56.46″ N 8° 47′ 19.27″ W	Seawall; classification[a]: "aceptable"; south of river Cavado mouth; agricultural/industrial effluents	Salvado 2009
18	Esposende	41° 32′ 30.22″ N 8° 47′ 36.20″ W	Seawall; classification[a]: "aceptable"; north of river Cavado mouth; agricultural/industrial effluents	Salvado 2009
19	Viana do Castelo	41° 40′ 39.48″ N 8° 50′ 14.32″ W	Seawall; classification[a]: "good"; north of river lima mouth; naval shipyards and metallurgic activities; domestic sewage and industrial effluents	Salvado 2009
20	Forte do Paçô	41° 45′ 41.89″ N 8° 52′ 42.80″ W	Rock/sand beach; classification[a]: "good"; near a wastewater treatment station	Salvado 2009

Table 1 (continued)

	Location	Geographical Coordinates	Description	Reference
21	Moledo	41° 50′ 50.12″ N 8° 52′ 4.24″ W	Rock/sand beach; classification[a]: "good"; south of river Minho mouth; domestic sewage and agricultural activities	Salvado 2009

[a] Classification of bath/recreation coastal water attributed by Portuguese Water Institute (INAG) based on physical, chemical and microbiological parameters, in accordance with national (Decree 236/98 of 1st August) and international (European Community Directive 76/170/EEC) laws

previously decontaminated. In situ, the main physicochemical parameters were measured as described by Reis et al. (2009): temperature, conductivity, total dissolved solids, redox potential, salinity and dissolved oxygen. In laboratory, samples were immediately filtered (Whatman cellulose nitrate membrane; 0.45 μm), acidified to pH below 2 and frozen at −8°C until analysis.

Metals (Cd, Cr, Cu, Fe, Mn, Ni and Zn) were determined by atomic absorption spectrometry (SpectrAA 220 FS, Varian) with flame atomization (Marck 7, Varian) and electrothermal atomization (Autosampler GTA 110, Varian), and with deuterium background correction system. The dissolved metal concentrations were analysed according to APHA (1998b, c). The metal extraction method used to pre-concentrate vestigial concentrations from seawater samples and to remove interfering ions was based in Chelex-100 (functional iminodiacetic acid groups) chelating resin. This final optimized method adapted from Vasconcelos and Leal (1997) consisted in the following steps:

1. Filling a solid phase extraction column (GracePure, 5 mL) with 1.0 g of Chelex-100 (Fluka, Na^+ form, 100–200 mesh) and adjust the flow rate of the extraction manifold system (Waters; model WAT200677) to 5 mL min^{-1};
2. Pre-cleaning the resin with 5 mL of 2 mol L^{-1} HNO_3 solution (two bed volumes) and 15 mL of ultrapure water (at least five bed volumes);
3. Regenerating the resin with 5 mL of 2 mol L^{-1} NH_3 solution (two bed volumes);
4. Conditioning the resin with 5 mL of 0.05 mol L^{-1} NH_4CH_3COO solution (two bed volumes);
5. Passing 250 mL of seawater sample at pH= 8 (adjusted with 7 mol L^{-1} NaOH solution and buffered with 4 mol L^{-1} NH_4CH_3COO solution);
6. Removing matrix sample from the resin with 5 mL of ultrapure water (two bed volumes);
7. Metal elution with 5 mL of 2 mol L^{-1} HNO_3 solution, which allows a 50-fold concentration factor (two bed volumes).

Seawater samples were spiked with known amounts of metals to check the suitability of this analytical procedure for coastal seawater. The analyses of the seawaters spiked revealed satisfactory mean recoveries for all elements, which were higher than 88% (Table 3).

C. montagui

Barnacles were simultaneously collected in the same sampling sites as seawater samples. At least 300 organisms of approximately the same size (1–5 mm rostro-carinal axis) were collected from intertidal rocks, placed in plastic bags and transported in refrigerated containers to the laboratory within 8 h.

The entire body (soft tissue) of *C. montagui* individuals was dissected and separated from their shell plates (hard tissue) and pooled to form three replicates of 100 individuals in each site. In accordance with similar studies, barnacle soft tissues were not depurated (Ruelas-Inzunza and Paez-Osuna 2000; Rainbow et al. 2000; Rainbow and Blackmore 2001; Morillo et al. 2005; Morillo and Usero 2008). However, shell plates were pre-cleaned with acetone for 16 min in an ultrasonic bath, as in Watson et al. (1995). Both types of tissues (soft and hard tissues) were dried until constant weight and homogenised. Among replicate samples, there were no significant differences ($p<0.05$) in the mean dry weight of ten pooled individuals, which means that the effect of body size on accumulated trace metals can be ruled out (Morillo et al. 2005; Morillo and Usero 2008).

Table 2 Physicochemical parameters measured in the coastal seawater in the 22 sampling locations

