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Effects of sewage effluent on the subtidal macrobenthic assemblage in an urban estuary of Argentina

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Abstract. We examined spatial variations in the biological descriptors of macrobenthic assemblage in relation to environmental parameters and metal distribution in the sediments along a channel of Bahía Blanca estuary affected by non-treatment sewage effluents. Along the channel, metal concentration values were higher than those in the internal estuary area, a sector considered as a reference site. The highest values of water turbidity and metal content in sediments were observed in the effluent discharge zone and at the site where sediments from dredging activities were deposited two years ago. The density of macrobenthic assemblages decreased towards the effluent discharge zone, but the richness and diversity reached to minimum values in both disturbed areas. Only two species of polychaetes were associated with these areas: *Laeonereis acuta* and *Aphelochaeta* sp.; the former being found in the effluent discharge zone, where Cd and Pb were most abundant, and the latter being the dominant species in the site characterized by dredging material and high concentrations of Cr and Ni. This study is the first approach to explore the impact of anthropogenic activities over the macrobenthic assemblage of the Bahía Blanca estuary, providing background data to use future management decisions.

[Keywords: human disturbance, subtidal assemblage, dredging sediments, Bahía Blanca estuary]

Resumen. Efecto de los efluentes cloacales sobre el ensamble macrobentónico submareal en un estuario urbano de la Argentina. Se analizó el efecto de los efluentes cloacales sin tratamiento sobre el ensamble macrobentónico submareal del estuario de Bahía Blanca. Se examinaron las variaciones espaciales en los descriptores biológicos en relación con los parámetros ambientales y la distribución de metales en los sedimentos de un canal afectado por efluentes urbanos. A lo largo del canal se registraron valores de concentración de metales mayores a los de la zona interna del estuario (sitio de referencia). Los valores más altos de turbidez del agua se registraron en el sitio próximo a la zona de descarga del efluente y en la zona media del canal, donde, además, se depositaron sedimentos de un dragado de la zona portuaria e industrial realizado dos años antes que este estudio. La densidad del ensamble disminuyó hacia la zona cercana a la descarga del efluente, mientras que la riqueza y la diversidad alcanzaron valores mínimos tanto en la zona de descarga como la zona media. Sólo dos especies de poliquetos se asociaron con estas áreas: *Laeonereis acuta* y *Aphelochaeta* sp.; el primero se encontró en la zona de descarga de efluentes, donde Cd y Pb fueron los metales más abundantes, y el segundo fue la especie dominante en el sitio caracterizado por la presencia de material de dragado y altas concentraciones de Cr y Ni. Este estudio proporciona datos de base con los cuales contrastar futuras medidas de manejo.

[Palabras clave: disturbio antrópico, comunidad submareal, sedimentos dragados, estuario de Bahía Blanca]

Introduction

Coastal marine ecosystems are increasingly affected by environmental stress and degradation due to pollution from a variety of human activities (Halpern et al. 2007). Wastewaters containing organic matter, inorganic nutrients, metals, hydrocarbons, pesticides and other toxic organic compounds are, in many cases, discharged untreated into the sea (Marcovecchio et al. 2008). Organic enrichment and heavy metal inputs cause important disturbances to marine species (Chariton 2005). Particularly, benthic invertebrates are very sensitive to physical and chemical perturbations and are, thus, considered good biological indicators of contamination (Warwick and Clarke 1993; Jongseong et al. 2011). Significant macrofaunal changes in response to stress due to detrimental environmental conditions are usually measured by alterations in benthic community parameters such as diversity, abundances, dominance and biomass, in both spatial and temporal patterns (Pearson and Rosenberg 1978; Rakocinski et al. 2000; de la Ossa Carretero et al. 2012).

