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Trace metal concentrations in *Spartina densiflora* and associated soil from a Patagonian salt marshYanina L. Idaszkin ^{a,b,*}, Pablo J. Bouza ^{a,b}, Carmen H. Marinho ^a, Mónica N. Gil ^{a,b}^a Centro Nacional Patagónico (CENPAT – CONICET), Boulevard Brown 2915, 9120 Puerto Madryn, Chubut, Argentina^b Universidad Nacional de la Patagonia San Juan Bosco, Boulevard Brown 3051, 9120 Puerto Madryn, Chubut, Argentina

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ABSTRACT

The objectives of this study were to (i) assess *in situ* trace metal concentrations in soil and in *Spartina densiflora* in a Patagonian salt marsh (Rawson, Chubut, Argentina) and (ii) investigate the relationship between trace metal concentrations in soils and in plants to improve our knowledge regarding the ability of *S. densiflora* to take up and accumulate trace metals from the soil within its native region. Our results indicate that the soil and *S. densiflora* exhibit low metal concentrations in the Rawson salt marsh. *S. densiflora* accumulates Zn in below- and above-ground plant structures and Cr in below-ground parts. These results suggest at the time of this study there is scarce human impact associated with metals in the Rawson salt marsh.

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The impact on coastal and estuarine environments of different pollutants such as trace metals, oil, pesticides, and fertilizers is an issue of great concern to ecologists and environmentalists (Mendelsohn et al., 2001; Marcovecchio and Ferrer, 2005). Although trace metals are natural elements in the environment, they may be enriched to toxic levels by human activities. Moreover, their concentration, behaviour and partitioning can be affected by different soil features such as pH, redox potential, salinity, texture, and organic matter content (Kabata-Pendias, 2011). Intertidal environments are subject to the input of contaminants discharged into seas or rivers and carried in water by the action of the tides. Salt marshes are coastal ecosystems characterized by the presence of vascular halophytes that have an important role in the dynamics of pollutants (Adam, 1990; Weis and Weis, 2004). In these environments, contaminants can be trapped in soils, remaining in biologically unusable forms (Hung and Chmura, 2007; Botté et al., 2010) or may be absorbed by vegetation (Reboreda and Caçador, 2007a,b; Hempel et al., 2008; Redondo-Gómez et al., 2009; Caçador et al., 2009; Duarte et al., 2010; Almeida et al., 2011). Furthermore, the physical and chemical soil conditions can be altered by plants that are able to produce micro-environmental changes, affecting metal mobility, speciation, bioavailability and in consequence their potential toxicity

(Reboreda and Caçador, 2007a,b). Plants have different mechanisms for regulating the uptake of metals (Greger, 2004). For example, some plant species can depress the uptake of metals increasing the pH in the rhizosphere, which decreases the mobility of the metals (Greger, 2004). Conversely, the acidification of the rhizosphere through the release of H⁺ or exudates (which can act as complexing agents) may favour metal mobility, thus increasing their bioavailability (Mucha et al., 2005). Plants living in environments subject to submerged periods, such as *Juncus maritimus* and *Spartina alterniflora*, can transport oxygen through the aerenchyma to the rhizosphere zone (Justin and Armstrong, 1987; Maricle and Lee, 2002), thus increasing oxygenation, creating a gradient that forces the metals to be concentrated toward the roots (Burke et al., 2000).

When metals are absorbed by the plants, they can be retained in their roots or rhizomes (Reboreda and Caçador, 2007b; Hempel et al., 2008; Duarte et al., 2010) or translocated to their shoots or leaves (Burke et al., 2000; Weis et al., 2003); depending on the physicochemical soil parameters, the plant species, and the metal (Weis and Weis, 2004). By absorbing metals, plants can act as a conduit, mobilizing them from the soil to the food web, either to be consumed directly by herbivores, or through plant debris that are incorporated in basal level of the food chain. The decomposition rate and the contribution of detritus are different among the plant species; therefore the recycling of metals and their return to the aquatic environment also depends on the species (Duarte et al., 2010). Thus, salt marshes can act as sink or source of metals for the surrounding aquatic environment.