Sampling location	Physicochemical parameter[a]				
	Temperature (°C)	Conductivity (mS cm^{-1})	Oxidation reduction potential (mV)	Salinity (psu)	Dissolved oxygen (mg L^{-1})
0	14.50±0.28	38.55±0.07	−198.50±0.71	30.35±0.49	10.12±0.17
1	14.95±0.21	39.95±0.07	−154.60±0.57	32.10±0.14	10.10±0.14
2	15.00±0.14	41.90±0.14	−143.85±0.21	33.50±0.71	10.11±0.15
3	13.70±0.10	36.85±0.21	−174.65±0.49	24.35±0.49	10.19±0.26
4	13.53±0.06	31.65±0.49	−163.65±0.49	24.25±0.35	10.23±0.32
5	13.63±0.06	30.95±0.07	−184.20±1.13	24.90±0.14	10.25±0.35
6	13.43±0.32	28.95±0.07	−255.80±0.28	23.15±0.21	10.22±0.30
7	13.55±0.35	29.10±0.14	−258.85±0.21	23.35±0.49	10.22±0.31
8	13.53±0.38	29.95±0.07	−228.45±0.78	24.10±0.14	10.25±0.35
9	14.00±0.14	32.70±0.42	−148.10±0.14	26.15±0.21	10.20±0.28
10	14.15±0.07	33.70±0.50	−170.85±0.21	27.55±0.64	10.34±0.48
11	14.25±0.05	38.85±0.21	−189.35±0.49	30.30±0.42	10.16±0.23
12	14.15±0.07	36.70±0.42	−178.15±0.21	29.95±0.07	10.21±0.30
13	13.20±0.14	14.55±0.08	−148.15±0.21	11.78±0.32	10.24±0.33
14	12.80±0.10	22.20±0.14	−341.25±0.35	2.00±0.40	10.24±0.34
15	14.03±0.06	36.20±0.15	−186.05±0.07	29.25±0.35	10.26±0.36
16	14.45±0.07	37.40±0.14	−141.15±0.21	30.15±0.21	11.14±0.19
17	14.30±0.10	38.80±0.14	−58.80±0.28	31.65±0.07	10.17±0.24
18	14.37±0.06	39.80±0.28	−99.05±0.07	32.47±0.15	10.41±0.04
19	13.37±0.06	21.23±0.10	−140.20±0.28	16.90±0.11	10.57±0.02
20	13.50±0.10	16.80±0.14	−65.05±0.07	36.40±1.27	10.15±0.21
21	13.80±0.10	13.20±0.14	−66.15±0.21	23.10±0.26	10.77±0.27

[a] Mean ± standard deviation (n=3)

For metal analyses, dried tissues (300 mg) were digested with diluted HNO_3 solution (27%, *v*/*v*) in an adapted domestic microwave system (model no. NE-1037, Panasonic) using Parr teflon bombs (Parr 4782), at high pressure, following the method described by Reis and Almeida (2008). Total metal concentrations (Cd, Cr, Cu, Fe, Mn, Ni and Zn) were determined by atomic absorption spectrometry with flame atomization (AAS-Flame) and electrothermal atomization (AAS-ET). The working parameters and matrix modifiers employed were those recommended by the Varian analytical methods for AAS-Flame and AAS-ET (Varian 1988, 1989).

C. montagui samples spiked with known amounts of metals and standard reference material (National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2976) certified for trace metals in mussel tissues were used to check, respectively, potential matrix effects and the suitability of the analytical procedure for barnacle tissues. The analysis of the spiked barnacle soft tissues, spiked barnacle hard tissues and NIST SRM 2976 also revealed satisfactory recoveries for all elements, which were above 90%, 86% and 88%, respectively (Tables 3 and 4).

For each solution analysed (seawater or tissue sample), Varian software was programmed to comply with a precision below 10% between readings, in a maximum of four readings per replicate, to assure reproducibility of the measurements. External calibrations were carried out with aqueous standards solutions. Blank solutions were prepared following the respective sample treatment, and metal levels in blank solutions were always below the limit of detection of the analytical procedure (Tables 3 and 4).

Table 3 Metal concentrations (nanograms per litre) in seawater in the 22 sampling locations

Sampling location	Element (ng L^{-1})						
	Cd	Cr	Cu	Mn	Ni	Zn	Fe
Limit of detection	2	4	151	287	2	2000	292
Sample spiked recoveries (%)	90±12	105±12	101±1	88±7	98±8	112±2	93±17
0	28±1	33±5	338±81	894±176	248±40	46,604±280	932±156
1	33±7	30±5	479±24	1,510±175	204±7	53,754±1,272	1,601±174
2	89±2	22±2	481±48	1,081±105	226±44	66,326±265	1,149±167
3	40±2	17±2	348±64	1,633±162	340±41	66,786±4,009	1,307±372
4	130±1	18±3	473±98	883±88	180±5	54,013±2,238	893±141
5	26±1	37±7	261±26	1,019±99	201±9	51,036±204	960±84
6	26±5	31±8	1,402±347	2,424±225	273±20	49,191±246	1,601±271
7	20±4	30±3	532±52	2,946±277	343±22	52,224±209	1,491±58
8	21±5	18±2	618±62	882±86	162±13	53,246±160	865±93
9	26±5	14±2	546±48	1,196±118	300±25	55,699±2,367	962±243
10	20±1	18±4	627±130	1,032±99	882±11	56,953±285	882±86
11	26±5	23±2	447±123	1,668±95	184±38	52,973±212	1,050±287
12	30±1	7±3	235±65	1,024±100	1,189±83	47,487±8,497	839±82
13	19±2	19±1	373±37	1,515±20	291±23	50,384±202	37,578±2,693[a]
14	21±2	26±3	581±56	6,135±979[a]	519±16	52,047±1,761	969±101
15	34±4	13±1	451±92	1,252±41	288±34	39,811±516	1,021±99
16	35±1	15±1	888±89	1,777±172	1,314±208	47,758±2,465	654±65
17	36±6	16±3	328±3	888±113	788±162	48,511±243	1,209±128
18	46±4	13±1	490±46	1,791±208	413±72	44,627±223	1,454±142
19	39±9	25±2	313±56	2,976±244[a]	586±39	49,531±6,722	1,307±123
20	32±5	26±3	480±56	651±87	273±25	39,733±119	1,360±210
21	77±4	10±1	636±59	2,600±624	521±84	51,527±309	903±89
Mean ± standard deviation	39±27	21±8	510±246	1,433±629	445±329	51,374±6,597	1,115±273

Limits of detection and recovery percentages of the samples spiked with known amounts of metals are also included. Mean ± standard deviation (n=3)

[a] Outlier value and not considered in calculation of mean ± standard deviation

The detection limits were calculated using the procedure recommended by APHA (1998d).

Data analysis

Three replicates per sample were treated and analysed separately, and the respective average and standard deviation were calculated (n=3). To compare similarity between locations, "Cluster" analyses based on Bray–Curtis similarity matrix and data logarithmic normalization were applied using PRIMER package (Clarke and Warwick 2001). Significant differences of metal concentrations between locations were tested using ANOVA and Student's t test after testing data normality with Shapiro–Wilk test. Two levels of significance were considered: p=0.05, significant and p=0.001, very significant.