Contaminants released into semi-enclosed systems such as estuaries or coastal lagoons

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tend to remain close to their sources (Dassenakis et al. 2003) and toxic pollutants such as metals rapidly bind to particulates and sink to the seafloor (La Colla et al. 2018). Sediments thus become repository for metals arose in natural or anthropogenic processes causing potentially adverse health effects on aquatic ecosystems (Singh et al. 1997) even after the source of contamination has been eliminated. Therefore, measurement of metal levels in coastal sediments should be included in monitoring programs designed to assess the environmental quality of ecosystems, in particular those with little connection to the open sea (Clements et al. 2000; Botté et al. 2010; Hasan et al. 2013). Though quality guidelines provide values that allow the quantification of sediment contamination, further comparisons are required to make an overall assessment of the degree of metal contamination in estuarine sediments (Burton et al. 2005). Tools such as the geoaccumulation index (Igeo) and the enrichment factor (EF) are also widely used to assess metal pollution in sediments (Burton et al. 2005; Klos et al. 2011; Hasan et al. 2013; Serra et al. 2017).

The Bahía Blanca estuary, is one of the most urbanized and industrialized coastal areas of Argentina (Arias et al. 2010; Biancalana et al. 2012). Over the last three decades the estuary has suffered severe perturbations as a result of strong demographic expansion accompanied by growing industrial activity and cumulative organic pollution due to the dumping of untreated sewage (Botté et al. 2007, 2010; Marcovecchio et al. 2008; La Colla et al. 2015). Since the estuary is home to the most important deep-water port system of the country, it is also affected by the continuous need for maintenance and dredging operations to accommodate maritime traffic. The physical and chemical changes in sediments produced by sewage effluents have been addressed in several studies (Tombesi et al. 2000; Marcovecchio et al. 2008; Serra et al. 2017) and their effects on the biota have been evaluated through bio-indicators such as bacteria (Baldini et al. 1999), planktonic communities (Barría de Cao et al. 2003; Biancalana et al. 2012; Dutto et al. 2012; Chazarreta et al. 2015) and commercial fishes (La Colla et al. 2017). Several studies have also been conducted on the concentration of metals in crabs and mollusks inhabiting the intertidal zone of Bahía Blanca estuary (Ferrer 2000; Ferrer et al. 2003; Simonetti et al. 2012; La Colla et al. 2018). However, the last ecological studies on benthic subtidal assemblages were carried out three decades ago, prior to the urban and industrial expansion of the area (Elías 1992; Elias and Bremec 1994).

The aim of this work was to assess the magnitude and nature of municipal wastewater impacts on Bahía Blanca estuary ecosystem. We therefore examined spatial variations in biological descriptors such as the abundance, composition and biomass of soft-bottom macrobenthic assemblages in relation to environmental parameters (organic matter, pH, turbidity and salinity) and metal distribution in the estuary sediments. We further explored the applicability of the contamination indices (geoaccumulation index and enrichment factor) to the studied ecosystem and its sensitivity for assessing pollution.

Materials and Methods

Study area

The Bahía Blanca estuary, located in the Argentinian coast (38° S - 62° W), has 2,300 km^2 and is formed by several tidal channels, extensive tidal flats with patches of low salt marshes and islands (Piccolo et al. 2008). The northern coast of the estuary is subjected to high anthropogenic impact as a result of human settlement, commercial ports and chemical industries (Arias et al. 2010; Botté et al. 2010). Two cities are established adjacent to the estuary, Bahía Blanca (35,000 inhabitants) and Punta Alta (60,000 inhabitants), generating urban sewage discharges of almost 84000 m^3/day that reach the estuary without appropriate treatment (CTE 2016; La Colla et al. 2015). The port area of Bahía Blanca city, located in the inner zone of the estuary, requires periodic maintenance dredging; final sediment disposal is carried out in other areas of the estuary (Schnegelberger et al. 2014).

Sampling design

This study was conducted in the middle part of the estuary along the Canal Vieja, a channel of 300 m wide with a mean depth of 6.5 m that receives the influence of untreated sewage from Bahía Blanca city and the dredging operations (Figure 1). This channel connects the small channel, non-navigable with the vessel available for sampling benthic organisms, which receives the discharge of the sewage pipe with the major channel of the estuary (Canal Principal). Subtidal sampling

