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# Assessment of the physicochemical conditions sediments in a polluted tidal flat colonized by microbial mats in Bahía Blanca Estuary (Argentina)

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ABSTRACT

The aim of this work is to assess the physicochemical conditions of the supratidal sediments colonized by microbial mats at two sites from Rosales Harbor (Bahía Blanca Estuary, Argentina) close to sewage discharge. Both sites differed in the size grain. No differences in pH, Eh and temperature were observed. Moisture retention and chlorophyll *a* concentration were significantly different between sites and sediment layers. Heavy metals and organic matter content were significantly higher in SII. No statistical differences were found in porewater nutrients concentration, being higher in SI (except DSi). The presence of *Escherichia coli* in water and sediment (1000 CFU/100 mL – uncountable and 35–40 CFU $g^{-1}$ dw, respectively) evidenced microbial contamination in the study area. The relationships between the physicochemical parameters evaluated and the influence of the sewage discharge allow defining two different areas in the Rosales Harbor despite the proximity and the presence of microbial mats.



## 1. Introduction

Estuaries have been recognized as dynamic, complex and unique systems, which are included among the most productive marine ecosystems in the world (Chapman and Wang, 2001). They frequently experience anthropogenic eutrophication, resulting in changes in the biogeochemical conditions (Buddemeier et al., 2001; Cloern, 2001; Rabalais and Nixon, 2002). Moreover, as they are sites of ports, industrial, urban and recreational developments, and also received the sewages discharges, they act as sink and/or transitional way of several chemical pollutants as well as nutrients between freshwater, land and the open sea.

Tidal flats are among the most productive components of shelf ecosystems because a variety of biogeochemical functions are performed in them, including sediment deposition, nitrogen and phosphorous removal and recycling of both terrestrially and marine-derived organic matter and nutrients (DeBusk, 1999). In addition, depending on the grain size, the sediments of tidal flats are usually rich in trace metals that easily bind to the large surface area of the clay particles. Physical, chemical, and biological interactions between tidal flats and saltwater estuarine system can have significant influences on the transport and distribution of nutrient, organic matter and trace metals.

Surface sediments of tidal flats may be colonized by different populations of phototrophic and chemotrophic microorganism. Microphytobenthos is the major component of intertidal sediment microbial communities in terms of biomass and production, and it is a primary source of fixed carbon for food webs and provides a food source for animals such as deposit feeders (Thornton et al., 2002 and reference therein). When the microphytobenthos is abundant, it can help to stabilize the surface of the sediments (Paterson et al., 1990) by decreasing its interstices and may produce macroscopically recognizable microbial mats, often dominated by the filamentous cyanobacteruim *Microcoleus chthonoplastes* or by visible biofilms of epipelic diatoms generally dominated by *Cylindrotheca closterium* (de Winder et al., 1999; Pan et al., 2013a; Noffke et al., 2002). Most components of the microphytobenthos live grow and are consumed in the upper millimeters of shallow ecosystems without vegetation. While this may seem restrictive, not be construed as the microphytobenthos is unimportant in the marshes (MacIntyre et al., 1996).

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Mats are ubiquitous in nature, they are found in a variety of different environments such as intertidal and supratidal sediments, marine salterm, hot springs, hypersaline ponds and deserts (Demergasso et al., 2003; Franks and Stolz, 2009; Lovelock et al., 2010; Guézennec et al., 2011). They commonly appear as floating masses in marine waters or over the sediments surfaces. Moreover, in shallow environments such as estuaries, they develop at the water sediment interface, where they stabilize the sediment (Bender and Phillips, 2004; García de Oteyza et al., 2006). Mats colonization is favored by clean, translucent and fine-grained quartz sand deposited at sites where hydrodynamic flow is sufficient to sweep clay minerals from mats surfaces but insufficient to erode bioestabilized laminae (Noffke et al., 2002). The vertical stratification and type of microorganism of these communities depend strongly on physiological requirements and physicochemical properties. Important physical properties include light, temperature and pressure. Key chemical parameters include oxygen, pH, redox potential, salinity, and available electron acceptors and donors, as well as the presence or absence of specific chemical species (Franks and Stolz, 2009; Guézennec et al., 2011). An important biological process of the mats is the secretion of extracellular polymeric substances (EPS) which are composed by carbohydrates (90%), proteins, lipids and lipopolysaccharide (Stal, 2010). This mucilaginous matrix in which the particles and organism are embedded is associated at motility of microorganism and stabilization of tidal flat (Stal and de Brouwer, 2003; Underwood et al., 2004).

Microbial mats play key geoactive roles in the biosphere, particularly in the areas of element biotransformations and biogeochemical cycling, metal and mineral transformations, decomposition, bioweathering, and soil and sediment formation (Gadd, 2010). Mats present in environments polluted by heavy metals have been the object of several research into metal biosorption (Incharoensakdi and Kitjaharn, 2002; De Philippis et al., 2003) and toxicity studies (Tripathi et al., 2003; Heng et al., 2004). Moreover, EPS generally contains high molecular weight compounds with charged functional groups and possess both adsorptive and adhesive properties. Due to the presence of charged moieties, EPS ideally serves as a natural ligand source, providing binding sites for other charged particles/molecules including metals (Decho, 1990).

Bahía Blanca Estuary (BBE) is integrated by a complex morphology composed by island, extensive muddy flats and channels subjected to a semidiurnal ebb and flood tidal action. At this site, there are good chances to introduce pollutants into the environment through man-activities. In the northern coast at the estuary is located the most important deep harbor system of Argentina, as well as several industries and populated cities as Bahía Blanca and Punta Alta. The middle zone of Bahía Blanca Estuary is characterized by extensive tidal flats (1000 m wide) covered by microbial mats in the upper intertidal and lower supratidal zones. The evolution of the microbial mat in the temperate estuary has been studied (Cuadrado et al., 2011) and also the relationship with seasonal changes, hidrodynamical and physical variables and the resulting sedimentary structures (Cuadrado et al., 2013a,b; Pan et al., 2013a,b). The domestic sewage dumping in estuarine channels has been classified as a potential source of pollution in Bahía Blanca estuarine system (Pierini et al., 2012); however, in the middle zone of the estuary this fact has not been studied yet. It is generally known that sewage contains in itself a diverse array of polluting agents including pathogens, organic substances, heavy metals and trace elements and so on, which pose direct and indirect effects on ecosystems and organisms (Islam and Tanaka, 2004).Thus, the main purpose of this study is to perform a physicochemical characterization of the sediments of a siliciclastic tidal flat colonized by microbial mats that received the input sewage discharge from Punta Alta City. Considering the hypothesis that the sewage discharge is affecting the characteristics and the quality of the supratidal sediments of the middle zone of the BBE, we analyze different sediment layers of two nearby sites and link physical and chemical variables to the spatial distribution of the sediments and to the development of microbial mats. In turn the influence of the domestic sewage discharge on the study area was evaluated by microbial analysis.

## 2. Materials and methods

### 2.1. Study site

The Bahía Blanca Estuary is located in the SW area of Buenos Aires province, Argentina (38°45′–39°25′S and 61°45′–62°30′W) (Fig. 1). A dry temperate climate is characteristic for this area, with a mean annual air temperature of 15.6 °C (mean temperatures range from 22.7 °C in January to 8.1 °C in July) and mean annual precipitation of 460.55 mm. The predominant wind direction is from the NNO, NO and N (mean velocity: 22.6 km/h) (Píccolo and Diez, 2004).

This estuary extends over about 2300 $km^2$ and comprises several tidal channels, extensive tidal flats (1150 $km^2$) with patches of low salt marshes, and islands (410 $km^2$) (Píccolo et al., 2008). It is categorized as mesotidal (Hayes, 1979); semi-diurnal tides predominate and the mean tidal amplitude is 2.5–3.4 m during neap and spring tides, respectively. At the northern boundaries of the estuary various ports (two are commercial ports), cities (Bahía Blanca, Punta Alta) and industries (oil, chemical, and plastic factories) are located. The soil is used mainly for agriculture and livestock development. Two freshwater tributaries enter the estuary from the northern shore: the Sauce Chico River (drainage area of 1600 $km^2$) and Napostá Grande Creek (drainage area of 1240 $km^2$) (Píccolo et al., 2008). Both tributaries behave similarly in spring and summer during maximum mean rainfall (Piccolo and Perillo, 1990). However, the largest input of freshwater, nutrients, and contaminants is provided by the sewage discharges from Bahía Blanca, Punta Alta, and Ingeniero White cities (Piccolo et al., 2008). Several articles have been published about physical and chemical parameters, dissolved nutrients in surface estuarine waters and porewater (Spetter, 2006; Negrin et al., 2013; Spetter et al., 2013 and references therein); and heavy metals in sediments (Marcovecchio and Ferrer, 2005; Botté et al., 2010) but mostly of them are from the inner part of the estuary. There are some recent works from the middle area of Bahía Blanca Estuary, but they were developed in the salt marshes (Negrin et al., 2011).

