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Metal biomonitoring in a Patagonian salt marsh

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Abstract Patagonian salt marshes are not affected by pollution, but historical mining wastes are a continuous source of metals to salt marsh in San Antonio Bay. The present study evaluated the concentration of metals in sediments and used the halophyte *Spartina* spp. and the crab *N. granulata* as biomonitors. The levels of metals in sediment and organisms in SAB remained at levels corresponding to a slight enrichment or contamination. The highest levels corresponded to innermost sites of the Encerrado channel and close to the mining wastes. *Spartina* is a phytostabilizer so its aboveground tissues do not reflect the concentrations in the sediment; although, it retains the metals in its belowground tissues and in the rhizosediment. *N. granulata* showed to be a useful biomonitor for Pb, but not for the other metals.

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Introduction

Metals are especially interesting given their geochemical, nutritional, and/or toxicological significance. Although metals exist in nature, their extraction and remobilization, mediated by man, generates a clear increase in the environment, which can alter the ecological and biogeochemical balance of the ecosystem (Sadiq 1992).

The use of total concentration as criterion for establishing the potential effect of contamination on soils and/or sediments, or for discussing their mobility, implies that all metal forms have the same impact on the environment; this shows not to be true (Tessier et al. 1979). The predominant physical and chemical conditions in the environment will determine the distribution of metals in the different geochemical fractions, which in turn will affect their availability. The bioavailability of a metal in the sediment does not necessarily imply that it reaches organisms, depending on the intrinsic characteristics of the organism itself (species, age, susceptibility, etc.). Biomonitoring is a scientific technique for assessing environment, exposures to natural and synthetic chemicals, based on sampling and analysis of an individual organism's tissues and fluids (Zhou et al. 2008).

Bioaccumulation is the net retention of a chemical in an organism across all exposure routes (diet, dermal, respiratory) and sources (water, sediment, food) as occurs in the natural environment (Spacie et al. 1995). On the other hand, bioconcentration is the process where a chemical is retained in an aquatic organism by absorption through the respiratory or dermal surface from its environment, not including the diet (Weisbrod et al.

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2007). Bioaccumulation (BAF) and bioconcentration (BCF) factors are usually used to refer to the relationship between the concentration of metals in the organism and in the sediment or in the water, respectively (Barron 2002; Zhou et al. 2008).

The terms bioindicator and biomonitor apply to an organism (or part of an organism or a community of organisms) that represents the occurrence of contaminants based on specific symptoms, reactions, morphological changes or concentrations (Phillips and Rainbow 1993; MacFarlane et al. 2000). When the organism provides qualitative information of the environmental quality, it is called bioindicator and when it contains quantitative information, it is called biomonitor (Mertens et al. 2005; Markert 2007).

In aquatic environments, plants and animals have been widely used as biomonitors. Macrophytes are particularly useful for monitoring metals, as they may have a large capacity to accumulate and tolerate high metal concentrations. However, the selection of plant species as biomonitors depends on local conditions and their availability (Bonanno and Lo Giudice 2010). Besides, invertebrates of the aquatic trophic chain, generally associated with sediments, are also useful as biomonitors for metal contamination. Among them, decapods are known to have an effective accumulation of non-essential metals, such as Cd and Pb, and reflect environmental levels (Rainbow 1990). In particular, the crab *Neohelice granulata*, a key specie within estuarine ecosystems, plays an important role in the transfer of pollutants to their predators, such as birds and fishes. These characteristics allow considering its use as a potential biological indicator (Beltrame et al. 2011).

The present study evaluated the concentration of metals in sediments and organisms, halophytic plants and crabs as biomonitors of contamination of San Antonio Bay, a macrotidal system affected by mining wastes.

Methodology

Study site

San Antonio Bay (SAB) is located in the northwest of San Matías gulf (40° 45′ S, 64° 55′ W), Río Negro province, Argentine Patagonia (Fig. 1). The scarce rainfall, the absence of freshwater inputs and high evaporation rates in the northern sector of the gulf, determine

higher salinities, which contrast with the colder and less saline waters of the southern sector of the gulf (Fucks et al. 2011). The bay has an approximate total surface of 12,772 ha and the dominant feature of its coasts is the large tidal range (7-m high), denominated macrotidal system.

In the intertidal zone, presents well-differentiated physiographic units with particular biological community (Escofet et al. 1978): sandy or muddy mud flats, salt marshes (*Spartina* or *Sarcocornia*), mussels (*Brachidontes rodriguezii*), and "cangrejales" (large muddy tidal flats covered by *N. granulata* burrows). The dominant halophyte species in salt marshes are *Spartina alterniflora* and *Sarcocornia perennis* with coverage percentages of 2068 ha and 2124 ha, respectively. There are also patches of *Spartina densiflora* with less coverage, compared to a tidal plain extension of 10,111 ha (Isacch et al. 2006). The littoral is used as a resting, feeding, and nesting place by resident and migratory birds. In 1993, this area was declared Western Hemisphere Shorebird Reserve Network, site of international importance.

Sampling

Sediments, plants, and crabs were collected seasonally during 2013 and 2014. Sampled sites are shown in Fig. 1. On salt marshes (MA), three replicates of sediment without vegetation (S patch sediment) and sediment surrounding the rhizosphere of *Spartina* (R patch rhizosediment) in blocks ($20 \times 20 \times 15$ cm^3) were taken. In addition, *Spartina* samples were collected at each site (aboveground and belowground tissues). Forty individuals of crab *N. granulata* were collected at four sampling sites in the marsh. Redox potential (Eh) and pH were also measured in situ in all sites, using an Altronix potenciometer.

Besides, seawater temperature (°C), salinity (psu), dissolved oxygen (DO%), pH, and Eh (mV) were measured with a multiparameter handheld instrument YSI model 556 MPS in tidal channel, within the Encerrado channel near the MA3 site and outside the channel near the MA8 site.

