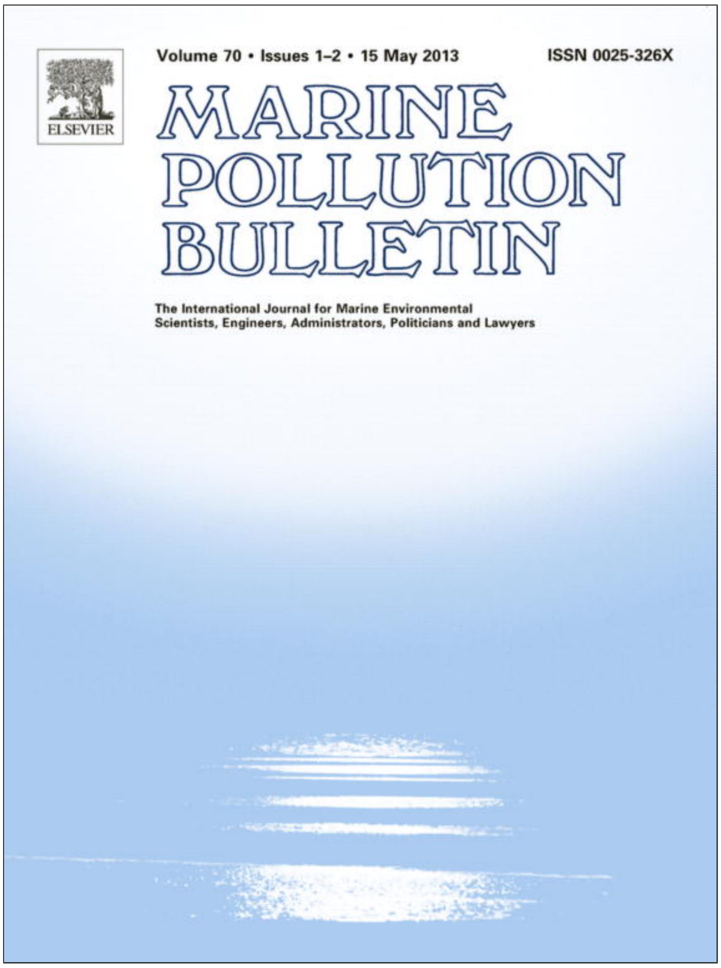
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Seasonal variation of metal contamination in the barnacles *Pollicipes pollicipes* in northwest coast of Portugal show clear correlation with levels in the surrounding water

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A B S T R A C T

The concentrations of metals were determined in northwest (NW) coast of Portugal seawaters and soft tissues of goose barnacles *Pollicipes pollicipes*. *P. pollicipes* can be used for monitoring metal contamination in these coastal seawaters, because there were significant correlations ($p < 0.05$) for all metals between soft tissues and seawaters during the four seasons. Metal concentrations in seawaters and *P. pollicipes* had significant ($p < 0.05$) spatial and seasonal variations and mean log BAFs for Fe and Cd were higher than for Cr, Cu, Mn and Zn. Regarding the metal concentrations obtained in the coastal seawaters, all NW coast of Portugal should be classified as "Class IV – Bad", except two locations (location 7 at Summer and location 10 at Winter), which should be classified as "Class III – Moderate". However, considering the metal concentrations bioaccumulated in *P. pollicipes*, all locations should be classified as "Class III – Remarkably Polluted" during all seasons of 2011.

The Water Directive is a Framework for the European Community Action in the Field of Water Policy. It requires the establishment of biomonitoring programs of aquatic ecosystems to assess their ecological quality and settles ecological goals, specific for each type of superficial water (Cortes and Oliveira, 2000). Several species are being used worldwide in Biomonitoring Watch Programmes such as in the United States of America, IndoPacific or Europe, using mussels – *Mytilus* and barnacles – *Amphibalanus* or *Balanus* (Lauenstein et al., 1990; Beliaeff et al., 1998; Cantillo, 1998; Rainbow and Blackmore, 2001; Rainbow et al., 2000, 2002, 2004; Reis at al., 2011). Philips (1977) suggested that biomonitor species should show significant correlations between the bioaccumulated and environmental concentrations in all locations, to avoid spurious ecological interpretations. In the particular case of Portugal, the actual Water Directive and European policies applied to assess the ecological quality of waters involve several parameters, which difficult their correct classification (Bordalo e Sá, 2001). The use of biotic indices and contaminants reference guidelines, such as Norwegian Pollution Control Authority SFT TA-2229/2007 (coastal seawaters) and SFT TA-1467/1997 (blue mussel *Mytilus edulis*) for metals, can be a useful solution, which allows an uniform ecological quality classification of coastal waters (Molvaer et al., 1997; SFT, 2007; Reis et al., 2012a,b).

Goose barnacles *Pollicipes pollicipes* were previously used by Reis et al. (2012b) to study metal contamination of these coastal waters but only during one season (Summer 2010). Thus, in this work, *P. pollicipes* was used again as biomonitor of metal contamination in coastal waters of the northwest (NW) coast of Portugal during one-year period (four sampling seasons). The main objectives were: (i) to assess the potential use of *P. pollicipes* as biomonitor of metal contamination in each season; (ii) to increase the information on metal concentrations in Portuguese seawaters and tissues of *P. pollicipes*; (iii) to assess spatial and seasonal variations of metal bioavailabilities; (iv) to establish relationships between metals in seawaters and *P. pollicipes* and (v) to calculate Bioaccumulation Factors (BAFs) of metals in *P. pollicipes*.

