

# Heavy Metal Concentrations Found in Seston and Microplankton from an Impacted Temperate Shallow Estuary along the Southwestern Atlantic Ocean

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## ABSTRACT

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Heavy metal concentrations (Cd, Cu, Cr, Fe, Mn, Ni, Pb, and Zn) were studied in the Bahía Blanca Estuary (BBE), one of the most anthropogenically disturbed estuaries in the SW Atlantic Ocean. This study evaluated metal concentrations in the microplankton and seston for the first time, as well as their role in the transport of contaminants in a disturbed coastal environment of the SW Atlantic. Spatial patterns of metals in suspended particulate matter (SPM; seston > 0.45 μm) and microplankton (20–200 μm) were analyzed at sampling sites located at sewage or industrial discharges and, also far from this area, during warm months in 2012 and 2013. Dissolved inorganic nutrients, particulate organic matter (POM), and chlorophyll *a* (Chl-*a*) were also analyzed. The POM and Chl-*a* concentrations were higher near to the sewage discharges; however, metals showed different concentrations between sampling site stations. Those located far from human disturbances showed similar levels to the sites at the sewage or industrial discharges in some cases. In general, the SPM had higher concentrations of metals than the microplankton. In particular, Cr presented important levels in this fraction, which was potentially associated with industrial discharges. On the other hand, a contrasting partition was found for Pb, Zn, and Cu that exhibited higher levels in the microplankton-net material. The high levels of Pb in the microplankton of the BBE may indicate a high availability of this metal in the environment and high uptake rates, with potential health risks to humans and marine life attributable to its toxic effects.

**ADDITIONAL INDEX WORDS:** *Suspended particles, pollution, estuarine system, persistent contaminants.*

## INTRODUCTION

Coastal environments are areas of significant biogeochemistry activity, with dynamic and complex processes resulting in highly productive ecosystems of ecological and commercial importance. They are transitional zones between the land and the ocean, with significant amounts of continental and marine materials (Prego *et al.*, 2013). Unfortunately, the contamination of these environments attributable to human development is a common issue. These ecosystems are increasingly more eutrophic and polluted as a result of high nutrient inputs of anthropogenic origin, and they receive the largest exposure to chemical and nutrient contamination because of their proximity to high-population density centers (Kennish, 1997).

Autotrophic and heterotrophic microplanktonic organisms are key steps in the biogeochemical cycling of metals as part of the marine food webs, participating in the transfer of these elements in aquatic ecosystems (Kehrig *et al.*, 2009a). Autotrophic organisms such as phytoplankton are the basis of the marine food webs; they play an essential role in nutrient

cycling and water quality, and they serve as food supply for heterotrophic organisms, such as zooplankton and filter feeders. Phytoplankton incorporate metals from the dissolved phase; the essential ones, such as Cu, Fe, Ni, Zn, and Mn, show greater penetration into the cytoplasm of the algal cells, while nonessentials, such as Cd and Pb, remain adsorbed on the cell surfaces (Michaels and Flegal, 1990). On the other hand, zooplankton accumulate them from ingested food as well as from the dissolved phase (Wang and Fisher, 1999). Once incorporated into the plankton, metals may be transferred to higher trophic levels through the marine food web, increasing or decreasing their concentrations (Caetano and Vale, 2003). Thus, direct measurements of metal quotas in plankton from natural environments are needed to advance in the understanding of metal biogeochemistry.

Another vital component in the cycling of heavy metals is seston, one of the main reservoirs for metals. Seston comprises the suspended particulate matter (SPM) of aquatic ecosystems and includes living/nonliving organisms and organic/inorganic particles of different quality and quantity. Silts and clay are generally the inorganic fraction of seston, while the organic seston corresponds to detritus and living material, such as plankton (Pedrosa, Magalhães de Souza, and Rezende, 2007). Trace metals are distributed in these major components of seston, and metal concentrations are usually high in this

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fraction, particularly in coastal areas. These particles are able to adsorb dissolved trace metals from the water column efficiently because of their high surface-to-volume ratio (Showell and Gaskin, 1992).

In this context, the study of heavy metals in the particulate fraction (seston) and the microplankton deserves special interest to better understand and predict the transfer of metals from the basis of the food chain in aquatic ecosystems. This kind of study is particularly essential in coastal areas, characterized by high levels of particulate material and metal discharges, in order to evaluate possible biomagnification from plankton to higher trophic levels. Numerous studies focused on heavy metals in seston and/or microplankton have been performed over the last decades (*e.g.*, Demina and Nemirovskaya, 2007; Demirak *et al.*, 2012; Jara-Marini, Soto-Jiménez, and Páez-Osuna, 2009; Laslett, 1995; Luoma *et al.*, 1998; Michaels and Flegal, 1990); however, most of them correspond to seston or SPM. In the case of microplankton, many of the studies come from laboratory culture rather than from natural systems. These studies emphasize the ability of seston and microplankton to adsorb or accumulate metals from the seawater, as well as their role in the trophic web through the regulation of metal transfer. Moreover, the aforementioned authors highlight the significant effects on the biogeochemical cycles and also their importance in interpreting fluctuations in heavy metal concentrations in aquatic systems. Despite the important properties of seston and microplankton, however, few studies contemplate their distribution in coastal zones and their relationships with inorganic nutrients and/or physico-chemical parameters of both fractions simultaneously (*e.g.*, Kehrig *et al.*, 2009a; Rossi and Jamet, 2008). Furthermore, information regarding heavy metals in seston and microplankton is scarce in coastal ecosystems, especially regarding coastal areas of the South Atlantic Ocean (Kehrig *et al.*, 2009a; Kehrig *et al.*, 2009b). Therefore, this work comprises one of the first studies to contemplate heavy metals in these significant fractions in the Bahía Blanca Estuary (BBE), one of the most important ecological coastal areas located in the SW Atlantic Ocean. The novelty of this contribution is the study of metal association with seston and microplankton and its relation with physico-chemical conditions in a coastal environment highly affected by human activities. The importance of this study relies on the key role these fractions play in the transport of contaminants through the aquatic food web in environments seriously threatened, such as estuaries.

In the particular case of the BBE, it is located in a temperate climate region on the SW Atlantic Ocean of Argentina and is characterized by both high concentrations of SPM and also significant plankton densities during some periods of the year. In recent decades, it has been seriously affected by human activities related to industries, ports, and urban development. It is one of the most essential estuaries in South America and is the main deep-water port of Argentina of 45 feet in depth. Because of this feature, several ports and significant industrial activities have been developed; in recent decades, it has been seriously affected by these activities. The estuary receives a large amount of discharges from different origins, such as raw sewage or inadequately treated sewage from the urban areas and effluents from chemical and petrochemical industries

(Arias *et al.*, 2010; Fernández Severini, Hoffmeyer, and Marcovecchio, 2013; Marcovecchio *et al.*, 2008). In this context, the BBE represents an area with urban and industrial effluents, including sewage-effluent waters and wastes, spills and effluents associated with shipping, docking, power generation, manufacturing, and chemical and petrochemical industries. Moreover, one of the most critical pollutants discharged into the estuary are heavy metals, which are rapidly adsorbed onto the particulate fraction of this turbid estuary (Fernández Severini, Hoffmeyer, and Marcovecchio, 2013), characterized by high concentrations of SPM and significant plankton densities during some periods of the year. Because of these singular features, BBE is a very interesting ecosystem for pollution studies in relation to heavy metal inputs from human activities. So, the research objectives of this study were broadly focused on the analysis of heavy metals in the total SPM (seston > 0.45  $\mu\text{m}$ ) and microplankton (particle size between 20 and 200  $\mu\text{m}$ ) at different sampling sites according to the pollutant-input levels. The relationships with nutrients and photosynthetic pigments, such as chlorophyll *a* (Chl-*a*), and physico-chemical parameters were also considered in order to analyze the influence of these parameters on the concentration of metals. In the present study, it is hypothesized that seston and microplankton show significant levels of metals as a consequence of the industrial and domestic wastes discharged into the BBE and that sampling sites associated with human discharges show higher metal concentrations.