The present study was conducted in the Rosales Harbor (38°55′S; 62°03′W) tidal flat, in the middle zone of the estuary. It is a siliciclastic depositional system with large tidal flat, 1000 m wide and with a very gentle slope, composed of fine sediments that range in size from fine sand to mud. The substrate of the tidal flats often experiences large fluctuations in water content, salinity and temperature, resulting in extreme conditions that limit the range of organisms able to inhabit this environment. The upper intertidal and supratidal zones are colonized by benthic microbial communities that form biofilms and microbial mats (Cuadrado et al., 2011; Pan et al., 2013a,b); and the intertidal area is vegetated by the cordgrass *Spartina alterniflora*, while patches of *Sarcocornia ambigua* are distributed on the supratidal zone. These tidal flats and salt marshes are also dominated by the burrowing crab *Neohelice (=Chasmagnatus) granulata* (Spivak, 1997; Iribarne et al., 2003).

Two sampling sites (SI and SII, Fig. 1) located ≈420 m apart from each other at the low supratidal area, were established on the basis of sedimentologic characteristics and preliminary field

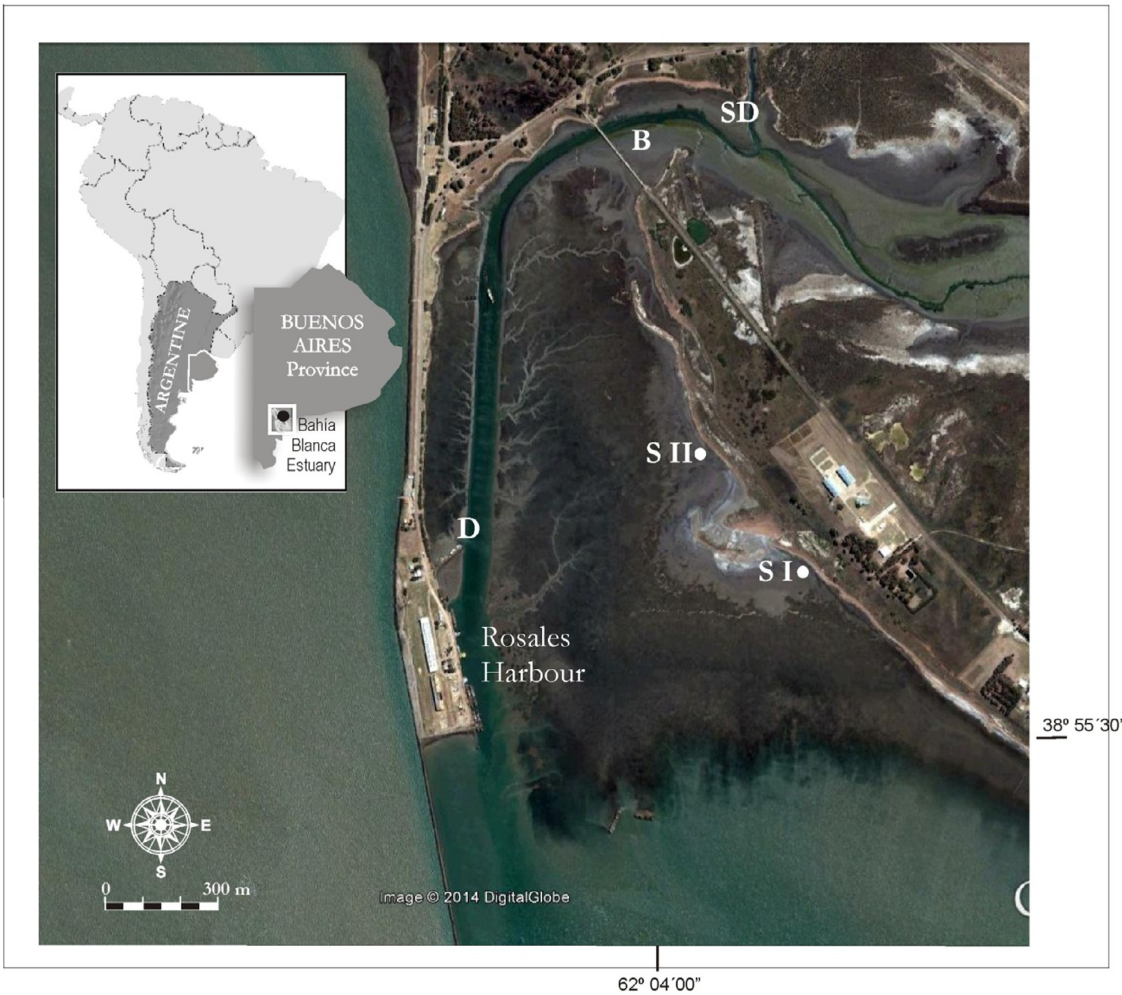


**Fig. 1.** General map showing the Bahía Blanca Estuary and the location of Rosales Harbor. The four sites selected are also shown as: SI: site I; SII: site II; B: bridge; D: dock and SD: sewage discharge of Punta Alta city.

observations. Both sites are unvegetated and flooded sporadically, in syzygy or in storm events, but SI (38°55′17″S; 62°03′42″W) is flooded more times than SII (38°55′07″S; 62°03′54″W) because it is in a lower level (difference in 0.3 m between both). SI is characterized by patches bioturbated by the burrowing crab *Neohelice granulata* which has strong effects on the sediment dynamics (Escapa et al., 2008), such as destabilization of cohesive sediments, directly affecting its porosity and permeability (Widdows et al., 1998; Escapa et al., 2008), while SII is undisturbed by macrozoobenthos and is nearer to the Punta Alta sewage discharge (SD) (Fig. 1). The sewage discharge of the city of Punta Alta (~60 thousand inhabitants) arrive raw and poor treatment to the estuary for over 15 years.

The influence of sewage discharge from the city of Punta Alta was assessed by analyzing the presence of *Escherichia coli*. For this, two other samples sites were chosen: the bridge (B), which is near to the discharge channel and the dock (D) (Fig. 1).

### 2.2. Sampling

Samples of undisturbed sediments were collected in the tidal flats of Rosales Harbor between June and December 2011, involving later fall, winter (cold period) and spring (warm period) seasons in the Southern Hemisphere. Sampling was consistently done at daytime hours and during low tide.

Sediments samples for determination of grain size, moisture, organic matter and heavy metal content were collected in duplicated with small cores of PVC (40 mm i.d; 120 mm long).

Sediment samples for chlorophyll *a* and pheopigments determination were taken in triplicated using mini cores (11 mm i.d; 40 mm long) and preserved in foil envelopes. Finally, porewater samples, between 0 and 30 mm depth, were *in situ* extracted in duplicated by a manual vacuum pump (Adams, 1994; Simpson et al., 2005) coupled to a PVC pipe (3.5 cm i.d and 15 cm long) with 500 μm pores (Martin et al., 2003).

All the samples were immediately transported to the laboratory in refrigerated boxes.

Simultaneously, temperature, pH and Eh in surface (0–5 mm) sediment were *in situ* measured using a Hanna Instruments probe (model HI991003).

In June 2011, sediment and water samples were collected in sterile containers in order to analyze the presence of *E. coli*. Samples of sediments from SI and SII and of water column from the bridge were taken at low tide; while at high tide samples of water column were collected from the dock and also from the bridge. All samples were placed in insulated containers to 4 ± 0.5 °C and taken to the laboratory within 6–8 h of collection (APHA, 2012; USEPA, 2000). The indicator of fecal contamination *E. coli* was counted in all the samples.

### 2.3. Laboratory analysis

In the laboratory sediment samples were separated into two layers, surface sediments (0–5 mm depth) and subsurface sediments (5–10 mm depth). Total heavy metals, moisture and organic matter, were analyzed in each layer. Samples were oven dried at 60 ± 5 °C to a constant weight for 4 days. Moisture content was calculated from weight differences before and after drying samples (Christie et al., 2000). Organic matter content (%OM) was calculated from weight loss on ignition (LOI) after drying samples and ashing/combusting at 450 ± 50 °C for 1 h in a muffle furnace (Dean, 1974).

According to the layered structure visible under a stereoscopic microscope the small cores of sediment collected were separated into three or four layers (layer thickness ranging from 4 to 10 mm). Sediment grain size was determined by laser diffraction using a Malvern-Mastersizer-2000 particle analyzer, for particles ranging between 0.02 and 2000 μm (i.e. colloids to sand).

To evaluate heavy metal concentration, large debris and fragments of biota were removed from the dried sediments before grounding. All the sediment subsamples were then ground in a porcelain mortar and stored in plastic ware within desiccators until analysis. Metal concentrations (Cd, Cu, Cr, Ni, Pb, Zn, Mn and Fe) in the total fraction (TF) were analyzed according to the methodology of Marcovecchio and Ferrer (2005). About 0.500 g of sediment samples were mineralized with a mixture $HNO_3/HClO_4$ (5:1) at 110 ± 10 °C. Then the extract (1 mL) was filled with 0.7% $HNO_3$ to a volume of 10 mL. Metals concentrations were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES Optima 2100 DV Perkin Elmer).

The detection limit of the method (MDL) was calculated as the standard deviation (SD) of 10 blank replicates (Federal Register 1984). The MDL for Cd, Cu, Pb, Zn, Mn, Ni, Cr and Fe were 0.0030, 0.0623, 0.0937, 0.1063, 0.0450, 0.0113, 0.0101 and 0.6139 mg $L^{-1}$, respectively.