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It is widely accepted that salt marshes are highly productive ecosystems critical to the maintenance of the regional integrity for both terrestrial and marine communities (Mitsch and Gosselink, 2000). Anyway, most studies evaluating the concentration of trace metals in salt marshes have been conducted in highly populated or highly industrialized areas (Redondo-Gómez et al., 2009). Thus, pristine or scarcely impacted environments have been poorly studied, despite the importance of having a reference study, which may permit early diagnosis of pollution and achievement of low cost environmental recovery practices, both economic as ecological and logistical.

The Argentinean Patagonia is usually considered an area with low human impact (Bortolus and Schwindt, 2007), considering its low urbanization and low port and industrial activities in relation to large cities. Although there are some studies in Patagonian intertidal environments (Gil et al., 1999; Vázquez et al., 2007; Marinho et al., 2013), to date there is no research evaluating the levels of trace metals in salt marshes. The objectives of this study were to (i) assess *in situ* trace metal concentrations in soils and in *Spartina densiflora* in a Patagonian salt marsh (Rawson, Chubut, Argentina) and (ii) investigate the relationship between trace metal concentrations in soils and in plants to improve our knowledge regarding the ability of *S. densiflora* to take up and accumulate trace metals from the soil within its native region.

The present study was conducted in "Rawson salt marsh", the *Spartina*-marsh located on the mouth of the Chubut River, Chubut Province, Argentina (65°04'S, 43°20'W, Fig. 1). This salt marsh is mostly inhabited by the cordgrass *S. densiflora*, accompanied by *Sarcocornia perennis* in a very low proportion (Bortolus et al., 2009). The Chubut River arises in the Andes mountains, crosses

from west to east of Chubut Province, and discharges its waters into the Atlantic Ocean at "Bahía Engaño" near a fishing port. In its lower course the river flows through the most populated area, where the largest riverside cities (Trelew: ~90,000 inhabitants and Rawson: ~34,000 inhabitants) and urban, agricultural, and industrial wastewaters are discharged. At the same time, the river is the principal source of water for several neighbouring cities of Chubut Province. Previous studies show moderate levels of pollution with Zn, Cu, and Pb in Bahía Engaño (Gil et al., 1999; Mohamed, 2008).

Two sampling sites were chosen in the Rawson salt marsh along a linear transect (running from east to west) to detect any contamination gradient: one named the 'RC' site, placed on the mouth of Chubut River, located nearest to Rawson City, and the second called 'RP' site located closest to the Rawson Port at the outlet of the river into the Atlantic ocean. In the spring of 2012, at each site, five core samples were collected at low tide from *S. densiflora* stands, all spaced 1 m from each other. Each sample (15-cm-diameter and 15-cm-depth) consisted of a block of soil with tillers, rhizomes and roots of *S. densiflora*. The redox potential (Eh) and pH of the soil in the root zone (0–10 cm) were determined in the field using a portable pH/Eh (ORP) meter and an electrode system Termo/pH meter Altronix TPA-IV. Samples were stored in polyethylene bags, transported to the laboratory, and frozen to –20 °C until analyzed.

The soil from the root zone (0–10 cm) was removed from the roots and rhizomes of each plant were dried at 85 °C until constant weight, and sieved through a 2 mm mesh to remove large stones and dead plant material. Electrical conductivity (EC) was measured with a conductivity meter after diluting 10 g of dried and sieved