Sediments: characterization and metals analysis

The sediments were dried to constant weight at 60 °C. The particle size distribution was determined using plastic mesh sieves of 63-μm and 2-mm pore size,
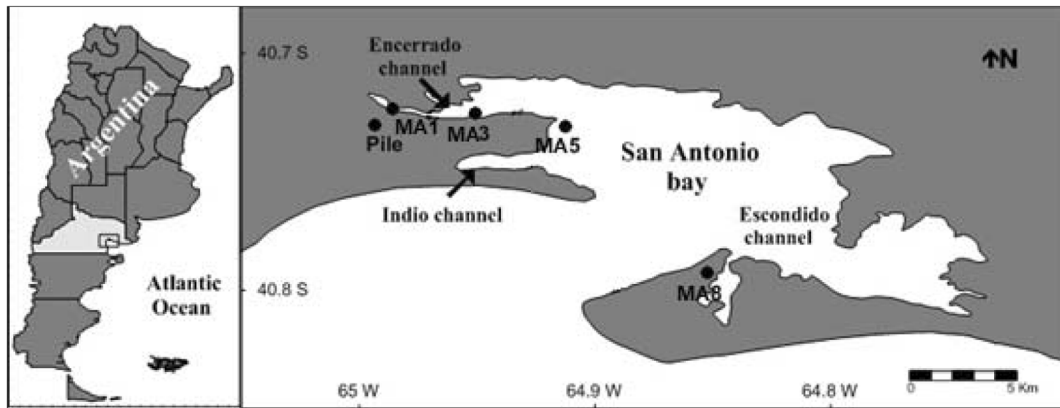
Fig. 1 Study site shown the pile (mining wastes) and MA1, MA3, MA5, and MA8 sampled sites. In each site, MA were sampled sediment without vegetation "S" and rhizosediment "R"

differentiating the fractions: gravel (> 2 mm), sand (between 2 mm and 63 μm), and silt-clay (< 63 μm). The organic matter content (OM) in the fraction < 63 μm was determined by loss on ignition at 450 °C for 4 h (Byers et al. 1978).

In order to determine the pseudototal content of Cd, Pb, Cu, Zn, and Fe in the sediment, aqua regia extraction was performed (MacGrath and Cunliffe 1985; ISO 1995). This type of extraction is suitable for sediments or matrices with OM < 20% (ISO 1995) and is used in assessing the potential of soils and sediments to exert negative effects on biota (CCME 2001a). About 1 g of dry sediment (fraction < 63 μm) was digested with 10 ml of aqua regia (3:1 ratio of HCl and HNO_3). It was evaporated in a hot plate at 85 °C for 2 h. The residue was recovered with 10 ml 5% HNO_3 and centrifuged at 8000 rpm for 20 min at room temperature. The supernatant was brought to a final volume of 25 ml with 5% HNO_3.

Plants: characterization and metals analysis

Plant biomass was separated in: aboveground tissue (AT: green leaves and stems) and belowground tissue (BT: roots and rhizomes). They were washed with tap water and rinsed with deionized water in ultrasonic bath for 2 min to promote the detachment of sediment adhered to the tissue.

Once plant tissues were dried to constant weight in an oven at 80 °C, they were grounded and homogenized with a butt-type grinder. About one gram of dry tissue was calcined in a furnace at 400 °C for 6 h, and digested with 3 ml of concentrated HNO3 at 80 °C to determine

the different metal concentrations. This process was repeated three to four times until complete digestion. The residue was resuspended in 3% HNO_3 and 6% HCl solution. It was centrifuged at 8000 rpm for 20 min and filled up to a final volume of 10 ml with a 3% HNO_3 and 6% HCl solution (BOE 1991; Yoong 1998).

Crabs: characterization and metals analysis

Crabs were washed with tap water and rinsed with deionized water. The morphometric measurements were as follows: width (WC) and length (LC) of the carapace, total weight (TW), and wet soft tissue weight (SW).

For each site, sample groups were formed with ten individuals each. They were dried at 80 °C until constant weight and the moisture percentage was determined. The ash percentage was calculated by calcining in a furnace at 550 °C for 12 h (AOAC 1995). Determination of metal concentration in soft tissues was done following the same procedure as in plant tissue.

The metal concentration in sediment extracts was determined using an inductively coupled plasma optical emission spectrometer (ICP-OES) Agilent 720. The instrument detection limits (DL) were 0.002 mg/kg Cd, 0.01 mg/kg Pb, 0.01 mg/kg Cu, 0.01 mg/kg Zn, and 0.02 mg/kg Fe. The metal concentrations in biological extracts were performed with an atomic absorption spectrometer (AAS) IL 457. The DL were 0.01 mg/kg Cd, 0.20 mg/kg Pb, 0.05 mg/kg Cu, 0.01 mg/kg Zn, and 0.10 mg/kg Fe.

All samples for metal analysis (soils, sediments, and plant tissues) were processed by duplicate including blanks. Analytical grade reagents (Merck or Baker)

were used. The accuracy of the methods was checked by the analysis of reference material. PACS-2 for sediments with recovery rates between 92 and 117%; BCR-060 for plants with recovery rates between 82 and 129%; and SRM-1566b for crabs with recovery rates between 98 and 106%. The variation coefficients tested for five replicates were always below 10%.

Statistical analyses

Statistical analyses were performed with the statistical package InfoStat (Di Rienzo et al. 2008). Non-parametric Mann Whitney (MW) and Kruskal Wallis (KW) tests were applied, as appropriate. In KW, pairwise comparisons were applied between the medians of the treatment ranges. The procedure used to judge the significance of multiple comparisons is the one described in Conover (1999). The Spearman coefficient was used for the correlation analyzes. A value of $p < 0.05$ was considered significant. In order to be able to include in the data matrix the largest amount of information, values < DL were replaced with the value corresponding to DL/2 (Gibbons and Coleman 2001; Tsakovski et al. 2009).

Results

Salt marsh sediments

According to the granulometry, the sediments were defined as sandy-gravel for MA3 and MA5-S and sandy for other sites. MA3 and MA5* presented the highest percentages of gravel (42% and 43%, respectively) and

MA1 presented the highest content of the silt-clay (6%) (Table 1). The percentages of OM in the silt-clay fraction varied between 3.3 and 4.7%, with the maximum in MA3. The pH values were neutral to slightly alkaline (7.1–8.2) and the Eh ranged between − 145 and 186 mV, evidencing suboxic to oxic conditions.

The pseudototal concentrations of metals showed the following pattern: Cd (0.22–0.45 mg/kg) < Cu (4.1–15.3 mg/kg) < Pb (3.5–35.1 mg/kg) < Zn (26.4–103.5 mg/kg) < Fe (0.98–1.56%). Cd concentrations were higher in MA1 and MA8 (KW $p < 0.05$). Pb, Cu, and Zn concentrations were higher in MA1 and MA3 than in others sites (KW $p < 0.05$), while Fe concentrations were higher in MA1 (KW $p < 0.05$) than in others sites (Fig. 2).