Porewater samples (10–25 mL) for nutrients determinations were immediately filtered through Whatman glass fiber grade F (GF/F 47 mm diameter and 0.7 μm) membranes and frozen (−20 °C) in plastic bottles until analysis. Samples for ammonium determination were preserved with a 7% $HgCl_2$ solution (Grasshoff, 1976). Nitrate ($NO_3^-$), nitrite ($NO_2^-$), ammonium ($NH_4^+$), phosphate ($PO_4^{3-}$), and silicates (DSi) were determined following the internationally standarized methods of Treguer and Le Corre (1975a,b), Grasshoff et al. (1983), Eberlein and Kattner (1987), and Technicon (1973), respectively. A Technicon AA-II Autoanalyzer expanded to five channels was used to perform the nutrient analyses. The quantification limit (QL) of the methods were 0.10 μM for $NO_3^-$, 0.02 μM for $NO_2^-$, 0.01 μM for $NH_4^+$, 0.01 μM for $PO_4^{3-}$ and 1.00 μM for DSi.

The sediment samples for pigments determination were fractioned in surface and subsurface and kept in dark at −20 °C until analysis. The chlorophyll *a* (Chl*a*) and phaeopigments content was analyzed using the method of Strickland and Parsons (1968). Sediment samples were extracted with acetone 90%, immersed in an ultrasonic bath at a controlled temperature and then refrigerated in the dark at −4 °C for 24 h. Then the samples were centrifuged and the supernatant separated. The pigments concentrations were determined by spectrophotometry (Jenway 6715 UV–Vis) using the equations of Lorenzen (1967). The results were expressed in μg $g^{-1}$ sediment dry weight.

For the evaluation of *E. coli* concentration, the double layer technique was used to recover the bacteria stressed due to the environmental conditions. The resuscitation of injured microorganisms was carried out during 2 h in agar plate count (PCA, Merck), and then dropping agar Endo (Merck) (Streitenberger and Baldini, 2010). The incubation was done at 44.5 ± 0.5 °C during 24 h. The results were expressed as CFU 100 $mL^{-1}$. Later typical colonies were selected, isolated in PCA (Merck) and biochemically identified (McFaddin, 2003).

### 2.4. Statistical analysis

The differences between sites were analyzed, combining all the data for the whole study period (i.e., ignoring seasonal/temporal differences) using *t*-Student test, while differences between sediments fraction in each site were evaluated using *t*-Student for paired samples. Data were transformed if required by assumptions or Man–Whitney non-parametric test was used, according to the characteristics of the data set. Statistical analyses were carried out with appropriate software, following Zar (1996). The acceptable level of statistical significance was 5%. Error values represent ± 1SE.

## 3. Results

### 3.1. Temperature, pH and Eh in sediments

Temperature in surface sediments (0–5 mm) varied from 2.1 °C to 31.3 °C showing the regular seasonal cycle, with low values in the cold period and high in the warm one. In addition no significant statistical differences were detected between the sampling sites ($p = 0.47$) (Fig. 2a).

The range of pH values at both sites varied between 6.1 and 7.8 and at each site they tended to be lower in the warm period. At SI it ranged between 6.1 and 7.6 and in SII between 6.5 and 7.8. In addition, no significant statistical differences were detected between the sampling sites, since pH values were very similar between them ($p = 0.08$) (Fig. 2b).

Eh ranged from −70 and 179 mV and displayed different seasonal pattern in sediments at SI and SII. In SI, Eh was positive throughout most of the study period, but with a decreasing pattern during it. It presented negative values as −55 mV during the warm period. In general it diminished with sediment temperature increment. In this site it varied between −55 and 179 mV, being 55 mV the mean value. Contrarily Eh values at SII were more variable, without a defined pattern and with lower values; it ranged between −70 and 94 mV, being 13.14 mV the mean value (Fig. 2c).

### 3.2. Microbiological analysis

All the samples collected showed the presence of *E. coli*. The water samples indicated the higher values, being 1000 CFU/100 ml, uncountable and 1600 CFU/100 ml in the water obtained from the dock at high tide, from the bridge at low and high tide respectively. The number of bacteria in sediments from SI and SII were 35 CFU $g^{-1}$ dw and 40 CFU $g^{-1}$ dw, respectively.

### 3.3. Moisture and organic matter content

The moisture retention of the sediments at both sites varied between 18.4% and 46.4%, being significantly higher at SII ($p < 0.01$) (Fig. 3a). Considering the different layers, moisture content tended to be lower at subsurface sediments. This pattern was more pronounced in the SII, presenting statistically significant differences ($p < 0.01$); with higher moisture content in the surface layer (Fig. 3a). Finally, the moisture content in the sediments of both sites decreased during the warm season.

The average %OM for the whole tidal flat was 4.60 ± 0.54% ($n = 14$) with a maximum of 8.51% in the surface sediments from SII and a minimum of 1.50% in the subsurface sediments from SI (Fig. 3b). The %OM were statistically different between the two

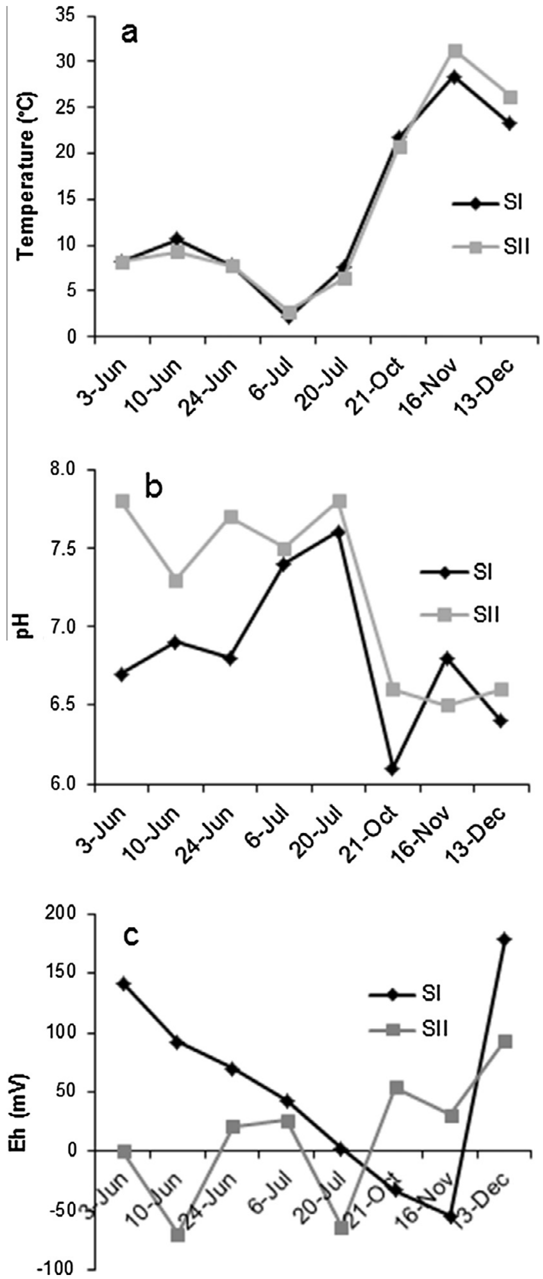


**Fig. 2.** Variation of temperature (a), pH (b) and Eh (c) in surface sediments from Site I (SI) and Site II (SII) at Rosales Harbor.

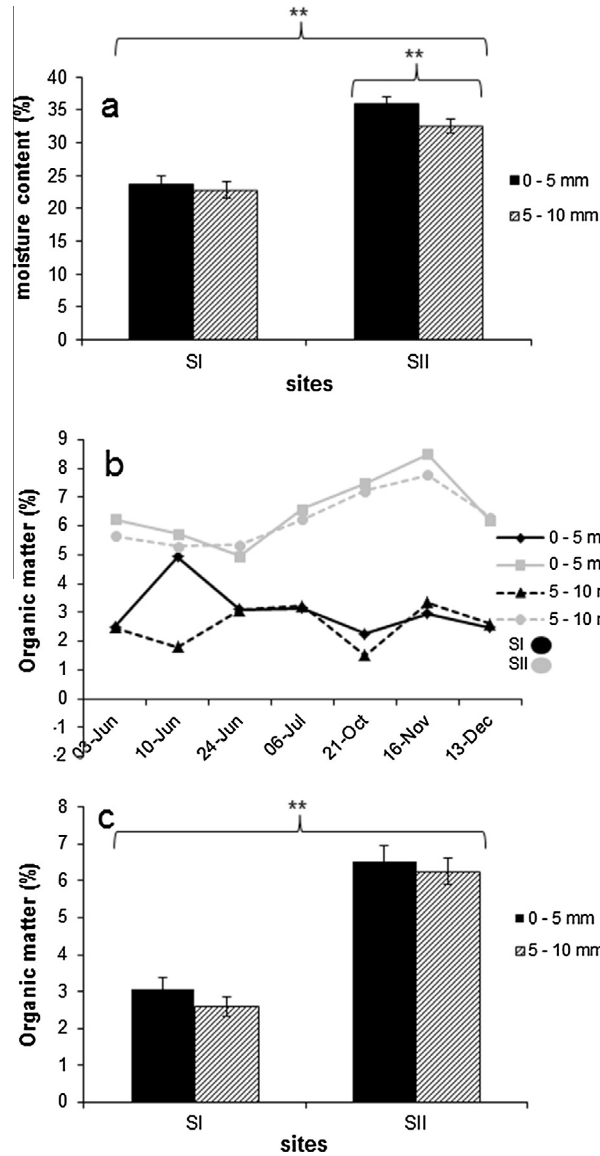


**Fig. 3.** (a) Average moisture content, (b) variation and (c) average of organic matter content from Site I (SI) and Site II (SII) throughout the sediment profile; values are means ± SE ($n = 14$ for figure a and c). **Highly significant differences ($p < 0.01$).

sampling sites, being higher in sediments from SII ($p < 0.01$) (Fig. 3c), although at SII it seemed that the organic matter content was higher in the oxic layer but finally, no significant differences were found between 5 mm and 10 mm depth at any of the sites evaluated ($p > 0.05$). The annual average %OM at SII was 6.40 ± 0.39%, more than twice higher than that at SI (2.82 ± 0.20%) (Fig. 3c).