## METHODS

This section comprises the general characteristics of the study area, the sampling procedures, and a detailed description of the analytical methods for chemical determinations, as well as the statistical analysis employed.

### Study Area

The BBE (38°44'–39°27' S; 61°45'–62°30' W) is a mesotidal, temperate, and turbid estuary, located in the SW Atlantic Ocean (Figure 1). It extends over approximately 2300 km<sup>2</sup> and comprises several tidal channels, extensive tidal flats, low marshes, and islands. The main navigation channel is funnel shaped, reaching 80 km in length, 3–4 km in width at the mouth, and 200 m in width near to the head. A quasistationary and semidiurnal tidal wave, which is the main energy input into the system, controls the water circulation in the BBE. The mean tidal amplitude ranges between 2.2 m at the mouth and 3.5 m at the head. Winds strongly modify the tidal wave, the N-NW wind reduces the sea level, and the SE wind generates the opposite effect (Piccolo, Perillo, and Melo, 2008).

Based on the salinity distribution, the BBE can be classified as a vertically mixed estuary during normal runoff conditions but with a strong tendency to become partially mixed during rainfall periods (Perillo *et al.*, 2004). Salinity varies from 17.9 to 41.3, depending on seasonal rainfall, winds, and air temperature (Freije and Marcovecchio, 2004). Freshwater inflow into the estuary is low, and it mainly comes from the Sauce Chico River and the Napostá Grande creek, which provide an annual mean runoff of 1.9 and 0.8 m<sup>3</sup> s<sup>-1</sup>, respectively (Perillo *et al.*, 2004). A series of small tributaries exist that may input minor quantities of runoff, influenced by local rainfalls (Perillo and

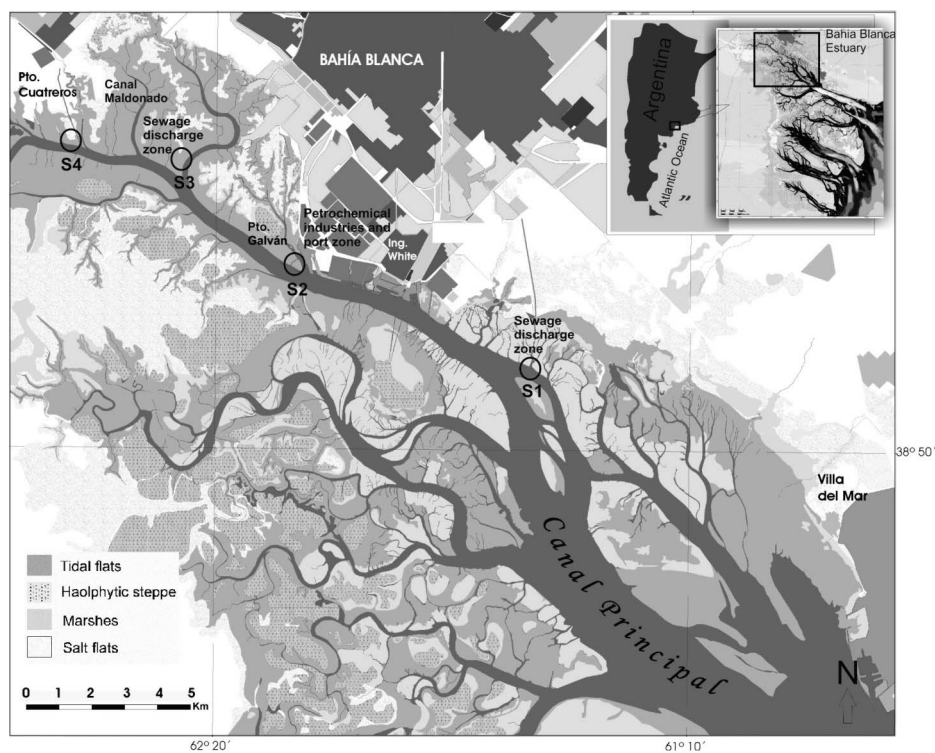


Figure 1. Map of the inner zone of the Bahía Blanca estuary (BBE) and location of the sampling stations (S1 to S4). In the upper right corner: general location of the BBE in Argentina.

Piccolo, 1999). The mean surface-water temperature varies from 7°C in winter to 23°C in summer (Freije *et al.*, 2008).

The BBE is recognized as a nutrient-enriched system because of the significant levels of inorganic nutrients (nitrate, nitrite, phosphate, and silicate) that are available during most of the year (Freije *et al.*, 2008; Spetter, 2015, and references therein). Mean dissolved oxygen is close to 7 mg L<sup>-1</sup>, reaching 13 mg L<sup>-1</sup> during periods of phytoplankton bloom (winter and late summer) (Freije *et al.*, 2008). The inner zone of the BBE is the most exposed to anthropogenic pressure as a consequence of human settlements, commercial ports, and diverse industries located on its northern coast (Arias *et al.*, 2010). Oil refineries and terminals, chemical and petrochemical industries, meat and fish factories, leather plants, textile and wool-washing plants, silos, and cereal mills discharge their processing residues into the streams or directly into the estuary. Also, the BBE is used extensively by fishing boats, oil tankers, and cargo vessels and requires regular dredging. Thus, this coastal ecosystem receives contaminant inputs from sewage discharges, industrial discharges, harbour-related operations, and runoff water that carries materials from land-development areas and also aerial fallout from atmospheric pollutants. The study area comprises four sampling points (S1–S4). The S1 corresponds to the main sewage discharge from Bahía Blanca and Ingeniero White cities (400,000 inhabitants). The S2 is next to industrial discharges of the petrochemical refineries and fertilizer plants as well as the deep water harbor. The S3 is near the secondary sewage

discharge of Bahía Blanca city, whereas S4, the innermost station, is far away from all these sources of contamination (Figure 1). These sampling sites were selected strategically along the estuary according to the different pollutant input levels.

### Sampling and Laboratory Procedures

Sampling campaigns were carried out monthly between November 2012 and March 2013, during the austral spring, summer, and late summer. Warm months were considered because of the higher plankton densities to ensure enough material for detecting heavy metals levels. The four sampling sites representative of the BBE and located at suitable points, easily accessible from a boat, were chosen to guarantee the quality and quantity of the samples and to ensure compliance with the proposed objectives. These sites are located from the standpoint of pollution sources and the density of planktonic individuals, as well as easy access and efficiency in terms of sampling efforts. Some of these sites (S1, S2, and S4) are permanent monitoring stations that serve to compare and analyze changes in metals concentrations over time. Surface-water samples for the analysis of metals in bulk-particulate material were collected manually at <1 m depth, using 1.5 L polyethylene-terephthalate (PET) bottles previously conditioned with ultrapure 5% HNO<sub>3</sub> during 2 days and rinsed gently several times with distilled water. To analyze metals, microplankton (20–200 μm) was sampled from a boat during ebb tide with a Nansen 20-μm-mesh size net, and two replicates

were taken per station (750 ml each). The tows were oblique (0.5–1 m depth) and at a constant speed (2 knots) for 5 minutes. Microplankton samples were collected with the Nansen net (20  $\mu\text{m}$ ) for qualitative analysis and with a Van Dorn bottle for the quantitative one. The microplankton samples for the analysis of metals were transferred to PET bottles (750 ml) previously cleaned with acid.

Water samples for the determination of Chl-*a*, POM, and dissolved inorganic nutrients (nitrite, nitrate, phosphate, silicate) were collected using a Van Dorn bottle. The physico-chemical variables (temperature, pH, conductivity/salinity, dissolved oxygen) were recorded at 1-m depth with a multi-sensor Horiba U-10. The samples were immediately transported to the laboratory and filtered by vacuum through acid treated Millipore® HAWP 04700 preweighed filters (47-mm diameter and 0.45- $\mu\text{m}$  pore) for the determination of metals in the particulate fraction and microplankton. All the sampling and laboratory materials were carefully cleaned with ultrapure 5%  $\text{HNO}_3$  during the 2 days prior to use and then rinsed gently with distilled water, following internationally recommended protocols (APHA, 1998). Water samples (250 mL) for nutrients, Chl-*a* and organic matter analysis were filtered through glass fiber grade F membranes (47-mm diameter and 0.7- $\mu\text{m}$  pore). The filters for nutrients and organic matter determinations were previously muffled (450–500°C, 1 hour). Samples for nutrient determinations were frozen (–20°C) in plastic bottles until analysis (APHA, 1998). The filters used for chlorophyll and organic-matter determinations were stored in aluminum envelopes and kept frozen in the dark until analysis.