Results and discussion

Coastal seawater—physicochemical parameters and dissolved metal concentrations

The characterization and classification of the coastal waters of each sampling location are shown in Table 1.

Table 4 Metal concentrations (milligrams per kilogram, dry wt.) in soft tissues of *Chthamalus montagui* in the 22 sampling locations

Sampling Location	Element (mg kg^{-1}, dry wt.)						
	Cd	Cr	Cu	Ni	Mn	Zn	Fe
Limit of detection	0.097	0.032	0.20	0.058	2.87	8.67	2.32
Spiked sample recoveries (%)	106±11	91±7	94±10	100±15	88±8	97±8	98±7
SRM 2976 recoveries (%)	93±5	102±3	94.7±0.2	112.7±0.1	95±2	101±7	98±3
0	1.0±0.2	3.2±0.5	1.7±0.4	1.1±0.2	11±1	336±28	369±28
1	1.2±0.1	2.7±0.5	2.4±0.2	1.3±0.2	17±1	556±3	713±47
2	1.7±0.1	1.5±0.1	2.1±0.2	1.2±0.2	11±1	956±26	385±61
3	1.1±0.2	1.4±0.3	1.9±0.3	1.4±0.2	17±1	843±35	507±6
4	1.9±0.3	1.7±0.3	2.2±0.2	1.0±0.1	14±1	523±28	269±41
5	0.9±0.1	3.2±0.1	1.4±0.1	1.0±0.1	9±1	336±13	216±18
6	0.91±0.04	2.8±0.7	3.3±0.4	0.81±0.01	23±1	418±12	785±111
7	0.7±0.1	2.2±0.1	3.4±0.6	1.0±0.2	26±1	457±11	649±34
8	0.9±0.2	1.3±0.2	6.7±0.8	0.72±0.03	13±1	702±4	279±36
9	1.0±0.1	1.1±0.1	3.7±0.3	1.4±0.1	12.1±0.2	778±20	397±39
10	0.6±0.1	1.3±0.2	3.7±0.5	2.4±0.2	13.0±0.3	729±13	276±15
11	0.9±0.2	1.9±0.3	1.9±0.2	0.8±0.1	20±1	361±16	456±27
12	1.0±0.1	0.5±0.1	1.2±0.1	4±1	14±2	368±28	356±81
13	0.59±0.03	1.6±0.3	2.5±0.2	1.2±0.2	17.7±0.1	537±84	301±23
14	0.9±0.2	2.4±0.6	3.6±0.5	2.1±0.4	22±1	521±40	426±14
15	0.9±0.1	1.2±0.2	2.7±0.4	0.9±0.1	12±1	313±4	388±21
16	0.8±0.2	1.4±0.3	4.7±0.7	5±1	17±2	434±34	214±9
17	0.8±0.2	1.4±0.2	1.9±0.5	3.0±0.1	12.6±0.2	178±1[a]	436±8
18	1.1±0.2	1.1±0.2	2.6±0.2	1.1±0.1	21±1	296±2	606±46
19	1.0±0.1	2.2±0.3	2.5±0.2	2.9±0.4	21±1	496±39	504±51
20	1.1±0.2	2.1±0.5	2.0±0.2	2.3±0.6	11±1	446±15	550±80
21	1.5±0.1	0.9±0.1	2.8±0.6	1.7±0.2	26±2	557±107	267±39
Mean ± standard deviation	1.0±0.3	1.8±0.7	2.8±1.2	1.7±1.1	16.3±5.0	522±184	425±159

Limits of detection and recovery percentages of the SRM 2976 Reference Mussel Tissue, certified for inorganic elements, are also included. Mean ± standard deviation (n=3)

[a] Outlier value and not considered in calculation of mean ± standard deviation

The physicochemical parameters and dissolved metal concentrations of the seawaters from NW coast of Portugal are shown, respectively, in Tables 2 and 3.

The abiotic parameters of seawaters followed the typical values of sampling season and geographical location: temperatures between 12.80°C and 15.00°C (spring), salinities between 2.00 and 36.40‰ (estuarine or marine area), dissolved oxygens above 10.10 mg L^{-1} (O_2 saturation), potential redox between −341.25 and −58.80 mV (estuarine or marine area) and conductivities between 13.20 and 41.90 mS cm^{-1} (estuarine or marine area). These values are similar to previous studies in the Portuguese coastal seawaters (Salvado 2009).

Metal distribution showed high spatial variation along the coast: 7–37 ng Cr L^{-1}; 20–130 ng Cd L^{-1}; 180–1,314 ng Ni L^{-1}; 235–1,402 ng Cu L^{-1}; 651–6,135 ng Mn L^{-1}; 654–37,578 ng Fe L^{-1} and 39,733–66,786 ng Zn L^{-1}. Some locations showed high metal concentrations, reflecting their proximity to urban areas: "Paramos", "Matosinhos", "Vila do Conde" and "Viana do Castelo" showed high concentrations of Cr, Fe and Mn, while waters near "Valadares", "Foz" and "Moledo" had high concentrations of Cd,