### 3.4. Grain size

The sediment composition showed differences in the 10 mm analyzed for both sites. The granulometric analysis evidenced that silt was the dominant sediment fraction in the surface sediments at SI (65%), but then coarser grain sediments (i.e. sand) content increased with depth (approximately 68%) (Fig. 4a). On the other hand, the sediment composition at SII was vertically and spatially more homogenous not showing any layer and presenting mainly silt and some clay in all the layers evaluated. In general, the values observed were between 40% and 60% of silt and up to 40% of clay (Fig. 4b).

### 3.5. Nutrients in porewater

Before considering the results we must clarify that it was unable to extract enough pore water during the warm period from both sites probably due to a higher evaporation rate. Because of this, the nutrients data in some cases are scarce or inexistent (Table 1).

During the cold season of the study period, the $NH_4^+$ concentration in pore water varied between 2.20 and 48.71 μM at SI and 5.75 and 40.36 μM at SII (Table 1). Although the results showed no significant differences between sites ($p > 0.05$) (Fig. 5a) $NH_4^+$ mean concentration at SI (27.86 ± 10.84 μM) was approximately two time higher than that at the SII (15.10 ± 8.44 μM) (Table 1). The $NO_3^-$ concentration did not show significant differences

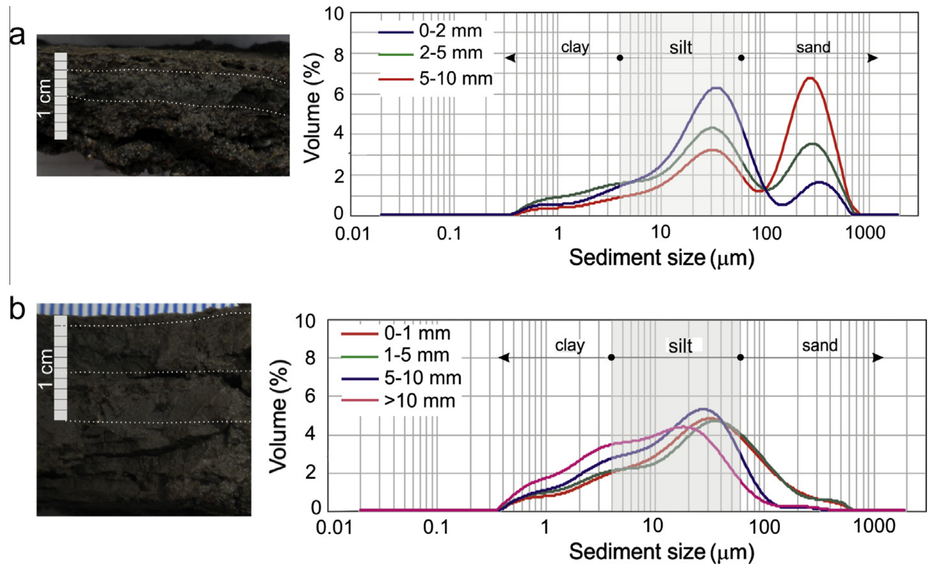


**Fig. 4.** Photographs of characteristic layering of sediments in Rosales Harbor, and granulometric analysis of surface and subsurface sediments for site SI (a) and SII (b).

**Table 1**
Summary of nutrient concentration (μM) in porewater from site I (SI) and site II (SII) (mean ± SE; $n = 8$ for cold season and $n = 6$ for warm season; minimum and maximum values).

| | $NO_2^-$ (μM) | $NO_3^-$ (μM) | $NH_4^+$ (μM) | $PO_4^{3-}$ (μM) | DSi (μM) |
|---|---|---|---|---|---|
| SI | | | | | |
| *Cold season* | | | | | |
| Mean ± SE | 0.57 ± 0.19 | 18.45 ± 5.92 | 27.86 ± 10.54 | 81.76 ± 48.56 | 158.42 ± 31.26 |
| Minimum | 0.25 (20 July) | 1.25 (24 June) | 2.20 (24 June) | 10.12 (3 June) | 85.62 (24 June) |
| Maximum | 1.06 (24 June) | 27.59 (6 July) | 48.71 (3 June) | 223.53 (6 July) | 216.95 (6 July) |
| *Warm season* | | | | | |
| Mean ± SE | 7.77 ± 6.56 | 67.18 ± 24.90 | 65.42 ± 8.54 | 21.27 ± 4.05 | 238.74 ± 70.31 |
| Minimum | 0.88 (21 October) | 17.59 (13 December) | 54.96 (13 December) | 14.00 (13 December) | 100.00 (13 December) |
| Maximum | 20.89 (16 November) | 95.92 (21 October) | 75.89 (21 October) | 28.02 (16 November) | 327.93 (16 November) |
| SII | | | | | |
| *Cold season* | | | | | |
| Mean ± SE | 0.39 ± 0.07 | 17.70 ± 7.45 | 15.10 ± 8.44 | 63.22 ± 10.10 | 239.31 ± 51.74 |
| Minimum | 0.21 (3 June) | 9.10 (24 June) | 5.75 (6 Juyl) | 43.24 (6 July) | 93.95 (6 July) |
| Maximum | 0.52 (20 July) | 40.02 (20 July) | 40.36 (3 June) | 81.76 (24 June) | 338.98 (20 July) |

between sites during the cold period ($p > 0.05$) (Fig. 5b). The $NO_3^-$ mean concentration was 18.45 ± 5.92 μM at SI and 17.70 ± 7.45 μM at SII (Table 1 and Fig. 5b). High $NO_3^-$ concentrations were found in both sites on July, 27.59 μM at SI and 40.02 μM at SII, and low concentrations were found also in both sites in later June, 1.25 and 9.10 μM at SI and SII, respectively (Table 1). The extremely high and low $NO_3^-$ concentrations in SII and SI probably masked the differences between sites. On the other hand, $NO_3^-$ concentration tends to increase in the warm period at SI (67.18 ± 24.90 μM), registering a maximum of 95.92 μM in October 2011 (Table 1).

$NO_2^-$ was the least abundant dissolved nitrogen nutrient, with an average concentration in the cold period of 0.57 ± 0.19 μM and 0.39 ± 0.07 μM at SI and SII, respectively (Table 1 and Fig. 5c). The $NO_2^-$ concentration presented a maximum of 20.89 μM at SI in November and a minimum of 0.21 μM at SII in June (Table 1).

The concentration of $PO_4^{3-}$ in porewater varied between 10.12 and 223.53 μM at SI and between 43.24 and 81.76 μM at SII (Table 1); however, no significant differences between sites were observed ($p > 0.05$) (Fig. 5d). In the cold season the mean value of $PO_4^{3-}$ concentration in SI (81.76 ± 48.56 μM) was higher than in SII (63.22 ± 10.10 μM) and show a greater variability. DSi ranged from 85.62 to 327.93 μM at SI and from 93.95 to 338.98 at SII (Table 1). Both nutrients behaved similarly on 6 July, showing a maximum peak at SI (223.53 μM and 216.55 μM, for $PO_4^{3-}$ and DSi respectively) and a decrease in their concentrations at SII (43.24 μM and 93.95 μM, for $PO_4^{3-}$ and DSi respectively) (Table 1). The average values of DSi concentration for the cold period were higher at SII (239.31 ± 51.74 μM) than SI (158.42 31.26 μM) although no significant differences were observed ($p > 0.05$) (Table 1 and Fig. 5d).

### 3.6. Chlorophyll a

The pigments concentration varied throughout the study period. In SI, Chl*a* increased in the cold period followed by a decrease in the warm one, while in SII was more irregular not showing a clear pattern (Fig. 6a and b).