At the laboratory, microplankton samples were prefiltered manually with a 200- $\mu\text{m}$ -mesh net to analyze only the microplanktonic fraction and to discharge particles out of the range. Then the samples were vacuum filtered (<20 cm Hg) following the same procedure as the SPM. In both cases, the filters with the retained material (SPM or plankton) were dried at  $50 \pm 5^\circ\text{C}$  to a constant weight, weighed in an analytical balance, and stored in a desiccator until their analytical treatment. Subsequently, each filter was digested with a mixed of 5 ml of  $\text{HNO}_3$  (70%, ultrapure) and 1 ml of  $\text{HClO}_4$  (70%, ultrapure) at  $110 (\pm 10^\circ\text{C})$  in a bath of glycerine on a hotplate to obtain an extract of about 1 ml. Each of these extracts were gently transferred to a graduated tube and completed with nitric acid 0.7% to a final volume of 10 ml. The same digestion procedure was performed for filters without particles to act as blanks, and all the fractions were analyzed in duplicate to ensure the reproducibility of the method. The metal concentrations in SPM and microplankton were determined by duplicate using ICP-OES Optima 2100 DV Perkin Elmer with a Cross-Flow nebulizer. The method detection limit (MDL) for each metal was calculated by performing the complete analytical procedure (digestion, cleanup, and instrumental analysis) on replicate samples (20) with clean filters Millipore® HAWP 04700 free from SPM and microplankton. The MDL were calculated by multiplying the standard deviation of these replicate ( $n$ ) measures by the Student's  $t$  value at the 99% confidence level (at  $n - 1$  degrees of freedom) (Federal Register, 1984). The MDL for Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were 0.003, 0.05, 0.09, 3, 0.23, 0.05, 0.04, and 0.11  $\mu\text{g g}^{-1}$ , respectively. For the analytical quality control, reagent blanks,

certified reference materials (CRMs [plankton, Certified Reference Material BCR No414, IRMM, Geel, Belgium]), and analytical grade reagents (Merck) were used. The recovery percentages for all trace metals in CRM were higher than 90%. Nitrate, nitrite, phosphate, and silicate concentrations were determined according to Treguer and Le Corre (1975); Grasshoff, Erhardt, and Kremling (1983); Eberlein and Kattner (1987); and Technicon Autoanalyzer® (1973), respectively. A Technicon AA-II Autoanalyzer® was used to perform the nutrient analyses. The quantification limit of the methods was 0.10  $\mu\text{M}$  for nitrates, 0.02  $\mu\text{M}$  for nitrites, 0.01  $\mu\text{M}$  for orthophosphates, and 1.00  $\mu\text{M}$  for silicates. Chl-*a* and phaeopigments were analyzed using the spectrophotometric method of APHA (1998) by extraction with acetone 90% during 24 hours at  $-4^\circ\text{C}$ , and the particulate organic matter was determined according to Strickland and Parsons (1968), both using a UV-Vis Jenway 6715 spectrophotometer.

### Statistical Analysis

Two-way analysis of variance (ANOVA) was used after logarithmic ( $\text{Log}_{10} X$ ) data transformation. The aim of this analysis was to detect the possible effects of the concentrations of metals in the SPM, on the microplankton at different sampling sites (factor 1), and in different sampling months (factor 2), and to see whether these effects were the same at each level of the other factor (interaction). If significant differences were detected between the sampling stations, Bonferroni's test was applied. Two-way ANOVA without replica was employed to evaluate seasonal and spatial differences in the physicochemical parameters. Spearman's correlations ( $r$ ) between metals, physicochemical parameters, nutrients, Chl-*a*, and particulate organic matter were tested for the seston and microplankton. Correlations between both fractions were also analyzed. Principal component analyses (PCA) with the covariance matrix were used to define the relationships between metals and their sources at each fraction. All statistical analyses were carried out using InfoStat (Di Rienzo *et al.*, 2015), following Zar (1996).

## RESULTS

This section describes the spatial and temporal dynamics of the physico-chemical parameters, such as water temperature, pH, salinity, dissolved oxygen, and the concentrations of POM, SPM, dissolved nutrients, and Chl-*a*, in the BBE. The levels of heavy metals in the SPM and microplankton, as well as the correlations between the different variables and PCA, are also analyzed.

### Physico-Chemical Parameters

The temperature in surface waters (0–1 m) varied from 17.1 to 25.3°C, showing the normal values of warm months at the BBE. Salinity values were high (30.87–36.87), in correlation with high temperatures, and the range of pH values was between 7.76 and 9.69. In addition, all these parameters were very similar between the sampling sites, and no statistical differences were detected between them (two-way ANOVA,  $p > 0.25$ ). The exception was the concentration of dissolved oxygen, which showed lower values ( $2.49 \pm 2.28 \text{ mg L}^{-1}$ ) at the sampling site located at the main sewage discharge ( $p < 0.01$ ).

Table 1. Mean values of the physico-chemical parameters, dissolved nitrogen nutrients ( $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{3-}$ ), silicates, particulate organic matter (POM), chlorophyll *a*, and suspended particulate matter (SPM) concentrations with their corresponding standard deviation (SD) and percentage of POM in the SPM in the surveyed sites.

	S1 Mean $\pm$ SD	S2 Mean $\pm$ SD	S3 Mean $\pm$ SD	S4 Mean $\pm$ SD
Temperature ( $^{\circ}\text{C}$ )	21.8 $\pm$ 2.8	20.8 $\pm$ 1.9	20.3 $\pm$ 2.3	21.1 $\pm$ 1.9
Salinity (psu)	31.41 $\pm$ 4.22	34.60 $\pm$ 1.71	34.23 $\pm$ 2.67	34.36 $\pm$ 2.17
DO ( $\text{mg L}^{-1}$ )	2.49 $\pm$ 2.28	3.98 $\pm$ 3.32	3.72 $\pm$ 3.01	4.04 $\pm$ 3.08
pH	8.6 $\pm$ 0.8	8.7 $\pm$ 0.8	8.9 $\pm$ 0.6	8.9 $\pm$ 0.9
$\text{NO}_2^-$ ( $\mu\text{M}$ )	1.58 $\pm$ 0.80	2.32 $\pm$ 0.43	1.72 $\pm$ 0.71	2.03 $\pm$ 0.45
$\text{NO}_3^-$ ( $\mu\text{M}$ )	6.84 $\pm$ 3.32	9.57 $\pm$ 3.17	10.05 $\pm$ 4.90	9.16 $\pm$ 2.45
$\text{PO}_4^{3-}$ ( $\mu\text{M}$ )	2.98 $\pm$ 1.85	2.03 $\pm$ 0.77	1.87 $\pm$ 0.78	4.38 $\pm$ 6.08
Silicates ( $\mu\text{M}$ )	47.48 $\pm$ 23.16	51.52 $\pm$ 15.46	40.39 $\pm$ 22.53	46.13 $\pm$ 17.04
Chlorophyll <i>a</i> ( $\mu\text{g L}^{-1}$ )	3 $\pm$ 6.59	0.44 $\pm$ 0.42	4.22 $\pm$ 7.35	2.21 $\pm$ 4.34
SPM ( $\text{mg L}^{-1}$ )	73,364.59 $\pm$ 65,172.62	124,199.49 $\pm$ 80,969.34	141,466.67 $\pm$ 62,814.22	76,646.99 $\pm$ 42,523.12
POM ( $\text{mgC m}^{-3}$ )	2282.57 $\pm$ 1152.54	1568.17 $\pm$ 402.56	2106.74 $\pm$ 511	1779.45 $\pm$ 303.15
(POM/SPM)*100	5.24	1.26	1.71	2.79

Table 1 shows the mean values of these physico-chemical parameters in the sampling sites.

