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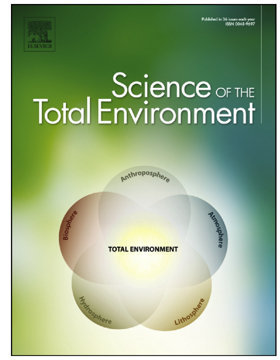

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Uptake and accumulation of metals in *Spartina alterniflora* salt marshes from a South American estuary

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Abstract

Salt marshes are capable of reducing metal pollution in coastal waters, but this capacity is highly dependent on the metal, the physico-chemical characteristics of the sediment, the plant species, the production of biomass, the time of the year, etc. The aim of this study was to assess the uptake and accumulation of Pb, Ni, Cu and Zn in *Spartina alterniflora* from three salt marshes within the Bahía Blanca estuary (BBE), a human-impacted Argentinean system. Metal concentrations in sediments and plants showed the same order at all sites: Zn> Cu> Pb≥ Ni. The site with lower organic matter and fine sediment content had lower metal concentrations in the sediments, but not a lower metal content in the plant tissues, meaning that the sediment characteristics influenced the metal concentrations in the sediment and their uptake by plants. Despite differences in sediment characteristics between sites, metals were always higher in the belowground tissues than in aboveground ones and, in general, higher in dead than in live tissues. Some metals were accumulated in plant tissues, but not others, and this is dependent on the metal and the sediment characteristics. Allocation patterns of metals in tissues of *S. alterniflora* were mainly dependent on metal concentrations, determining higher belowground pools, but the aboveground pools were important in some cases due to higher biomass. Partitioning of metals in above or belowground pools determines their fate within the estuarine system, since tissues can decompose *in situ* (belowground) or be exported (aboveground). Seasonal dynamics were important for some variables but were less noticeable than the differences between sites and tissues. Our results indicate that *S. alterniflora* from the BBE is efficient in accumulating some metals, despite usually low metal concentrations in sediments and plants. This accumulation capacity has implications for the whole system through the fate of the tissues.

Keywords: *halophytes; wetlands; Bahía Blanca; bioconcentration; sediments*

1. Introduction

Salt marshes are recognized worldwide for the various ecosystem services they provide (Barbier et al., 2011). One of these services is the reduction of metal pollution in coastal waters due to the capacity of halophytes to sequester metals in their tissues, although this capacity is highly variable (Anjum et al., 2013). Halophytes uptake metals during growth at a rate that is dependent on metal mobility and availability in the sediment, which is in turn determined by the physico-chemical characteristics of the sediment (redox potential, pH, organic matter content, grain size, etc.) (Kabata-Pedias, 2011). The uptake of metals also depends on the metal, the plant species and the tidal energy, among others (e.g., Weis and Weis, 2004).

Once metals are absorbed by halophytes, they can be retained in the belowground tissues or be translocated to the shoots and leaves, depending on the metal, the plant species, the time of the year, etc. (Weis and Weis, 2004; Anjum et al., 2013; Phillips et al., 2015; Petranich et al., 2017). The allocation of metals in the below or aboveground tissues determines whether metals are retained within the salt marsh (belowground) or whether they are likely to be exported to other areas of the coastal system through the action of tides and waves (aboveground) (Windham et al., 2003; Caçador et al., 2009; Duarte el al., 2010; Duarte et al., 2017). In addition, the differential metal distribution in live and senescent aboveground tissues and the biomass production of each type of tissues are critical factors that control the amount and rate of release of metals (Caçador et al., 2009; Duarte et al., 2010; Couto et al., 2013; Song and Sun, 2014; Lian et al., 2017). The release of metals back to the environment through decomposition varies with the plant species, the chemical composition of the tissues and environmental factors (Liao et al., 2008; Negrin et al., 2012a; Song and Sun, 2014; Lian et al., 2017). Moreover, detritus produced from senescent aboveground tissues is the base for most marine food webs, resulting in the introduction of metals to higher trophic levels (Moore et al., 2004; Weis and Weis, 2004; Bergamino and Richoux, 2014). Hence, the partitioning of metals between different tissues ultimately determines their fate within the coastal environment.

One of the most studied halophytic species regarding metal accumulation is *Spartina alterniflora* Loisel., including both field and experimental laboratory studies (Redondo-Gómez, 2013). *S. alterniflora* is a perennial C_4 grass which reproduces mainly by rhizomes and is native of the Atlantic coast of North America but has been introduced worldwide, mainly for erosion control (Mobberley, 1956; Bortolus et al., 2015; Global Invasive Species Database, 2018). *S. alterniflora* usually accumulates metals in belowground tissues (Alberts et al., 1990; Windham et al., 2003; Quan et al., 2007; Marinho et al., 2017; Lian et al., 2017), as most salt marsh plants (e.g., Caetano et al., 2008; Caçador et al., 2009; Cuoto et al., 2013; Idaszkin et al., 2014; Son and Sun, 2014; Phillips et al., 2015; Petranich et al., 2017), although some authors have found the opposite pattern (Pang et al., 2017). Despite the importance of this species in salt marshes throughout the world, most of the information comes from studies in North America and, more recently, in Asia (Redondo-Gómez, 2013). In the Southern Hemisphere, *S. alterniflora* is usually the dominant species from southern Brazil to the northern coasts of Argentina (Isacch et al., 2006), but only a few studies deal with metal accumulation in its tissues. To the best of our knowledge, there are only two studies on the topic from Argentina, both from the Bahía Blanca estuary (BBE; Botté, 2005; Hempel et al., 2008), and two reviews partially based on them (Negrin et al., 2016; Marcovecchio et al., 2016). However, the importance of the sediment characteristics and the seasonal dynamics of metals and biomass production were not evaluated in these previous studies.

The aim of this study was to assess the uptake and accumulation of Pb, Ni, Cu and Zn in *S. alterniflora* in three salt marshes within the BBE, a human-impacted Argentinean coastal system. We conducted seasonal sampling and evaluated the concentration of these metals in the sediment and plant tissues together with the biomass production and the physico-chemical characteristics of the sediment at each site. We hypothesized that: 1) sediment characteristics greatly influence the concentration of metals in sediment, which in turn determine different accumulation and distribution patterns in the different populations of *S. alterniflora*; 2) seasonal dynamics in plant growth and the uptake of metals influence metal distribution within salt marsh compartments.

2. Materials and methods

2.1. Study area

The BBE (Figure 1) is a mesotidal shallow system that extends over 2,300 km^2 and is formed by a series of northwest to southeast oriented tidal channels separated by extensive tidal flats, salt marshes and islands. It has a main navigation channel, the Canal Principal, of 60 km long (Piccolo et al., 2008). Tides and winds are the main inputs of energy to the estuarine circulation. Tidal energy is provided by a quasi-stationary semidiurnal tidal wave (Perillo and Piccolo, 1991). Winds are persistent all year round and their annual mean velocity is 22.5 km/h. The climate of the region is temperate (mean temperature is 15 ºC), with a large spatial and temporal viariability in the precipitation, but usually higher in spring and summer (Piccolo, 2008). Rainfall patterns affect the freshwater input to the BBE, which is usually low (mean discharge of 241,000 m^3/day) due to the scarce discharge of the only two permanent tributaries: the Sauce Chico and the Napostá Grande streams (Limbozzi and Leitão, 2008).

The water column of the BBE is characterized by high salinity and turbidity, and high levels of nutrients and particulate organic matter are found most of the year (Freije et al., 2008). Dissolved metal concentrations are usually low, but the particulate forms of some metals are in higher concentrations than in other polluted estuaries (Botté et al., 2007; La Colla et al., 2015; Fernández Severini et al., 2017; La Colla et al., submitted). Regarding metals in sediments, the concentrations in tidal flats were lower than those reported for other estuarine systems (Botté et al., 2010; Serra et al., 2017; Simonetti et al., 2017). The anthropogenic impact depends on different local pressures: cities, industries, cattle production and cropping. The most important deep-water port system of Argentina (Ingeniero White) is located in association with these industries. Due to the circulation of big ships, the Canal Principal is regularly dredged with a considerable removal of sediments.

Salt marshes are distributed along the margins of the channels and islands of the BBE, and *S. alterniflora* is one of the most abundant species (Isacch et al., 2006). Pure stands of *S. alterniflora*, which cover an area of 196 km^2, are commonly restricted to marshes in the middle and outer zones of the BBE, and rarely appear in the inner area (Piovan, 2016). *S. alterniflora* marshes are expanding their cover in the estuary in a process that involves sedimentation –which is mainly related to dredging activities- and mudflat colonization by plants (Pratolongo et al., 2010, 2013). The present study was conducted in three natural salt marshes within the BBE: Maldonado (hereafter site 1), Villa del Mar (hereafter site 2) and Puerto Rosales (hereafter site 3) (Figure 1). The intertidal zones from the chosen sites are mainly covered by *S. alterniflora*, which can be up to 1.50 m in height (Lamberto et al., 1997).