During the four seasons of 2011 (Winter; Spring; Summer and Autumn), 10 locations were selected and monitored along the NW coast of Portugal (Fig. 1). These locations showed consistent mega-populations of *P. pollicipes* during all year and are under different levels of metal contamination (Reis et al., 2012a,b).

The reagents were at least of pro analysis (p.a.) grade: HNO_3 (Fluka, 35% (w/w), Suprapure); resin Chelex-100 (Fluka, Na^+ form, 100–200 mesh, p.a.); NH_3 (Panreac, 25% (w/w), p.a.) and NH_4CH_3COO (Panreac, p.a.). The metal standard solutions were daily prepared by weight from stock solutions of 1000 mg L^{-1} (Fluka, p.a.)

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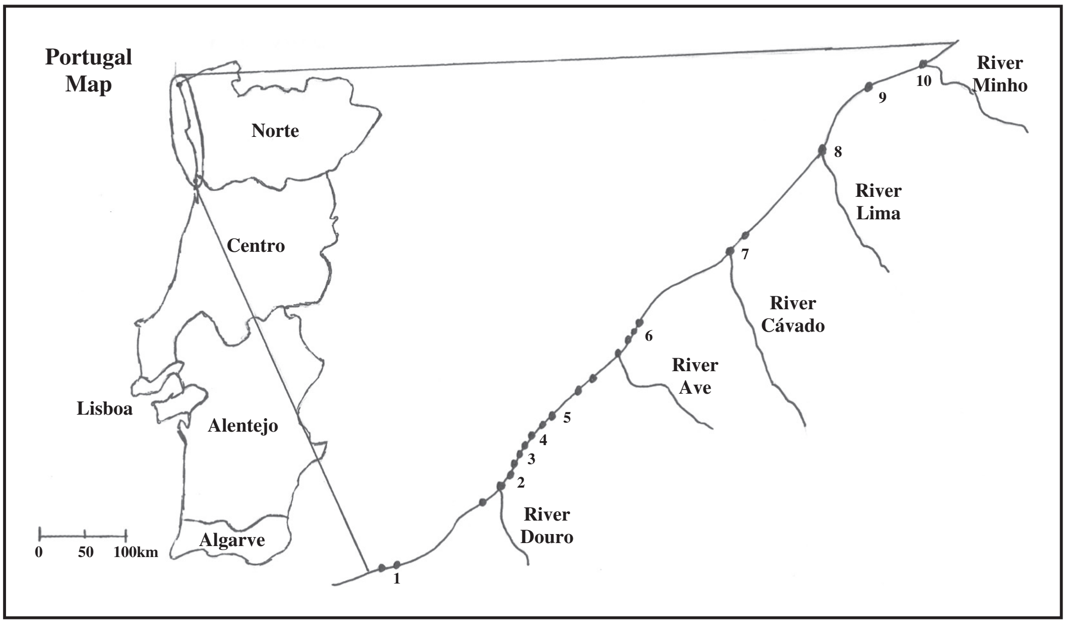

Fig. 1. Geographical distribution of the 10 sampling locations along the northwest coast of Portugal.

with ultra-pure water from Milli-Q system (conductivity: 0.054 μS cm^{-1} at 25 °C). Materials were pre-decontaminated in nitric acid solution (20%, v/v) and washed with deionised water (conductivity: <0.066 μS cm^{-1} at 25 °C; Elix-3 system), according APHA recommendations (APHA, 1998a).

In each sampling location and season, three independent replicates ($n = 3$) of coastal seawaters were collected with polyethylene bottles. In laboratory, seawater samples were filtered (Whatman, cellulose nitrate membrane filter, 0.45 μm), acidified (pH < 2) and frozen (−8 °C) until analyses (Reis et al., 2011b,c). Their dissolved metal concentrations (Cd, Cr, Cu, Fe, Mn and Zn) were analysed by Atomic Absorption Spectrometry (SpectrAA 220 FS, Varian) with flame atomization (Marck 7, Varian) or electrothermal atomization (Autosampler GTA 110, Varian) and with deuterium background correction system, according to APHA recommendations (APHA, 1998b,c). A solid phase extraction (SPE) method was applied to concentrate metals (concentration factor: 50) and remove interfering ions (Reis et al., 2012a,b). This SPE method was tested with seawater samples spiked with known amounts of each metal, which showed mean percentages of recovery higher than 93% for all elements (Cd: 105 ± 18%; Cr: 102 ± 17%; Cu: 111 ± 12%; Fe: 102 ± 22%; Mn: 93 ± 17% and Zn: 98 ± 18%).