### Nutrients, Chl-*a*, and Particulate Organic Matter Concentrations

In general, nutrients (nitrite, nitrate, phosphate, and silicate) showed similar concentrations between the sampling sites, and no statistical differences were detected between them (two-way ANOVA,  $p > 0.25$ ); however, S1 and S3 (located at sewage discharges) showed the highest values of POM. Moreover, S1 presented the highest POM concentrations (2283  $\text{mg C m}^{-3}$ ), followed by S3 (2107  $\text{mg C m}^{-3}$ ). Additionally, S1 and S3 showed the highest mean concentrations of Chl-*a*, indicating that SPM and microplankton at these sites had more phytoplankton than the other sites. On the other hand, the ratio between POM and SPM was calculated to establish the contribution of the organic fraction to the total particulate fraction. In this case, S1 and S4 presented the highest percentages (Table 1).

### Metal Contents in the Microplankton and Total SPM

Figure 2 shows the concentrations of Cd, Cu, Cr, Fe, Mn, Ni, Pb, and Zn in the microplanktonic fraction at the surveyed sites (S1 to S4) in the BBE. Microplankton showed the highest mean concentrations of Cd (0.32  $\mu\text{g g}^{-1}$ ), Cu (43.26  $\mu\text{g g}^{-1}$ ), Zn (84  $\mu\text{g g}^{-1}$ ), and Pb (16.91  $\mu\text{g g}^{-1}$ ), particularly at S1. The SPM also presented the highest concentrations of Cd (0.40  $\mu\text{g g}^{-1}$ ), Cu (33.89  $\mu\text{g g}^{-1}$ ), and Zn (72.61  $\mu\text{g g}^{-1}$ ) at this sampling site (Figure 2). On the other hand, all metals in the microplankton and SPM were high at S2, close to the industrial discharges. A two-way ANOVA for each metal was performed to detect these differences and to find the effects on the metal concentrations in the SPM and microplankton attributable to the sampling station (factor 1) and attributable to the sampling month (factor 2) and to see whether these effects are the same at each level of the other factor (interaction). Metal concentrations in the microplankton were significantly different between the sites ( $p < 0.05$ ) for all metals except for Cd and Cr ( $p > 0.25$ ). Statistical differences were also detected because of the sampling month ( $p < 0.05$ ) for all metals except Cd and Cu ( $p > 0.25$ ). On the other hand, significant statistical differences were detected in the SPM between the sampling stations ( $p < 0.05$ ) for all metals except Pb and Zn ( $p > 0.25$ ). Considering the

months, significant differences were detected for all the metals ( $p < 0.05$ ) with the exception of Cu ( $p > 0.25$ ). Figure 2 also shows the mean concentrations of metals at each sampling site and the statistical differences between them. An analysis of these results showed no clear differences between the sites located near the discharges and the site with least human influence (S4). For example, Cr, Zn, and Cd in the microplankton at all sampling sites showed similar concentrations at high levels, and Pb and Zn showed the same pattern in the SPM.

Taking each fraction into account, Cu, Pb, and Zn were higher in the microplankton than in the SPM at all the sampling stations which could indicate a more effective transfer of these metals from water to microplankton. The remaining metals were slightly higher in the SPM. Considering the general mean concentrations of each metal, the differences between SPM and microplankton were not so obvious. Figure 3 represents a visual summary of the proportions of metals in SPM and microplankton considering all the sampling sites. In this figure, metals are quite similar in each compartment. Both fractions mainly comprised Fe and Mn and at similar percentages, while the percentages of the rest of the metals were lower. In the case of Cd, the value was very low.

Considerable seasonal differences in the levels of metals in the SPM and microplankton were also observed, showing generally higher levels of all the metals in summer (February–March) than in spring (November–December) (Figure 4a,b). The exception was Fe, which was the only metal with higher levels in spring. Moreover, the levels in summer exceeded those in spring at all the sampling sites.

### Correlation Analysis

Table 2 shows the significant ( $p < 0.05$ ) Spearman correlations to analyze relationships among metals, physico-chemical parameters, nutrients, Chl-*a*, SPM, and POM concentrations. No significant correlations were found between metals and most of these parameters; however, some correlations were detected between POM and SPM (Cd in SPM/SPM:  $r = -0.66$ ; Cd in SPM/POM:  $r = 0.65$ ; Cd in micro/POM:  $r = 0.82$ ; Cu in micro/POM:  $r = 0.65$ ). In addition, some significant correlations were detected between metals in the microplankton and the SPM, which could indicate the same origin or source.

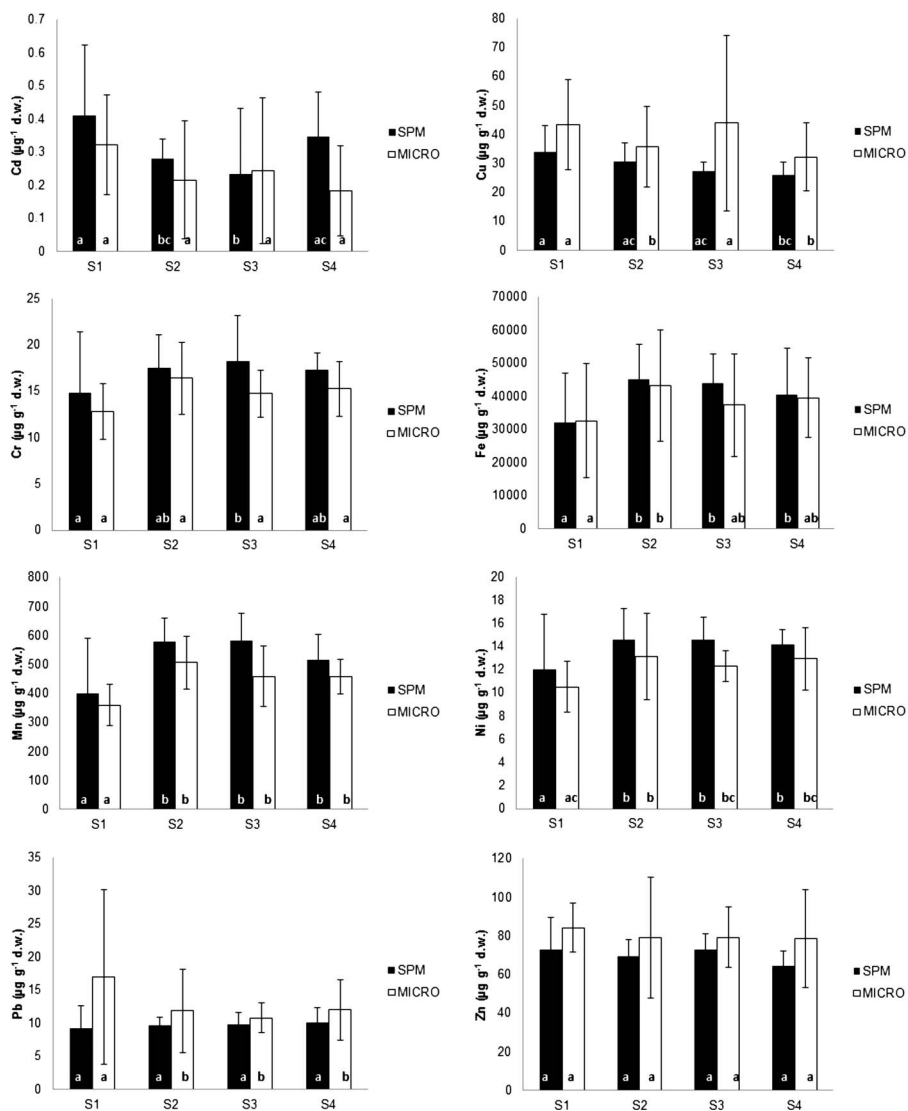


Figure 2. Spatial distribution of heavy metals in seston (or SPM) and microplankton ( $\mu\text{g g}^{-1}$  dry weight). Sampling sites with different letters mean significant statistical differences ( $p < 0.01$ ).