Both study sites significantly differed in chlorophyll and phaeopigments concentration ($p < 0.0001$). At SII surface sediments chlorophyll *a* (Chl*a)* and phaeopigments values ranged from 6.25 to 28.4 μg g$^{-1}$ and 6.53 to 59.9 μg g$^{-1}$ respectively and were higher than in SI (4.50 to 12.1 μg g$^{-1}$ and 0.40 to 14.5 μg g$^{-1}$, respectively). No significant differences were observed in phaeopigments concentrations between sediments fractions ($p = 0.4409$) at SI but

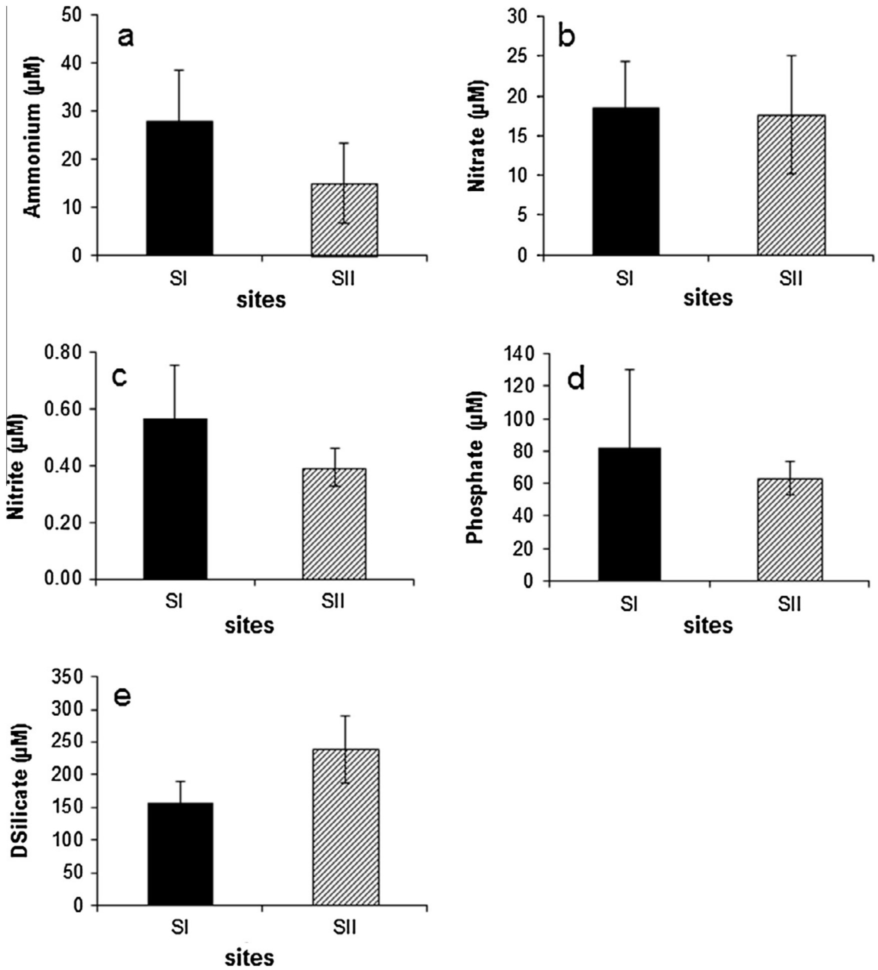


**Fig. 5.** Average concentration of nutrients in porewater (mean ± SE; $n = 8$) from site I (SI) and site II (SII) at Rosales Harbor during later fall and winter 2011. Not significant differences in nutrient concentration were found between sites ($p > 0.05$).

there were significant differences at SII being higher in the surface layer.

Surface and subsurface sediments Chl*a* concentration differed significantly in each site of the study area ($p < 0.0001$), being this difference ($11.2 \pm 7.7\ \mu g\ g^{-1}$) higher at SII. Along the entire study period Chl*a* content was lower in the subsurface layer at both sites. The highest Chl*a* value in the upper 5 mm were registered in winter for SI ($12.1 \pm 3.1\ \mu g\ g^{-1}$) and spring for SII ($28.4 \pm 2.0\ \mu g\ g^{-1}$) while minimum values were registered in later fall ($4.57 \pm 1.63\ \mu g\ g^{-1}$) and winter ($6.25 \pm 0.90\ \mu g\ g^{-1}$) for each site respectively.

### 3.7. Heavy metals

Heavy metals concentration in sediments from Rosales Harbor showed no significant vertical change at any of the studied sites ($p > 0.05$). Because of this all the other analyses were carried out using the mean value of heavy metal concentration of the surface and subsurface sediments. The ranges for the total concentrations of Cd, Cu, Pb, Zn, Mn, Ni, Cr and Fe were: not detectable–0.13, 6.78–18.56, 3.34–6.75, 21–47.33, 171.6–452.53, 3.85–7.56, 6.20–10.66 $\mu g\ g^{-1}$, 14.22–26.36 $mg\ g^{-1}$ dry weight respectively. In all cases the lowest metal concentrations were recorded at SI while the highest at SII. The minimums and maximums of heavy metals in the sediment cores for each site are summarized in Table 2.

The average concentrations of heavy metals in sediments of all the fractions at both sites revealed the following order: Fe > Mn > Zn > Cu > Cr > Ni > Pb > Cd.

Statistical analysis in metal concentration resulted significantly higher in SII than in SI ($p < 0.01$) for all the heavy metals analyzed; especially, Cu and Pb which were twice higher at SII than at SI (Fig. 7).

In order to reveal the correlation between the trace elements, statistical analysis was carried out to the whole data set of the metals concentrations in sediment cores from each site. The correlation coefficient matrix between heavy metals is listed in Table 3. Strong positive correlation was observed between Cr and Fe, Cr and Mn; and Cu and Ni at SI. On the other hand, Cd, Cu, Fe and Pb; and Pb and Zn showed strong correlation at SII. Note that in both places Cd and Pb presented a strong positive correlation.

## 4. Discussion

### 4.1. Temperature, pH and Eh in sediments

Temperature is one of the key regulators influencing biogeochemical processes in wetlands, by influencing the growth, activity, and survival of organisms (Reddy and DeLaune, 2008). In this work, sediment temperatures were similar at both sites, following seasonal fluctuations with air temperature.

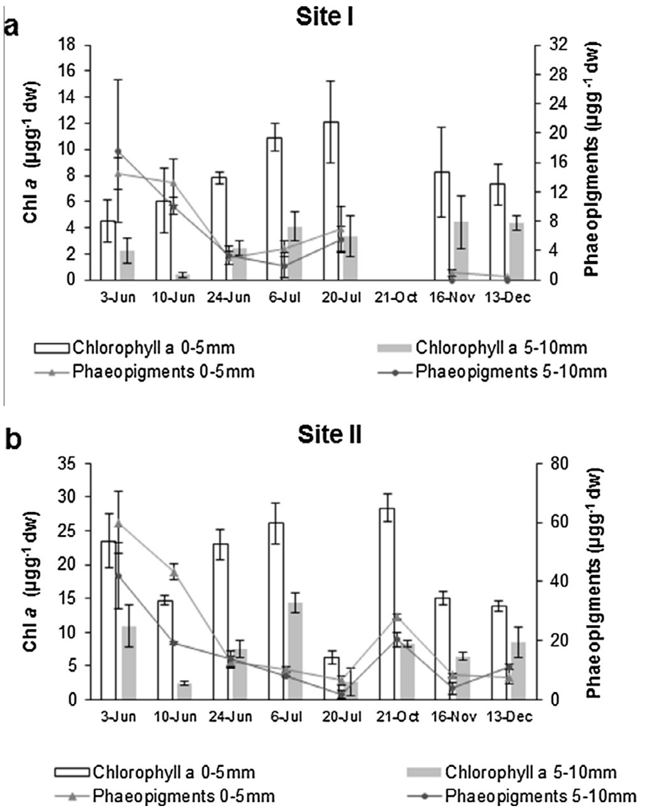


**Fig. 6.** Variation of chlorophyll *a* and phaeopigments concentration in surface (0–5 mm) and subsurface (5–10 mm) sediments from site I (SI) and site II (SII) at Rosales Harbor during later fall, winter and spring 2011. (Mean ± SE; n = 3).

pH and Eh of soil are important electrochemical properties affected by a wide variety of different factors, such as presence of vegetation, tidal flooding, benthic primary productivity, the dominating respiratory pathway/s, sediment composition, etc. (Reddy and DeLaune, 2008); however in this case the pH of the sediments practically did not vary between sites and were near to neutral (7.0). They were similar to that found in other areas, and in particular in non vegetated sediments, of the same estuary (Negrin et al., 2011; 2013). Moreover, the pH values registered were among the normal range values for estuarine sediments (5–7) (Caçador et al., 2004; Reddy and DeLaune, 2008).