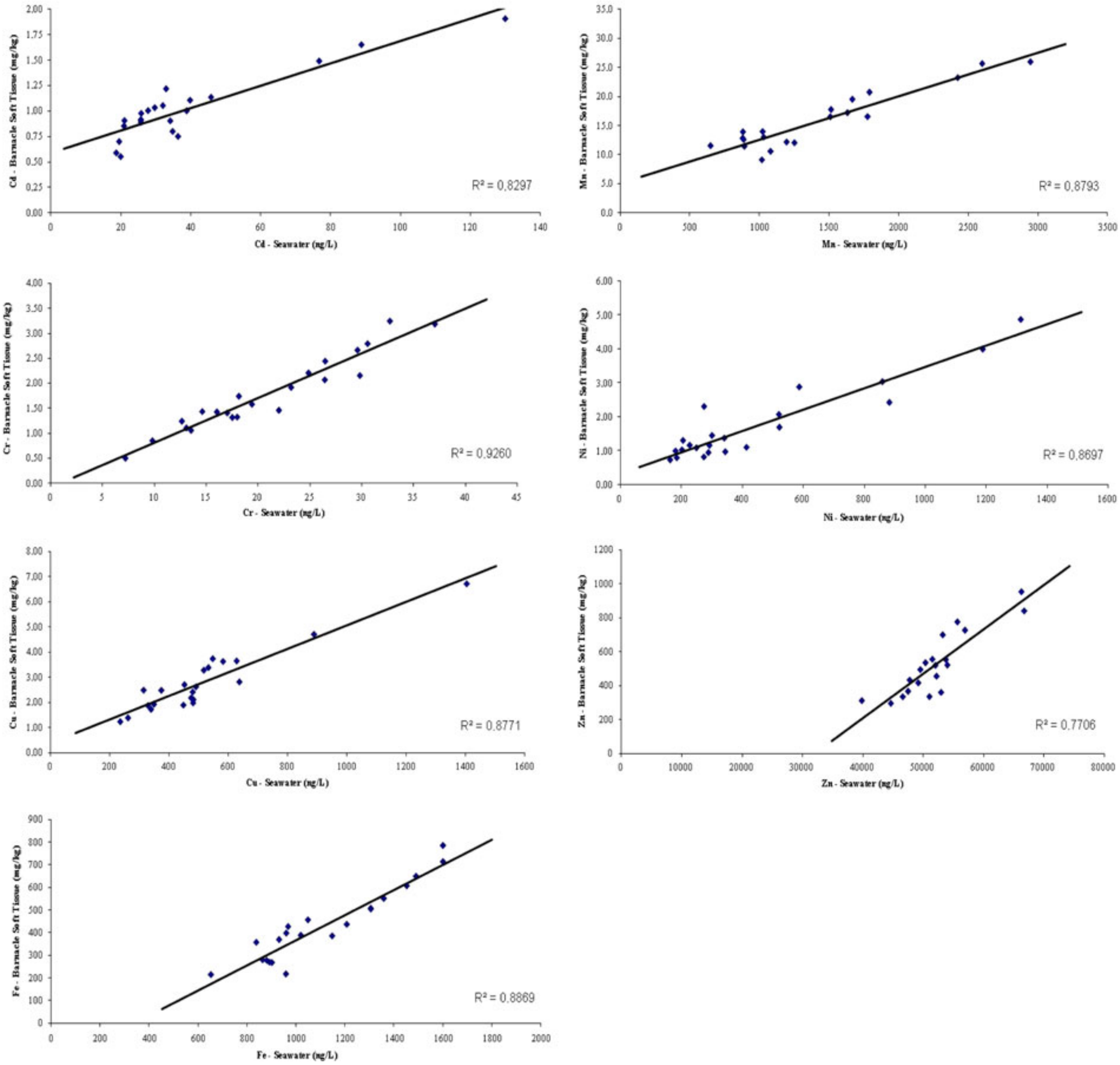

Fig. 2 Linear regressions between metal concentrations in *C. montagui* soft tissues (milligrams per kilogram, dry wt.) and coastal seawaters (milligrams per litre)

Zn as well as Mn. On the other hand, coastal seawaters near “Foz”, “Aterro”, “Vila Chã” and “Moledo” showed low concentrations of Cu and Cr, “Vila do Conde” and “Forte do Paçô” showed low concentrations of Mn and Zn, and “Póvoa de Varzim” showed minimum concentrations of Fe.

Comparing these results with Norwegian Pollution Control Authority guidelines for metals in coastal seawaters (SFT TA-2229/2007), all locations along NW coast of Portugal can be classified as “class I–background level” or “class II–good”, except for Zn. Regarding Zn concentrations (>6,000 ng L^{-1}), all coastal seawaters should be classified as “bad/very bad” (SFT 2007).

Barnacle soft tissues—total metal concentrations

Total metal concentrations obtained in *C. montagui* soft tissues are shown in Table 4. They showed high spatial variations along NW coast of Portugal, and their

Table 5 Metal bioaccumulation factor (log BAF) in the soft tissues of *Chthamalus montagui* of the 22 sampling locations

Sampling location	Bioaccumulation factor (log BAF)						
	Cd	Cr	Cu	Ni	Mn	Zn	Fe
0	4.56	5.00	3.71	3.64	4.11	3.86	5.60
1	4.57	4.96	3.70	3.80	4.04	4.01	5.65
2	4.27	4.82	3.64	3.71	3.99	4.16	5.52
3	4.44	4.92	3.74	3.60	4.02	4.10	5.59
4	4.17	4.98	3.66	3.74	4.20	3.99	5.48
5	4.54	4.94	3.73	3.70	3.95	3.82	5.35
6	4.55	4.96	3.80	3.47	3.98	3.93	5.69
7	4.55	4.86	3.80	3.45	3.95	3.94	5.64
8	4.61	4.88	3.68	3.65	4.16	4.12	5.51
9	4.58	4.89	3.84	3.68	4.01	4.15	5.62
10	4.44	4.87	3.77	3.44	4.10	4.11	5.50
11	4.55	4.92	3.63	3.63	4.07	3.83	5.64
12	4.54	4.84	3.72	3.53	4.13	3.89	5.63
13	4.50	4.91	3.82	3.60	4.07	4.03	3.90
14	4.63	4.97	3.80	3.60	3.55	4.00	5.64
15	4.42	4.99	3.78	3.51	3.98	3.89	5.58
16	4.36	4.99	3.72	3.57	3.97	3.96	5.51
17	4.31	4.95	3.76	3.55	4.15	3.56	5.56
18	4.39	4.93	3.73	3.42	4.06	3.82	5.62
19	4.41	4.95	3.90	3.69	3.85	4.00	5.59
20	4.52	4.89	3.62	3.92	4.25	4.05	5.61
21	4.29	4.94	3.65	3.51	3.99	4.03	5.47
Mean ± standard deviation	4.46±0.12	4.93±0.05	3.74±0.07	3.61±0.12	4.03±0.14	3.97±0.14	5.49±0.36

ranges were: 0.59–1.7 mg Cd kg^{-1}; 0.5–3.2 mg Cr kg^{-1}; 0.72–3.0 mg Ni kg^{-1}; 1.2–6.7 mg Cu kg^{-1}; 9–26 mg Mn kg^{-1}; 214–785 mg Fe $kg^{-1}$and 178–956 mg Zn kg^{-1}.