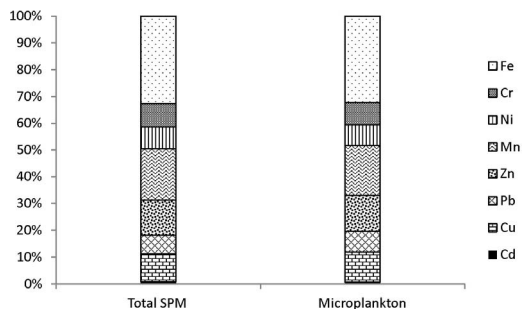


Figure 3. Percentage of the metals at each fraction.

### Metals Relationships and Sources Analysis

The PCA and a covariance matrix were applied to each fraction to analyze metal associations and sources of heavy metals. This type of matrix was employed because the variables have the same unit ( $\mu\text{g g}^{-1}$ ). Figure 5a shows the results of the PCA of metals in SPM, where the first two components (F1 and F2) explained 83.85% of the total variability. Pb, Mn, Ni and Cr are clustered together near the right side of the  $x$  axis and contributed to F1 with high positive loadings. On the other hand, Cd, Cu, and Zn had moderate to low negative loadings to F1. Fe contributed with a moderate loading to F2. In the case of the microplankton, the first two components explained 74.95% of the total variability (Figure 5b). Cu, Pb, and Zn showed high positive correlations with F1 and clustered together near the

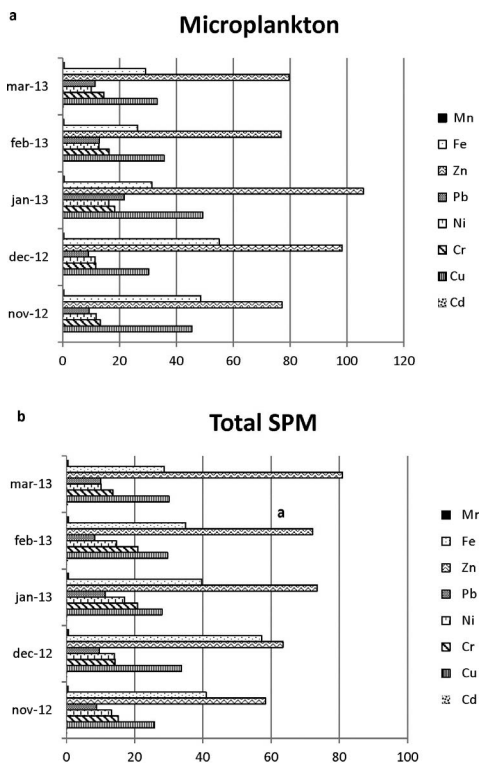


Figure 4. Mean concentrations of metals ( $\mu\text{g g}^{-1}$ ) in the SPM (a) and microplankton (b) in each sampling month. Mn and Fe in  $\text{mg g}^{-1}$ .

right side of the  $x$  axis. F2 showed high correlations with Fe and Mn.

### DISCUSSION

It is well known that physico-chemical parameters, such as temperature, pH, and salinity, influence the biogeochemical cycles of metals in aquatic environments (Morel and Price, 2003). In this study, the temperature, pH, and salinity were within the typical range of the BBE during warm months, and they did not vary between sampling sites. In addition, they were similar to the previous records in the estuary (Andrade,

Table 2. Spearman's correlation matrix with the significant correlations ( $p < 0.001$ ). In all of the cases,  $n=38$ .

	SPM			SPM	POM	Microplankton	
	Fe	Ni	Cr			Zn	Pb
SPM							
Cd	-0.69						
Cr		0.65					
Mn	0.64						
Fe				0.76			
Ni							
Microplankton							
Cd					0.82		
Cu					0.65	0.73	0.65
Fe							
Zn							0.77
Ni		0.74					
Cr			0.79				

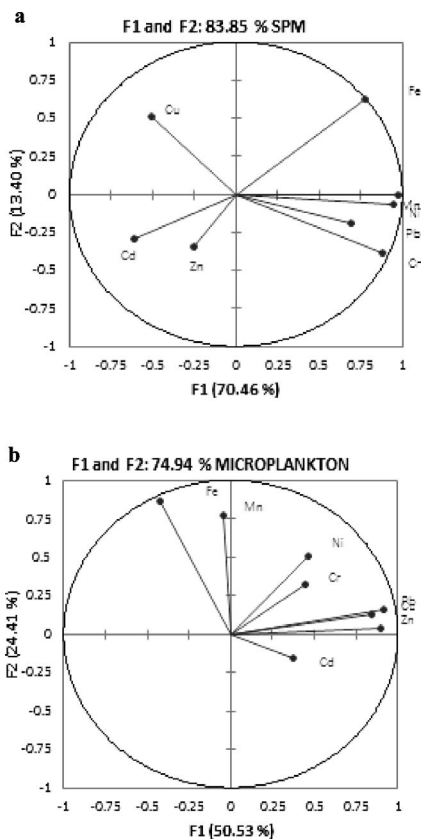


Figure 5. Principal component analysis loading plots of metals. (a) SPM (suspended particulate matter); (b) microplankton.

2001; Freije *et al.*, 2008). In the particular case of salinity, the BBE became hypersaline because of both low rainfall and water evaporation as a consequence of high temperatures especially during late spring and summer.

The low values of dissolved oxygen recorded in S1 are within the expected concentrations because this area corresponds to sewage discharges that favor algal and bacterial activity and the subsequent decrease of dissolved oxygen concentrations. Also, these values are similar to the historical records of the estuary (Botté, 2004; Freije *et al.*, 2008). On the other hand, S1 and S3 showed the highest levels of particulate organic matter because of the closeness to the sources of sewage. The precise cause of these values is the raw sewage or inadequately treated sewage that reaches these sampling sites. Both sites also showed the highest concentrations of the photosynthetic pigment Chl-*a*, and this could be attributable to the sewage, which favors algal development as a nutrient supply. The high percentages of POM in the SPM at S1 and S4 indicate that POM constituted an essential fraction in the SPM fraction, as it likely comprises photoautotrophic organisms because of the higher concentrations of Chl-*a* recorded at these sites.

The BBE is a naturally nutrient-enriched environment, maintaining significant levels of inorganic nutrients during most of the year and low concentrations during the presence of the phytoplankton bloom (Guinder *et al.*, 2013; Marcovecchio

and Freije, 2004; Spetter *et al.*, 2015, and references therein). The results of the dissolved nutrients in this study showed the typical behavior, with maximum values during spring–summer. In spring, after the bloom, the concentration increases, and a recovery phase takes place (Guinder *et al.*, 2013; Marcovecchio and Freije, 2004; Marcovecchio *et al.*, 2008; Spetter, 2006). This phase is associated with the remineralization of the organic matter generated in the bloom and also with the freshwater and rainfalls, which supply nutrients. Nutrient regeneration processes resulting from water-sediment interactions, the increase of zooplankton activity during the post-bloom period (Hoffmeyer, 1994), and presumably other factors, such as adjacent salt marshes, may also contribute to the nutrient increase, which occurs during spring and summer (Popovich and Marcovecchio, 2008).

Spatially, it was hypothesized that stations next to the industrial, or at the sewage, discharges would present higher metal levels; however, relative little differences were found between the sampling sites, and no spatial trend was detected. This pattern can be explained by the short distance between the stations and the points of discharge. As previously mentioned, most of the sites are located in areas of sewage and industrial effluents, and one of them (S4) is located at a more distant area, far from the direct influence of human inputs, but only a few kilometers (4 km) further away. Thus, the high levels also found at S4 can be explained by the water movement attributable to the effect of tides because the samples were taken at low tide. S4, the least affected, is in the innermost zone of the estuary, and during high tide it receives water from human discharges. Then, at low tide, the water begins to withdraw, but during the sampling it may still contain water from the sewage and industrial effluents.

As previously mentioned, the SPM and microplankton adsorbed or accumulated metals from the dissolved phase and thus influenced metal transfer through the aquatic food chain because these fractions serve as a food source for predators, such as mesozooplankton, bivalves, and fishes. Therefore, studies focused on the metal quota at different trophic levels are essential for understanding bioaccumulation processes and biogeochemical cycling of heavy metals in marine ecosystems (Fisher and Reinfelder, 1995). Moreover, the analyses of metals at the basis of the food chain become necessary because seston and microplankton are critical keys in the transfer of carbon and trace elements through marine food webs.