Simultaneously with seawater, three independent replicates of *P. pollicipes* of at least 25 individuals (adult commercial-size: >2 cm, total length) were collected in each sampling location and season and transported in refrigerated plastic bags to laboratory within 8 h (Reis et al., 2012b). The peduncle muscle and entire body (soft tissues) of *P. pollicipes* were lyophilized, and homogenised (Rainbow et al., 2000; Rainbow and Blackmore, 2001; Morillo et al., 2005; Morillo and Usero 2008; Reis et al., 2012a,b). No differences among replicates ($p < 0.05$) in the mean dry weights of 25 pooled adult individuals were obtained, thus no significant effects of body size on accumulated metals are expected (Morillo et al., 2005; Morillo and Usero, 2008; Reis et al., 2012b). For metal analyses (Cd, Cr, Cu, Fe, Mn and Zn), soft tissues samples were digested following the method described by Reis and Almeida (2008). Total metal concentrations were determined by Atomic Absorption Spectrometry with flame atomization and electrothermal atomization, using the APHA recommendations (APHA, 1998b,c). The working parameters of the equipment and the matrix modifiers were those recommended by Varian Methods (Varian, 1988, 1989). Some samples were spiked with known amounts of metals, which allowed studying potential matrix effects in the determination of metals in soft tissues of *P. pollicipes*. These analyses showed mean recoveries above 95% for all elements (Cd: 98 ± 10%; Cr: 108 ± 18%; Cu: 102 ± 14%; Fe: 95 ± 13%; Mn: 106 ± 13% and Zn: 106 ± 7%). Finally, standard reference material certified for trace metals in mussel tissues (NIST SRM 2976) was used to study the suitability of the entire analytical procedure. These analyses showed mean recoveries above 90% for all elements (Cd: 90 ± 16%; Cr: 113 ± 23%; Cu: 97 ± 10%; Fe: 94 ± 14%; Mn: 102 ± 8% and Zn: 106 ± 3%).

Varian software was programmed to work with precisions below 10% between readings, in a maximum of four readings per replicate, to assure reproducibility of measurements. For each metal, external calibrations were carried out with aqueous standards solutions and blank solutions were prepared following the same treatment of samples. Metal concentrations in blanks were below the limit of detection of the analytical procedure for all elements in seawaters (Cd: 0.30 ng L^{-1}; Cr: 1.35 ng L^{-1}; Cu: 18 ng L^{-1}; Fe: 25 ng L^{-1}; Mn: 36 ng L^{-1} and Zn: 446 ng L^{-1}) and in soft tissues (Cd: 0.076 mg kg^{-1}; Cr: 0.035 mg kg^{-1}; Cu: 0.304 mg kg^{-1}; Fe: 7.99 mg kg^{-1}; Mn: 1.996 mg kg^{-1} and Zn: 26.6 mg kg^{-1}). The limits of detection were calculated using APHA recommendations (APHA, 1998d).

The statistical analyses of metal concentrations in seawaters and soft tissues of *P. pollicipes* consisted in a sequence of tests (Underwood, 1997; Rainbow et al., 2004; Silva et al., 2006): (i) normality of all data was checked with Shapiro–Wilk test, considering one level of significance ($p < 0.05$), using SPSS software; (ii) as some data failed normality test, all metal concentrations were logarithmically (base 10) transformed to create additive data sets with reduced deviations from normal distribution; (iii) homogeneity of variances was checked with Cochran test using WinGMAv 5 software (EICC, University of Sydney), considering one level of significance ($p < 0.05$); (iv) one-way analyses of variance (ANOVA) were used to identify significant differences ($p < 0.05$) in metal concentrations among locations (spatial variations) and seasons (seasonal variations), using WinGMAv 5 software (EICC, University of Sydney); (v) finally, unplanned comparisons (*post hoc* tests) were performed to establish groups of locations/seasons which