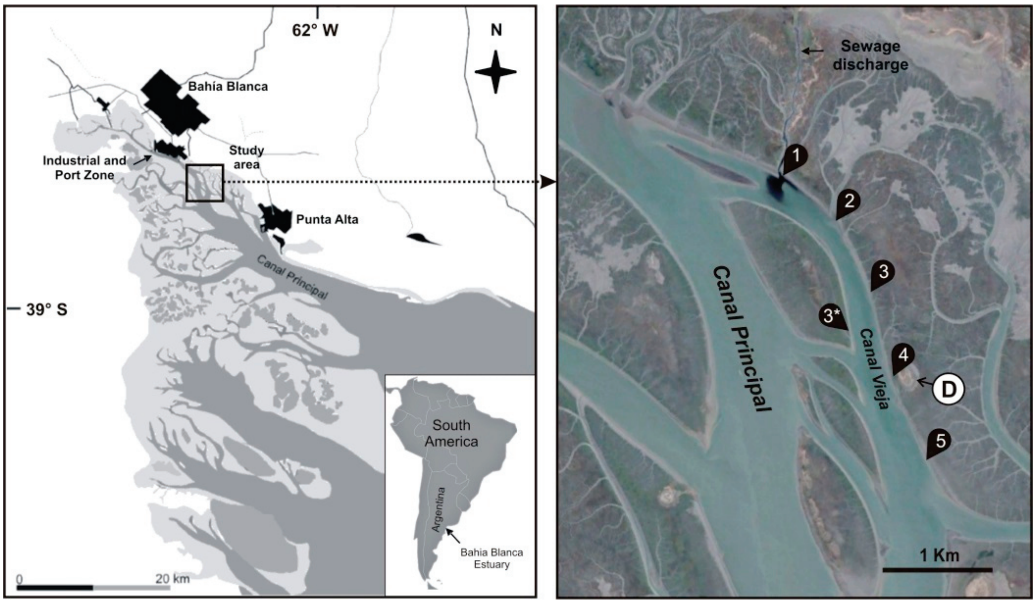

Figure 1. Map of Bahía Blanca Estuary showing the location of sampling sites (numbers) along the sewage channel (Canal Vieja) of Bahía Blanca estuary. D=Area of deposit of dredging material.

Figura 1. Mapa del estuario de Bahía Blanca donde se muestra la ubicación de los sitios de muestreo (números) a lo largo del canal que recibe los efluentes cloacales (Canal Vieja) en el estuario de Bahía Blanca. D=Área donde se deposita el material de dragado.

was conducted on board of an oceanographic coastal vessel during May 2015. In order to know the influence of the sewage effluent along the northern coast of Canal Vieja, five sampling sites were established equidistant from each other (500 m), and an additional sampling site was established on the opposite side of the channel (site 3*) to analyze the influence of the effluent across the channel. At each site,four biological and sediment samples were taken with a van Veen dredge (0.165 m^2). A single in situ measurement of temperature (°C), turbidity (UNT), salinity (practical salinity scale) and pH of water were obtained with a digital multisensor (Horiba U-10).

Lab routines. Sediment samples (N=24) were used to perform analyses of sedimentology, organic matter content and concentration of metals (Cd, Cu, Pb, Ni, Zn, Mn and Fe). Grain size was determined using laser diffraction (Malvern Masterziser 2000), and Shepard's classification system (Shepard 1954) was used for the characterization of sediments. The percentage of the total organic matter content (TOM) was determined by the calcination method (Byers et al. 1978).

To determine metal content, sediment samples were cleaned of debris, biota fragments and clasts, before drying at 50±5 °C to constant weight. Next, they were sifted through stainless steel meshes to obtain fine particles (63 μm). Sediment subsamples from the <63 μm sediment fraction (0.5±0.01 g) were mineralized in HNO_3:$HClO_4$ (5:1) mixture (Merck) at 110±10 °C in a glycerin bath until complete mineralization was reached. After cooling, 0.7% nitric acid was added to the residue up to 10 mL into centrifuge tubes prior to undergoing metal concentration analysis (Botté et al. 2010). The concentration of Cd, Cu, Zn, Cr, Pb, Ni, Fe and Mn was measured using an inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optima 2100 DV). The limit detection of the method (MDL) was calculated as three times the standard deviation of 12 blank replicates with α= 0.01. The MDL for each metal (μg/mL) was: Cd: 0.04, Cu: 0.01, Pb: 0.06, Zn: 0.12, Mn: 1.78, Ni: 0.0024, Cr: 0.0015, Fe: 2.68. All of the relative SDs of the duplicate samples were <12%. For the analytical quality control, reagent blanks, certified reference materials (CRM; Pond Sediments, R.M. No. 2, NIES, Japan), and analytical-grade reagents (Merck or Carlo Erba) were used. The recovery percentages for all trace metals in CRM were above 75% Cu and 147% Fe. The material used for metal measurement during sampling and in the laboratory was cleaned according to internationally recommended protocols (APHA 1998).