When comparing metals between patches in each of sampling site, for MA1 Pb, Cu, and Zn concentrations were higher in R patch than in S patch (MW $p < 0.05$). The same pattern was observed for Zn in MA3 (MW $p < 0.05$), for Pb in MA5 (MW $p < 0.05$), for all metal in MA5* (MW $p < 0.05$) and for Pb and Cu in MA8 (MW $p < 0.05$) (Fig. 2).

In seawater, the temperature varied according to the seasonal period. It presented ranges between 7.9 and 23.7 °C in MA3 and ranges between 10.0 and 29.9 °C in MA8. The salinity was 36.1 ± 1.8 psu in MA3 and 37.6 ± 2.4 psu in MA8. The dissolved oxygen (DO) was always higher than 100% and the pH was similar in both sites (8.2–8.3).

Salt marsh plants

At sites MA1, MA3, and MA5, the data refer to *Spartina densiflora*, whereas in MA5* and MA8, they

Table 1 Salt marsh sediments characteristics from SAB (mean ± SD, $N = 24$)

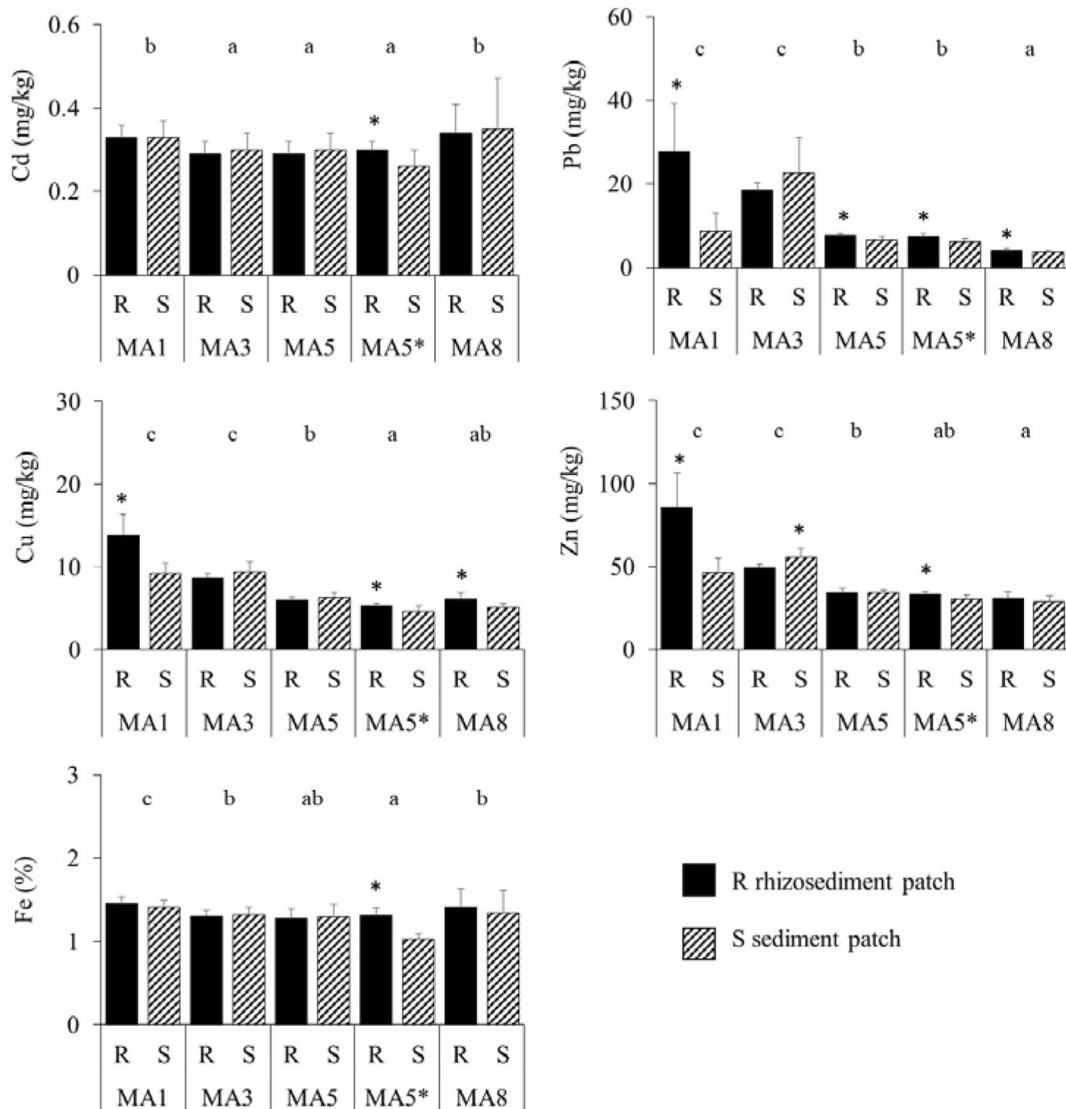| Site	Patch	Gravel (%)	Sand (%)	Silt-clay (%)	OM (%)	Eh (mV)	pH
MA1	R	4 ± 3	85 ± 12	6 ± 1	4.5 ± 0.3	186 ± 53	7.9 ± 0.1
	S	7 ± 2	83 ± 11	6 ± 2	4.0 ± 0.4	173 ± 40	8.2 ± 0.2
MA3	R	30 ± 9	65 ± 9	3 ± 1	4.7 ± 0.3	155 ± 53	7.9 ± 0.3
	S	42 ± 20	55 ± 19	3 ± 1	4.7 ± 0.6	175 ± 49	8.0 ± 0.3
MA5	R	20 ± 5	72 ± 8	4 ± 1	3.6 ± 0.4	139 ± 46	7.9 ± 0.2
	S	25 ± 5	69 ± 7	4 ± 1	3.6 ± 0.4	152 ± 37	8.0 ± 0.3
MA5*	R	30 ± 14	67 ± 14	3 ± 1	4.3 ± 0.3	23 ± 53	7.3 ± 0.2
	S	43 ± 12	54 ± 11	3 ± 1	3.5 ± 0.9	− 87 ± 126	7.3 ± 0.4
MA8	R	13 ± 12	82 ± 12	3 ± 2	3.6 ± 0.6	− 145 ± 113	7.2 ± 0.4
	S	15 ± 6	80 ± 8	2 ± 1	3.2 ± 0.5	− 76 ± 182	7.1 ± 0.5

Fig. 2 Pseudototal concentrations of metals in sediments of the SAB (mean ± SD, $N = 24$). Asterisk*indicates significant differences (MW $p < 0.05$) between rhizosediment and sediment from each site. Letters indicate significant differences among sites (KW $p < 0.05$)

correspond to *Spartina alterniflora*, according to the species found at each place. For that reason, comparisons among sites were performed without considering the species.