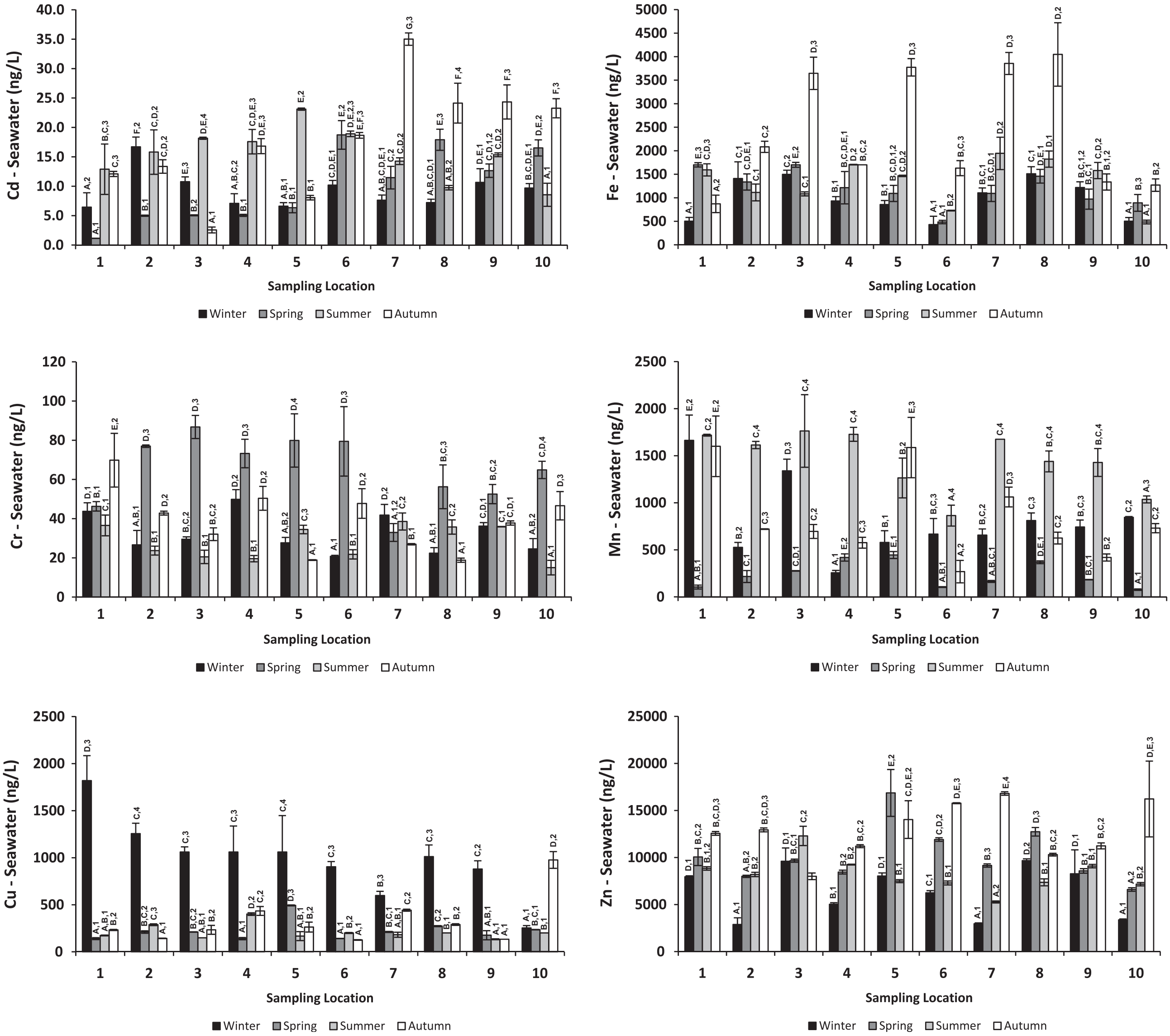

Fig. 2. Metal concentrations (ng L^{-1}) in seawater of the 10 locations in each season of 2011. In each season, different letters shows significant spatial differences ($p < 0.05$): increasing order of concentrations is A < B < C, etc. In each location, different numbers shows significant temporal differences ($p < 0.05$): increasing order of concentrations is 1 < 2 < 3, etc.

did not differ significantly ($p < 0.05$), using Student–Newman–Keuls (SNK) test for ANOVAs, from WinGMAv 5 software (EICC, University of Sydney).

Dissolved metal concentrations (Cd, Cr, Cu, Fe, Mn and Zn) obtained in coastal seawaters along the NW coast of Portugal in each season are shown in Fig. 2. Significant spatial variations ($p < 0.05$) of metal concentrations were obtained along the NW coast of Portugal during the four seasons. Coastal seawaters showed the highest concentrations of: (i) Cd: in location 2 at Winter, in location 6 at Spring, in location 5 at Summer and in location 7 at Autumn; (ii) Cr: in location 4 at Winter, in location 3 at Spring, in location 7 at Summer and in location 1 at Autumn; (iii) Cu: in location 1 at Winter, in location 5 at Spring, in location 4 at Summer and in location 10 at Autumn; (iv) Fe: in location 8 at Winter and Autumn, in location 3 at Spring and in location 7 at Summer; (v) Mn: in location 1 at Winter and Autumn, in location 5 at Spring and in location 3 at Summer; (vi) Zn: in location 8 at Winter, in location 5 at Spring, in location 3 at Summer and in location 7 at Autumn. Independent of season, the highest concentrations of: (i) Cd were obtained in location 7; (ii) Cr and Mn in location 3; (iii) Cu in location 1; (iv) Fe in location 8 and (v) Zn in location 5.

Significant seasonal variations ($p < 0.05$) of metal concentrations in seawaters were also obtained along the NW coast of Portugal during the four seasons. Seawaters showed the highest concentrations at: (i) Winter: for Cu and Mn in location 1, for Fe and Zn in location 8 and for Cd and Cr, respectively, in locations 2 and 4; (ii) Spring: for Cr and Fe in location 3, for Cu, Mn and Zn in location 5 and for Cd in location 6; (iii) Summer: for Mn and Zn in location 3, for Cr and Fe in location 7 and for Cu and Cd, respectively, in locations 4 and 5; (iv) Autumn: for Cr and Mn in location 1, for Cd and Zn in location 7, for Fe and Cu, respectively, in locations 8 and 10. The order of metal concentrations in each sampling season, were at: (i) Winter: Cd < Cr < Mn < Cu ~ Fe < Zn; (ii) Spring: Cd < Cr < Cu ~ Mn < Fe < Zn; (iii) Summer: Cd < Cr < Cu < Fe < Mn < Zn and (iv) Autumn: Cd < Cr < Cu < Mn < Fe < Zn. Along the NW coast of Portugal, Winter–Spring seasons showed the lowest concentrations of Cd, Fe, Mn and Zn (except locations 3 and 10 for Cd; locations 2, 3 and 10 for Fe and locations 3, 5 and 8 for Zn) and highest concentrations for Cr and Cu (except