All biological samples (N=24) were sieved through a 500 µm mesh; organisms retained were fixed in 4% formalin and identified to the lowest taxonomic level possible. The total wet weight for each sample and each species were measured to the nearest of 0.1 g. All mollusks obtained were weighed with shell.

Data processing and statistical analyses

Environmental variables and metal distribution. Differences in environmental variables of sediments (TOM and metal concentration) among sites were evaluated with one-way ANOVA tests and post hoc Tukey test. The results were compared with the background levels for Bahía Blanca estuary (Grecco et al. 2006) and the information available of a reference site, located in the inner zone of the estuary, not perturbed by anthropogenic activities (IADO, 2016). Since is no legislation at the national, regional or local levels on the maximum permitted levels of metals in estuarial surface sediments, results were compared with the Canadian Sediment Quality Guidelines (SQG) for the Protection of Aquatic Life, specifically LEL (lowest effect level: indicates a level of contamination which has no effect on the majority of the sediment-dwelling organisms) (Persaud et al. 1993).

Two indexes were used to assess the presence and intensity of metal in bottom sediment: enrichment factor (EF) and geoaccumulation index (Igeo). The extent of metal contamination compared to the background levels according Villa (1988) (content of metals in pre-industrial sediments) in the area was assessed using the enrichment factor (EF) (Hasan et al. 2013), which provides information about the origin (natural or anthropogenic) of the metals following the scale proposed by Sutherland (2000). Metal concentrations were normalized to the textural characteristic of sediments with respect to Fe. The EF was calculated according to the following equation:

$$EF = [(Me/Fe)_{sediment}/(Me/Fe)_{crust}] \quad (1)$$

where $(Me/Fe)_{sediment}$ represents the Fe-normalized metal concentration (Me) in the sediment sample, while ratio $(Me/Fe)_{crust}$ the concentration in a suitable background or baseline reference material (Grecco et al. 2006). The Geoaccumulation Index (I_{geo}) is a quantitative measure of pollution in marine sediments; it was calculated using Muller's (1969) expression:

$$I_{geo} = Log_2[C_n/1.5B_n] \quad (2)$$

where Cn is the concentration of the metal "n" in the sediment and 1.5 is the correction factor of lithogenic effects. Bn is the background value of metal in fossil argillaceous sediments. The Bn data of Turekian and Wedepohl (Salomons and Förstner 1984) were used for all Igeo values.

Macrobenthic assemblage. In order to know the spatial variation of parameters of benthic assemblage along the sewage channel, the following variables were estimated for each sampling site: density (individuals/m^2), biomass (g/m^2), richness and diversity. Assemblage richness was estimated by Margalef index (d), and diversity was analyzed by applying the Simpson (λ), Shannon-Wiener (H´) and Pielou (J) indices. Biological indices were compared between sites by a one-way ANOVA or Kruskal-Wallis nonparametric test (H test). Significant results were analyzed using a post hoc Tukey tests, after an ANOVA, or with Conover scores, after a Kruskal-Wallis.

Non-metric multidimensional scaling analysis (nMDS) was used to represent the assemblage under study. The technique was based on a triangular matrix using the Bray Curtis similarity index on transformed data $log_{10}(X+1)$. The stress was calculated to indicate the degree of reliance of the bidimensional representation of the intersample distances (Clarke and Warwick 1994). Assemblages were compared between sites along the channel and the hypothesis of significant differences in assemblage structure between sites was tested by ANOSIM permutation test (Clarke 1993; Clarke and Warwick 1994). Similarity percentage analysis (SIMPER) was used to determine the organisms that most contributed to the differences observed. Statistical analyze and biological indices were performed using the statistical package PRIMER-E® 6 (Clarke and Gorley 2006).