In general, the metals accumulated in barnacle soft tissues seem to reflect the concentrations of metals in seawaters: Zn, Fe and Mn were the metals more accumulated, and Cd was the least accumulated. The relationships between metal concentrations in coastal seawaters and in *C. montagui* soft tissues were tested using a linear regression model shown in Fig. 2. Normality of metal data was confirmed with Shapiro–Wilk test ($p>0.1705$). The sampling locations considered outliers (Primer software package: metal values that changed linear correlation coefficients (R) more than 0.1) were not used in this linear regression model (Reis et al. 2009).

There were very significant positive correlations ($p<0.001$, $R^2>0.7706$) between the concentrations of Cd, Cr, Cu and Ni in coastal seawaters and in *C. montagui* soft tissues of the 22 sampling locations along the NW coast of Portugal. However, some locations showed very high concentrations of Fe, Mn and Zn in barnacle soft tissues and were excluded from the linear regression model as outliners: Fe in location 13, Mn in locations 14 and 19 and Zn in locations 17 and 20. Particularly, barnacles from location 20 showed higher Zn concentrations in their soft tissues than expected, regarding the linear regression model and the available Zn in this seawater, which suggested a memory effect of previous episodes of Zn anthropogenic contamination (Barber and Trefry 1981; Morillo et al. 2005).

The regression line slopes reflect the bioaccumulation of metals by barnacles: if one particular metal

Table 6 Range of metal concentrations (milligrams per kilogram, dry wt.) in soft tissues of different barnacle species from various ecosystems around the world

Species	Location	Range of metal concentrations in barnacle soft tissues (mg kg^{-1}, dry wt.)							Reference
		Cd	Cu	Zn	Cr	Fe	Mn	Ni	
Chthamalus montagui	NW coast of Portugal	0.59–1.7	1.2–6.7	178–956	0.50–3.2	210–780	9.0–26.0	0.72–3.0	This work
Chthamalus stellatus	S–SW coast of Portugal and Tinto river estuary (Huelva, Spain)	6.0	6.3–11.3	100–237	–	–	–	–	Stenner and Nickless (1975)
Amphibalanus amphitrite	La Jolla (California, USA)	–	–	910	–	–	–	–	Alexander and Rowland (1966)
	S–SW coast of Portugal and Tinto river estuary (Huelva, Spain)	12	550–600	1780–3,300	–	–	–	–	Stenner and Nickless (1975)
	Adriatic Lagoons (Croatia)	–	41–109	–	2.1–3.9	–	–	–	Barbaro et al. (1978)
	Zuari estuary (India)	–	847	1,938	–	–	–	–	Anil and Wagh (1988)
	Hong Kong (China)	0.69–30.9	29.0–6,317	1,476–23,300	0.22–28.0	313–5,929	14.5–277	1.25–98.9	Blackmore (1996); Blackmore et al. (1998); Philips and Rainbow (1988, 1990); Rainbow and Smith (1992); Rainbow et al. (1993); Rainbow and Blackmore (2001)
	Salton Sea (California, USA)	6–58	40–3,750	620–37,900	–	450–1,150	–	–	Fialkowski and Newman (1998)
	Huelva estuary (Spain)	21–168	3,720–9,430	19,900–48,500	–	–	168–469	3.7–8.3	Morillo et al. (2008)
	Algeciras Bay (Spain)	2.1–3.6	94–225	852–4,170	–	–	26–154	2.7–26	Morillo et al. (2008)
	Ross Creek (Australia)	3.0–8.4	–	–	–	–	–	–	da Silva et al. (2005)
	Curimataú estuary (Brazil)	5.1	23.6	1185	–	466	9.6	9.1	Silva et al. (2006)
Semibalanus balanoides	Woods Hole (Massachusetts, USA)	–	68–104	–	–	–	–	–	Clarke (1947)
	Cardigan Bay, Wales (UK)	–	46–3,750	1,028–30,000	–	–	–	–	Ireland (1974); Walker et al. (1975)
	Huelva estuary (Spain)	24–330	2,496–21,800	11,520–101,000	–	2,020–13,700	85–816	4.1–18	Morillo et al. (2005)

Table 7 Metal levels (milligrams per kilogram, dry wt.) in hard tissues of *Chthamalus montagui* in the 22 sampling locations