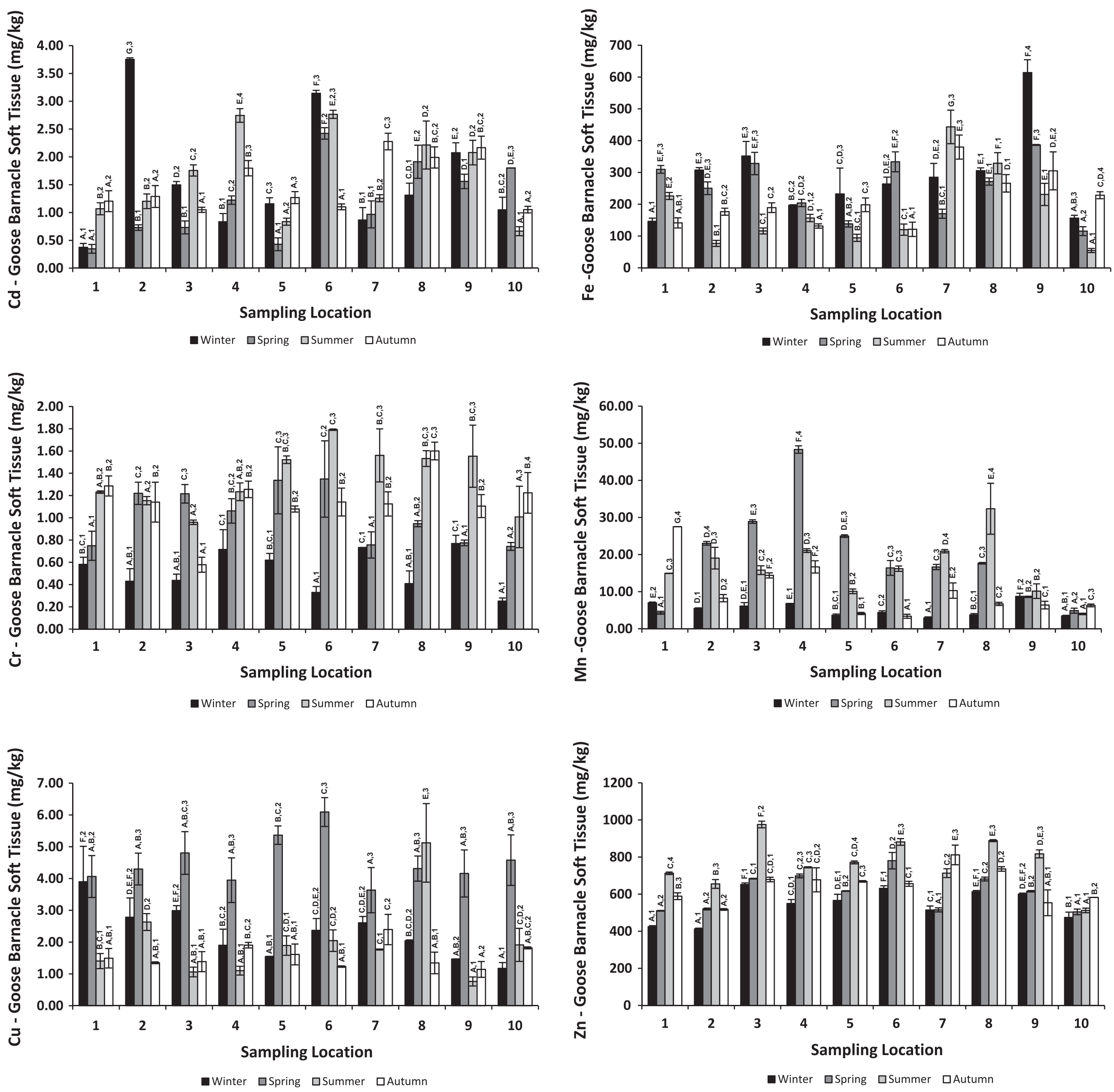

Fig. 3. Metal concentrations (mg kg^{-1}, dry wt.) in soft tissues of *Pollicipes pollicipes* of the 10 locations in each season of 2011. In each season, different letters shows significant spatial differences ($p < 0.05$): increasing order of concentrations is A < B < C, etc. In each location, different numbers shows significant temporal differences ($p < 0.05$): increasing order of concentrations is 1 < 2 < 3, etc.

locations 1 and 10, respectively, for Cr and Cu). The Summer-Autumn seasons showed the lowest concentrations for Cr (location 6) and Cu (locations 1 and 4) and highest concentrations for Cd, Fe, Mn and Zn in all locations (except location 1 for Fe, location 2 for Cd and locations 5 and 8 for Zn).

Independent of season or location, the concentrations of each metal in seawaters ranged: (i) Cd: 1.2–35 ng L^{-1}; (ii) Cr: 15–87 ng L^{-1}; (iii) Mn: 77–1763 ng L^{-1}; (iv) Cu: 126–1819; (v) Fe: 430–4048 ng L^{-1} and (vi) Zn: 2889–16,867 ng L^{-1}.

Following the Norwegian Pollution Control Authority guidelines (SFT TA-2229/2007) for metals in coastal seawaters, the ecological quality of seawaters from the NW coast of Portugal can be classified as "Class I/II - Background Level or Good" during the four sampling seasons of 2011, except for Cu and Zn (SFT, 2007). Regarding Cu (Class III: 640–800 ng L^{-1} and Class IV: 800–7700 ng L^{-1}) and Zn (Class III: 2900–6000 ng L^{-1} and Class IV: 6000–60,000 ng L^{-1}) concentrations, all seawaters should be classified as "Class IV – Bad", except location 7 at Summer and location 10 at Winter, which should be classified as "Class III – Moderate" (SFT, 2007). Comparisons with Reis et al. (2012a,b) showed that the ecological quality of seawaters in 2011 remained similar to 2009 and 2010. However, some positive indications of less metal contamination in Portuguese coastal seawaters are evident, mainly for Zn: Summer 2009 (51,374 ng L^{-1}) > Summer 2010 (24,874 ng L^{-1}) > Summer 2011 (8230 ng L^{-1}) (Reis et al., 2012a,b).