Principal component analyses (PCA) by Spearman's rank correlation matrix were applied to detect relationships among the biological and environmental analyzed variables. The variables were standardized prior to the PCA to level their weight. Accordingly, the mean of each variable was subtracted from each value and then divided by the standard deviation of the variable along the samples. Temperature, pH, turbidity and salinity had only one measurement per site, so it was necessary to replicate times 4 the value of the site measurement, the same number of replicas of the other variables. However, since these variables do not meet the assumption of independence between the samples, associations were explored between variables to allow to characterize sites, and therefore the centroids were calculated to locate the position of each site in the drawings of the main components. This concession was made (i.e., the absence of an independence assumption) for 4 variables out of a total of 17 variables, in exploratory PCA with the objective of looking for differences between sites and not the variability within each site. These analyses were performed with PRIMER-E® 6 (Clarke and Gorley 2006).

Results

Environmental variables

Temperature, salinity and pH were similar along the channel (Table 1), turbidity values were higher in the site closest to the sewage discharge zone (site 1) and at site 4. The percentage of TOM in the sediment varied between sampling sites ($H_{(5)}$=13.55; P=0.01), higher values being found in sites 3, 3* and 5. In general, the physical variable values at site 3*, located on the opposite side of the

Table 1. Environmental parameters and sedimentological characterization of the sampling sites along the Vieja Channel.

Tabla 1. Parámetros ambientales y caracterización sedimentológica de los sitios de muestreo ubicados a lo largo del Canal Vieja.

Site	1	2	3	3*	4	5
Temperature (°C)	16.80	17.00	16.70	16.45	16.40	15.43
Salinity (PSU)	3.16	3.21	3.15	3.15	3.18	3.14
Turbidity (UNT)	80.00	43.13	17.04	37.1	70.00	23.92
TOM (%)	4.45±1.84	3.75±0.61	5.57±0.46	3.87±0.28	5.16±0.20	6.16±0.27
pH	7.97	8.00	7.84	7.8	7.83	7.87
Clay (%)	6.88	16.32	13.65	11.16	8.74	10.02
Silt (%)	43.13	48.99	60.94	45.96	58.48	58.21
Sand (%)	49.95	34.61	25.40	42.40	32.78	31.49

Table 2. Summary of metal concentration (ug/g dry weight) of sediments along the Canal Vieja (sites 1 to 5 and range of metal concentration), results of previous studies made in Bahía Blanca estuary and LEL (Lowest Effect Level) according to Guidelines for the Protection and Management of Aquatic Sediment Quality in Ontario in 1993. ANOVA were detailed. SD=standard deviations, Nd=not detectable. Significance codes: ns =not significant *P*-value; *=significant difference,**=highly significant difference.

Tabla 2. Concentración de metales (ug/g de peso seco) de sedimentos a lo largo del Canal Vieja (Sitios 1 a 5 y rango de concentración de metales), resultados de estudios previos realizados en el estuario de Bahía Blanca y LEL, de acuerdo con las Pautas para la Protección y Gestión de la Calidad de los Sedimentos Acuáticos en Ontario en 1993. Se detallan los resultados de las pruebas realizados para comprar los valores medios de los metales (ANOVA y Tukey). SD=desviaciones estándar. Nd=no detectable. Códigos de significancia: ns=no significativo, *=diferencia significativa, **=diferencia altamente significativa.

Sites	Cd	Cu	Pb	Zn	Mn	Ni	Cr	Fe
1	0.09±0.01	16.86±2.72	9.98±1.66	48.70±8.07	297.46±9.54	9.39±0.32	11.22±0.69	27869±6487
2	0.07±0.01	14.47±1.82	8.03±1.41	45.79±6.00	362.90±27.24	10.34±1.08	11.93±1.09	27870±3786
3	0.06±0.01	12.78±0.95	6.80±0.48	38.13±2.19	397.34±29.23	9.60±0.46	11.67±0.92	28624±2065
3*	0.05±0.01	12.28±0.71	8.25±0.64	40.06±3.10	348.73±22.05	9.45±0.52	11.14±1.15	25715±1586
4	0.05±0.01	16.40±1.72	8.02±0.77	46.53±3.54	378.73±2.89	10.45±0.70	13.12±0.87	30370±1320
5	0.05±0.01	11.15±0.65	6.08±0.52	34.98±3.13	322.71±13.50	8.81±0.63	11.17±0.85	25483±1175
Range of metal concentration	0.03-0.10	9.94-18.89	5.53-11.32	32.80-50.30	284.40-434.30	8.34-11.01	10.12-14.25	23140-3225
Reference Site (CTE 2016)	Nd	10.00	4.70	25.00	Sin datos	5.75	11.50	17500
Background (Villa 1988)	0.56±0.08	15.28±5.46	19.9	51.08±11.72	550.50±53.97	9.60	7.18	22375±1362
LEL	0.6	16	31	120	460	16	26	20000
ANOVA $F_{(5,18)}$	6.88**	8.25**	6.93**	5.12**	13.64**	3.50*	2.63 (ns)	1.10 (ns)
Tukey test	1>3=3*=4=5 1=2	1>3=3*=5 3=2=4	1>3=5 1=2=3*=4	1>5	3>1=5=3* 3=2=4	4=2>5		