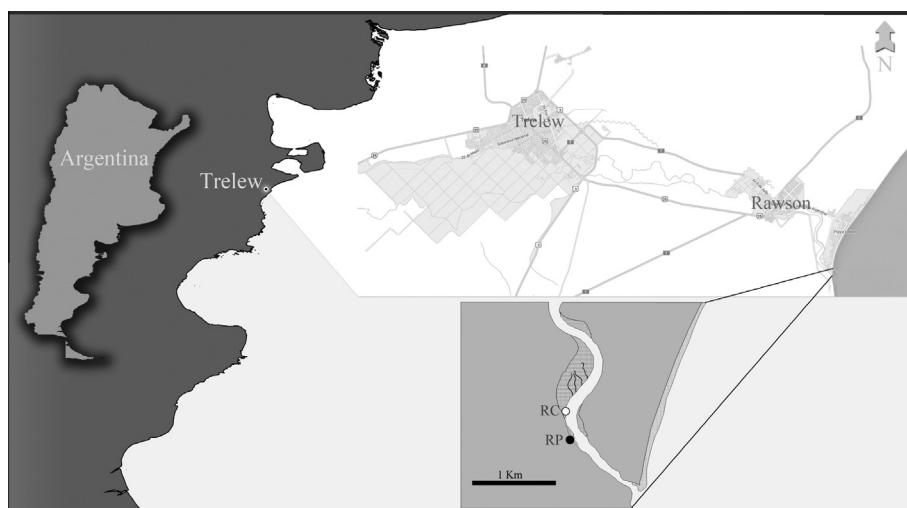


Fig. 1. Location of the sampling sites in the Rawson salt marsh.

Table 1
Trace metal concentrations in Reference Material and Recovery Percent.

Element	PACS-2			BCR-060		
	Media ± SD (n = 5)	% R	LD ($\mu\text{g g}^{-1}$)	Media ± SD (n = 5)	% R	LD ($\mu\text{g g}^{-1}$)
Cd ($\mu\text{g g}^{-1}$)	1.8 ± 0.1	88	0.25	2.1 ± 0.2	93	0.1
Cr ($\mu\text{g g}^{-1}$)	55.7 ± 5.1	61	2.50	31.6 ± 0.9 ^a	122	1.0
Cu ($\mu\text{g g}^{-1}$)	275.6 ± 10.1	89	1.25	46.2 ± 1.3	90	0.5
Pb ($\mu\text{g g}^{-1}$)	158.0 ± 9.3	86	5.00	54.1 ± 4.9	84	2.0
Ni ($\mu\text{g g}^{-1}$)	33.1 ± 1.4	84	2.50	40.3 ± 1.2 ^a	101	1.0
Zn ($\mu\text{g g}^{-1}$)	327 ± 14	90	0.25	441.7 ± 34.1 ^a	141	0.1
Fe (mg g ⁻¹)	3.29 ± 0.14	81	2.50	0.23 ± 0.03 ^b	99	1.0

^a Values no certified.

^b Fe as Fe_2O_3 .

Table 2

Physicochemical properties of soils.

Site	RP		RC	
	Media ± S.E.	(min–max)	Media ± S.E.	(min–max)
Clay %	10.52 ± 1.2	(6.63–14.1)	9.56 ± 1.53	(6.29–14.2)
Silt %	27.43 ± 6.13	(7.69–39.7)	40.43 ± 10.6	(15.65–66.08)
Sand %	62.06 ± 6.67	(46.2–80.85)	50.01 ± 9.59	(27.63–75.82)
Eh (mV)	−96.81 ± 46.16	(−265 to −0.07)	−179 ± 42.31	(−302 to −66)
pH	7.42 ± 0.04	(7.29–7.53)	7.23 ± 0.06	(7.09–7.39)
EC (mmhos cm ^{−1})	6.43 ± 0.95	(2.76–8.27)	6.47 ± 1.24	(4.07–10.06)
OM%	3.14 ± 0.34	(1.89–3.89)	3.92 ± 0.75	(2.61–6.12)

PR = Port Rawson; CR = City Rawson.

Table 3

Pseudototal trace metal concentrations measured in soils.

Site	RP		RC	
	Media ± S.E.	(min–max)	Media ± S.E.	(min–max)
Cd ($\mu\text{g g}^{-1}$)	Undetectable		Undetectable	
Cr ($\mu\text{g g}^{-1}$)	17.12 ± 1.65	(10.81–20.01)	18.64 ± 1.6	(12.7–22.18)
Cu ($\mu\text{g g}^{-1}$)	7.48 ± 0.61	(5.22–8.56)	8.05 ± 0.5	(6.85–9.66)
Ni ($\mu\text{g g}^{-1}$)	11.68 ± 0.93	(9.92–14.89)	10.94 ± 0.6	(9.93–12.44)
Pb ($\mu\text{g g}^{-1}$)	7.20 ± 0.61	(4.97–8.68)	6.21 ± 0.39	(4.97–7.43)
Zn ($\mu\text{g g}^{-1}$)	38.79 ± 2.85	(28.72–44.91)	41.84 ± 2.76	(34.5–49.13)
Fe (mg g ^{−1})	17.93 ± 1.28	(13.04–20.46)	20.16 ± 1.072	(17.10–22.61)

PR = Port Rawson; CR = City Rawson.