Sampling location	Element (mg kg^{-1}, dry wt.)							Ca (g kg^{-1}, dry wt.)
	Cd	Cr	Cu	Ni	Mn	Zn	Fe	
Limit of detection	0.0033	0.067	0.330	0.067	0.387	0.667	6.67	0.001
Spiked sample recoveries (%)	111±7	87±11	86±19	91±10	86±5	89±8	98±5	102±11
SRM 2976 recoveries (%)	94.6±0.4	118±2	102±10	88±7	96±2	97±15	102±8	97±12
0	0.092±0.012	1.11±0.03	1.29±0.10	1.02±0.02	8.9±1.8	32±6	164±36	546±50
1	0.103±0.010	0.65±0.13	1.25±0.25	0.62±0.01	8.9±0.6	20±2	350±44	476±24
2	0.064±0.012	0.32±0.02	1.08±0.03	0.55±0.05	5.0±0.7	30±6	70±7	538±24
3	0.087±0.006	0.65±0.04	1.04±0.07	0.76±0.05	9.3±0.3	29±2	156±6	529±17
4	0.093±0.002	0.61±0.04	0.90±0.15	0.49±0.02	3.6±0.7	35±7	55±8	539±4
5	0.072±0.005	0.49±0.02	0.67±0.18	0.42±0.11	3.2±0.3	24±1	47±4	554±39
6	0.086±0.005	0.70±0.03	1.18±0.15	0.46±0.09	14.3±1.4	59±10	278±28	511±50
7	0.107±0.024	1.20±0.11	1.57±0.29	0.78±0.08	18.2±1.0	87±18	357±11	494±51
8	0.084±0.001	0.64±0.02	1.82±0.17	0.60±0.06	8.0±0.2	62±6	132±2	540±36
9	0.104±0.014	0.74±0.02	1.56±0.12	0.67±0.03	6.4±0.7	43±2	154±17	548±14
10	0.069±0.008	0.62±0.04	1.11±0.13	1.34±0.19	8.2±0.3	68±1	104±7	507±45
11	0.073±0.008	1.00±0.20	0.65±0.01	0.79±0.11	8.7±1.2	22±5	177±19	476±81
12	0.029±0.004	0.38±0.08	0.80±0.12	0.66±0.09	3.7±0.2	42±2	106±8	490±18
13	0.020±0.001	0.25±0.01	1.03±0.12	0.68±0.10	10.4±0.4	40±8	79±4	460±11
14	0.025±0.004	0.47±0.09	0.94±0.18	0.90±0.06	12.2±1.3	44±11	96±16	490±48
15	0.045±0.004	0.41±0.04	1.01±0.07	0.63±0.02	5.9±0.2	48±1	96±6	488±47
16	0.053±0.002	0.47±0.07	1.62±0.10	0.76±0.02	3.6±0.3	64±5	114±2	455±52
17	0.031±0.007	0.38±0.02	0.49±0.05	0.70±0.12	5.3±0.4	17±2	100±7	460±20
18	0.036±0.002	0.44±0.05	0.84±0.09	0.81±0.09	12.0±1.6	28±1	230±1	439±5
19	0.034±0.005	0.59±0.04	0.68±0.01	0.78±0.09	8.7±0.8	30±2	165±23	463±16
20	0.053±0.013	0.46±0.04	0.64±0.15	0.70±0.04	4.5±0.6	19±3	86±22	494±74
21	0.059±0.012	0.74±0.09	0.41±0.05	0.53±0.06	7.0±1.4	26±7	68±14	474±24
Mean ± standard deviation	0.064±0.028	0.61±0.25	1.03±0.38	0.71±0.20	8.0±3.8	39±18	145±88	499±35

Limits of detection and recovery percentages of the SRM 2976 Reference Mussel Tissue, certified for inorganic elements, are also included. Mean ± standard deviation (n=3)

has a higher slope than another, the same variation in seawater concentrations will provoke a greater increase in barnacle soft tissues concentration, and vice versa (Morillo et al. 2005). The metals with the highest regression line slopes were Fe, Cr and Zn, respectively, with values of 555,205, 89,433 and 26,093 L of coastal seawater per kilogram of soft tissue. The lowest slopes were obtained for Cd, Mn, Cu and Ni, with values of 10,995, 7,456, 4,675 and 3,144 L of coastal seawater per kilogram of soft tissue, respectively. The significant correlations obtained for all metals between coastal seawaters and barnacle soft tissues ($p<0.05$) also suggest that the dissolved metal concentrations describe well the process of metal accumulation in barnacle soft tissues, and they will give good estimates of metal bioavailabilities in the different locations.

The metal bioaccumulation factor (BAF) is an adequate approach to assess the efficiency of barnacle species to accumulate metals in their soft tissues (Morillo et al. 2005). The log BAF values are shown in Table 5 and were calculated as the log-transformed ratio between metal concentrations in the barnacle soft tissue (milligrams per kilogram, dry mass) and in

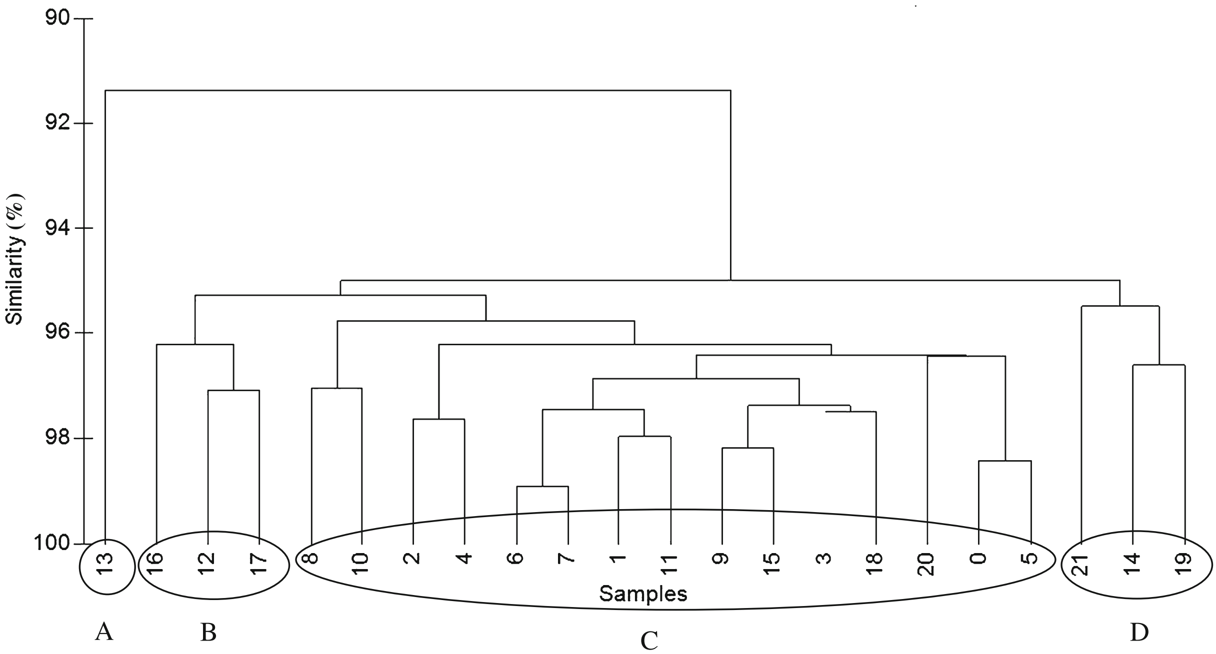

Fig. 3 Bray–Curtis similarity diagram showing the association of the sampling locations according to metal concentrations in soft tissues of *C. montagui* and the physicochemical parameters of the seawater