With regard to seasonal variation, Cd concentration in AT was detected in summer and autumn in MA5* and in autumn and winter in MA8 and it was < DL in all the other cases. In BT, Cd concentration ranged from < DL to 0.38 mg/kg. Comparing among sites, Cd concentration was higher in MA5* and MA8 than in others sites (KW $p < 0.05$). Pb concentration in AT varied between < DL and 2.40 mg/kg, being higher in MA1 and MA3

than in others sites (KW $p < 0.05$). In BT, Pb concentration varied between < DL and 7.50 mg/kg, being MA1 higher than MA3, and both of them higher than the rest (KW $p < 0.05$). Considering the seasonal variation, in summer, it presented highest Pb concentration in MA3, MA5, MA5*, and MA8 (KW $p < 0.05$). Cu concentration in AT varied between 1.38 and 4.32 mg/kg, being higher in MA5* than the rest (KW $p < 0.05$). Seasonal variation was found in MA3, with the minimum concentration in winter. Cu levels in BT varied between 2.4 and 8.2 mg/kg, being maximum in MA1 and minimum in MA5 (KW $p < 0.05$). Significant seasonal variation
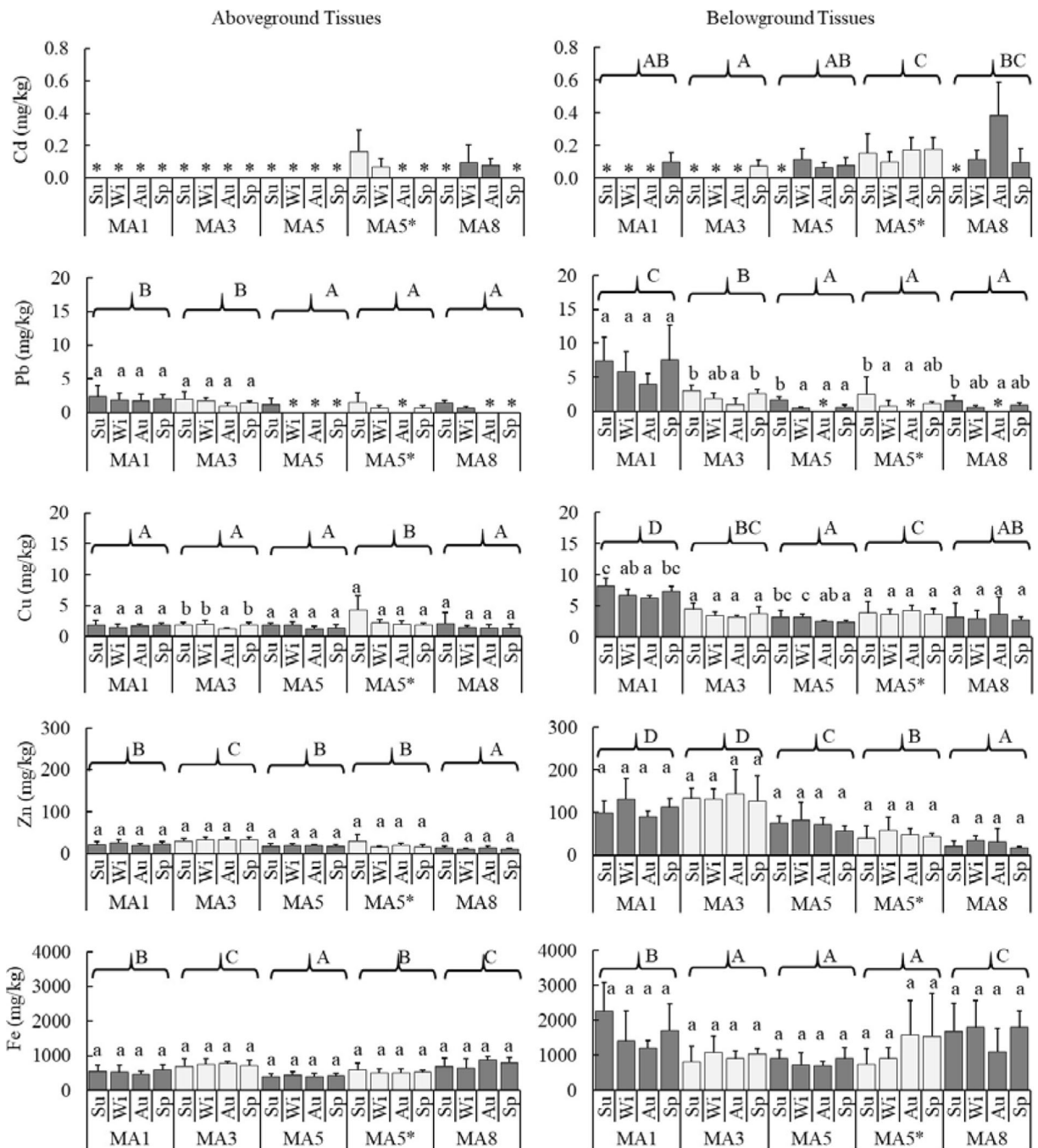
Fig. 3 Metals concentrations in aboveground and belowground tissues of *Spartina* (mean ± SD, N = 6). Su, summer; Wi, winter; Au, autumn; Sp, spring. Small letters indicate significant differences among seasons for each site (KW $p < 0.05$). Capitals letters indicate differences significant differences among sites (KW $p < 0.05$)

was found in MA1 being the highest concentrations measured in summer and spring and, in MA5 in summer and autumn. Zn concentrations in AT varied between 10.2 and 34.0 mg/kg, being maximum in MA3 and minimum in MA8 (KW $p < 0.05$). Zn concentrations

in BT ranged from 18.1 to 145.2 mg/kg, with the highest values in MA1 and MA3 and the lowest one in MA8 (KW $p < 0.05$). Finally, Fe concentrations in AT ranged between 384.4 and 875.1 mg/kg and between 713.0 and 2257.0 mg/kg in BT. In AT, Fe was higher in MA3 and

MA8 than the other sites (KW $p < 0.05$), whereas in BT, it was highest in MA8 followed by MA1 (KW $p < 0.05$) (Fig. 3).