Total metal concentrations (Cd, Cr, Cu, Fe, Mn and Zn) obtained in *P. pollicipes* soft tissues from the NW coast of Portugal during the four sampling seasons of 2011 are shown in Fig. 3. Significant spatial variations ($p < 0.05$) of metal concentrations were obtained along the NW coast of Portugal during the four seasons. Goose barnacles *P. pollicipes* bioaccumulated the highest concentrations of: (i) Cd: in location 2 at Winter, location 6 at Spring and Summer and location 7 at Autumn; (ii) Cr: in location 9 at Winter, location 6 at Spring and Summer and location 8 at Autumn; (iii) Cu: in location 1 at Winter; location 6 at Spring, location 8 at Summer and location 7 at Autumn; (iv) Fe: in location 9 at Winter and Spring and in location 7 at Summer and Autumn; (v) Mn: in location 9 at Winter, location 4 at Spring, location 8 at Summer and location 1 at Autumn; Zn: in location 3 at Winter and Summer, location 6 at Spring and location 7 at Autumn. Globally, independent of season, the highest concentrations of: (i) Cd were obtained in location 2; (ii) Cr and Cu in location 6; (iii) Fe in location 9; (iv) Mn in location 4 and (v) Zn in location 3.

Significant seasonal variations ($p < 0.05$) of metal concentrations in *P. pollicipes* were also obtained along the NW coast of Portugal during the four seasons. Goose barnacles *P. pollicipes* showed the highest concentrations at: (i) Winter: for Cu in location 1, for Cd in location 2, for Zn in location 3 and for Cr, Fe and Mn in location 9; (ii) Spring: for Mn and Fe, respectively, in locations 4 and 6 and for Cd, Cr, Cu and Zn in location 6; (iii) Summer: for Zn and Fe, respectively, in locations 3 and 7, for Cd and Cr in location 6 and for Cu and Mn in location 8 and (iv) Autumn: for Mn and Cr, respectively, in locations 1 and 8 and for Cd, Cu, Fe and Zn in location 7. The metal concentrations in soft tissues of *P. pollicipes* followed similar orders during the four seasons: (i) Winter: Cr < Cd ~ Cu < Mn < Fe < Zn; (ii) Spring: Cr ~ Cd < Cu < Mn < Fe < Zn and (iii) Summer and Autumn: Cr ~ Cd ~ Cu < Mn < Fe < Zn.

Comparisons with the metal concentrations bioaccumulated by *P. pollicipes* in Summer 2010 showed that significant decrease occurred during 2011, mainly for Zn (Reis et al., 2012b).

For all locations, Winter-Spring group showed the lowest concentrations of Cd, Cr, Mn and Zn (except location 6 for Cd and Mn; location 9 for Mn and Zn and locations 10 for Cd) and the highest concentrations of Cu and Fe (except location 7 for Fe; location 8 for Cu and Fe and location 10 for Fe). Summer-Autumn group showed the lowest concentrations of Cu and Fe (except locations 5 and 10 for Cu and location 7 for Fe) and the highest concentrations of Cd, Cr and Zn (except location 2 for Cd and Cr; location 3 for Cr; locations 6 and 10 for Cd).

Independent of season or location, the metal concentrations obtained in *P. pollicipes* soft tissues (mg kg^{-1}, dry wt.) ranged: (i) Cd: 0.35–3.75 mg kg^{-1}; (ii) Cr: 0.25–1.79 mg kg^{-1}; (iii) Cu: 0.76–6.09 mg kg^{-1}; (iv) Fe: 55–614 mg kg^{-1}; (v) Mn: 2.89–48.33 mg kg^{-1} and (vi) Zn: 413–976 mg kg^{-1}.

The correlations between metal concentrations in coastal seawaters and *P. pollicipes* soft tissues were performed for each season by linear regression models shown in Table 1. There were significant positive correlations ($p < 0.05$, $R^2 > 0.6191$) for all metals during the four seasons of 2011. These results suggested that dissolved metal concentrations of seawaters describe well the processes of metal accumulation by *P. pollicipes*, which will give representative information about the bioavailable metal fractions of each location. However, using SPSS software, the metal values that changed linear correlation coefficients (R) more than 0.1 were not used in the linear regression models and the respective locations were considered outliners (Reis et al., 2009, 2012a,b). However, this work showed that *P. pollicipes* soft tissues can be used for monitoring metal bioavailability of seawaters along the NW coast of Portugal during the four seasons.

The regression line slopes reflect the bioaccumulation of metals in *P. pollicipes*: for one particular metal with higher slope than another, the same variation in seawater concentration will provoke a greater increase in bioaccumulated concentration in tissues, and vice versa (Morillo et al., 2005; Reis et al., 2012a,b). These regression line slopes (L of coastal seawater kg^{-1} of soft tissue) of each metal were significantly different ($p < 0.05$) during the four seasons and followed these orders:

(i) Winter: Cd (281,407) > Fe (176,715) > [Zn (23,898) ~ Cr (14,250)] > [Mn (3465) ~ Cu (1785)].
(ii) Spring: Fe (263,011) > Mn (122,918) > Cd (100,388) > Zn (40,209) > [Cr (12,360)] > Cu (3774)].
(iii) Summer: Fe (231,623) > Cd (196,608) > Zn (71,465) > [Cr (22,692) ~ Mn (20,310) ~ Cu (11,361)].
(iv) Autumn: [Cd (43,808) ~ Zn (43,527) ~ Fe (33,746)] > Mn (16,625) > [Cr (4215) ~ Cu (2939)].