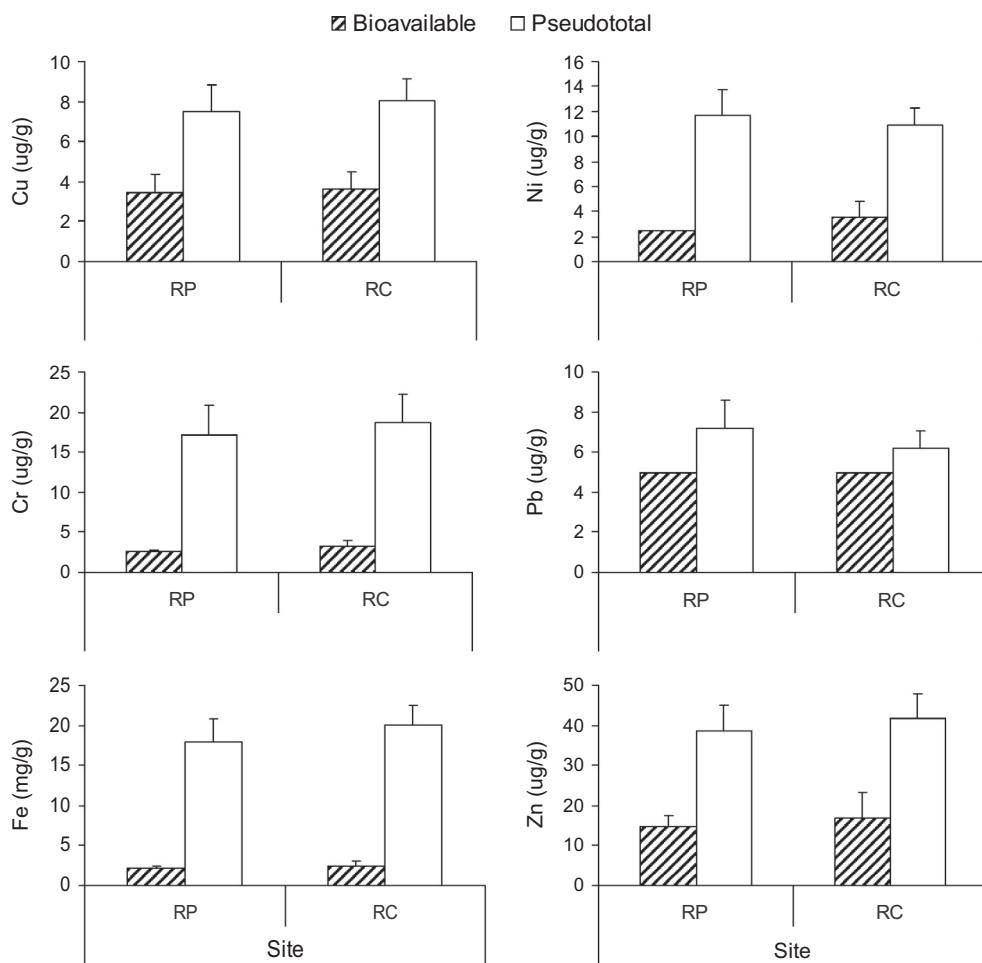
**Fig. 2.** Pseudototal and potentially bioavailable concentration of studied trace metals in soil from each sample site in the Rawson salt marsh. Data are means + SD, n = 5.

Table 4

Pearson correlation coefficients (r) between physicochemical variables and pseudototal trace metal concentrations in soils.