(insert Figure 1) Figure 1. Location of the sampling sites within the Bahía Blanca estuary (Argentina)

2.2. Sampling

Sampling was conducted at low tide from November 2011 to August 2012, on a seasonal basis, in pure stands of *S. alterniflora* at each site. The aboveground tissues were harvested by clipping the vegetation at the sediment surface in randomly established 25x25 cm plots. All standing tissues were removed and placed in plastic bags. The belowground tissues (roots+rhizomes) were collected using PVC cores (15 cm long x 11 cm diameter); cores were taken from the centre of each plot after the aboveground parts had been collected. Sediment cores (15 cm long x 6 cm diameter) were also collected from the centre of each plot to evaluate the sediment associated with the belowground tissues. This depth was chosen since the majority of belowground tissues in *S. alterniflora* are in the upper 15 cm (Lacerda and Abrão, 1984; Lima et al., 1989). At each sampling date and site, 4 replicates of each type of sample were collected. All samples were transported to the laboratory in a cooler. Sediment samples were kept refrigerated (4ºC) and plant samples were frozen (-20ºC) until processing. At each sampling site, several physico-chemical characteristics of the sediment were evaluated. pH and redox potential (Eh, mV) were measured *in situ* on the surface of the sediment using a pH/mV meter (HANNA HI991003) with a HI 1297D probe (n=4). Organic matter (OM) content was evaluated in the same sediment samples collected for metal analyses (n = 4) and samples for grain size characterization were obtained in May 2012 (n=3 in each site).

2.3. Sample processing

Plant samples were washed carefully with tap water and rinsed twice with deionized water. In the aboveground tissues, dead and senescent leaves and shoots (hereafter dead) were identified by their yellowish or brownish color and separated from the living material (hereafter live). Belowground tissues were washed in a 500 µm sieve to avoid the loss of small roots. All plant samples (aboveground live, aboveground dead and belowground) were treated separately in the analyses. They were dried at 60 ºC until constant weight and weighed to the nearest 0.01 g for later determination of the biomass (g dry weight/m^2). Finally, each plant sample was ground into a fine powder in a stainless steel grinder and homogenised. Sediments were cleaned free of roots and shell fragments with tweezers and dried (at 60 ºC until constant weight). After that, the sediment samples were ground with a porcelain mortar and homogenised. OM content was obtained by loss on ignition (LOI) in a muffle furnace (450 ºC, 4h) (Hieri et al., 2001). Grain size composition was determined with a Malvern Mastersizer 2000 laser diffractometer, with previous treatment of the samples with hydrogen peroxide in order to remove OM.

2.4. Metal analyses

Concentrations of Pb, Ni, Cu and Zn were determined in ground samples of plants and sediments following the methodology described by Botté et al. (2010), with a further modification annexed to the procedure (La Colla et al., 2015). Sub-sample portions of 0.4 ±0.01 g (dry weight) were put in test tubes, spiked with 5 ml of concentrated nitric acid (65%, Merck) and left in predigestion overnight or for 2-3 hours. Later, 1 ml of concentrated perchloric acid

(70–72%, Merck) was added and the tubes placed in a glycerine bath at 120 ± 5°C for 72 h or until the volume was reduced to less than 1 ml. After the acid digestion, 0.7 % nitric acid was added to the residue up to 10 ml in centrifuge tubes. Metal concentrations were measured with an inductively coupled plasma-optical emission spectrometry (ICP OES) Perkin Elmer Optima 2100 DV with axial view. The results are expressed in µg/g dry weight.

Analytical grade reagents, reagent blanks and certified reference materials (CRM) were used to ensure analytical quality control. Estuarine sediments (LGC6137), provided by the Laboratory of the Government Chemist (UK), were used as CRM for sediments, and pepperbush (R.M. N º1), provided by The National Institute for Environmental Studies (Japan), for plants. The recovery percentages for all metals in their corresponding CRM were between 75-115 %. In addition, all samples were analyzed in duplicate and the coefficient of variation was <10%. The analytical method detection limit (MDL) for each metal (µg/g) was: 4.14 for Pb; 0.82 for Ni; 1.99 for Cu and 5.17 for Zn.

2.5. Cleaning procedures

All material used during sampling and in the laboratory was conditioned according to internationally recommended protocols (APHA, 1998). The cleaning procedure included washing the material with non-ionic detergent, rinsing it with tap water and then three times with deionized water. The material was then soaked for 24 h in a diluted nitric acid solution (5%) and finally rinsed three times with deionized water.

2.6. Data processing and statistical analyses

One-way analysis of variance (ANOVA) followed by Tukey or a non-parametric test (Kruskal-Wallis) followed by non-parametric multiple comparison tests were performed to assess differences, between sites, in the physico-chemical characteristics of the sediment (OM content, percentage of fine sediment (<63µm), Eh and pH) and in the metal concentrations in sediments, using the data for the whole study period. In addition, one-way ANOVAs or Kruskal-Wallis tests (followed by the appropriate multiple comparison test) were performed to evaluate differences between sampling dates, within each site, in the above mentioned sediment characteristics and in metal concentrations.

Two-way ANOVAs were performed, within each site, to evaluate differences in the metal concentrations between plant tissues (dead, live and belowground) and sampling dates. Bioconcentration (metal in belowground/metal in sediments; BCF) and translocation (metal in live/metal in belowground; TF) factors were also calculated. In case of BCF >1, there is an active accumulation; otherwise, BCF<1 means that no accumulation can be stated. Similarly, TF> 1 indicates that the metal has been translocate to the aerial part of the plant. Differences in biomass, within each site, between tissues (live, dead and belowground) and sampling dates were evaluated by two-way ANOVAs. In all cases, after two-ways ANOVAs, multiple comparisons were performed with Tukey test. To compare sites regarding the content of metals in plant tissues, we performed one-way ANOVA or Kruskal-Wallis using the data from the whole study period. Metal pools in each plant tissue (live, dead and belowground) were calculated multiplying the metal concentration at each sampling date and the corresponding biomass value; this was done for each site. Since all aboveground tissues are ultimately exported as detritus, live and dead pools were finally added to obtain aboveground pools.

All statistical analyses were carried out using STATISTICA 7.0 (StatSoft, Inc.), following Zar (2010). If necessary, data was previously ln-transformed (except biomass that was square-root-transformed) to meet the required assumptions of homogeneity of variances and normality for the parametric tests. The acceptable level of statistical significance was less than the 5%. Data presented in figures or tables were not transformed. Error values, either in figures, tables or in text, represent standard deviation.

3. Results

3.1. Sediment characteristics

Sampling sites differed significantly in the OM content and the grain size composition of the sediment, but not in the rest of the physico-chemical characteristics analyzed, considering the data from the whole study period (annual mean, Table 1). The content of OM was lower at site 2 (~2.4 %) than at the rest of the sites, which showed no differences between them (~ 8.7 % in average) (Kruskal-Wallis: $p<0.001$). The percentage of fine sediment (silt+clay) was also significantly lower at site 2 (~32 %) than at the other sites, which were similar (80-96 %) (ANOVA: $p=0.0016$; Tukey: $p<0.05$). Neither pH nor Eh differed significantly between sites (ANOVA: $p= 0.36$ and Kruskal Wallis: $p=0.077$, respectively). However, a trend of higher Eh values was observed at site 2 with respect to sites 1 and 3; significant differences could be masked by the high variability of this parameter at all sites (Table 1).

Variation in the physico-chemical characteristics of sediments over the study period is also shown in Table 1. OM content and pH did not vary over the study period at any site (ANOVA: $p>0.085$), except pH at site 3 (ANOVA: $p= 0.046$). Atthis site, pH was significantly higher in May 2012 than in February 2012 (Tukey: $p<0.05$). On the other hand, Eh showed significant differences between sampling dates at the three sites (ANOVA: $p<0.001$ in all cases). At site 1, Eh values were lower in February 2012 than in May 2012, whereas site 2 exhibited significantly lower Eh values in February 2012 than on the rest of the sampling dates analyzed (November 2011 and August 2012) (Tukey: $p<0.05$ in all cases). At site 3, Eh values in both November 2011 and February 2012 were lower than in May 2012 (Tukey: $p<0.05$).

(insert Table 1) Table 1. Physico-chemical characteristics (mean ± SD) of the sediments vegetated by *Spartina alterniflora* at three sites within the Bahía Blanca estuary. Different letters indicate significant differences between sites considering the whole study period. Note: annual mean values of fine sediment correspond to three replicates from May-12; md: missing data due to malfunction of the equipment.

3.2. Metal concentrations in sediments

Concentrations of the studied metals in sediments showed the same decreasing order at all sites: Zn> Cu> Pb ≥ Ni. Concentrations of the studied metals differed significantly between sites (ANOVA: $p<0.001$ for Pb, Ni and Zn, and Kruskal-Wallis: $p<0.001$ for Cu). The concentrations of all metals were lower at site 2, compared to the other sites;

in addition, for Pb and Ni, there were significant differences between the concentrations found at the sites 1 and 3 (Tukey or non-parametric multiple comparison, as appropriate: $p<0.05$) (Figure 2).

(insert Figure 2) Figure 2. Concentration (mean ± SD) of Pb, Ni, Cu and Zn (µg/g dry weight) in sediments vegetated by *Spartina alterniflora* at three sites within the Bahía Blanca estuary, in average for the whole study period. Different letters indicate significant differences in the same metal between sites (n=16, except for Pb at site 2 where n= 14).

Comparing metal concentrations in sediments between sampling dates within each site, we also found some significant differences (Figure 3). The concentrations of metals at sites 1 and 2 did not show any variations in any metal over the study period (Kruskal Wallis for Cu and Zn and ANOVA for Pb and Ni; $p>0.15$ in all cases). However, at site 3, the concentration of Pb was significantly higher in February and in May 2012 than in August 2012 and the concentrations of Ni, Cu and Zn were significantly higher in February 2012 than on the rest of the sampling dates (ANOVA and Tukey: $p<0.05$ in all cases).