At sites where it was possible to compare between aboveground and belowground tissues, Cd, Pb, Cu, Zn, and Fe concentrations were higher in BT than in AT (MW $p < 0.05$).

In this regard, metal uptake by the plants was assessed through the bioaccumulation factor (BAF), which was calculated as the ratio between metal concentration in BT and in rhizosediment (Yoon et al. 2006). It was higher than 1 only for Zn, meanwhile for the rest of the elements, BAF was lower than 1 and in the following order: Cu > Cd > Pb > Fe (Table 2). In addition, translocation factor (TF) was calculated to relate metal concentration in AT and in BT (Yoon et al. 2006). This factor was always lower than 1 for the essential elements Cu, Zn, and Fe, close to 1 for Pb in all sites except for MA1, and greater than 1 for Cd in MA5* (Table 2).

Crab *N. granulata*

The adult male crabs presented sizes between 27.6 and 30.0 mm of WC and between 23.0 and 24.9 mm of LC. The TW ranged from 11.7 to 15.8 g and SW between 1.4 and 1.7 g. The moisture percentage varied between 79 and 82%, and the ash percentage between 19 and 24% (Table 3).

The metal concentrations in soft tissue of *N. granulata* presented the following order Cd < Pb < Zn < Cu < Fe (Fig. 4). Cd levels ranged from 0.52 to 1.7 mg/kg and showed seasonal variation only in MA1,

being highest concentrations in winter (KW $p < 0.05$). Among sites, Cd concentrations were higher in MA5 and MA8 than in MA1 and MA3 (KW $p < 0.05$). Pb concentrations ranged from < DL to 6.4 mg/kg. With respect to seasonal variation for each site, Pb concentration in MA1 was lower in spring than in the rest of the seasons (KW $p < 0.05$), in MA3 was higher in summer and autumn (KW $p < 0.05$), in MA5 greater in autumn and winter (KW $p < 0.05$), and in MA8 did not present significant differences. Among sites, Pb concentrations were highest in MA1, intermediate in MA3, and lowest in MA5 and MA8 (KW $p < 0.05$). Cu concentrations ranged from 192 to 415 mg/kg. In general, the levels were maximum in winter and minimum in spring (KW $p < 0.05$), except in MA8 that did not show significant differences. Among sites, MA1 and MA3 presented the highest Cu concentrations and MA5 and MA8 the lowest ones (KW $p < 0.05$). Zn concentrations varied between 90 and 147 mg/kg. In MA1 and MA3, the highest concentrations were found in autumn-winter (KW $p < 0.05$), in MA5, the concentration was lowest in spring (KW $p < 0.05$), and in MA8, the concentration was highest in autumn (KW $p < 0.05$). Among sites, Zn concentrations were higher in MA1 than in other sites (KW $p < 0.05$). Finally, Fe concentrations ranged from 493 to 1613 mg/kg. In MA1, higher values were obtained in autumn-winter than in spring (KW $p < 0.05$); in MA3, no significant differences were found; in MA5, concentrations were highest in autumn and winter (KW $p < 0.05$); and in MA8, they were higher in autumn than in summer (KW $p < 0.05$). Among sites, Fe concentrations were highest in MA1, followed by MA8 and MA5, and lowest in MA3 (KW $p < 0.05$).

Table 2 Bioaccumulation (BAF) and translocation (TF) factors (mean ± SD, $N = 24$)

	Site	Cd	Pb	Cu	Zn	Fe
BAF	MA1	< DL	0.3 ± 0.2	0.5 ± 0.2	1.3 ± 0.5	0.1 ± 0.1
	MA3	0.3 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	2.7 ± 0.8	0.1 ± 0.0
	MA5	0.3 ± 0.2	0.1 ± 0.1	0.5 ± 0.1	2.1 ± 0.8	0.1 ± 0.0
	MA5*	0.5 ± 0.3	0.2 ± 0.2	0.8 ± 0.2	1.4 ± 0.7	0.1 ± 0.1
	MA8	0.6 ± 0.8	0.2 ± 0.1	0.5 ± 0.3	0.9 ± 0.7	0.1 ± 0.1
TF	MA1	< DL	0.4 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.2
	MA3	< DL	1.0 ± 0.9	0.5 ± 0.2	0.3 ± 0.1	0.9 ± 0.3
	MA5	< DL	0.9 ± 0.7	0.6 ± 0.2	0.3 ± 0.1	0.6 ± 0.3
	MA5*	1.8 ± 0.9	1.0 ± 1.0	0.7 ± 0.6	0.6 ± 0.7	0.7 ± 0.6
	MA8	0.7 ± 0.2	1.1 ± 0.9	0.6 ± 0.3	0.7 ± 0.5	0.6 ± 0.5

< DL below detected limit

Table 3 Characteristics of crab *N. granulata* (mean ± SD, *N* = 270–300)

Sitie	WC (mm)	LC (mm)	TW (g)	SW (g)	Moisture (%)	Ash (%)
MA1	28.3 ± 3.1	23.6 ± 2.6	13.3 ± 4.8	1.5 ± 0.6	79 ± 3	19 ± 4
MA3	28.2 ± 3.0	23.5 ± 2.5	13.5 ± 4.6	1.6 ± 0.6	80 ± 3	22 ± 3
MA5	27.6 ± 2.3	23.0 ± 1.9	11.7 ± 3.5	1.4 ± 0.4	82 ± 3	24 ± 5
MA8	30.0 ± 2.3	24.9 ± 2.0	15.8 ± 4.1	1.7 ± 0.5	81 ± 4	22 ± 4

The biota accumulation factor (BAF) for crabs is the ratio of the concentration of an element in the organism to the concentration of the same element in the sediment. BAF for Pb and Fe were smaller than 1, while those of Cd, Cu, and Zn were higher (Table 4). Cu and Zn were significantly higher at sites MA5* and MA8 than at the other sites (KW $p < 0.05$).