Metal	Cr	Cu	Fe	Ni	Pb	Zn	FF%	OM%	EC	Eh
Cu	0.88*									
Fe	0.93*	0.92*								
Ni	-0.03	0.24	-0.05							
Pb	0.33	0.27	0.3	-0.12						
Zn	0.87*	0.76*	0.88*	-0.07	0.37					
FF%	0.66*	0.6	0.79*	-0.14	0.09	0.85*				
OM%	0.71*	0.76*	0.79*	0.35	0.16	0.85*	0.82*			
EC	0.79*	0.81*	0.81*	0.26	0.46	0.88*	0.77*	0.91*		
Eh	-0.6*	-0.68*	-0.62	-0.25	0.09	-0.6	-0.6	-0.8*	-0.68*	
pH	-0.36	-0.24	-0.33	0.07	0.4	-0.41	-0.39	-0.47	-0.2	0.71*

* $p < 0.05$.

Table 5

Potentially bioavailable trace metal concentrations measured in soils.

Site	PR		CR		
	Potentially bioavailable metal	Media ± S.E.	(min–max)	Media ± S.E.	(min–max)
Cd ($\mu\text{g g}^{-1}$)	Undetectable			Undetectable	
Cr ($\mu\text{g g}^{-1}$)	2.58 ± 0.1	(2.48–2.98)		3.21 ± 0.35	(2.48–4.12)
Cu ($\mu\text{g g}^{-1}$)	3.45 ± 0.4	(2.24–4.59)		3.64 ± 0.36	(2.86–4.49)
Ni ($\mu\text{g g}^{-1}$)	2.48 ± 0.0024	(2.48–2.49)		3.49 ± 0.61	(2.48–4.99)
Pb ($\mu\text{g g}^{-1}$)	4.97 ± 0.0037	(4.96–4.98)		4.98 ± 0.01	(4.97–5)
Zn ($\mu\text{g g}^{-1}$)	14.82 ± 1.22	(12.08–19.17)		16.93 ± 2.88	(8.49–26.32)
Fe (mg g^{-1})	2.093 ± 0.166	(1.66–2.67)		2.46 ± 0.24	(1.83–3.26)

PR = Port Rawson; CR = City Rawson.

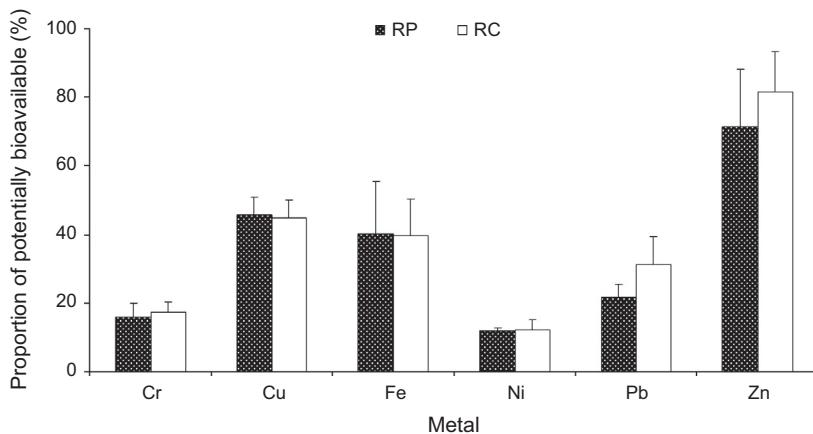


Fig. 3. Potentially bioavailable proportion of studied trace metals in soil from each sample site in the Rawson salt marsh. Data are means + SD, $n = 5$.

soil with 50 ml of distilled water. Percentages of sand, silt, and clay were estimated using the Bouyoucos hydrometer method (Bouyoucos, 1927). Organic matter (OM) was determined by the loss on ignition method (4 h at 450 °C) (Davies, 1974). For analysis of metals, 1 g of dried and sieved soil was digested using 10 ml of aqua regia at 85 °C and evaporated on a hot plate to near dryness. The digest was cooled to ambient temperature and recollected taking it to final volume of 25 ml with 5% nitric acid (MacGrath and Cunliffe, 1985; ISO, 1995). This digestion is often referred to as pseudototal analysis and extracts between 70% and 90% of the total contents of metals (Hung and Chmura, 2007). In addition, to extract the labile or potentially bioavailable metals, 1 g of dried and sieved soil was used to make a cold extraction with 25 ml of 0.5 N HCl (Ageomian and Chau, 1976). Cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), Ni (nickel), zinc (Zn) and iron (Fe) were measured in the extracts using an Atomic Absorption Spectrophotometer (Instrumentation Laboratory, USA) with N₂O/acetylene flame for Cr and air/acetylene flame for other metals. All metal

analyses were carried out in duplicate, and blanks were processed as the samples. Results are reported on a dry weight.