(Insert Figure 3) Figure 3. Seasonal variations in the concentration (mean ± SD) of Pb, Ni, Cu and Zn (µg/g dry weight) in sediments vegetated by *Spartina alterniflora* at three sites within the Bahía Blanca estuary. Different letters indicate significant differences between sampling dates within each site (n= 4, except for Pb at site 2 in February where n=2)

3.3. Concentration of metals in *S. alterniflora*

Concentrations of the studied metals in tissues of *S. alterniflora* followed the same order as in the vegetated sediments (*i.e.* Zn> Cu> Pb ≥ Ni). While Cu and Zn were always above the MDL, most samples showed non-detectable values of Pb and Ni. Indeed, Ni was only detectable in belowground tissues and at times in dead tissues, whereas Pb was only detectable in belowground tissues from sites 1 and 2. At each site, the concentrations of all detectable metals always differed significantly between tissues and sometimes between sampling dates (Figure 4).

Metal concentrations in plant tissues at site 1 are shown in Figure 4a. Pb concentrations were only detectable in belowground tissues; hence, only seasonal dynamics in this tissue could be evaluated, showing no significant differences between sampling dates ($p=0.926$). Ni concentrations were significantly higher in belowground tissues than in dead ones ($p<0.001$) (using only data from November 2011 and May 2012 due to the lack of information from February and August 2012 for dead tissues). In addition, belowground tissues did not differ significantly between sampling dates ($p=0.079$). Cu and Zn concentrations in belowground tissues were approximately 5 times higher than in aboveground ones. Levels of Cu in belowground tissues were always significantly higher than in dead and live tissues; moreover, Cu concentration in dead tissues was usually (except in May 2012) lower than in live ones, but this difference was only significant in November 2011 (Tukey: $p<0.05$). No significant differences were observed between sampling dates for belowground tissues, but differences were detected for both live and dead ones: in both types of tissues, the concentration was highest in May 2012, but the difference was only significant in respect to a single sampling date (February 2012 for live tissues and November 2011 for dead ones) (Tukey: $p<0.05$). Zn concentrations followed the same order (live<dead<belowground tissues) on all sampling dates, although the differences were only significant between belowground tissues and both live and dead tissues (Tukey: $p<0.05$). Significant differences between sampling dates were only observed for belowground tissues, with higher concentration in May 2012 than in November 2011 (Tukey: $p<0.05$).

Metal concentrations in plant tissues at site 2 are shown in Figure 4b. Pb was only detectable in belowground tissues in November 2011 and August 2012; therefore, no statistical comparison was performed for this metal. Ni levels were higher in belowground tissues than dead ones, but the differences were only significant in August 2012 (Tukey: $p<0.05$). No seasonal variation was appreciated in the belowground tissues, but some significant differences were observed for the dead ones: Ni concentration in August 2012 was significantly lower than in February 2012 and November 2011 (Tukey $p<0.05$). Cu and Zn concentrations in belowground tissues were at least twice as high as in the aboveground ones, being as much as 10 times higher on some dates in the case of Zn. Cu concentrations were always higher in belowground tissues than in dead and live ones, always with significant differences in respect to live tissues, whereas significant differences between belowground and dead tissues were only found on some sampling dates (*i.e*. November 2011 and May 2012); between both types of aboveground tissues, the Cu concentration was always lower in live ones, although the difference was only significant in February 2012 (Tukey: $p<0.05$). No statistical differences were detected between sampling dates for either type of tissue ($p>0.50$). Zn concentrations were always significantly higher in belowground tissues than in dead and live ones; between live and dead, there is a trend of lower values in live tissues, although the differences were only significant in November 2011 and February 2012 (Tukey: $p<0.05$). Regarding seasonal variation, it was only observed for belowground and dead tissues: Zn concentration was highest in November 2011 and May 2012 for both types of tissues, with significant differences compared to August 2012 for dead tissues and in respect to February 2012 for belowground ones (Tukey: $p<0.05$).

Metal concentrations in plant tissues at site 3 are shown in Figure 4c. Pb was non-detectable in all plant samples. Ni concentrations were significantly higher in belowground tissues than in dead ones ($p<0.001$) (using only data from November 2011 and February 2012 due to the lack of information about May and August 2012 for dead tissues). Moreover, belowground tissues did not differ significantly between sampling dates ($p=0.886$). Cu and Zn concentrations in belowground tissues were always several times higher than in aboveground tissues, mainly in November 2011 when Cu levels in belowground tissues were 50 times higher than in the aboveground ones. Cu concentrations in belowground tissues were significantly higher than those found in dead and live ones; between live and dead, there was a trend of lower values in live tissues (except in August 2012) although the differences were always non-significant (Tukey: $p<0.05$). Regarding seasonal variation, it was only found for belowground tissues, with the Cu concentration in November 2011 being significantly higher than in the rest of the study period (Tukey: $p<0.05$). Zn concentration was always significantly higher in belowground tissues than in aboveground ones; between live and dead, levels were usually higher in dead tissues (except in August 2012), although the difference was only significant in November 2011 (Tukey: $p<0.05$). The only significant difference in Zn concentration between sampling dates was observed for dead tissues, with values significantly higher in November 2011 in respect to August 2012 (Tukey: $p<0.05$).

(Insert Figure 4) Figure 4. Concentration (mean ± SD) of Pb, Ni, Cu and Zn (μg/ g dry weight) in live, dead and belowground tissues of *Spartina alterniflora* at three sites within the Bahía Blanca estuary over the study period ($2\leq n\leq 4$)

Given that the concentrations of all metals were higher in the belowground tissues, we compared the concentrations of Ni, Cu and Zn in this tissue between sites (not enough data was available for Pb). Ni concentration was significantly higher at site 3 than at site 1 (ANOVA: $p=0.011$; Tukey: $p<0.05$) whereas the opposite situation was observed for Zn concentration: levels were significantly higher at site 1 than at site 3 (ANOVA: $p<0.001$; Tukey: $p<0.05$). Cu concentrations did not show any significant differences between sites (Kruskal-Wallis: $p= 0.248$).

3.4. Bioconcentration and translocation factors

BCFs were calculated for Pb, Ni, Cu and Zn for all sites, when data was available (Figure 5). For Pb, BCF presented a different behavior at each site: at site 1 BCF was below 1 for all sampling dates, whereas at site 2 this factor could only be calculated for two sampling dates, being above 1 in November 2011 and below 1 in August 2012; at site 3 BCF could never been calculated. For Ni, BCF varied over the study period but was always below 1, at the three sites and on all sampling dates. For Cu, BCF varied the most, both between sampling dates and between sites. It was always below 1 at site 1 and always above 1 at site 2; at site 3 it was above 1 in November 2011 and below 1 on the rest of the sampling dates. For Zn, BCF was always above 1, for all sites and sampling dates. BCFs for all metals were always higher at site 2 than at the rest of the sites over the study period.

(Insert Figure 5) Figure 5. BCF (bioconcentration factor) (mean ± SD) of Pb, Ni, Cu and Zn of *Spartina alterniflora* at three sites within the Bahía Blanca estuary over the study period ($2\leq n\leq 4$). The red line indicates BCF=1

TFs were only calculated for Cu and Zn since Pb and Ni were always below MDL in live tissues. TFs for both metals were always below 1 at the three sites and on all sampling dates. For Cu, TF varied between 0.099 ± 0.024 (November 2011, site 2) and 0.38 ± 0.41 (November 2011, site 1). For Zn, TF ranged between 0.074 ± 0.007 (November 2011, site 2) and 0.19 ± 0.14 (November 2011, site 1).

3.5. Biomass and metal pools

Both live and dead aboveground biomass was usually higher than belowground biomass at all sites; moreover, some differences between sampling dates were also observed within each site. However, the differences were only significant in some cases, probably due to the variability of the data (Figure 6). At site 1, dead and live aboveground biomass were only significantly higher than belowground biomass in February 2012 and seasonal variation was only reported for live tissues, with higher biomass in February than in May and August 2012 (Tukey: $p<0.05$). At site 2, significant differences were never detected, either between tissues ($p=0.063$) or between sampling dates ($p=0.074$). At site 3, the seasonal pattern was similar to site 1, but with significant differences between February 2012 and the rest of the sampling dates for live biomass; with respect to differences between tissues, live biomass was significantly higher than belowground biomass on several sampling dates (November 2011, February 2012 and August 2012) (Tukey: $p<0.05$).

(Insert Figure 6) Figure 6. Live, dead and belowground biomass (mean ± SD) of *Spartina alterniflora* at three sites within the Bahía Blanca estuary over the study period (n=4)

Pools of Pb, Ni, Cu and Zn in *S. alterniflora* are shown in Table 2. Metal pools were highly variable between sites, tissues and sampling dates. Within each site, belowground pools of Ni, Cu and Zn were usually higher than aboveground ones, in agreement with higher concentration of metals in this tissue. However, in some sites and sampling dates, aboveground pools were higher than belowground ones, especially at site 3 and in February 2012.