Discussion

Pseudototal concentrations of metals were generally higher in sediments of the Encerrado channel than in the other sites, as indicated by Pb, Cu, and Zn values. Concentrations of these elements in the innermost site of the channel presented the following values close to the limits for the protection of the aquatic life according to the Canadian legislation (CCME 2001b): 30.2 mg/kg, 18.7 mg/kg, and 124 mg/kg, respectively. The R patches of the site presented remarkable higher concentrations of Pb, Cu, and Zn than the S patches, suggesting probable influence of vegetation.

Metal uptake by the plants was assessed through BAF. This factor proved to be above one only for Zn, probably due to its importance as a component of enzymes for protein synthesis and energy production and
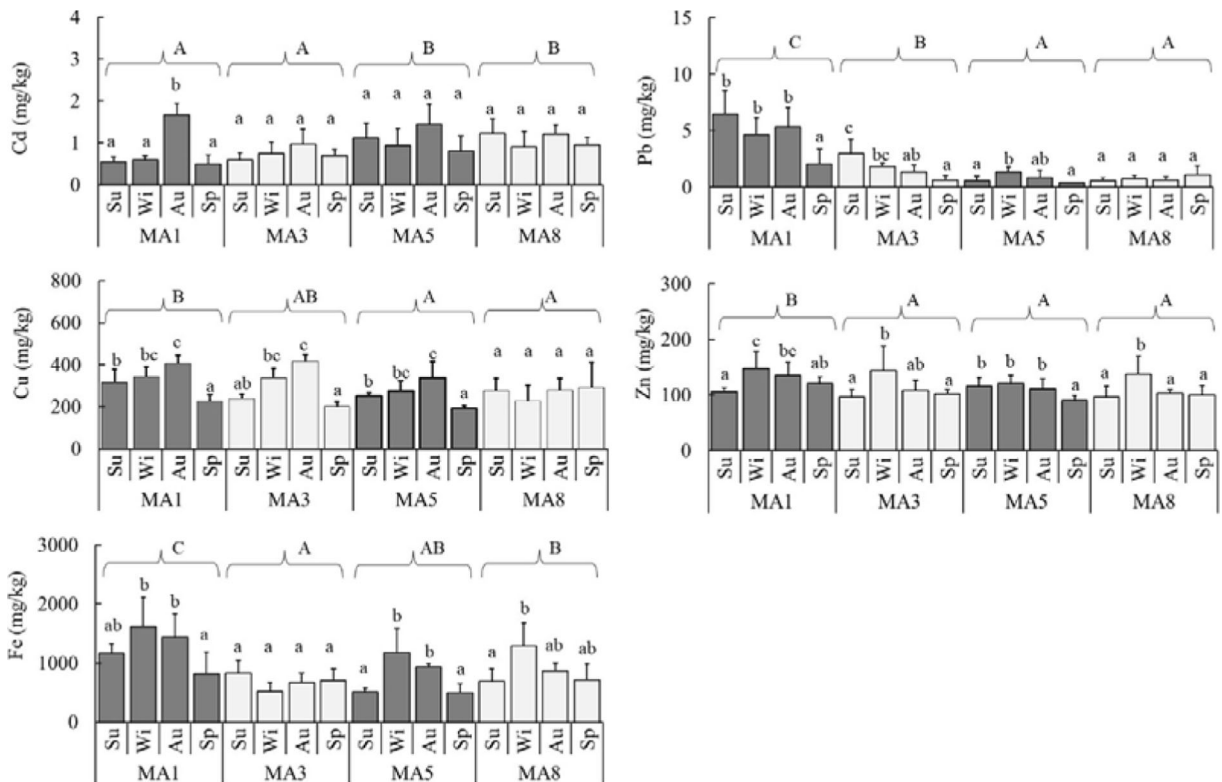

Fig. 4 Metals concentrations in soft tissues of crab *N. granulata* (mean ± SD, mg/kg, *N* = 6–8). Su, summer; Wi, winter; Au, autumn; Sp, spring. Small letters indicate significant differences among seasons for each site (KW $p < 0.05$). Capitals letters indicate significant differences among sites (KW $p < 0.05$)

Table 4 Bioaccumulation factor (BAF) for *N. granulata* (mean ± SD, $N = 24$)

Sitio	Cd	Pb	Cu	Zn	Fe
MA1	2.4 ± 1.5	0.1 ± 0.1	28 ± 7	1.9 ± 0.3	0.1 ± 0.0
MA3	2.5 ± 0.4	0.1 ± 0.0	33 ± 10	2.2 ± 0.5	0.1 ± 0.0
MA5*	3.8 ± 0.8	0.1 ± 0.1	54 ± 15	3.4 ± 0.6	0.1 ± 0.0
MA8	3.1 ± 0.7	0.2 ± 0.1	48 ± 8	3.6 ± 0.7	0.1 ± 0.0

maintains the structural integrity of biomembranes (Hänsch and Mendel 2009). The Fe biogeochemistry plays an important role in the control of its own absorption and other elements (Feng et al. 2017). In this sense, low BAF for Fe are consistent with the high concentrations found in rhizosediments, probably in the form of oxyhydroxides. Plants capable of releasing oxygen from the roots promote the formation of Fe-rich rhizoconcretions. One of the major consequences of radial oxygen release from plant roots is the oxidation of Fe (II) (mainly as iron sulfides) and the precipitation of oxihydroxides as rhizoconcretions. These may also constitute a retention substrate for the other elements such as Mn, Zn, Cd, and Pb, limiting their entry into the tissues (Sundby et al. 1998; Vale et al. 2003; Redondo Gómez 2013). In general, low metal BAF are expected in plants capable of releasing oxygen from the roots, as in the case of Spartina (Vale et al. 1990; Williams et al. 1994).

Translocation factor was greater than 1 for Cd in MA5*. It must be taken into account that the compounds released by the plants to facilitate the absorption of Zn can promote the entry of Cd, since both elements possess similar chemical properties (Almeida et al. 2011). In general, it was observed that in places where the concentrations in rhizosediments were higher, BAF and TF were lower (MA1 and MA3).