coastal seawater (nanograms per litre). The metals that *C. montagui* bioaccumulated more efficiently were Fe, Cr and Cd with mean log BAF values of 5.49, 4.93 and 4.46, respectively, reflecting barnacles' high sensitivity to these metals. Much lower mean log BAF values were obtained for Mn, Zn, Cu and Ni reaching only 4.03, 3.97, 3.74 and 3.61, respectively, which may be due to lower bioavailability of some of these metals in the coastal seawaters or the organism's ability to eliminate them. In the particular case of Zn, the high regression line slope (26,093 L of coastal seawater per kilogram) was not followed by a high log BAF value (4.93), which can represent potential anthropogenic contamination of the seawaters. These preliminary results showed that *C. montagui* soft tissues can be used for monitoring metal bioavailabilities in coastal seawaters along the NW coast of Portugal.

Ecological quality of the coastal seawaters

Metal concentrations obtained in barnacle *C. montagui* soft tissues collected along the NW coast of Portugal were lower or similar to the metal concentrations reported for other barnacle species sampled in different locations around the world (Table 6). Comparing these results with metal concentrations in *Chthamalus stellatus* soft tissues from the S–SW coast of Portugal and Rio Tinto estuary, *C. montagui* exhibited lower concentrations of Cd and Cu, but higher concentrations of Zn, probably in their metal-rich granules (Stenner and Nickless 1975). Extending this comparison to the other barnacle species reported in Table 6, Portuguese *C. montagui* soft tissues accumulated the lowest metal concentrations, even Fe and Cr, which are the metals with highest BAF. Indeed, our results suggested that metal bioavailabilities in seawaters from the NW coast of Portugal are low, except for Zn.

In terms of food consumption safety and according to the European Community Commission Regulation No 629/2008 of 2 July 2008 (EU 2008), the maximum Cd concentration allowed in foodstuff, particularly in crustaceans soft tissues, is 0.50 mg Cd kg^{-1} (wet wt.), which is equivalent to 2.50 mg kg^{-1} (dry wt.) assuming 80% of water in barnacle soft tissues. The Cd concentrations determined in *C. montagui* soft tissues in all locations were lower than this reference value, which proves that the NW coastal waters of Portugal have low Cd contamination. However, applying the Norwegian Pollution Control Authority metal concentration guidelines (SFT TA-1467/1997) for blue mussel *Mytilus edulis*, all coastal seawaters analysed can be classified as "class I–unpolluted/slightly polluted" for