Regarding plants, they were carefully washed with tri-distilled water and separated into below-ground tissues (rhizomes and roots) and above-ground tissues (stems and leaves). All samples were dried at 80 °C until a constant weight was reached and subsequently pulverized in a mill. Aliquots of 1 g of powder were calcined in muffle (6 h at 400 °C) and treated on a hot plate (85 °C) with concentrated HNO₃ until dryness. These two steps were repeated until white ashes were obtained (BOE, 1991). All plant samples were processed by duplicate and blanks were processed as the samples. Metal analyses were carried out using an Atomic Absorption Spectrophotometer (Instrumentation Laboratory, USA). Results are reported on a dry weight.

Reagents of analytical grade were used for the blanks and for calibration curves. Quality assurance of soils and plants was done through analysis of standard reference marine sediment PACS-2 and BCR-060 aquatic plant (*Lagarosiphon major*), respectively

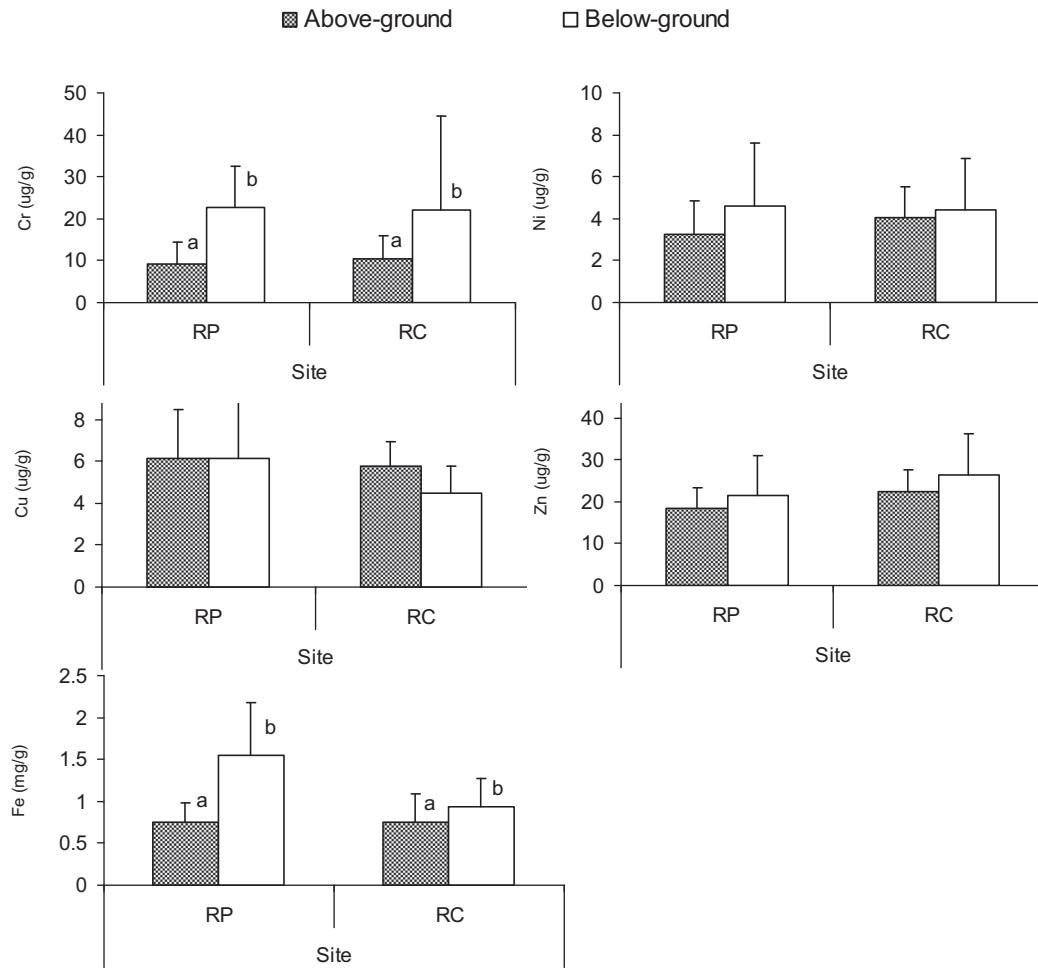


Fig. 4. Trace metal concentrations in below- and above-ground structures of *Spartina densiflora* from each sample site in the Rawson salt marsh. Data are means + SD, $n = 5$. Different letters indicate significant differences ($p < 0.05$) between structures in two-way ANOVA test.

(Table 1). The recovery was >80% for all metals except for Cr (60%). Detection limits of the method are seen in Table 1. The variation coefficients tested for five replicates of the same samples were always below 10%.

One-way Analysis of Variance (ANOVA) was used to evaluate differences in soil measures and trace metal concentrations between sites and two-way ANOVA with interactions to test the effect of sites and plant structure on plant metal concentration. Before the analyses, data were tested for normality with the Shapiro-Wilk test and for homogeneity of variance with the Levene test. To correct for nonnormality and heterogeneity of variance, the OM content, Cr plant concentration, and Cr bioenrichment factors (BAF: [metal in plant structure]/[pseudototal metal in soils]; Mason, 2013) variables were ln (X)-transformed. Being that soil bioavailable Ni and Cr did not satisfy the assumptions of parametric analysis, differences in these variables between sites were evaluated using Kruskal Wallis nonparametric ANOVA test. Also, Pearson coefficients (r) were calculated to evaluate correlation between different variables.