The metals with the highest regression line slopes during the four seasons were Cd and Fe and with the lowest was Cu. Thus, an increase of Fe or Cd concentrations in seawaters will provoke a much higher bioaccumulation in *P. pollicipes* than the same increase of Cu. The regressions line slopes of the metals had different patterns during the four seasons, which suggests that more complex processes are involved in their bioaccumulation by goose barnacles, such as different metal assimilation efficiencies and efflux rates along the year. This is in accordance with the results obtained in 2010 by Reis et al. (2012b). However, these seasonal variations should be clarified in future studies with metals in goose barnacles *P. pollicipes*.

Metal Bioaccumulation Factors (BAFs) is an adequate approach to assess the efficiency of metal accumulation in goose barnacles soft tissues (Morillo et al., 2005; Reis et al., 2012a,b). The log BAF values (basis 10) shown in Table 2 were calculated as log transformed ratio between metal concentrations in *P. pollicipes* soft tissues (mg kg^{-1}, dry mass) and in coastal seawaters (ng L^{-1}). The mean log BAF values of each metal showed significant seasonal differences ($p < 0.05$) during all seasons of 2011 and followed these orders:

Table 1
Correlations between metal concentrations in coastal seawaters (ng L^{-1}) and *Pollicipes pollicipes* soft tissues (mg kg^{-1}, dry wt.) performed by linear regression models: Y [*Pollicipes pollicipes* tissue] = $a \cdot$ X [coastal seawater] + b.

Correlations between metal concentrations in coastal seawaters and *P. pollicipes* soft tissues	Linear regression model: Y = $a \cdot$ X + b											
	Winter			Spring			Summer			Autumn		
Element	a	b	R^2	a	b	R^2	a	b	R^2	a	b	R^2
Cd	0.2814	−1.1535	0.8718**	0.1004	0.2104	0.8335**	0.1966	−1.2070	0.7582*	0.0438	0.8833	0.8891**
Cr	0.0143	0.0669	0.6191*	0.0124	0.2124	0.7275*	0.0227	0.6504	0.7233*	0.0042	0.9900	0.7470*
Cu	0.0018	0.3967	0.7012*	0.0038	3.4751	0.6240*	0.0114	−0.4362	0.8158*	0.0029	0.7905	0.8000*
Fe	0.1767	63.94	0.9204**	0.2630	−121.5	0.9637**	0.2316	−100.1	0.8114*	0.0337	89.41	0.7293*
Mn	0.0035	1.212	0.8619*	0.1229	−4.878	0.8859**	0.0203	−16.54	0.7798*	0.0166	−2.320	0.7970*
Zn	0.0239	398.8	0.8254*	0.0402	231.5	0.6667*	0.0715	104.7	0.7181*	0.0435	27.90	0.7466*

* $p < 0.05$, the linear coefficient correlations (R^2) are statistically significant correlations.
** $p < 0.001$, the linear coefficient correlations (R^2) are statistically very significant correlations.

Table 2
Metal Bioaccumulation Factor (log BAF) in the soft tissues of *Pollicipes pollicipes* of the 10 locations in each season of 2011. Mean log BAF values obtained by Reis et al. (2012b) in 2010 are also included.

Season 2011	Mean log BAF values in soft tissues of *P. pollicipes* of NW coast of Portugal[a]					
	Cd	Cr	Cu	Fe	Mn	Zn
Winter	5.13 ± 0.18	4.21 ± 0.10	3.35 ± 0.15	5.39 ± 0.07	3.69 ± 0.08	4.99 ± 0.17
Spring	5.12 ± 0.19	4.20 ± 0.08	4.30 ± 0.13	5.21 ± 0.07	4.92 ± 0.20	4.81 ± 0.06
Summer	5.03 ± 0.13	4.67 ± 0.09	3.94 ± 0.10	5.13 ± 0.16	3.94 ± 0.17	4.92 ± 0.05
Autumn	5.06 ± 0.25	4.47 ± 0.15	3.81 ± 0.13	4.88 ± 0.17	4.11 ± 0.13	4.66 ± 0.04
2010 (Reis et al., 2012b)	5.47 ± 0.50	4.18 ± 0.33	3.98 ± 0.42	5.57 ± 0.38	4.14 ± 0.24	4.41 ± 0.34
2011 (Annual)	5.09 ± 0.19	4.37 ± 0.23	3.85 ± 0.37	5.16 ± 0.22	4.18 ± 0.49	4.86 ± 0.16

[a] Mean ± standard deviation ($n = 10$).

(i) Winter: Fe (5.39) > Cd (5.13) > Zn (4.99) > Cr (4.21) > Mn (3.69) > Cu (3.35).
(ii) Spring: Fe (5.21) > Cd (5.12) > Mn (4.92) > Zn (4.81) > Cu (4.30) > Cr (4.20).
(iii) Summer: Fe (5.13) > Cd (5.03) > Zn (4.92) > Cr (4.67) > Mn (3.94) ~ Cu (3.94).
(iv) Autumn: Cd (5.06) > Fe (4.88) > Zn (4.66) > Cr (4.47) > Mn (4.11) > Cu (3.81).