The lower metal concentration in aerial parts compared to that in roots could be related to the development of mechanisms such as compartmentation, which would control ion transport into leaves, thereby improving plant tolerance to heavy metals (Mateos Naranjo et al. 2008). This would indicate that plants avoid entering excess of metals and the translocation to aerial tissues, as it has been reported for different *Spartina* species (Windham et al. 2003; Duarte et al. 2009; Cambrollé et al. 2011; Almeida et al. 2011; Redondo Gómez 2013). Considering the high capacity to immobilize the metals by accumulation in the roots or precipitation in the

rhizosediment, *Spartina* has been suggested, by the latter authors, as a phytostabilizer genus. Although metals remaining in the roots are generally considered "out of trouble" as far as release to the environment is concerned, further studies are needed regarding the turnover of nutritive roots and the potential release of metals from decomposing roots (Weis and Weis 2004).

Metal accumulation in *Spartina* depends on the species, metal content in sediment and their bioavailability. In the case of SAB, the values recorded in *Spartina* did not exceed those that can be phytotoxic (5–30 mg/kg Cd, 30–300 mg/kg Pb, 20–100 mg/kg Cu, and 100–400 mg/kg Zn) (Prasad et al. 2006; Kabata Pendias 2011). Despite Zn concentration in belowground tissues that was within the phytotoxic range, (145.2 mg/kg), it must be taken into account that the content was determined without removing rizhoconcretions. Zn would be associated to rizhoconcretions and not inside of the root. This agree with a low translocation of Zn towards aboveground tissues.

By contrast, they were comparable to those reported in sites with low or moderate pollution, such as the Rawson marsh (Idaszkin et al. 2014), Bahía Blanca estuary (Hempel et al. 2008) and even SAB (Idaszkin et al. 2015, 2017) (Table 5). In highly contaminated areas such as the estuary of rivers Odiel and Tinto in Spain, levels of metals such as Pb, Cu, and Zn in tissues of *S. densiflora* and *S. maritima* are 10 to 100 times greater than those found in this study (Cambrollé et al. 2008). In the case of *S. alterniflora* from Yangtze River estuary in China, metal levels were much higher than those from SAB (Quan et al. 2007).

Salinity influences the uptake and excretion of metals in plants; however, there is controversy about how this mechanism works. The increase of the salinity of the overlying water could increase the excretion of salts by *S. alterniflora*, associated to an increase in the excretion of metals (Weis and Weis 2004). This mechanism has been proposed for the excretion of metals such as Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn in *Spartina* (Burke et al. 2000; Windham et al. 2001). On the other hand, in response to saline stress, the halophytes excrete a greater amount of Na and decrease the uptake and the excretion of Ca. As a consequence of the above, a lower excretion of divalent metals, such as Pb and Zn, could occur (Brown et al. 2006; Mahon and Carman 2008). Also, studies in *S. densiflora* report that salinity favors tolerance to the toxic action of Zn (Redondo Gómez et al. 2011).

Table 5 Metals concentrations in aboveground tissues (AT) and belowground tissues (BT) of *Spartina* (mg/kg)

Species	Tissue	Cd	Pb	Cu	Zn	Fe	Site (reference)
S. densiflora	AT	< 0.10	< 0.7–4.9	2.4 ± 0.9	15.2 ± 5.8	453 ± 139	SAB (this study)
	BT	< 0.10–0.11	1.1–11.8	4.8 ± 0.7	39.8 ± 21.9	801 ± 729	
	AT	< 1	< 2	6	18–22	0.0075	Rawson (Idaszkin et al. 2014)
	BT	< 1	< 2	4–6	20–25	1–1.5%	
	AT	< 0.1	2–12	1–3	22,190	200–500	SAB (Idaszkin et al. 2015)
	BT	< 0.1	6–18	3–17	20–120	500–2800	
	Young leaves	nd	< 10–20	< 100–150	< 100–150	nd	Odiel and Tinto salt marshes (Cambrollé et al. 2008)
	Old leaves	nd	25–60	200–600	150–250	nd	
	Shoots	nd	25–80	100–300	100–200	nd	
	BT	nd	100–150	300–2000	500–1000	nd	
S. alterniflora	AT	< 0.10–0.16	< 0.7	2.2 ± 0.8	24.0 ± 11.9	384 ± 200	SAB (this study)
	BT	< 0.10–0.38	< 0.7	5.2 ± 2.6	64.1 ± 16.0	1042 ± 995	
	Shoots	0.10–0.8	< 0.12	41,395	19–27	199–365	Bahía Blanca estuary (Hempel et al. 2008)
	Roots	< 0.01–0.1	< 0.12–2.0	13,850	46–63	239–510	
	Leaves	nd	1.6 ± 1.1	3.7–6.5	4.2–26.5	nd	Hackensack Meadowlands, New Jersey (Windham et al. 2003)
	Shoots	nd	0.9 ± 0.8	0.9–7.1	12.9–32.2	nd	
	Roots	nd	120.6 ± 54.8	100.8–212.3	236–898	nd	
	Rhizomes	nd	19.7 ± 20.8	6.3–52.8	29.5–168.0	nd	
	Shoots	nd	2–10	5–30	25–80	nd	Yangtze River estuary (Quan et al. 2007)
	Roots	nd	5–25	20–100	25–200	nd	

nd no data, is not present values

Salinity in SAB generally is 36 psu, which may be restricting the metal uptake and its translocation. *Spartina* is a phytostabilizer plant that maintains metals in the rhizosediment with very low translocation towards aerial tissues. This results in low transfer in the food chain. However, it should be mentioned that in this study, the content of metals in dead or dry tissue was not measured, which could contain concentrations higher than those measured in living tissue (shoots and leaves green). Senescent tissues usually accumulate contaminants in greater proportion than photosynthetic or young leaves (Cambrollé et al. 2008; Salla et al. 2011).

Regarding decapod crustaceans, sex is a biological factor that can affect the metal concentrations in their tissues (MacFarlane et al. 2000). For *N. granulata*, some studies have reported no sexual differences for Cu and Cd (Beltrame et al. 2011), while others indicated differences between sexes for Cu, Zn, Ni, and Pb (Ferrer 2001; Simonetti et al. 2011, 2013). In this study, metal levels were evaluated in male crabs, in order to decrease the variability during different stages of the reproductive cycle. Cd and Cu levels were higher in winter in most sites, while for Zn and Fe, these were higher in autumn.