There were no significant differences between "RC" and "RP" sites in soil Eh, EC and OM content. Although the pH was statistically different, it was neutral in both sites (Table 2). Regarding soil texture, sand was the major fraction in both sites, followed by silt and clay fractions (Table 2), the texture being principally sandy loam in both sites.

Pseudototal metal concentrations in soils did not show differences between sites. The highest value was measured for Fe, fol-

lowed by Zn, Cr, Ni, Cu, and Pb. Cd was always undetectable ($<0.25 \mu\text{g g}^{-1}$) (Table 3 and Fig. 2). Unlike in the other intertidal environments of Argentina, research evaluating the levels of trace metals in salt marshes is scarce, being mainly concentrated in Bahía Blanca (Buenos Aires, Argentina) and, to our knowledge, there are no previous studies in Patagonian salt marshes. The concentrations found in this study are lower than those reported by Hempel et al. (2008) in soils associated with *S. alterniflora* within Bahía Blanca salt marshes. Moreover, all metal concentrations that we found are below the reference points of Marine Sediment Quality Guidelines (ISQG) established for The Canadian Council of Ministers of the Environment (2002), denoting scarce human impact.

Significant positive correlations were observed among Fe, Zn, Cu, and Cr (Table 4), which also correlated positively with fine fraction (FF, clay + silt), OM, and EC and negatively with Eh, all with $p < 0.05$, except for Cu-FF, Fe- and Zn-Eh. On the other hand, no correlations were observed for Ni and Pb. These patterns suggest a similar biogeochemical behaviour for Fe, Zn, Cu, and Cr, in addition to probable common external inputs. In that sense, river contributions upstream from the port (where most urban and industrial activities are developed), as well as the port itself, would be considered. Regarding Pb and Ni instead, anthropogenic sources would mainly be related to the port.

Similar to the other variables, potentially bioavailable metal soil concentrations did not show differences between sites. They followed the order Fe, Zn, Pb, Cu, and Cr or Ni (Table 5 and Fig. 2)

Table 6

Bioaccumulation factors ([metal in plant structure]/[pseudototal metal in soils]) of Cr, Cu, Ni, Zn, and Fe in below- and above-ground parts of *Spartina densiflora*.