At all the sampling sites, Cu, Pb, and Zn in the microplankton were higher than in the SPM, which could indicate a more effective transfer of dissolved metals from water to microplankton. This fraction mainly comprised diatoms (90%) as part of the phytoplankton and tintinids, ciliates aloricates, rotifers, and dinoflagellates at a lower percentage as part of the microzooplankton. Therefore, most of the metal quota in microplankton corresponded to diatoms, which can adsorb dissolved metals efficiently onto their cell surfaces or intracellularly through passive and active uptake. So, the adsorbed metals became part of the microplanktonic fraction.

In the particular case of microplankton, metal levels represent biological demand and also environmental availability (Twining and Baines, 2013). According to the present study,

the higher levels of Pb and Zn in the microplankton in relation to SPM are noteworthy. These metals deserve special attention because they are extremely toxic at high concentrations and they can be transferred to other organisms in the aquatic food chain, such as copepods that feed on microplankton, and then to top predators like fishes, affecting human health. Within the microplankton, the phytoplankton mainly comprising diatoms was the most representative fraction in this study. Thus, phytoplankton was the main autochthonous source of these metals in the BBE during the sampling period of the present study. It is well known that phytoplankton take up dissolved trace metals from the environment, either by adsorption onto the cell surface or by assimilation (Hassler, Slaveykova, and Wilkinson, 2004), and diatoms especially possess a great ability to acquire and store metals (Twining *et al.*, 2015). Moreover, Martin and Knauer (1973) have previously emphasized the capacity of phytoplankton to bioaccumulate metals. In particular in the case of lead, Fisher (1985) found that phytoplankton can scavenge and concentrate this metal from seawater, which represents the main source of Pb for the zooplankton; moreover, Showell and Gaskin (1992) also found a high lead-phytoplankton association. Pb is a nonessential toxic element, and Zn is essential, but at low concentrations, for CO<sub>2</sub> acquisition as well as for silica uptake by large diatoms (Morel and Price, 2003). Moreover, Pb is not essential for any living organisms, and it is toxic at low concentrations and persists in the environment, so its concentrations may increase in water, sediments, or in biological tissues to levels above the natural background. This metal is an essential source of Pb for organisms at many trophic levels in the dissolved phase as well as in the planktonic one (Soto-Jiménez *et al.*, 2008). Under this scenario, the high concentrations of this metal in aquatic systems, such as the BBE, may involve risks to marine life (*e.g.*, primary producers, zooplankton, filter feeders, and fish) and ultimately to humans (Bryan and Langston, 1992). Fernández Severini *et al.* (2010) and Marcovecchio *et al.* (2009) found elevated levels of Pb at the BBE associated with anthropogenic activities in different biotic and abiotic compartments, and they warned about the ecological risks. For example, artisanal fishing in the estuary is a very common activity that might be affected. With an unloading of 40,000 tons of species from deep waters and open sea, such as striped weakfish, Patagonian smooth hound, white croaker, silver side, and flounder, these species are consumed by humans (Pizarro and Piccolo, 2008) who can potentially be affected as a consequence of Pb inputs from industrial and or domestic discharges.

Zinc, the other metal found at high levels in the microplankton of the BBE, is essential; however, at high levels it may affect the oxidizing side of PSII by noncompetitively inhibiting Ca21 and Mn21 binding at their native sites in the water oxidizing complex (Miao, Wang, and Juneau, 2005). The levels found in this study must be taken into account and can be related to an anthropogenic source because cellular concentrations of Zn in the phytoplankton increase in direct proportion to free-metal concentrations (Twining and Baines, 2013). High concentrations in microplankton can be driven by high levels of dissolved zinc, which several authors (Collier and Edmond, 1984; Cullen *et al.*, 2003) have found in Southern Ocean phytoplankton. In addition, it is worth mentioning that some



authors (e.g., Campbell *et al.*, 2003; Quinn *et al.*, 2003) reported Zn biomagnification in several types of aquatic food webs, and essential elements such Zn usually increase the concentrations in planktonic food webs (Nfon *et al.*, 2009).

Copper also showed important concentrations in the microplankton at the BBE. Caetano and Vale (2003) found higher concentrations of Cu in microplankton-net material than in SPM, which they attributed to the micronutrient character of this element that may contribute to this pattern. Petit *et al.* (2013) also found copper enrichments in particles associated with primary production or anthropogenic contamination. It is well known that it is a limiting micronutrient in all living organisms and that also it is an essential metal for human activities. It is one of the most toxic inorganic contaminants (de Oliveira-Filho, Lopes, and Paumgartten, 2004) found in all types of aquatic ecosystems. For this reason, it has been the most intensively studied trace metal for its role as a micronutrient and as a toxic element to phytoplankton in marine systems when present at high concentrations. For example, diatoms may be tolerant of copper, an excess of which may cause teratological formations in the frustules and a reduction in the production of phytoplankton and chlorophyll (Balczon and Pratt, 1994; Viana and Rocha, 2005). Also, the combination of high levels of free Cu and low levels of chelators, Fe and Mn, is inhibitory to phytoplankton (Sunda, Barber, and Huntsman, 1981), and many other studies have found negative effects of Cu on the photosynthetic system (PSI and PSII) (e.g., Miao, Wang, and Juneau, 2005). Luoma *et al.* (1998) and Wang (2002), however, argued that as Cu and Zn are essential metals required by physiological processes, it is probable that there may be an internal regulation in phytoplankton cells.

At the BBE, the remaining metals, such as Cd, Cr, Fe, Mn, and Ni, were slightly higher at the SPM or seston reflecting a major affinity of these metals for the SPM and also suggested recent discharges and/or strong mobilization and transport throughout the estuarine system. It is worth mentioning that seston comprises several biogenic and lithogenic particles from different sources (Huang, Kreeger, and Newell, 2003), with diverse affinities for pollutants. This estuary is characterized by a high turbidity because of the high levels of silt and clay (Gelos *et al.*, 2007). Thus, a high percentage of seston comprises these types of lithogenic particles rich in metals, such as Cd, Cr, Ni, Fe, and Mn. Moreover, this indicates that accumulation of these metals by microplankton rarely exceeds levels in the detrital particles of seston with a higher lithogenic proportion. In addition, the highest concentrations of Fe and Mn in the seston are within the expected results because these metals are typically associated with terrigenous particles or authigenic oxides. Twining *et al.* (2015) also found significant higher levels of Fe, Mn, and Ni in the particulate fraction than the phytoplankton in the North Atlantic Ocean, which indicates a significant scavenging or detrital component in the particulate pool.

Considering the seasonal behavior during the sampling period (November 2012–March 2013), the higher levels of metals were recorded in summer (January–March 2013) at all the stations. This could be associated with the higher levels of pH recorded during these months, which favors the adsorption of metals onto the particulate phase. In this sense, there was a

noticeable increase of pH from 8 in November and December to 9.6 in January–March.

Analyzing the correlations, the significant correlation between POM and Cd and Cu in the microplankton is interesting. The POM (especially at the sampling sites located near the sewages), which is formed by biogenic matter produced as a consequence of algal development, had high Chl-*a* values. On the other hand, the significant relationship between Cd in SPM and POM has already been detected in studies such as the one by Showell and Gaskin (1992), in which they found a strong association with the organic detrital component of the POM. Also, the existence of significant correlations between some metals in the microplankton (Cu-Zn, Pb-Cu, Pb-Zn) can be related to the same source of metal and geochemical processes, resulting in the accumulation of metals in the plankton. Some significant correlations were detected between Ni in the SPM and microplankton, as well as Cr, which could be associated with the same source of metals.