The higher Eh in SI could be related to the presence of *Neohelice granulata*, their burrowing activity may oxygenate tidal soil, enhance soil drainage, and modify sediment meiofaunal abundance (Iribarne et al., 1997). On the other hand, the lower redox condition of the sediments at SII could be influenced by the discharge of oxidizable organic matter from Punta Alta City urban sewage discharge (Values of particulate organic matter between 1130–3150 mgC m$^{-3}$ were detected in the Bridge, Spetter unpublished data). The consequence of this could be a drastic increase in the density of aerobic and facultative anaerobic heterotrophic bacteria. This in turn, causes an increase in respiratory activity and a concomitant decrease of dissolved oxygen. In this way, the reduction of other elements with redox potential lower than the oxygen is favored. Even more, the smallest porosity due to the smaller grain size of the sediment at SII could be influencing the lower oxygen content. Anyway, flat laminated microbial mats are often the sites of pronounced gradients of oxygen; it can range from saturation below the sediment–water interface to a complete anoxia in less than a millimeter (Dupraz and Visscher, 2005). The net production of oxygen usually occurs in the middle of the day (Visscher et al., 1998) but in some cases it can occur at different day hour depending for example of the intensity of the noon-day sun (Miller et al., 1998; Jonkers et al., 2003). Despite the difference found in this study, sediments at both sites are considered moderately reduced (Cronk and Fennessy, 2001). Eh values between 0 and 300 mV represent moderately anaerobic conditions typical of wetlands in a transition zone (Reddy and DeLaune, 2008). The variability found in Eh values during the analyzed period, may be related to the content of organic matter, because as we mentioned before, the redox potential is also influenced by frequent additions of organic substrates. Another important factor to consider is the activity of the oxygenic phototrophs (e.g., cyanobacteria) which can also affect the Eh of the sediment, resulting in Eh values as high as 400 mV in the oxic zone (Visscher and Stolz, 2005). Eh values of sediment could be a good indicator of the reaction(s) involved in the decomposition of organic matter, which in turn is related to the available electron acceptors. At an Eh between 400 and 600 mV, oxygen is the most preferred electron acceptor for microorganism. Once the oxygen is depleted, other electron acceptors are used, at different Eh values. The Eh reported in this study (−70 to 179 mV) would indicate that the oxidation of the organic matter is being performed by the reduction of iron (at 120 mV) (Cronk and Fennessy, 2001; Reddy and DeLaune, 2008), as has been previously reported in both marine such as the mats from Loihi Seamount (Gao et al., 2006) and terrestrial such as Iron Mountain (Bond et al., 2000a,b; Baker and Banfield, 2003); and not of sulphate (−75 to −150 mV) (Cronk and Fennessy, 2001; Reddy and DeLaune, 2008), as usually found in this kind of environment (Canfield and Des Marais, 1991; Visscher et al., 1998).

### 4.2. Microbial contamination

Domestic and municipal sewage contains a large number of pathogenic microorganisms originating from humans who are infected or who are carriers of a particular disease. The most common pathogens found in sewage are those that cause typhoid fever, dysentery and gastroenteritis (Soller et al., 2010). The faecal

**Table 2**
Summary of trace metal concentration (µg g$^{-1}$ dw; Fe in g g$^{-1}$ dw) in the total fraction of the sediments from different areas of Bahía Blanca Estuary (mean ± SD).

| AREA | Cd | Zn | Cu | Cr | Pb | Fe | References |
|---|---|---|---|---|---|---|---|
| Principal Channel (Rosales Harbor) | 0.15 ± 0.08 | 25.73 ± 4.92 | 5.88 ± 1.73 | 3.56 ± 1.45 | 8.53 ± 2.12 | 12.34 ± 2.38 | Marcovecchio and Ferrer (2005) |
| Access Channel (Rosales Harbor) | 0.125 | 82.25 | – | 17.30 | 11.00 | – | Pizani (2008) |
| Cuatreros Harbor (intertidal sediments) | 0.87 ± 0.08 | – | – | 9.81 ± 1.58 | 15.50 ± 1.37 | – | Botté et al. (2010) |
| Maldonado (intertidal sediments) | 0.80 ± 0.10 | – | – | 8.58 ± 1.45 | 13.80 ± 1.78 | – | Botté et al. (2010) |
| Galván Harbor (intertidal sediments) | 0.98 ± 0.11 | – | – | 10.16 ± 2.12 | 15.15 ± 1.10 | – | Botté et al. (2010) |
| SI | 0.057 ± 0.008 | 27.405 ± 1.279 | 7.500 ± 0.553 | 6.737 ± 0.408 | 3.681 ± 0.201 | 15.210 ± 0.885 | This study |
| SII | 0.089 ± 0.023 | 42.002 ± 2.745 | 15.811 ± 1.347 | 10.461 ± 0.510 | 6.140 ± 0.401 | 23.790 ± 1.283 | This study |

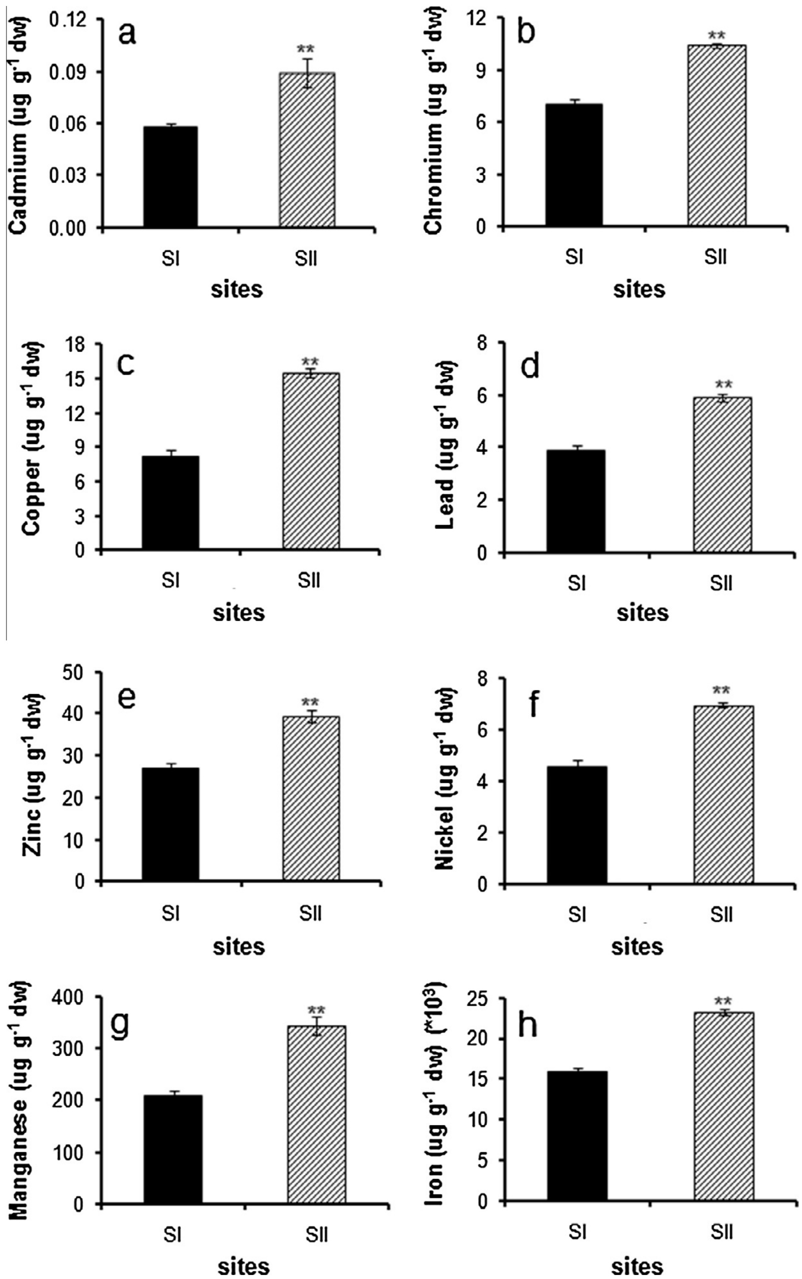


**Fig. 7.** Average concentration of heavy metals in sediments from site I (SI) and site II (SII) (mean ± SE; $7 < n < 12$) at Rosales Harbor during later fall, winter and spring 2011. **Highly significant differences ($p < 0.01$).

**Table 3**
Correlation matrix (Pearson correlation) for heavy metals concentration in sediments from Site I (SI) and Site II (SII) (bold numbers indicate significative correlation, p < 0.05, and in grey squares indicate lineal graph).

| | SI | | | | | | | | SII | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cd | Cu | Pb | Zn | Mn | Ni | Cr | Fe | Cd | Cu | Pb | Zn | Mn | Ni | Cr | Fe |
| Cd | 1 | | | | | | | | 1 | | | | | | | |
| Cu | 0.54 | 1 | | | | | | | **0.90** | 1 | | | | | | |
| Pb | **0.89** | 0.64 | 1 | | | | | | **0.85** | **0.86** | 1 | | | | | |
| Zn | 0.64 | 0.48 | 0.65 | 1 | | | | | 0.57 | 0.54 | −0.1 | 1 | | | | |
| Mn | 0.26 | 0.31 | 0.30 | 0.64 | 1 | | | | −0.70 | −0.70 | −0.40 | −0.50 | 1 | | | |
| Ni | 0.67 | **0.85** | 0.75 | 0.62 | 0.69 | 1 | | | **0.71** | **0.77** | 0.58 | **0.72** | −0.50 | 1 | | |
| Cr | 0.38 | 0.42 | 0.45 | 0.72 | **0.83** | **0.72** | 1 | | 0.69 | **0.86** | 0.45 | **0.73** | −0.60 | **0.92** | 1 | |
| Fe | 0.21 | 0.42 | 0.12 | **0.83** | 0.68 | 0.60 | **0.81** | 1 | **0.90** | **0.91** | **0.83** | 0.63 | −0.60 | **0.89** | **0.86** | 1 |

coliform group and particularly the bacteria *E. coli* are usually used as indicator organism of microbial contamination resulting from domestic and municipal sewage (Fries et al., 2006).