Site	RP		RC	
	Below-ground	Above-ground	Below-ground	Above-ground
Cr	1.25 ± 0.53 ^a	0.62 ± 0.12 ^b	1.34 ± 0.42 ^a	0.51 ± 0.13 ^b
Cu	0.63 ± 0.12	0.78 ± 0.06	0.8 ± 0.21	0.79 ± 0.17
Ni	0.41 ± 0.13	0.36 ± 0.08	0.45 ± 0.14	0.31 ± 0.08
Zn	4.02 ± 1.14	3.19 ± 0.35	3.59 ± 0.79	2.98 ± 0.31
Fe	0.05 ± 0.01 ^a	0.04 ± 0.01 ^b	0.08 ± 0.02 ^a	0.04 ± 0.01 ^b

PR = Port Rawson; CR = City Rawson. Different letters indicate significant differences ($p < 0.05$) in two way ANOVA test.

and represented between ~12% and ~80% of the pseudototal concentration (Fig. 3), indicating that a significant amount of the latter could be available to be assimilated by the biota. In agreement, except for Cd ($<0.1 \mu\text{g g}^{-1}$) and Pb ($<2.0 \mu\text{g g}^{-1}$), all these metals were found in the plants in concentrations high enough to be quantifiable (Fig. 4). Fe, Zn, Cu, and Ni are essential elements for plant growth, but can be toxic at high concentration. Levels found in this study were lower than the potential phytotoxic levels (Chen et al., 2009; Mateos-Naranjo et al., 2008a,b; Redondo-Gómez et al., 2011) for all tissues. Cambrollé et al. (2008, 2011) studied the distribution of several metals in tissues of *S. densiflora* and in soil from polluted salt marshes where it was introduced (Odiel, SW Spain). They found levels of the trace metals in soils higher than we found in the Rawson salt marsh, and an elevated range of concentrations of Pb, Zn, and Cu, a range similar to Ni, and lower Cr than we found in *S. densiflora*.

Only Fe and Cr plant concentrations were significantly different between structures, both being higher in the below-ground than in the above-ground structures (Fe, site: $F_{1,16} = 2.91$, $p = 0.1$; structure: $F_{1,16} = 7.22$, $p = 0.01$; interaction: $F_{1,16} = 2.83$, $p = 0.11$; (ln(Cr)-transformed, site: $F_{1,16} = 0.1$, $p = 0.75$; structure: $F_{1,16} = 5.78$, $p = 0.03$; interaction: $F_{1,16} = 0.84$, $p = 0.37$). Several studies found that *Spartina* species minimize the translocation of metals to the photosynthetic tissues (Reboreda et al., 2008; Mateos-Naranjo et al., 2008a, 2011), as previously reported for Cr, Ni, Cu, Pb and Zn, in *S. densiflora* from Odiel salt marshes (Cambrollé et al., 2008, 2011). Instead, in a greenhouse experiment, Cr concentrations in *Spartina argentinensis* were greater in the shoots than in roots (Redondo-Gómez et al., 2011). Anyway, it is well established that the degree of uptake and the distribution of metals within plant structures differ upon the metal ion uptake routes, the metal speciation and bioavailability, and the plant species (Williams et al., 1994; Redondo-Gómez, 2013). In this regard, it would be considered that metal concentrations in soils from the Rawson salt marsh are considerably lower than those reported in the literature, which could explain the different pattern of metals distribution within the plants.

To estimate the capability of *S. densiflora* of the studied salt marsh to accumulate trace metals actually present in the soil, bioaccumulation factors (BAF) were calculated (Table 6). In agreement with the other studied variables, metal BAF did not show differences between sites. Only for Cr and Fe BAF was higher in the below-ground than in the above-ground structures (Fe, site: $F_{1,16} = 1.36$, $p = 0.26$; structure: $F_{1,16} = 5.69$, $p = 0.03$; interaction: $F_{1,16} = 2.16$, $p = 0.16$; (ln(Cr)-transformed, site: $F_{1,16} = 0.0003$, $p = 0.98$; structure: $F_{1,16} = 4.82$, $p = 0.04$; interaction: $F_{1,16} = 0.78$, $p = 0.89$; Table 6). In addition, results suggest that *S. densiflora* in the Rawson salt marsh is able to accumulate (BAF > 1) Zn in below- and above-ground structures and Cr in below-ground parts (Table 6).

According to previous results, it may be concluded that at the time of this research the Rawson salt marsh was very little impacted by trace metal pollution.

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