Taking into account the PCA of metals in the SPM, the first two components (F1 and F2) explained more than the 83% of the total variability. Pb, Mn, Ni, and Cr were clustered together and contributed to F1 with high positive loadings, indicating the same origins of these elements. Therefore, F1 was characterized by elements of anthropogenic sources associated with industrial and domestic discharges. Fe contributed a moderate loading to Factor 2, which indicated a natural source. In the case of microplankton, the first two components explained the nearly 75% of variability. Metals such as Cu, Pb, and Zn presented high positive correlations, with F1 and clustered together near the right side of the *x* axis. These metals, which are toxic to microplankton at high concentrations, were found at critical levels in this study; therefore, F1 represented sources associated with industrial and or wastewater discharges. In addition, F2 presented high correlations with Fe and Mn. These metals were recorded at regular concentrations in this study, which indicated a natural source because it is well known that Fe and Mn are commonly considered as micronutrients elements for microplankton and considered as natural elements in the environment (Twining and Baines, 2013).

The authors of the present manuscript have been performing studies on the levels of heavy metals in suspended material of the BBE since 2001. In particular, the most studied sampling sites are S2 (industrial discharges), S3 (secondary sewage discharge), and S4 (far from any discharges), whereas S1 located at the main sewage discharge was incorporated in 2012. The levels of Cd, Cu, Cr, Ni, Pb, and Zn of the current study were generally lower than in previous trials (Table 3), although Fe and Mn concentrations were higher. Thus, particulate metals of the BBE exhibit seasonal variability, and these temporal changes may result from anthropogenic effects as well as natural factors (*i.e.* oceanographic and hydrographic conditions), typical of dynamic coastal environments. Moreover, the concentration and composition of seston varies seasonally, as well as on a daily basis (Huang, Kreeger, and Newell, 2003; Prins *et al.*, 1996). Circulation associated with water density, tides, and wind-driven currents participates in exchange processes and has a significant role in the ecology, chemistry, water quality, sedimentary, and resuspension

Table 3. Heavy metal concentrations ( $\mu\text{g g}^{-1}$  dry weight) in suspended particulate matter (SPM) and microplankton in different marine environments around the world.

Study Area	Cd	Cu	Cr	Fe	Mn	Ni	Pb	Zn	Reference
SPM									
English Channel (Dover)	0.1–4.27	13.02–17.98				20.02–24.01	14.50–63.30	75.97–319.83	James <i>et al.</i> (1991)
Western Bay of Fundy (Canada)	1.46 (0.37–2.82)						2.9 (0.91–61.50)		Showell and Gaskin (1992)
North Sea	0.1–1.4	20–42.95				0.99–62.99	15.95–88.06	72.96–1099.69	Laslett (1995)
North Atlantic	2.69–16.86	9.53–41.94				5.87–20.54	4.15–5.80	16.34–62.11	Kuss and Kremling (1999)
Port Pirie, Wills Creek, and Barker Inlet (South Australia)	<0.05–5.39	19.65–62.12					10.01–141.07		Edwards <i>et al.</i> (2001)
White Sea	2.76	12.3	1.38	353.6	21.24	6.67	89.15	186	Demina and Nemirovskaya (2007)
SE Gulf of California Coast Estuary in Bayness Sound (Canada)	2.36–2.40	115–430					25–128.7 7.96–8.55	76.2–110	Soto-Jiménez <i>et al.</i> (2008) Widmeyer and Young (2008)
Guanabara Bay (Brazil)	0.17–0.96	2.08–11.42					3.98–17		Kehring <i>et al.</i> (2009)
Portuguese coast	0.01–1.69	3.17–62.91				1.29–86.36	2.07–71.89	27.19–717.94	Santos-Echeandía <i>et al.</i> (2012)
Tagus estuary (Portugal)	26	16	15			50	150	8689	Duarte and Caçador (2012)
Gokova Bay (Turkey)	0.03–7.81	0.42–7.85		2445–60,455 7 ± 2 g kg <sup>-1</sup>		12 ± 7	0.017–88.55	30–987 324 ± 67	Demirak <i>et al.</i> (2012) Prego <i>et al.</i> (2013)
NE Atlantic coastal zone of Fimisterre (Iberian Peninsula)	<0.2–8	12–60	<0.6–28	11,540–29,360	92.79–404.29	0.5–50	<0.5–5.5	98–395	Andrade (2001)
Bahía Blanca Estuary	0.9–4.9	13.3–31	8.52–30.54			6.67–27.27	<0.5–50	31.89–299.92	Botté (2004)
Bahía Blanca Estuary	0.01–32.83	0.04–62.78					0.5–68.6		Fernández Severini, Hoffmeyer, and Marcovechio (2013)
Bahía Blanca Estuary	0.11–7.53	7.34–85.67	7.18–51.54	8141–41,760	221.1–1293	4.89–31.05	0.5–53.76	30.18–631.2	Fernández Severini (unpublished)
Bahía Blanca Estuary	0.07–0.63	21.87–49.06	4.99–23.98	15,670–64,590	11.57–702.75	4.91–18.14	5.34–14.14	50.6–93.42	Present study
Microplankton									
Ria de Aveiro (Portugal)	0.22–8.43	31.77–508.37		19,546–36,300			3–22	130.76–1438.36	Monterroso <i>et al.</i> 2003
Portuguese Coast	2.76	12.3	1.38	353.6	21.24	6.67	36.05	186	Caetano and Vale (2003)
White Sea									Demina and Nemirovskaya (2007)
Guanabara Bay (Brazil)	0.0028–0.01	0.38–5.25					1.75–3.8		Kehring <i>et al.</i> (2009)
Estero de Urrías (SE Gulf of California, Mexico)	0.25 ± 0.03	2.12 ± 0.35					1.65 ± 0.15	3.75 ± 0.74	Jara-Marini, Soto-Jiménez, and Páez-Osuna (2009)
Baltic Sea	0.27 ± 0.07	2.48 ± 0.2		29.88 ± 5.46	12.07 ± 3.00	0.15 ± 0.03	2.93 ± 1.48	3.87 ± 0.61	Nfon <i>et al.</i> (2009)
Taihu Lake, China	0.02 ± (w.w.)	0.58 ± 0.17	0.20 ± 0.04			72.60 ± 7.31	0.09 ± 0.03	11.49 ± 2.50	Tao <i>et al.</i> (2012)
NE Atlantic coastal zone of Fimisterre (Iberian Peninsula)	1.34 ± 0.32	151.45 ± 25.13	57.33 ± 5.96	3.6–9.6 (g kg <sup>-1</sup> )		7.1–14.6	56.67 ± 4.34	727.66 ± 24.63	Prego <i>et al.</i> (2013)
Bahía Blanca Estuary	0.3–3.23	9.4–30					19–24	0.58–0.60(g kg <sup>-1</sup> )	
Bahía Blanca Estuary	<0.2–3.7	8–55	3–16	3000–5000	197.15–1146	6–22	0.5–50	50–350	Andrade (2001)
Bahía Blanca Estuary	<0.11–1.64	16.16–65.25	6.81–50.24	11,343–51,440		5.31–29.31	6.06–66.68	56.2–612.30	Fernández Severini (unpublished)
Bahía Blanca Estuary	0.06–0.73	23.45–97.78	9.69–22.4	16,450–71,370	280.55–593.30	7.61–19.72	7.37–39.99	51.73–132.93	Present study

Abbreviations: w. w. = wet weight

processes in coastal environments (Geyer and Signell, 1992; Park and James, 1990). Also, estuarine environmental variability plays a vital role in the changing seston composition, mainly because of sediment resuspension caused by tidal hydrodynamics. Therefore, seston and metal concentrations within this fraction will vary between environments (Nfon *et al.*, 2009) and also within the same environment. So all these factors must be considered in the analysis of metal levels in the particulate fraction in the same study area, as well as when considering several coastal environments.