The faecal pollution in the Bahía Blanca Estuary has been reported by Cabezali et al. (1994), Baldini et al. (1999), Streitenberger and Baldini (2010), and Pierini et al. (2012). However, this is the first report of the faecal pollution in the tidal flats at Rosales Harbor. In this way, high abundance of *E. coli* was detected in water column and sediments in all samples analyzed. This fact demonstrates the "biological pollution" (Islam and Tanaka, 2004) evidencing the impact originated by sewage discharge on this ecosystem.

### 4.3. Grain size, moisture and organic matter content

Moisture retention by the different sediment layers was related to sediment granulometry. All layers at SII, where silt was the most abundant fraction, comparatively retained a higher proportion of moisture than those at SI, where the sediments were sandier. This difference in moisture content was observed despite the SII was flooded with lesser frequency (Fig. 8).

Organic matter is added to these environments from both external (i.e. domestic and municipal sewage) and internal (i.e. detrital matter from algal and microbial mats) sources. In addition, benthic invertebrates and soil microorganisms may also contribute significant amounts of organic carbon to soil (Reddy and DeLaune, 2008). Thus, the highest content of organic matter at SII is not only related to the higher clay and silt content, as has been seen that finer sediments are rich in organic matter (Reddy and DeLaune, 2008), but also to the greater contribution of terrestrial matter by the urban sewage inputs. Considering this last factor, we must add that SII is located closer to the discharge channel of the urban wastewater, for this reason its influence could be higher. Furthermore, as we mentioned before, SII was lesser flooded than SI, thus we can assume that the organic matter dissolution or the mineralization rate were lower. Considering the internal sources, deposit or detritus feeders can decrease the amount of entrained organic matter in the sediment (Bird 1982; Suchanek, 1985), and control sediment grain size, transport, mixing and deposition (Robertson and Pfeiffer, 1982; Suchanek, 1983; Tudhope and Scoffin, 1984). This suggests that the presence of the burrowing crab *Neohelice granulata* could influence the content of organic matter and its arrival to the sediments of SI. It has been demonstrated that this species is a deposit feeder when inhabiting mudflats (Iribarne et al., 1997) and that the funnel-shaped burrow entrances efficiently trap organic reached sediments and detritus from the water column at zones with low current speeds (Botto et al., 2006), being in this way the content of organic matter higher than nearby areas without crabs (Botto and Iribarne, 2000; Escapa et al., 2004; Botto et al., 2006).

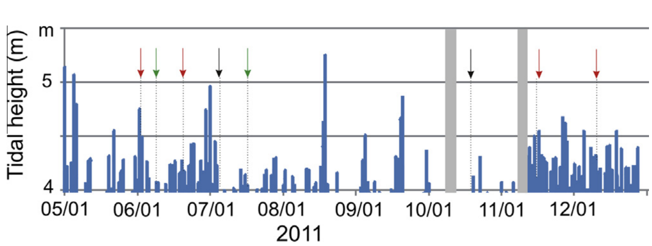


**Fig. 8.** Daily frequency of tidal flat inundation at Rosales Harbor based on semidiurnal tidal height data from a tidal gauge located 5 km into the inner Bahía Blanca Estuary from the study site. Black arrows: any site flooded; Green arrows: Site I flooded; Red arrows: Site I and Site II flooded; Grey bars: no data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 4.4. Nutrients in porewater

Coastal zone and shallow marine areas are among the most productive systems within the world (Mateus et al., 2008). It is widely accepted that shallow costal sediments (mainly in the first millimeters) are important site for the mineralization of organic matter and nutrient cycling in coastal marine environments (Borum, 1996; Srithongouthai et al., 2003).

Much of the biogeochemical cycling that is critical to organic matter decomposition is controlled by the microbial communities (Atlas and Bartha, 2002; Galloway, 2005). Microbial biomass regulates the transformation and storage of nutrients (Reddy and DeLaune, 2008).

Moreover the benthic fluxes of nutrients and finally their spatial and temporal variability in sediment–water exchange are affected by environmental factors such as organic matter, temperature, oxygen availability and sediment grain size (Borum, 1996; Wilson and Brennan, 2004; Arndt et al., 2013; Ospina-Alvarez et al., 2014). Nutrient availability can affect decomposition rate by limiting the growth rate of microbial decomposers. Growth-limiting nutrients may be obtained by microorganisms from the organic substrates or from the water column or soil porewater (Reddy and DeLaune, 2008). Anyway, this is not the problem in the study area, since the Bahía Blanca Estuary is considered a highly eutrophic ambient (Spetter et al., 2013 and references therein). Moreover, freshwater discharge during rainy periods has been recognized as an important source of external nutrients into the coastal area (Spetter, 2006; Melo and Limbozzi, 2008).

Both sites behaved differently as regards porewater nutrient concentration. Considering $NH_4^+$ and $NO_3^-$, it can be seen that both nutrients showed a similar behavior during the cold period at SI, with minimum values in late June. This may be associated with the dynamics of nutrient uptake by benthic microalgae during photosynthesis (Sullivan and Currin, 2000). Moreover, $NH_4^+$ concentration in porewater at this site and in surface estuarine water in the dock (Spetter unpublished data), presented the minimum and maximum values in the same dates (24-Jun and 3-Jun respectively). Instead, at SII where the organic matter content was high, $NH_4^+$ was not the only dominant fraction of the dissolved inorganic nitrogen, as it is usually found in intertidal areas (e.g. Windham-Myers, 2005; Lillebø et al., 2006; Negrin et al., 2011), but something similar was observed in another site of this estuary (Negrin et al., 2013). Engelsen et al. (2008) suggests that the flow of nutrients in porewater is related to the trophic status rather than the organic matter content of the sediment. On the opposite, others authors suggest that it depends on the amount of organic matter produced, since nutrients are released during decomposition (Burdige, 2006). $NH_4^+$ concentrations in porewater recorded here are lower than those found in other works from the Bahía Blanca Estuary (Spetter, 2006; Marcovecchio et al., 2009; Negrin et al., 2011) and from others tidal flats in the world (Magni and Montani, 2006; Sin et al., 2009). On the contrary, $NO_3^-$ values from both sites were in general higher than the ones usually found in other sites of the estuary without microbial mats (Spetter, 2006; Marcovecchio et al., 2009).The high $NO_3^-$ concentrations could be associated with the possible input of nutrients from groundwater (Galloway, 2005), which has not been studied in the area so far, but the groundwater presence has been reported by Cuadrado et al. (2013a) at the study site.

The $PO_4^{3-}$ concentrations in porewater reported at both sites were higher than those recorded in other sites of Bahía Blanca Estuary where the microbial mats were absent (Spetter, 2006; Marcovecchio et al., 2009; Negrin et al., 2011; 2013). Both nitrogen and phosphorus have been identified as microbial growth-limiting nutrients in wetlands. In the estuaries, over 90% of particulate phosphorous is remineralized in the sediments and released through the porewater (Benitez-Nelson, 2000). However, much of the phosphorus in the estuarine sediment is adsorbed on soil particles or to form compounds with other elements because of the great sensitivity of ion $PO_4^{3-}$ to form complexes with organic compounds, to be adsorbed on iron oxides and aluminum, and to precipitate in the presence of divalent metal ($Ca^{+2}$, $Mg^{+2}$) or ferric ion ($Fe^{+3}$) at neutral or alkaline pH (Atlas and Bartha, 2002; Bally et al., 2004). Then, the phosphorous tends to remain in the sediments, but changes in sediment conditions related to flooding, such as increase of pH, decrease of Eh or depletion of oxygen concentration, induce the release of phosphate from the soil particles increasing the levels in porewater (e.g. Lillebø et al., 2002; Coelho et al., 2004; Palmer-Felgate et al., 2011). According to Jensen et al. (1995), the release of phosphates would be linked to sulfate reduction, where the sulfides would be responsible for the reduction of ferric oxy-hydroxides (FeOOH) and after the release of P bound to Fe and precipitation of FeS and $FeS_2$, which restricts migration of $Fe^{+2}$ but not of the ortho-phosphates ($PO_4^{3-}$) dissolved which results in a saturation of adsorption sites on $PO_4^{3-}$ (FeOOH) in the surface layer of sediments, which exerts a strong control over the dissolved phosphorous in interstitial water. High concentrations of phosphorus found in this study could be due to such, since studies carried out in sediments at SI showed that the phosphorous concentrations bound at iron, calcium and the labile fraction or adsorbed was higher than in other tidal flats in the world (Pérez, 2013). Moreover, the excess nutrients inputs are mainly human-related and are due to high coastal population density, various agricultural practices, the burning of fossil fuels, and sewage discharge. In this way, the high levels of nitrogen and phosphorous nutrients found in this area could be related to the proximity to the sewage discharge (Punta Alta City).