(Insert Table 2) Table 2. Live, dead, above (live + dead) and belowground pools of Pb, Ni, Cu and Zn (mg/m^2; mean ± SD) of *Spartina alterniflora* at three sites within the Bahía Blanca estuary. Values in bold indicate a higher pool (between above and belowground) on each sampling date and site for each metal. Note: Ni aboveground pool is only represented by the dead pool, since Ni was always undetectable in live tissues

4. Discussion

4.1. Physico-chemical characteristics and metal concentration in sediments

Sediments vegetated by *S. alterniflora* at three sites within the BBE differ in both the physico-chemical characteristics and the metal concentrations. The contents of OM and fine sediments were lower at site 2, where the concentrations of all analyzed metals were also lower. The lower content of OM at site 2 might be associated with the lower biomass of *S. alterniflora* at this site (see Figure 6), since the contribution of this species to the soil carbon pool is significant (Liao et al., 2008; Wang et al., 2018). Sediments with a high content of OM and fine sediments are capable of binding metal ions (Kabata-Pedias, 2011), and several studies have shown that intertidal sediments with these characteristics certainly have higher levels of metals (e.g., Reboreda et al., 2008; Wang et al., 2013; Idaszkin et al., 2014; 2017; Serra et al., 2017; Simonetti et al., 2017). However, in some salt marshes, or for a particular metal, no such relationship was observed (e.g., Idaszkin et al., 2014; Marinho et al., 2017). This emphasizes the need for studying the physico-chemical characteristics of the sediments in different environments, and concerning different metals, in order to avoid misleading generalizations.

pH and Eh, other two key variables related to metal mobility in sediments, did not show any significant differences between sites, although a trend to higher levels of Eh was observed at site 2. This is also in agreement with the higher content of sand found at this site, which allows better penetration of oxygen into the sediments. Eh, in particular, is very important since under anoxic conditions bacterial sulphate reduction is the dominant process in sediments, which sequesters metals in insoluble precipitates (Reddy and DeLaune, 2008). Hence, under lower Eh, metals are immobilized in sediments, which is consistent with the higher concentrations reported at sites 1 and 3 compared to site 2. Similar results were achieved by other authors (e.g., Lima et al., 1989; Reboreda et al., 2008; Wang et al., 2013; Idaszkin et al., 2014; Pang et al., 2017).

Values of the physico-chemical variables studied in sediments were, in general, within the ranges reported for other *S. alterniflora* salt marshes (OM content ~1-13%, fine sediments ~50-95%, reducing conditions and neutral pH) (Lacerda and Abrão, 1984; Lima et al., 1989; Wang et al., 2013; Chai et al., 2014; Pang et al., 2017). However, the values of all the metals studied are usually lower than in sediments vegetated by this species in other coastal systems, as well as in previous studies in the BBE (Appendix, Table A1). The only exception was the higher levels of Cu compared to previous studies in the BBE (Botté, 2005; Hempel et al., 2008) and to a study from Brazil performed 3 decades ago (Lacerda and Abrão, 1984). Moreover, Cuoto et al. (2013), studying sediments vegetated by *S. maritima* in Mondego estuary (Portugal) ,which is considered as low-polluted, reported levels of Pb and Zn higher than ours, but the levels of Cu were in the same range as those reported in the present research. Summarizing, the relatively high values of Cu found in this study, and the trend to increase in salt marsh sediments from the BBE over the years - a trend also observed for unvegetated intertidal sediments (Simonetti et al., 2017) - set up an alarm with respect to the Cu concentrations in the BBE and emphasizes the role of regular monitoring of metals in sediments.

Regarding seasonal dynamics, differences between sampling dates were observed for some sediment characteristics (Eh at all sites and pH at site 3) and for all metals at site 3. The lowest Eh values were reported in February 2012 (summer) at all sites, and this might be related to the input of OM. The presence of OM (either as plant detritus or as organic compounds) stimulates anaerobic respiration and, hence, promotes reductive conditions in sediments (Reddy and DeLaune, 2008). In this study, live biomass of *S. alterniflora* was usually higher in February 2012 (see Figure 6) and, moreover, the release of organic compounds by salt marsh plants was found to be higher in summer (Mucha et al., 2010). In addition, the lowest Eh recorded in February 2012 was consistent with the highest metal concentration in sediments at site 3. High concentration of metals at that site in February 2012 is also coincident with the lowest pH on that date, and this might be explained by the fact that acidic conditions promote metal release from OM complexes (Reddy and DeLaune, 2008). Seasonal variations for metals and pH were only observed at site 3, which might be related to the specific characteristics of this site. Site 3 is located in the proximity of a channel that transports urban sewage discharge and near to a 200 m-long breakwater which restricts the circulation in this area (Cuadrado et al., 2005). This might imply that the dynamics of this site is more affected by the variations of local discharges, which are usually higher in summer.

The generally lower values of metals in sediments in the BBE might be related to the lower human impact in this estuary in comparison to other polluted systems, like estuaries and bays from China (Appendix, Table A1). Furthermore, since the BBE is highly affected by tides and winds, pollutants that come from industries and cities are redistributed with the general circulation within the estuary, making it difficult to identify point sources of metal pollution. This has already been observed for metals in unvegetated sediments (Simonetti et al., 2017) and for dissolved and particulate metals in seawater (La Colla et al., 2015; La Colla et al., submitted). Taking this into account, we infer that metal concentrations in sediments at the sites studied were not related to dissimilar pollution sources, as many authors have pointed out (e.g., Reboredo et al., 2008; Marinho et al., 2017; Pang et al., 2017). On the other hand, we consider that only the striking differences between sites in the OM content and grain size composition and, to a lesser extent Eh, rule the metal concentration patterns between the salt marshes in the BBE. This, in turn, has a significant effect on the uptake and bioconcentration of metals by the species studied, as discussed later (see sections 4.2 and 4.3).

4.2. Variation in metal concentration in tissues of S. *alterniflora*

Concentrations of Pb, Ni, Cu and Zn were higher in the belowground tissues than in the aboveground ones for all sites and sampling dates. This higher allocation of metals in belowground tissues is in agreement with the usual pattern in *S. alterniflora* (Appendix, Table A1) and other *Spartina* species (e.g., Caetano et al., 2008; Reboreda et al., 2008; Caçador et al., 2009; Cuoto et al., 2013; Idaszkin et al., 2014; Phillips et al., 2015). Regarding the aboveground tissues, the levels of metals were usually higher in dead tissues than in live ones. In general, the concentration of metals in aboveground tissues of *S. alterniflora* has been evaluated by separating the leaves from the stems and the concentration in live and dead tissues was only evaluated separately in a few studies (Appendix, Table A1). In those studies, metal concentrations were usually higher in dead than in live tissues, in agreement with our findings. Moreover, Weis et al. (2003) found higher accumulation of Cu, Zn and Pb in older than in young leaves of *S. alterniflora* in a greenhouse study in New Jersey (USA), which shows an accumulation of metals over the lifespan of the plant, suggesting the existence of a possible detoxification mechanism through leaf fall in *S. alterniflora.* Our results indicate that detritus of *S. alterniflora* should have a higher content of some metals relative to live standing aboveground tissues, which has important implications in detritus-based food webs (Weis et al., 2003; Weis and Weis, 2004), which are the dominant energy pathways in salt marshes (Moore et al., 2004; Bergamino and Richoux, 2014).

Seasonal dynamics in metal concentration was observed for all types of tissues, but not at all sites nor for all metals and, hence, seasonal patterns were usually not clear. The only consistent pattern was that the highest Cu and Zn concentrations were in May 2012 (autumn) at site 1. In most studies, metal concentrations in salt marsh plants have been evaluated on one sampling date and the few studies that performed seasonal sampling showed a trend of higher values in winter and autumn, which has been explained by a “dilution effect” during the growing season (Whidham et al., 2003; Cuoto et al., 2013; Son and Sun, 2014). At site 1, the biomass of *S. alterniflora* in May 2012 was low (Figure 6), which is coincident with the highest metal concentrations; thus, seasonal dynamics at site 1 could be explained by the dilution effect. However, no such relationship was found for sites 2 and 3. Seasonal dynamics of physico-chemical characteristics of the sediments may also be affecting the seasonality of metals in plant tissues. High Eh values, which imply a release of metals from the sediment and a greater availability for plant uptake, were observed in May 2012 at site 1, and this might be another explanation for the high values of metals in tissues in autumn at this site. Once again, no such a relationship was observed for the other sites, not even at site 3, where both pH and metal concentrations in sediments varied seasonally. This shows the complexity of seasonal patterns in dynamic environments like estuaries and, at least in this study, seasonality was less marked than patterns of metal distribution between tissues and between sites.

Comparing sites, the lower concentration of Ni, Cu and Zn found in sediments of site 2 were not reflected in lower concentrations in plants, as concentrations of these metals in belowground tissues from site 2 were never lower than those from sites 1 or 3. Once again, physico-chemical characteristics of the sediments might explain this. At site 2, the lower OM content and higher sand content implies that fewer metals are bound to the sediment matrix and, thus, more available for plant uptake (Kabata-Pedias, 2011). On the other hand, the higher Ni concentration in tissues at site 3 than at site 1 is in agreement with the concentrations of this metal in sediments. However, the higher Zn concentration at site 3 with respect to site 1 is not linked to the concentrations in sediments, highlighting the different behaviors of each particular metal under the same sediment conditions.