In comparison to the seston in other coastal areas around the world (Table 3), the levels of Cd, Pb, and Zn in the BBE were generally lower, whereas Fe was higher (Demina and Nemirovskaya, 2007; Demirak *et al.*, 2012; Duarte and Caçador, 2012; Kehrig *et al.*, 2009a; Prego *et al.*, 2013; Santos-Echeandía *et al.*, 2012; Soto-Jiménez *et al.*, 2008; Widmeyer and Bendell-Young, 2008). The rest of the metals (Cu, Ni, and Mn) showed more variation, although it is very common to find these highly variable concentrations of heavy metals among different environments. Several authors (*e.g.*, Bilos, Colombo, and Rodriguez Presa, 1998; Fang *et al.*, 2006) recommend caution when the metal content in the SPM of different regions of the world is compared directly. They also suggested considering the characteristics of the SPM (particle size, mineralogy, *etc.*) and the particular environment where the samples were obtained. As mentioned in the previous paragraph, particulate metals are highly dynamic and variable even in the same estuary, which is attributable to the specific characteristics of these and many others coastal environments: significant gradients in water chemistry, variable concentration of SPM, and complex hydrodynamic processes (Millward, 1995; Salomons and Förstner, 1984). In the particular case of Cr in seston, the levels in the BBE are high when compared with other environments (*e.g.*, Demina and Nemirovskaya, 2007; Duarte Caçador, 2012), so the high concentrations found in this study should not be overlooked. The two common oxidation states of Cr in the environment are Cr(III) and Cr(VI), and this metal is commonly found in a wide range of industrial activities, such as metallurgical, refractory, and chemical (Kotaś and Stasicka, 2000), as well as from sanitary landfill leaching, water cooling towers, *etc.* Abundant quantities of Cr compounds in liquid, solid, and gaseous wastes are discharged into the environment and can ultimately have significant adverse biological and ecological effects. Moreover, Cr is not biodegradable, and some chemical species such as Cr(III) and Cr(VI) are extremely toxic at high levels (Richard and Bourg, 1991). They are also commonly found associated with particulate forms in the water column. Previous studies in the BBE indicated a continuous (or *quasi continuous*) source of Cr, which is probably associated with industrial discharges (Marcovecchio *et al.*, 2009), and once in the water column, the dissolved Cr immediately joins the particulate fraction. Thus, the results of the present study agree with these previous records and reaffirm the continuous (or *quasicontinuous*) inputs of dissolved chromium.

Studies on microplankton in the BBE also began in 2001 (Andrade, 2001); an interruption occurred, for a long time, and they were restarted in 2012 (Fernández Severini, unpublished). In general, these previous results showed higher or similar concentrations to the present study (Table 3); however,

compared with other coastal environments of the world, the levels at the BBE are also as variable as the SPM. The concentrations of metals in marine organisms, such as plankton, depend on many factors like the physico-chemical properties of the environment (*e.g.*, pH, water chemistry, metal speciation, *etc.*), photoperiod, nutritional status, the strategy of metal handling, and storage of the species in the organism, as well as the species itself. So, it is very common to find variations between the environments. In a review, Twining and Baines (2013) discussed the regional differences in the metal quota of ocean phytoplankton, for example, they found that Zn and Cd are highest in the Southern Ocean, whereas Fe levels are highest in the North Atlantic, lower in the North Pacific, and lowest in the Southern Ocean. On the other hand, comparing the results of the present study with the pristine area of the Sound system in the coastal zone of Finisterre, Iberian Peninsula, in Spain (Prego *et al.*, 2013) the levels are lower; however, Pb in the BBE presented high concentrations, with a maximum of  $40 \mu\text{g g}^{-1}$  dry weight (d.w.). Similar levels were found by Demina and Nemirovskaya (2007) in the White Sea; with a mean concentration of  $36.05 \mu\text{g g}^{-1}$  d.w., these authors argued that the cause was related to anthropogenic activity, more specifically to aerosols. Vilhena *et al.* (2014) found a mean concentration of  $36 \mu\text{g g}^{-1}$  d.w. for lead at the Mocajuba estuary (Eastern Amazonia), similar to the maximum values in this study area; the authors also correlated the high levels with anthropogenic inputs. As previously mentioned, Pb is a nonessential metal and is toxic even in trace amounts. The main source of lead in industrial waste is attributable to the use in paints, explosives, and gasoline and also exposure to the nonproper disposal of municipal sewage sludge that has a large percentage of lead. In the BBE, there is inadequate treatment of industrial effluents and sewage, so the high level of Pb found in the microplankton should be considered as a warning. The high toxicity of Pb can threaten several consumers in the aquatic food chain, such as copepods, bivalves, fish larvae, and their adults, all organisms that commonly inhabit the BBE.

Another toxic metal that showed higher levels in the BBE was cadmium. Compared with microplankton of other aquatic environments, the maximum concentrations recorded in the BBE ( $0.73 \mu\text{g g}^{-1}$  d.w.) was higher. Jara-Marini, Soto-Jiménez, and Páez-Osuna (2009), Kehrig *et al.* (2009a), and Vilhena *et al.* (2014), found values of 0.27, 0.01, and  $0.5 \mu\text{g g}^{-1}$  d.w., respectively. Thus, Cd levels found in the estuary must be taken into account because they may potentially be transferred to the next level in the aquatic food chain, such as the mesoplankton. Reinfelder and Fisher (1991) demonstrated a linear relationship (1:1) between cadmium assimilated by copepods fed with diatoms and the metal incorporated in the cytoplasm of the ingested diatom and once ingested by copepods; it can be transferred to filter-feeder organisms and ichthyoplankton and finally to fish.

Table 3 also presents metal levels in the SPM and the microplankton in the BBE in different study periods, including the results of the present one. According to these results, the metal levels in the BBE have been changing over the years. Some metals, such as Fe in seston and Cu in microplankton, presented higher concentrations, whereas others were lower. These variations are mainly attributable to changes in the

seasonal environmental conditions, plankton dynamics, and also changes in the industrial or domestic discharges and in the dredging that frequently occurs in the estuary.

## CONCLUSIONS

This study includes the analysis of levels of heavy metals in seston and microplankton and their relationship with the dissolved nutrients and physico-chemical parameters in a coastal area of the SW Atlantic, specifically in a highly impacted coastal environment such as the BBE. The novelty of this manuscript is precisely the integrate analysis of all these variables, analyzed for the first time in the SW Atlantic and with a worldwide perspective. Therefore, this study enabled interesting conclusions to be made. The BBE was characterized by important levels of toxic heavy metals, especially Cd, Pb, Cr, and Zn. Spatial comparisons of metal concentrations showed no differences between all the sampling stations. Despite their different locations, all sites had significant heavy metal concentrations, thus, the entire study area was influenced by sewage and industrial discharges. The highest concentrations of POM and Chl-*a* were recorded at the sites located at the urban discharges. So, the organic matter of these sites came from internal sources (autotrophic organisms), as well as from external ones (domestic and industrial sewage). Furthermore, the increase of phytoplankton, represented by high levels of Chl-*a*, was attributable to the contribution of these sewage discharges, rich in nutrients for phytoplankton.

The Pb, Zn, and Cu levels in the microplankton were higher than in the SPM, indicating a more effective transfer of these metals from water to plankton. Moreover, important levels of Pb have been detected in the microplankton, which represents an environmental problem because of its toxic effects and the possible transfer to higher levels in the food chain. From a temporal point of view, metals in the microplankton and SPM present a highly seasonal variability. In summer, both fractions showed higher levels of metals, and this behavior is associated with an increase in pH, which favors metal adsorptions onto particles. Large diatoms account for more than 90% of the microplankton analyzed at the BBE during warm months and may represent an essential source of metals for the estuary. In addition, the high levels of Cr in the particulate fraction or seston showed necessary inputs of dissolved Cr, especially from industrial discharges that commonly use this metal for multiple purposes in the BBE.

It is evident that many environmental factors and processes affect metal-seston and metal-plankton interactions in dynamic ecosystems such as estuaries. Indeed, all such factors and processes must be taken into account when studying metal concentrations in seston and plankton in aquatic systems. The results of the present study showed that the concentrations of metals in seston and microplankton could be critical factors for interpreting variations in metal levels in the BBE, as well as in other coastal environments.

These results showed that seston and microplankton adsorbed and/or incorporated metals in relatively high concentrations. Further studies that involve different particle size and also key components at the basis of the aquatic food chain, such as plankton (micro-, meso-, and macrozooplankton), will be taken into account. Studies that include the trophic transfer of

metals from primary producers and consumers throughout the food chain are critical for evaluating the potential effects at the top consumer level in the marine environmental and finally to humans. In addition, phytoplankton as part of the microplankton have a great capacity to accumulate trace metals, and for this reason they are potentially excellent bioindicators for the evaluation of the pollution at the BBE.

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