Silicates were always present in porewater of the two sites throughout the study period in agreement with those reported in previous studies in other sites of the estuary (Spetter, 2006; Marcovecchio et al., 2009). High concentrations of this element found in porewater may be due to the dissolution of silica compounds present in the sediment such as feldspars, quartz, pyroxenos, and amphibole (Cuadrado and Pizani, 2007). In addition the maximum values are found in the warm period where higher temperatures favor the dissolution of Si (Mortimer et al., 1998). The reported values may indicate the availability of this nutrient for the production of benthic diatoms, which are involved in the microbial mat development (Parodi and Barria de Cao, 2003).

### 4.5. Chlorophyll a

Chlorophyll *a* (Chl*a*) is widely employed to estimate the biomass of benthic microalgae, which present high degree of spatial and temporal heterogeneity. Factors such as resuspension, nutrient, light availability, organic matter, grazing, grain size and desiccation have been suggested to control microphytobenthic biomass (Underwood and Kromkamp, 1999; Moreno and Niell, 2004). As photosynthesis process is carried out by microphytobenthos in a thin photic zone, the 68–70% of pigments was concentrated in the topmost sediments layer. The photic zone is defined as the depth at which light intensity is reduced to 1% of incident intensity which is $\leqslant$2 mm (Pinckney, 1994; de Winder et al., 1999). The results showed that the concentration of surface Chl*a* for SI (4.57–12.09 $\mu g\ g^{-1}$) was similar to that found in others temperate estuaries where microbial mats are absent (Moreno and Niell, 2004 and references therein). A similar concentration range was found by Dupré (2012) in the inner zone of the Bahía Blanca Estuary where biofilms of diatoms are present. On the other hand, SII presented lower values of Chl*a* than other tidal flats covered by cyanobacterial mats (de Winder et al., 1999).

The higher Chl*a* content in SII could be attributed to the type of habitats preferred by the microorganism that form microbial mats. In Rosales Harbor the microphytobenthic community that integrated the supratidal flat consists of epipelic diatoms and filamentous cyanobacteria (Pan et al., 2013a,b). The biofilm of diatoms occurred on relatively fine-grained sediment, whereas the mat of cyanobacteria developed on much coarser sediment. It has been suggested that cyanobacteria cannot cope with the more mobile and dynamic cohesive sediments and develop only in the translucent, fine-grained, non-cohesive quartz sediments (Stal, 2010). Previous reports carried out in these sites have shown that the composition of the two phototrophic communities does no differs between sites (Pizani, 2008; Pan et al., 2013a,b) despite the different granulometric composition of the sediments. According to Wachendörfer et al. (1994) the microorganisms themselves alter the sediment, including its grain size and porosity. All this suggests that another factor controls the biomass in the study area as it was observed by Pan et al. (2013b) who attributed the temporal distribution of microphytobenthos to seasonal variation in irradiance and sediment temperature. Similar behavior was observed by Cartaxana et al. (2006) in the Tagus Estuary in muddy and sandy sediments where the microphytobenthic assemblages were dominated by diatoms.

Microbial mats are taxonomically complex, metabolically interactive, self-sustaining communities. They are frequently found in environments where extreme conditions in temperature, hydrology, salinity, or nutrient availability limit the occurrence of grazing invertebrates (Jørgensen et al., 1983). In this way, the absence of predators at SII could also be influencing the higher concentration of Chl*a*, and therefore the difference in organic matter content might probably relate to a difference in microalgal biomass between sites.

In the upper 5 mm, the highest Chl*a* value found in winter for SI was supported by previous available nutrients stock. On the other hand, SII showed a high value of Chl*a* in the cold season in coincidence with an important decline of $NH_4^+$, $PO_4^{3-}$ y DSi concentrations. These nutrients could be uptaken by benthic microalgae which would be causing the decrease in their concentrations. Furthermore, the $NH_4^+$ concentration plays an important role in determining the estuarine benthic diatom species compositions of epipelic algae (Underwood et al., 1998).

The ratio of pigments/organic matter (mg $g^{-1}$) can be used as an index of sediments quality (Niell, 1980). In this study the ratio was <1 in the surface and subsurface layer, suggests a low sediment quality and possible organic matter refractory and allochthonous origin probably related to the influence of the sewage discharge.

### 4.6. Heavy metals in sediments

Sediments are an important sink for metals and other pollutants; they have been described as a non-point source for metals and sediment-bound metals can be released into overlying waters and adversely affect aquatic organisms (Wang et al., 2004). The concentration of heavy metals depends on the sources, the chemical characteristics of the elements, physicochemical conditions and complex reaction such as adsorption, flocculation and redox condition of the estuarine sediments (Establier et al., 1984). In this case, the distribution of heavy metals in the sediment was not uniform over the whole tidal flat analyzed, though in both sites evaluated are developing microbial mats. The charges distributed along the filamentous cyanobacteria provide an enormous surface area for binding of positively charged metals (Ahuja et al., 1999; Blanco et al., 1999). Probably the higher mat biomass at SII must be affecting the higher content of heavy metals observed at this site. Moreover, EPS ideally serves as a natural ligand source, providing binding sites for metals (Decho, 1990), and fine-grained sediments present high amounts of EPS (de Brouwer et al., 2003), so we can suggest that the finer sediment found at SII throughout the EPS could be involved in the different levels of metals recorded. In the same way finer sediments are observed to be rich in organic matter content, which is fundamental in the fixing or capture of elements (Chandra Sekhar et al., 2003). The increment of organic matter deposition to coastal waters has been related to intensive use of inorganic fertilizers, the population growth (Cornwell et al., 1996) and the increasing sewage inputs of particulate sedimentary matter (Stull et al., 1986). Therefore, the enrichments of organic matter as well as trace metals found in SII probably reflect the direct discharge of untreated or primary treatment domestic wastes which must be acting as good binding sites for metals. The urban wastewater contains the pollutants introduced by run-off rainwater, domestic and commercial sources. Domestic sources include the potentially toxic elements discharged from the household and, in addition, corrosion from materials used in distribution and plumbing networks, tap water and detergents. Fecal matter, body care products, pharmaceuticals, cleaning products and liquid wastes are some of the principal sources of metals. Despite their higher values, Fe and Mn are not discussed here as both metals are not considered potential hazardous pollutants. Then considering the order of heavy metals levels in the studied sediments we must emphasize that Zn may enter urban wastewater from corrosion and leaching of plumbing, water-proofing products, anti-pest products, wood preservatives, deodorants and cosmetics, medicines and ointments, paints and pigments, health supplements, among others. In the same way, Cu comes mainly from corrosion and leaching of plumbing, fungicides, pigments, wood preservatives, larvicides and antifouling paints related to the proximity to Rosales Harbor.

Alternately, sediments enriched with metals may be diluted by the landward incursion of less enriched marine sediments into the estuaries (Forstner and Muller, 1974). In this case, the vegetation of both sites and especially the cordgrass *S. alterniflora* acts as a shallow barrier, producing wave attenuation (Koch et al., 2009), and reducing the capability of currents to erode and transport sediments (Wolanski et al., 2009). The analysis of tidal heights at Rosales Harbor in the year 2011, evidences that the tidal flat at this site was less inundated compared to 2010 (Cuadrado et al., 2013a), anyway SI was flooded longer than the SII (Fig. 8), which presented higher heavy metal content. Therefore the heavy metal content distribution may be affected by water dynamic, as revealed Yu and Zou (2013) in sediments from Jiangsu coastal wetland where the contents of heavy metals got lower from high tidal zone to low tidal zone.

Previous reports performed in sediments from the Principal Channel in vecinity of Rosales Harbor (Marcovecchio and Ferrer, 2005) and in sediments from the Access Channel (Pizani, 2008) showed higher concentrations of Cd and Pb and lower of Cu and Fe, while the levels of Zn and Cr were similar. Comparing with other sites of this estuary and other estuaries, but in vegetated or unvegetated sediments of intertidal areas (Botté et al., 2010 and references therein; Buggy and Tobin, 2008; Serafim et al., 2013), we observed that especially the Cd, Pb and Cr were similar or lower to those reported.

The heavy metals concentration detected are in general higher than the ones found in other supratidal area but from hypersaline environments colonized by microbial mats (Taher et al., 1994). We must highlight that the information linked to this topic is poor and ever more that one related to microbial mats in supratidal areas.

The correlations observed, indicate that these metals are associated with each other and may have a common anthropogenic and natural sources in the sediments of the Rosales Harbor tidal flat. So, from the above discussion it could be said that their source was almost the same and which may be derived from the port activity and sewage discharge.

## 5. Conclusion

It is worthy to note that this is the first time that physicochemical parameters are evaluated in the supratidal sediments colonized by microbial mats from the middle zone of Bahía Blanca Estuary. The evaluated parameters showed differences between the two studied sites, despite the proximity and the presence of microbial mats in both. The interaction among all factors analyzed influences the microphytobenthonic development in the tidal flat of Rosales Harbor.

We must emphasize that the high nutrients and heavy metals levels, and specially the "biological pollution" evidenced by the presence of *E. coli* at both sites indicate a strong influence of the sewage discharge of Punta Alta City in the study area. This is an important risk factor for human health and for the entire ecosystem.

Finally, these results allow us to define specific characteristics (organic matter, chlorophyll, nutrient and heavy metal content among others) that make these tidal flats a propitious place for the development of microbial mats, while contributing to a better understanding of the sediments dynamics of these areas from Bahía Blanca Estuary as well as from other tidal flats in the world.

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