Pb, which is highly toxic (Nagajyoti et al., 2010), was found in sediments but it was not always uptaken by plants since it was undetectable in most plant samples. This metal was only reported in belowground tissues and values were usually lower than in the corresponding tissues of *S. alterniflora* in other salt marshes (Appendix, Table A1) or in other *Spartina* species (e.g., Reboreda and Caçador, 2007; Reboreda et al., 2008; Duarte et al., 2010; Cuoto et al., 2013; Phillips et al., 2015). Nonetheless, Pb concentrations in belowground tissues were higher than levels reported in previous studies in the BBE (Botté, 2005; Hempel et al., 2008) and in a Patagonian bay (Argentina) (Marinho et al., 2017). However, the values of Pb in sediments in this study are lower than in previous studies in the BBE and similar to those reported by Marinho et al. (2017); this would imply a greater efficiency in the uptake of this metal by *S. alterniflora* in the present study.

Ni was detected in below and dead aboveground tissues of *S. alterniflora* in the BBE. Ni levels reported here were, in general, lower than those found in other *S. alterniflora* marshes worldwide and than those reported in previous studies in the BBE (Appendix, Table A1). They were also lower than in other *Spartina* species (Caetano et al., 2008; Idaszkin et al., 2014). Ni^{2+} can increase due to human activities, such as sewage discharge and use of fertilizers, and the excess of Ni^{2+} causes various physiological alterations in plants (Nagajyoti et al., 2010). The undetectable values of Ni in live tissues in all samples might be related to an efficient detoxification mechanism for this metal. For Ni, there are many less studies available than for other metals which implies that there is a need for information on the distribution of this metal in plants, as has been pointed out opportunely by Gomez-Redondo (2013) in relation to *Spartina* species. Thus, the values of Ni reported here may constitute a reference level for researchers on the topic.

Cu and Zn concentrations in this study were, in general, in approximately the same range as the levels found in *S. alterniflora* salt marshes worldwide (Appendix, Table A1); this is stated not considering the unusual very high Cu concentration in November 2011 at site 3, which needs further research. However, in respect to Pang et al. (2017), Cu and Zn concentrations in belowground tissues were in general higher, whereas the values for aboveground tissues were lower. This is related to the fact that in salt marshes from the Hangzhou Bay most metals were translocated to aboveground tissues, in opposition to our findings (see section 4.3) and to the usual behavior in salt marsh species. Moreover, we found that both above and belowground values in this study were lower than in the Patagonian bay (Marinho et al., 2017), which is in agreement with the lower values of these metals in sediments in that salt marsh. Considering other *Spartina* species, Cu and Zn levels in the BBE were in general in the range reported worldwide (e.g., Caetano et al., 2008; Reboreda et al., 2008; Caçador et al., 2009; Duarte et al., 2010; Cuoto et al., 2013; Phillips et al., 2015).

4.3. Metal partitioning in *S. alterniflora* salt marshes within the BBE

Bioconcentration factors showed different patterns across metals, sites and sampling dates. Differences in BCFs are usually related to the metal considered (for instance, essential versus non-essential ones) but the physico-chemical characteristics of sediments can also have implications to determine the accumulation or not of metals in plant tissues (Reboreda et al., 2008; Marinho et al., 2017; Pang et al., 2017; Petranich et al., 2017). As already stated, sediment characteristics in site 2 makes metals more available for plant uptake than in the other sites, and this is in line with BCFs always higher at site 2. Moreover, at site 2, the BCFs were higher than 1 for all metals except Ni, meaning that the conditions at this site are favourable for accumulating Pb, Cu and Zn. Differences about metals need further explanation, which is given below.

Pb is non-essential and Ni is essential but only needed in very low concentrations for plant growth (Nagajyoti et al., 2010). In agreement with that, Ni showed limited uptake by plants, as appreciated by BCF always below 1, which is the usual pattern for this metal in *Spartina* salt marshes worldwide (Idaszkin et al., 2014; Pang et al., 2017). BCF for Pb was sometimes below 1 (always at site 1 and in August 2012 at site 2), sometimes higher than 1(November 2011 at site 2, when BCF ~2.5) and sometimes could not be calculated (site 3 and two sampling dates at site 2). Hence, BCF for Pb did not show a clear pattern, in agreement with other studies in *Spartina* marshes (Reboredo et al., 2008; Idaszkin et al., 2017; Marinho et al., 2017; Pang et al., 2017). Pang et al., (2017) also found different patterns at different sites when studying *S. alterniflora* in the Hangzhou Bay, and the authors related this to both the degree of pollution at each site (BCF >1 in polluted sites) and to the physico-chemical characteristics of the sediments. Consequently, Pb accumulation in salt marshes is not clear and further research is needed. Future studies about Pb in the BBE should be focused on site 2, since this site has different physico-chemical characteristics of sediments in respect to the other sites and the BCFs there were above 1, at least on one sampling date. Monitoring the dynamics of Pb is particularly important in the BBE due to the trend of increasing concentrations in tissues of *S. alterniflora* in comparison with previous studies.

Cu and Zn are essential metals with several functions in plants (Nagajyoti et al., 2010). BCF for Cu was always below 1 for site 1 and always above 1 for site 2; for site 3 BCF was below 1 except for the high value in November 2011. Thus, the capacity of *S. alterniflora* from the BBE to accumulate Cu seems to be highly variable with the sediment conditions. Comparing with *Spartina* marshes worldwide, BCF for Cu was sometimes higher and sometimes lower than 1 (Reboreda et al., 2008; Idaszkin et al., 2014; 2017; Pang et al., 2017). As far as Zn is concerned, BCF was always above 1, for all sites and sampling dates. This implies that this metal is easily accumulated in *S. alterniflora* tissues in the BBE despite the specific sediment conditions. Reported BCFs for Zn for *Spartina* marshes worldwide vary greatly, from below 1 to values even several times higher than ours (~20) (Reboreda et al., 2008; Idaszkin et al., 2014, 2017; Marinho et al., 2017; Pang et al., 2017). As for Pb, Pang et al., (2017) also reported differences between sites regarding BCFs for Cu and Zn, which were also attributed to the degree of pollution and the sediment characteristics.

Translocation factors were always below 1, meaning that there is an exclusion of metals from aboveground tissues at all sites, regardless of the sediment characteristics. This has been suggested as a metal tolerance strategy in wetland plants (Baker, 1981) and is the usual pattern in *Spartina* species (Redondo-Gómez, 2013). TFs lower than 1 were also reported for *S. alterniflora* in salt marshes in the USA (Whidman et al., 2003) and in the Patagonian salt marsh (Marinho et al., 2017).

4.4 Allocation patterns of biomass and metals in *S. alterniflora* salt marshes within the BBE

It is believed that *Spartina* has the majority of its biomass allocated in the belowground tissues (Redondo-Gómez, 2013). In fact, the belowground biomass of several *Spartina* species from different salt marshes was reported to be as high as 80% of the total biomass (Whidman et al., 2003; Caçador et al., 2009; Duarte et al., 2010; Shao et al., 2013; Tripathee and Schäfer, 2014; Lian et al., 2007). A previous study in the BBE (site 2) also reported slightly higher belowground biomass (Negrin et al., 2012b). However, in this study we did not find that pattern: both live and dead aboveground biomass was higher than belowground biomass at all sites, although in most cases the differences were not significant, especially at site 2, probably due to the greater variability in this parameter. As for the metal concentrations, seasonal patterns were limited and were only found for live biomass, with the highest levels in summer, especially for sites 1 and 3.

Element pools are dependent, by definition, on both the element concentrations in tissues and on biomass. However, the extent to which the pools rely on each factor is variable. For example, metal pools of *S. alterniflora* in New Jersey (USA) were more influenced by element concentrations (Whidman et al., 2003) whereas metal pools in *S. maritima* in the Tagus estuary reflected mainly biomass dynamics (Caçador et al., 2009). On the other hand, Lian et al. (2017) found differences in the importance of each factor in relation to the metal considered. Moreover, in a previous study in the BBE (site 2) with *S. alterniflora*, carbon, nitrogen and phosphorus pools showed a higher influence of biomass (Negrin et al., 2012b). In the present research, despite the variability between sites, tissues and sampling dates, belowground pools of Pb, Ni, Cu and Zn were usually higher than aboveground ones showing, in general, a greater influence of metal concentration in pools. Higher belowground pools imply a greater release of metals *in situ*, through decomposition. However, in certain sites and sampling dates, aboveground pools were higher than belowground ones, with the consequent higher production of metal-containing detritus. This would impact on the detritus-based food webs in the salt marsh of origin and also in other areas within the BBE through exportation, which is usually significant in mesotidal systems, especially during spring tides (Duarte et al., 2017).

5. Conclusions

The physico-chemical characteristics of sediments played a key role in the metal concentrations in the sediments from the BBE, especially OM content and grain size composition, showing that the site with lower OM and fine sediment contents (site 2) also had lower levels of Ni, Pb, Cu and Zn in sediments. Nevertheless, site 2 did not have lower metal concentrations in plant tissues, showing that the sediment conditions were not only critical for metal concentrations in sediments but also for the capacity of metal uptake by plants. Despite the differences in physico-chemical characteristics in sediments and in metal concentrations in sediments and plant tissues between sites, metals followed approximately the same pattern of partitioning between tissues at the three sites: all metals were in higher concentrations in belowground tissues than in aboveground ones and, in general, higher in dead tissues than in live ones. Some metals (especially Zn) at some sites (especially site 2) were accumulated in *S. alterniflora* tissues in respect to the sediments, whereas others (Ni) showed limited uptake by plants and none of the metals studied were translocated to aboveground tissues.