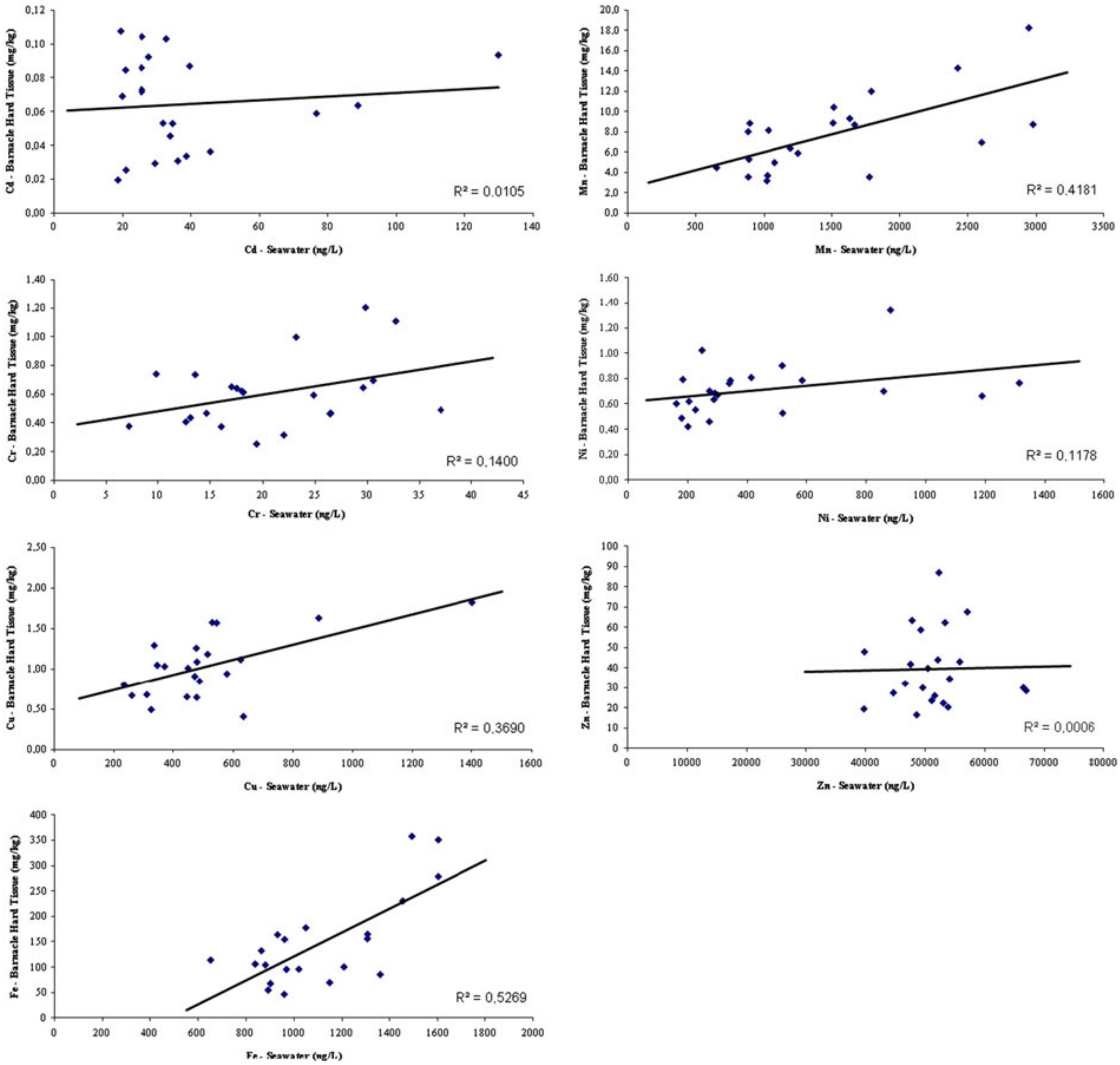

Fig. 4 Linear regressions between metal concentrations in *C. montagui* hard tissues (milligrams per kilogram, dry wt.) and coastal seawaters (milligrams per litre)

Cd and Cu, but "class II/III–moderately/remarkably polluted" for Zn (200–1,000 mg Zn kg^{-1}; Molvaer et al. 1997). Therefore, Zn is the metal of major concern since its bioavailability in seawater and its bioaccumulation in *C. montagui* soft tissues were high.

Barnacle shells—total metal concentrations

Total metal concentrations obtained in *C. montagui* shell plates are shown in Table 7. Their distribution along the NW coast of Portugal showed a wide range of concentrations: 0.020–0.107 mg Cd kg^{-1}; 0.25–1.20 mg Cr kg^{-1}; 0.42–1.34 mg Ni kg^{-1}; 0.41–1.82 mg Cu kg^{-1}; 3.2–18.2 mg Mn kg^{-1}; 17–87 mg Zn kg^{-1}; 47–357 mg Fe kg^{-1} and 439–554 g Ca kg^{-1}.

Figure 3 shows the linear regressions between metal concentrations in coastal seawaters and *C. montagui* hard tissues (shell plates). Normality of metal data was confirmed with Shapiro–Wilk test ($p > 0.05$).

Unlike the good correlations observed between metals in seawater and barnacle soft tissues, there were no significant correlations ($p<0.05$) between Cd, Cr, Ni and Zn concentrations in seawater and *C. montagui* shells. Although significant correlations ($p<0.05$) were obtained for Cu, Fe and Mn, the squared correlation coefficient values (R^2) were very low to be accepted, ranging 0.3387–0.5267. These results mean that barnacle shells do not reflect adequately metal uptake from seawater and should not be used to biomonitor or predict metal contamination in the environment. The bioaccumulation of metals in barnacle hard tissues involves the metabolic mechanisms related with the equilibrium of metal assimilation from phytoplankton and zooplankton diets and metal efflux rates, but probably also the mineralization processes during shell plates formation, when replacement of Ca by other bioavailable metals in the seawater may occur (da Silva et al. 2004, 2005, 2009a; Hockett et al.,1995, 1997; Ng et al. 2005; Rainbow 2007; Rainbow and Wang 2001, 2005; Rainbow and White 1989; Rainbow et al. 2003, 2004b; Viarengo and Nott 1993; Wang and Rainbow 2000, 2005, 2008; Wang et al. 1999; Watson et al. 1995). Thus, bioaccumulation of metals in barnacle hard tissues may be masked by mineralization (Fig. 4).

These preliminary results showed that *C. montagui* shell plates cannot be considered an ideal matrix for monitoring metal bioavailability in coastal seawaters. The same conclusion was reported by Watson et al. (1995) in *S. balanoides* shell plates due to significant variation of metal concentrations over time.

Conclusions

The results of this study showed that:

1. *C. montagui* proved to be a sensitive species to spatial variation of metal bioavailabilities in the water, and the average metal contamination in the water followed the order Cr<Cd<Ni<Cu<Fe<Mn<Zn, while the accumulation in their soft tissues was: Cd<Ni<Cr<Cu<Mn<Fe<Zn. In the shell plates, metal concentrations were lower than in soft tissues: Cd<Cr<Ni<Cu<Mn<Zn<Fe and were not correlated with the water concentrations;
2. *C. montagui* soft tissues, but not their shell plates, may be used for monitoring metal bioavailabilities in seawater of the NW coast of Portugal since there were significant positive correlations ($p<0.05$) between the concentrations of the metals Cd, Cr, Cu, Fe, Mn, Ni and Zn in seawaters and their concentrations in *C. montagui* soft tissues;
3. The mean metal bioaccumulation factors (log BAF) in *C. montagui* soft tissues were higher for Fe, Cr and Cd, with values of 5.49, 4.93 and 4.46, respectively, and lower for Mn, Zn, Cu and Ni, with values of 3.93, 3.83, 3.74 and 3.61, respectively. It seems that there is a discriminating bioaccumulation process which will require further investigation;
4. In terms of water quality regarding metal concentrations, all locations of the NW coast of Portugal sampled should be classified as coastal seawater of "class I/II–background level/good" or "unpolluted/slightly polluted", except for Zn. The very high concentrations of Zn in the seawaters and *C. montagui* soft tissues provided a final ecological classification of "class II/III–moderately/remarkably polluted".

This work intends to contribute to the increase of data on metal concentrations in coastal seawaters and in soft/hard tissues of *C. montagui* of the NW coast of Portugal, which will allow constructing a useful database for comparison purposes in future works. Additionally, it must be stressed out that these preliminary results need to be strengthened with a more complete metal monitoring programme using *C. montagui* soft tissues along the NW coast of Portugal in different seasons.

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