Pb levels, meanwhile, were maximum in summer in the innermost sites of the Encerrado channel. During autumn and winter, crabs are less active and have a lower metabolic rate (Ituarte et al. 2004, 2006). The higher concentrations of metals in this period could be the result of less excretion and a change in their mode of feeding (higher debris content), due to their lower activity outside the burrows.

Since decapod crustaceans are not able to regulate non-essential metals such as Cd and Pb, they accumulate them in amounts directly proportional to external concentrations (MacFarlane et al. 2000; Rainbow 2002). Pb concentrations were highest in individuals from the innermost site of the Encerrado channel, according to the sediment concentrations. While Cd concentrations were highest outside the channel. On the other hand, *N. granulata* regulates essential metals such as Cu and Zn. Cu is part of the respiratory pigment hemocyanin and Zn is part of numerous enzymes (Rainbow 1997).

Non-essential metals as well as the excess of essential metals must be detoxified. They may be regulated in the hepatopancreas and detoxified by several pathways: by

Table 6 Metals concentrations in soft tissue of *N. granulata* in Argentina (mg/kg)

Site (reference)	Cd	Pb	Cu	Zn	Fe
SAB (this study)	0.3–1.7	0.4–8.0	174–438	81–168	399–1970
SAB (Giarratano et al. 2016)	0.2–2.3	< 0.2–8.2	150–380	78–130	380–1800
SAB (Gil et al. 2006)	< 0.08	10–13.2	87–102	68–86	nd
Bahía Blanca estuary (Ferrer et al. 2006)	5.2 ± 0.8	3.1 ± 0.7	14.3 ± 2.0	74.1 ± 5.6	nd
Bahía Blanca estuary (Simonetti et al. 2011, 2013)	8.2–9.1	< 0.29–4.66	135–137	33	nd

nd no data, is not present values

binding to proteins (metallothioneins); by accumulation in vacuoles in the form of sulfide granules; by elimination in feces and urine or accumulated in hemolymph or other detoxification organs (Rainbow 2007; Sa et al. 2008; Henry et al. 2012). Therefore, metals are regulated to a certain threshold, above which they are accumulated proportionally to the external levels. In this study, except for individuals from the innermost site of the Encerrado channel, there were similar among sites, which could be related to the fact that sediment concentrations did not exceed thresholds established for the protection of the aquatic life (CCME 2001b).

Cu and Zn are essential metals and decapod crustaceans have several routes of detoxification of excess, tolerating relative high concentrations. Thus, in the sites with higher concentrations of metals in sediments, the BAF remained low. In the case of Cd, it is a non-essential metal and its detoxification is slow, so that at small concentrations in the environment, a net accumulation in the organisms is observed. Studies have shown that accumulated concentrations of Cd in crabs increase with dissolved Cd exposure without significant increase in excretion (Rainbow 2002, 2007).

Metals concentrations in *N. granulata* were similar to those reported by Giarratano et al. (2016) and relatively higher than those reported by Gil et al. (2006) in organisms of the tidal plain of this bay, except in the case of Pb that was slightly higher (Table 6). Concentrations of the same order of magnitude for this species have been reported in the estuary of Bahía Blanca (Buenos Aires), a site with moderate pollution (Simonetti et al. 2013), being smaller in most metals except Cd.

Essential and non-essential metals possess the ability to produce reactive oxygen species (ROS) that cause DNA damage, lipid peroxidation, and enzymatic inhibition. Studies in *N. granulata* of the BSA did not present a differentiated oxidative stress between organisms of the Encerrado channel and outside of it

(Giarratano et al. 2016). The toxicity of metals in the organism is not directly related to the total accumulated concentration since the metals can be stored in unavailable forms, such as granules or metallothionein complexes (Rainbow 2007). It is also possible that crabs from contaminated areas have developed a genetic tolerance to metals, indicating strong ecotoxicological selective pressure (Luoma and Rainbow 2008).

It has been reported for *N. granulata* that the increase in salinity decreases the toxicity and accumulation of Cd (Vitale et al. 1999) and Cu (Lauer et al. 2012). Also, in other crabs such as *Carcinus maenas*, Zn accumulation decreased with increasing salinity (Niyogi et al. 2015). These authors indicate that higher salinity increases the tolerance to these metals. SAB receive low fresh water input from rainfalls and evaporation rate is high. In this study, the surrounding seawater had salinity between 36 and 37 psu, and salinities of 39 psu have been also reported in the bay (Montemayor et al. 2014). The high salinity of the environment could favor the metal tolerance of crabs from SAB.

Conclusions

The levels of metals in sediment and organisms in SAB remained at levels corresponding to a slight enrichment or contamination. The highest levels correspond to innermost sites of the Encerrado channel and close to the mining wastes.

Spartina is a phytostabilizer plant so its aboveground tissues do not reflect the concentrations in the sediment; instead, it retains the metals in its belowground tissues and in the rhizosediment. The metal levels in the rhizosediment were higher than in the adjacent sediment evidencing the influence of the vegetation, but only in the innermost site of the channel.

The pools of metals bioaccumulated by *Spartina* were much lower in the aboveground tissues, as seen by the lower translocation rates and by the metal concentrations. The necromass from root system becomes important to metal budget of the sediment, not only due to its input of metals, but also due to the increase of organic matter (Duarte et al. 2010). On the other hand, since crabs' herbivory is mainly on young leaves would not represent a significant source of metals for these organisms (Salla et al. 2011).

N. granulata showed to be a useful biomonitor for Pb, but not for the other metals. This may be because the concentrations in the environment do not reach threshold levels being the crabs able to regulate the uptake and detoxification of excess metals. In this work, metals have been measured in total soft tissues. It is possible that different results could be obtained when specific tissues are analyzed. Crustaceans use physiological and biochemical detoxification and storage mechanisms in particular organs to protect other tissues and organs from the harmful effects of the toxic metals. There are several internal and external factors affecting the patterns of metal accumulation in crustaceans, such as those described in Marsden and Rainbow (2004).

Present information is of interest in order to have an update of the metal levels in different matrices of the ecosystem (sediments and organisms) of the SAB. Currently, a remediation plan is being carried out at the study site, which consists in the removal of mining wastes and affected soil. If movement of soils is not carried out with care, it is possible that dispersion of metal-enriched particles towards the Bay occurs. It is necessary to have an environmental control that monitors this situation, during and after this process has been completed.

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