Seasonal dynamics were important in some cases for the physico-chemical characteristics of the sediments, and for the metal concentrations in sediments at one site. Regarding the plants, differences between sampling dates were observed for live biomass and for metal concentrations in plant tissues in some cases. However, seasonal differences were always less noticeable and consistent than the differences between sites and tissues. Therefore, the partitioning of each metal between sediments, belowground tissues and aboveground tissues is mainly dependent on the sediment characteristics and the considered metal and, to a lesser extent, on the time of the year.

Both the live and dead aboveground biomass was usually higher than the belowground biomass at all sites. However, allocation patterns of each metal in tissues of *S. alterniflora* were mainly dependent on the metal concentrations, with the belowground pools usually higher than the aboveground ones, which implies a greater release of metals within the salt marsh of origin. Nevertheless, on some specific sampling dates and at some sites the aboveground pools were higher than the belowground ones due the very high aboveground biomass. The importance of aboveground pools in those cases should be considered in the context of this mesotidal estuary with high tidal energy and strong winds, since metal-containing detritus can be exported to other areas of the estuary, even to the southern salt marshes which are less impacted by cities and industries.

Our results indicate that *S. alterniflora* from the BBE is efficient in accumulating some metals, which implies that this species could be used for phytoremediation purposes. The accumulation capacity was observed despite the generally low metal concentrations in sediments and plant tissues, in comparison with other salt marshes worldwide. Further research is needed to expand our understanding of the influence of the physico-chemical characteristics of sediments in metal accumulation in this estuary. Moreover, laboratory studies on the addition of metals under controlled conditions would allow us to predict the probable performance of this species in a scenario of incidental loads of metals in the system, and this is the next step of our research. Furthermore, monitoring of metals in salt marsh sediments and plants should continue in the BBE, especially regarding Pb and Cu, given the trend of increasing levels that both metals have shown in respect to previous studies in this estuarine system.

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Table and figure captions (color should NOT be used for any figures in print)

Figure 1. Location of the sampling sites within the Bahía Blanca estuary (Argentina)

Figure 2. Concentration (mean ± SD) of Pb, Ni, Cu and Zn (µg/g dry weight) in sediments vegetated by *Spartina alterniflora* at three sites within the Bahía Blanca estuary, in average for the whole study period. Different letters indicate significant differences in the same metal between sites (n=16, except for Pb at site 2 where n= 14).

Figure 3. Seasonal variations in the concentration (mean ± SD) of Pb, Ni, Cu and Zn (µg/g dry weight) in sediments vegetated by *Spartina alterniflora* at three sites within the Bahía Blanca estuary. Different letters indicate significant differences between sampling dates within each site (n= 4, except for Pb at site 2 in February where n=2)

Figure 4. Concentration (mean ± SD) of Pb, Ni, Cu and Zn (µg/ g dry weight) in live, dead and belowground tissues of *Spartina alterniflora* at three sites within the Bahía Blanca estuary over the study period ($2 \leq n \leq 4$)

Figure 5. BCF (bioconcentration factor) (mean ± SD) of Pb, Ni, Cu and Zn of *Spartina alterniflora* at three sites within the Bahía Blanca estuary over the study period ($2 \leq n \leq 4$). The red line indicates BCF=1

Figure 6. Live, dead and belowground biomass (mean ± SD) of *Spartina alterniflora* at three sites within the Bahía Blanca estuary over the study period (n=4)

Table 1. Physico-chemical characteristics (mean ± SD) of the sediments vegetated by *Spartina alterniflora* at three sites within the Bahía Blanca estuary. Different letters indicate significant differences between sites considering the

whole study period. Note: annual mean values of fine sediment correspond to three replicates from May-12; md: missing data due to malfunction of the equipment.

Table 2. Live, dead, above (live + dead) and belowground pools of Pb, Ni, Cu and Zn (mg/m^2; mean ± SD) of *Spartina alterniflora* at three sites within the Bahía Blanca estuary. Values in bold indicate a higher pool (between above and belowground) on each sampling date and site for each metal. Note: Ni aboveground pool is only represented by the dead pool, since Ni was always undetectable in live tissues

Table A1. Metal concentrations (µg/g) in sediments vegetated by *Spartina alterniflora* and in tissues of this species from natural coastal systems worldwide and from previous studies in the Bahía Blanca estuary. Values are given as a range of mean values (± standard deviation (SD) or ± standard error (SE)) or as a range of values (minimum and maximum). Note: *: SD; $: SE; nd: non-detectable; ": data taken from figure; ¶: unusual very high level at site 3 in November 2011.

Table 1. Physico-chemical characteristics (mean ± SD) of the sediments vegetated by *Spartina alterniflora* in three sites within the Bahía Blanca estuary. Different letters indicate significant differences between sites considering the whole study period.

Site	Sampling date	Fine sediments (%)	OM (%)	pH	Eh (mV)
Site 1	Nov-11		10.75±0.92	6.85±0.22	-66.5±118
	Feb-12		8.11±1.55	7.03±0.56	-237.32±85.9
	May-12		9.86±1.16	7.87±0.33	37.25±58.33
	Aug-12		9.21±1.95	7.59±1.19	-131.4±62.9
	Annual mean	**80±12^{b}**	**8.9±1.75^{b}**	**7.26±0.62^{a}**	**-100±119^{a}**
Site 2	Nov-11		2.05±0.64	7.28±0.24	43.12±35.43
	Feb-12		2.21±0.81	6.97±0.33	-225.75±48.7
	May-12		2.42±0.86	md	md
	Aug-12		2.79±1.02	6.71±0.78	104.32±42.5
	Annual mean	**32±9.6^{a}**	**2.36±0.75^{a}**	**6.99±0.51^{a}**	**-26±157^{a}**
Site 3	Nov-11		9.02±0.30	7.01±0.41	-191.9±16.4
	Feb-12		8.44±0.27	6.86±0.11	-162.6±42.4
	May-12		7.90±0.27	7.38±0.10	-92.5±21.32
	Aug-12		7.88±0.34	md	md
	Annual mean	**96.9±0.84^{b}**	**8.56±0.45^{b}**	**8.56±0.45^{a}**	**-149±51^{a}**

Note: annual mean values of fine sediment corresponds to three replicates from May-12; md: missing data due to malfunction of the equipment.

Table 2. Live, dead, above (live + dead) and belowground pools of Pb, Ni, Cu and Zn (mg/m^2; mean ± SD) of *Spartina alterniflora* in three sites within the Bahía Blanca estuary. Values in bold indicate higher pool (between above and belowground) in each sampling date and site for each metal

		Pb	**Ni**		**Cu**				**Zn**			
Site	**Sampling date**	**Belowg. pool**	**Aboveg. Pool**	**Belowg. pool**	**Live pool**	**Dead pool**	**Aboveg. pool**	**Belowg. pool**	**Live pool**	**Dead pool**	**Aboveg. pool**	**Belowg. pool**
site 1	Nov-11	1.30±0.50	0.42±0.13	**0.69±0.15**	1.25±0.79	0.60±0.14	1.86±0.92	**5.39±0.38**	6.61±3.70	11.98±2.40	18.6±5.3	**41.7±16.4**
	Feb-12	0.87±0.15	-	**0.31±0.01**	1.74±0.85	1.16±0.33	**2.91±0.98**	1.90±0.59	10.66±2.97	12.38±5.99	23.0±7.5	**24.3±5.7**
	May-12	1.11±0.37	0.39±0.10	**0.42±0.20**	1.44±0.36	1.82±0.42	3.26±0.68	**4.63±1.71**	6.16±1.79	10.57±2.37	16.7±3.7	**55.9±16.7**
	Aug-12	1.51±0.11	-	**0.38±0.17**	0.55±0.09	0.94±0.15	1.49±0.16	**3.47±2.03**	2.78±0.80	7.29±2.22	10.1±2.9	**39.5±22.2**
site 2	Nov-11	1.25±1.14	**0.26±0.09**	0.25±0.24	0.39±0.09	0.40±0.14	0.79±0.19	**2.43±2.67**	2.10±0.59	3.30±1.09	5.4±1.6	**13.9±9.4**
	Feb-12	-	0.09±0.05	**0.49±0.22**	0.34±0.21	0.26±0.05	0.59±0.26	**1.90±0.92**	1.54±1.20	0.99±0.50	2.5±1.7	**11.1±6.2**
	May-12	-	0.07±0.04	**0.15±0.04**	0.23±0.09	0.25±0.15	0.47±0.23	**1.37±0.52**	1.32±0.60	1.17±0.51	2.5±1.1	**11.4±2.5**
	Aug-12	0.33±0.13	0.08±0.04	**0.17±0.05**	0.23±0.10	0.34±0.07	0.57±0.16	**1.28±0.03**	0.89±0.53	1.35±0.29	2.2±0.8	**10.0±1.8**
site 3	Nov-11	-	0.14±0.04	**0.19±0.13**	1.06±0.78	0.37±0.28	1.42±0.52	**15.0±5.16**	4.49±1.79	2.87±0.73	7.4±2.2	**8.54±3.5**
	Feb-12	-	**0.16±0.04**	0.12±0.09	1.87±0.44	0.44±0.13	**2.31±0.46**	0.65±0.28	11.83±1.75	2.49±0.54	**14.3±1.7**	6.58±2.8
	May-12	-	-	**0.24±0.26**	0.82±0.32	0.78±0.17	1.59±0.46	1.59±1.50	4.84±2.29	5.23±1.60	10.1±3.8	**22.5±23.6**
	Aug-12	-	-	**0.06±0.06**	0.58±0.23	0.58±0.38	**1.16±0.41**	0.55±0.74	3.15±0.85	2.88±2.38	**6.10±2.2**	4.7±6.3