The metals that *P. pollicipes* bioaccumulated more efficiently during the four seasons were Fe and Cd, reflecting their high sensitivity to these metals, which means that Fe and Cd should be continuously monitored in future works. The lowest mean log BAF values were obtained for Cu and Mn during all year, except in Spring, which may be due their low bioavailability in coastal seawaters and *P. pollicipes* physiological abilities to regulate or eliminate them. Comparing with the BAFs values obtained by Reis et al. (2012b), *P. pollicipes* at Summer 2011 followed the same order of Summer 2010 (Table 2): Fe (5.57) > Cd (5.47) > Zn (4.41) > Cr (4.18) > Mn (4.14) > Cu (3.98). Comparing annual results of different years, goose barnacle *P. pollicipes* had been showing much higher sensitivity and efficiency to bioaccumulate Cd and Fe from seawaters than Cu, Cr, Mn and Zn (Table 2).

Comparisons with other goose barnacle species (*Capitulum mitella*, *Lepas anatifera* and *Pollicipes polymerus*) from different locations around the world, resumed by Reis et al. (2011), showed that Portuguese *P. pollicipes* have much lower metal accumulation, suggesting much lower metal bioavailabilities in seawaters.

The European Community Commission Regulation No. 629/2008 established rules for food consumption safety and the maximum concentration of Cd allowed in soft tissues of crustacean species is 0.50 mg Cd kg^{-1} (wet wt.) or 2.50 mg kg^{-1} (dry wt., assuming 80% of water) (EU, 2008). Goose barnacle *P. pollicipes* is an important economic commercial species in Portugal and Spain, thus Cd concentrations should be controlled during all year. All locations showed Cd concentrations lower than this reference value, which confirmed that NW coast of Portugal have low Cd contamination, except locations 2 (at Winter), 4 (at Summer) and 6 (at Winter and Summer). The capture of *P. pollicipes* in locations 2 (''Foz''), 4 (''Leça'') and 6 (''Vila do Conde'') should be interdicted until these high Cd concentrations decrease to acceptable values or *P. pollicipes* should be depurated before human consumption.

Norwegian Pollution Control Authority (SFT TA-1467/1997) established metal concentrations guidelines for blue mussel *M. edulis*, which allowed to classify the ecological quality of seawaters (Molvaer et al., 1997). Comparisons with these guideline values showed that the NW coast of Portugal can be classified as ''Class I – Unpolluted'' during the four seasons of 2011, considering Cu concentrations (<10 mg Cu kg^{-1}, dry wt.). However, considering Cd (''Class I – Unpolluted'': <2 mg Cd kg^{-1} and ''Class II – Moderately Polluted'': 2–5 mg Cd kg^{-1}) and Zn (''Class III – Remarkably Polluted'': 400–1000 mg Zn kg^{-1}), all locations should be classified as ''Class III – Remarkably Polluted'' during all seasons of 2011 (Molvaer et al., 1997).

Comparing with the ecological quality classifications obtained with *P. pollicipes* in Summer 2010, all locations showed better or similar classifications in 2011, confirming the our previous positive indications obtained with seawaters (Reis et al., 2012b).

It should be stated that although Norwegian Pollution Control Authority guidelines are used to classify the ecological quality of Portuguese Coastal Seawaters (Region IV), the differences of Portuguese abiotic characteristics of seawater (such as, local estuarine freshwater inputs, regional seawater circulation and lower seawater depths than Norwegian) and different biomonitor species (such as *Mytilus galloprovincialis*, *Chthamalus montagui*, and *P. pollicipes*) should imply future improvements and adaptations to these guidelines.

This work showed that goose barnacle *P. pollicipes* can be used as biomonitor of metal contamination along the NW coast of Portugal during all seasons of the year, since there were significant positive correlations ($p < 0.05$) between all metal concentrations in seawaters and *P. pollicipes*. Metal concentrations in coastal seawaters and soft tissues of *P. pollicipes* showed significant ($p < 0.05$) spatial and seasonal variations. The mean log BAF values of each metal were also significantly ($p < 0.05$) different during the four seasons and the metals that *P. pollicipes* bioaccumulated more efficiently were Fe and Cd, reflecting their high sensitivity to these metals. The lowest mean log BAF values were obtained for Cu and Mn during all year, except in Spring, due their low bioavailabilities in coastal seawaters and *P. pollicipes* physiological abilities to regulate or eliminate them. These discriminating bioaccumulation processes will require further investigation in future works. In terms of ecological quality classification based on their metal concentrations in seawaters, all NW coast of Portugal should be classified as ''Class IV – Bad'', except location 7 at Summer and location 10 at Winter, which should be classified as ''Class III – Moderate''. Considering the metal concentrations bioaccumulated in soft tissues of *P. pollicipes*, all locations of NW coast of Portugal should be classified as ''Class III – Remarkably Polluted'' during all seasons of 2011. Indeed, due the high Cd concentrations bioaccumulated in *P. pollicipes* from locations 2, 4 and 6, the capture of individuals to human consumption in these locations should be interdicted or individuals should be previously depurated. However, all locations showed better or similar ecological quality classifications in 2011 than the classifications obtained in Summer of 2010. These results reinforced the need of further investigations with more complete metal monitoring programs, which should include other marine species and be extended to entire coast of Portugal.

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