Note: Ni aboveground pool is only represented by dead pool, since Ni was always undetectable in live tissues

Highlights

Metal concentrations in sediments and plants were always in this order: Zn>Cu>Pb ≥Ni>Cd

Site 2 achieved the lowest values for OM, fine sediments and all metals in sediments

All metals were in higher concentrations in belowground than in aboveground tissues

Bioconcentration factors for all metals were higher in site 2

Belowground pools were usually higher than aboveground pools for all metals

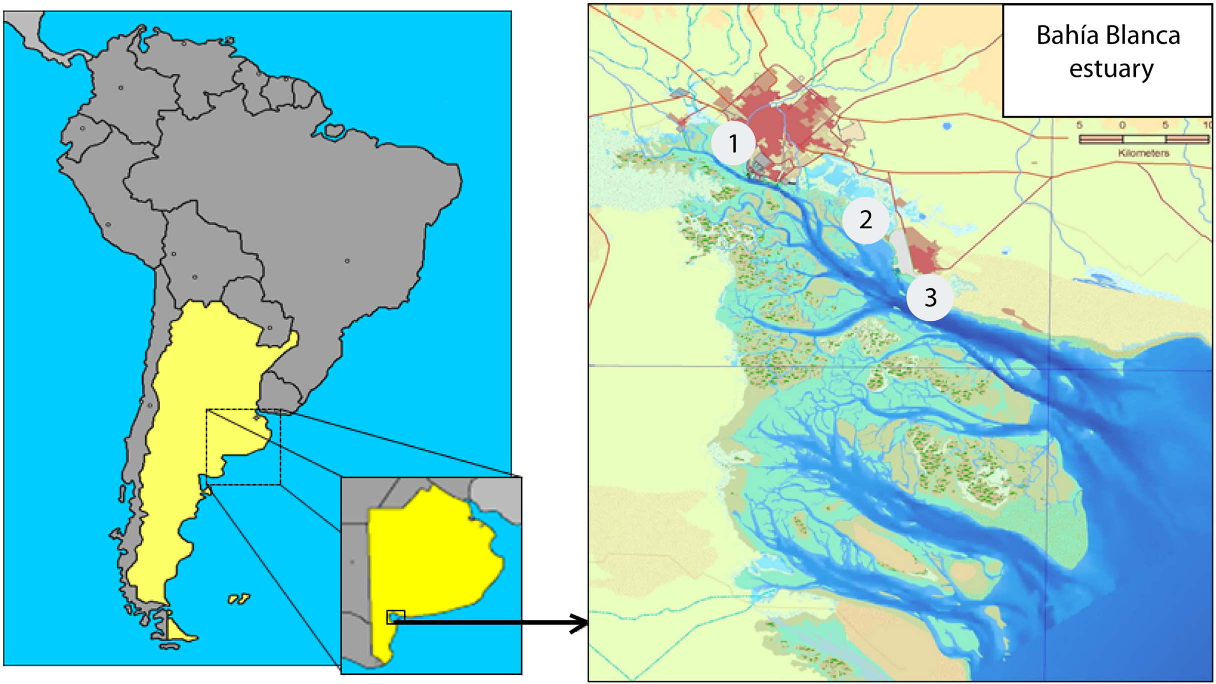
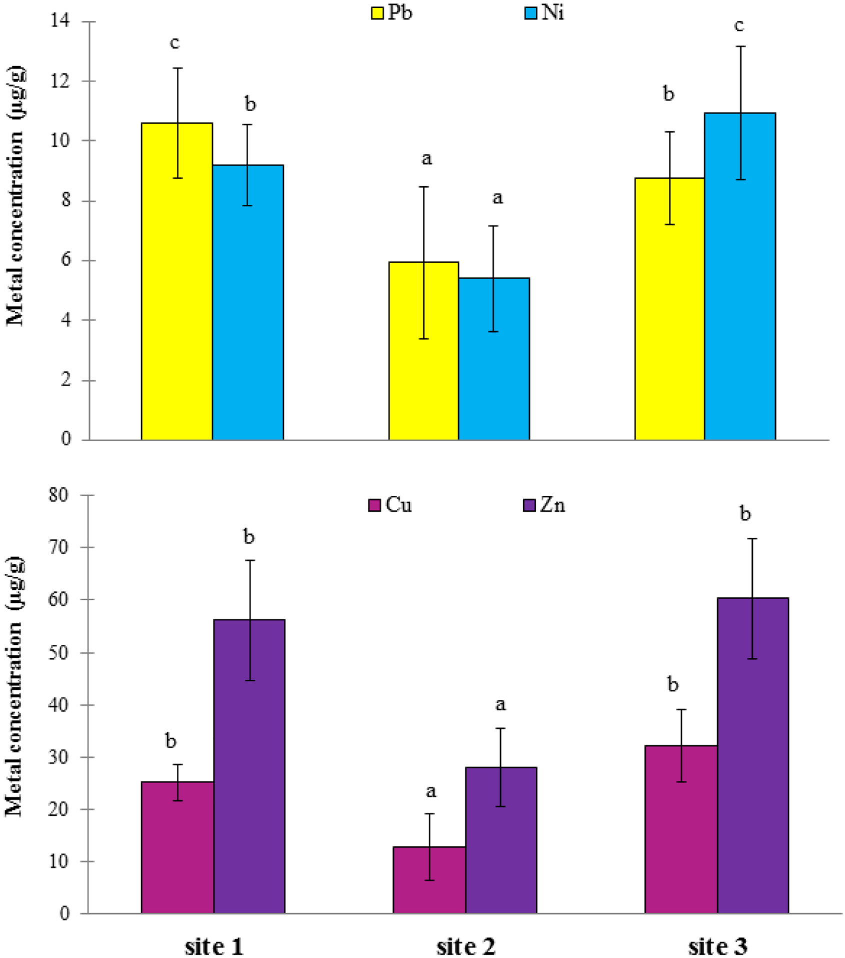
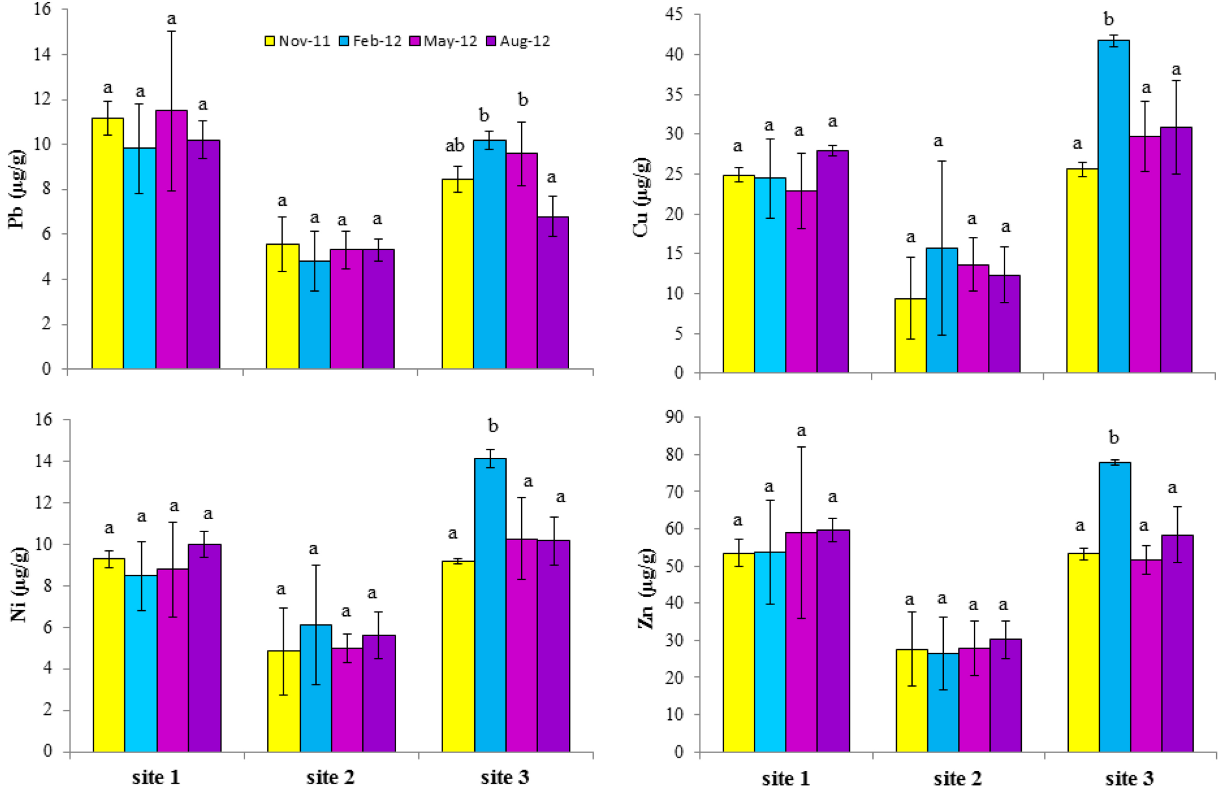


Figure 1



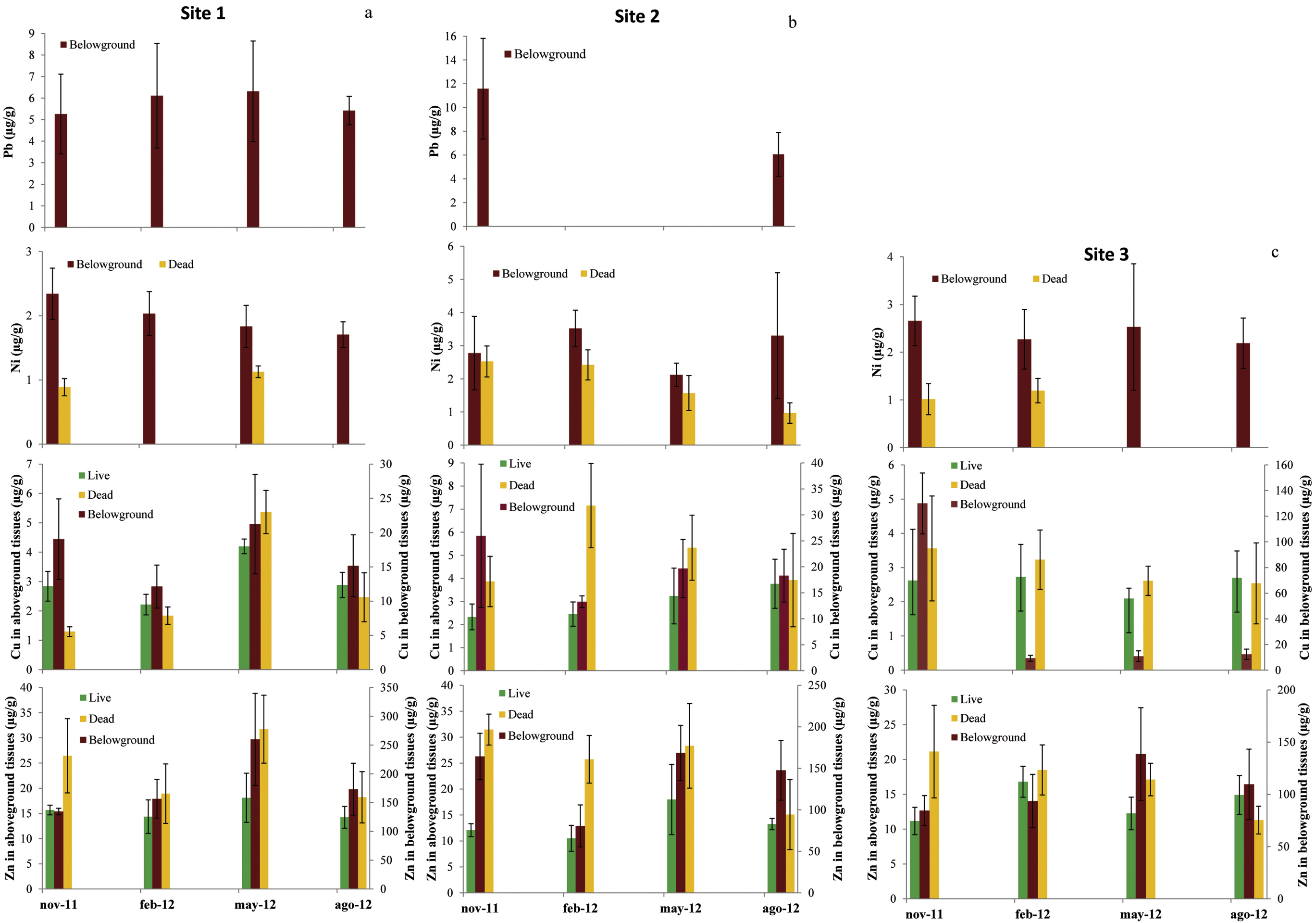
Pb
Ni
Cu
Zn
Metal concentration (µg/g)
Metal concentration (µg/g)
0
2
4
6
8
10
12
14
0
10
20
30
40
50
60
70
80
a
b
c
site 1
site 2
site 3

Figure 2



Nov-11
Feb-12
May-12
Aug-12
Pb (µg/g)
Cu (µg/g)
Ni (µg/g)
Zn (µg/g)
site 1
site 2
site 3

Figure 3



Site 1
a
Site 2
b
Site 3
c
Belowground
Dead
Live
Pb (µg/g)
Ni (µg/g)
Cu in aboveground tissues (µg/g)
Cu in belowground tissues (µg/g)
Zn in aboveground tissues (µg/g)
Zn in belowground tissues (µg/g)
nov-11
feb-12
may-12
ago-12

Figure 4

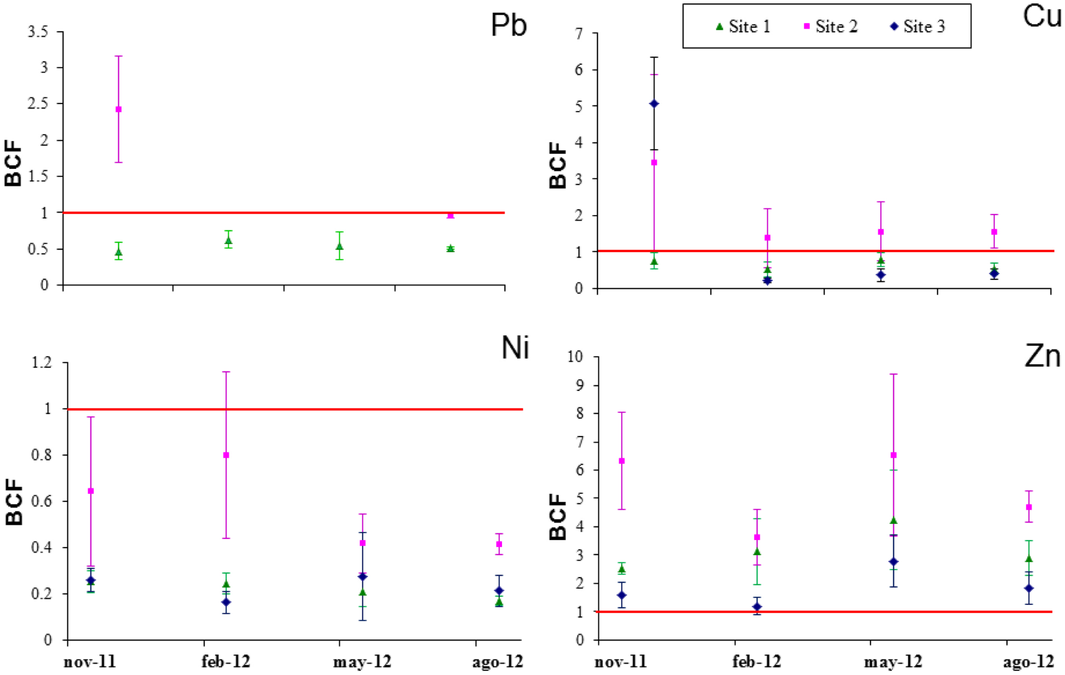
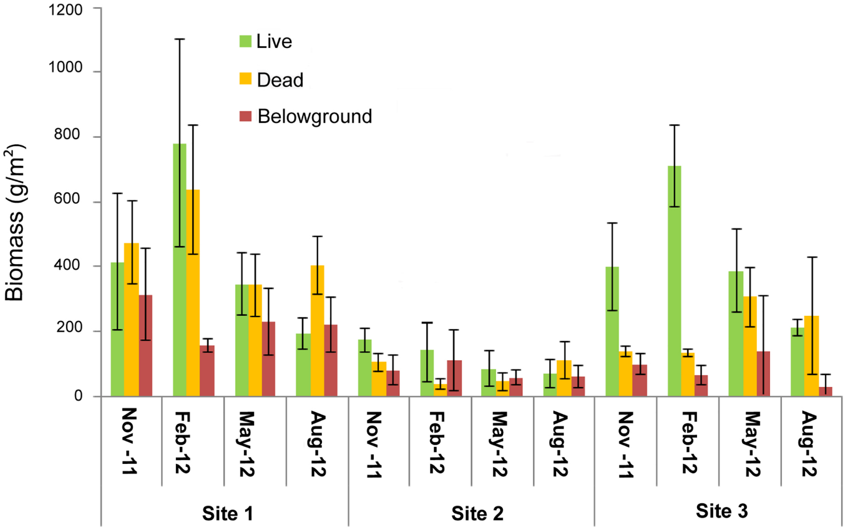

Pb
Cu
Ni
Zn
Site 1
Site 2
Site 3
BCF
BCF
BCF
BCF
3.5
3
2.5
2
1.5
1
0.5
0
7
6
5
4
3
2
1
0
1.2
1
0.8
0.6
0.4
0.2
0
10
9
8
7
6
5
4
3
2
1
0
nov-11
feb-12
may-12
ago-12
nov-11
feb-12
may-12
ago-12

Figure 5



Biomass (g/m^2)
1200
1000
800
600
400
200
0
Live
Dead
Belowground
Nov -11
Feb-12
May-12
Aug-12
Nov -11
Feb-12
May-12
Aug-12
Nov -11
Feb-12
May-12
Aug-12
Site 1
Site 2
Site 3

Figure 6