channel, were intermediate between those recorded for sites 3 and 4 (Table 1). The texture of sediments was predominantly sand-silty; samples from sites 2 and 4 showed the highest dispersion, varying from silty-sand to clayey-silt (Supplementary Materials, Figure 1). Site 1 showed a higher proportion of fine ($H_{(5)}$=20.18, P<0.01) and medium sands ($H_{(5)}$=18.94; P<0.01) than the other sites.

Metal distribution

The variation in metal concentration differed among sites depending on the metal considered. The ANOVA test of mean metal concentration showed significant differences between sites for almost all metals analyzed (Table 2). Tukey's post hoc comparison indicated that the Cd was significantly higher in site 1 than in most of the other sites and had no statistical difference with site 2. The Cu was significantly higher in sites 1 and 4 than in the other sites and had no statistical difference with site 2. The Zn was significantly higher in sites 1, 2 and 4 than in site 5; sites 1, 2, 3* and 4 do not differ in Pb concentration. In the case of Mn, mean concentration was significantly higher in site 3 than in sites 1, 3* and 5. Concentration of Cr and Fe were significantly higher in site 4 than sites 3* and 5. Finally, no significant difference in Ni between sites was found. The overall trend observed was as follows: in site 1, it was found the average highest concentration of Cd, Pb, Cu and Zn, and these last two were also high in site 4 where Cr also presented a value slightly higher than in the rest. Almost all mean values along the sewage channel were higher than concentration of metals registered for references site. Also, concentration of Cu, Mn, Cr and Fe were higher than those that as background values, also Cu showed higher values than LEL. The Igeo value for Cd was <0 (uncontaminated), whereas the values for Cu, Pb, Zn, Mn, Ni, Fe and Cr belonged to class 1 (uncontaminated to moderately contaminated) (Table 3). The EF values for Cd, Cu, Pb, Zn, Mn and Ni revealed that the sediments at all sites were <1 (no enrichment). The EF values for Cr and Fe along the channel belong to class 1 (minor enrichment) (Table 3).

Macrobenthic assemblage

A total of 59 taxa (2753 individuals) were identified from the subtidal assemblage along the studied channel. Only 11 taxa exceeded 1% of abundance (more than 29 individuals/taxa). Polychaeta was the most diverse group, with 27 species belonging to 14 different families. Mollusca were represented by Bivalvia (11 species) and Gastropoda (4 species). Arthropoda (Crustaceans) included 9 species belonging to Decapoda (4 species), Amphipoda (3 species), Isopoda (1 species) and Tanaidacea (1 species). Other less represented groups were Platyhelminthes, Nemertea and Cnidaria. Polychaeta was also the most abundant group, comprising 92.45% of total abundance, followed by Mollusca (5.58%) and Arthropoda (1.71%).

Mean density of the macrobenthic assemblage, driven by the abundance of Polychaeta, varied widely along the sewage channel, significant differences ($F_{(5,18)}$=4.63; P<0.01) were found between the sites, according Tukey pos-hoc contrast, where the effluent is discharged (sites 1 and 2) and the end of the channel (site 5). The value recorded at site 5 (ca. 251.50±69.92 individuals/m^2) was 9 times higher than that at site 1 (26.25±23.75 individuals/m^2) (Figure 2a). There were no significant differences ($F_{(5,18)}$=2.59; P>0.05) in total biomass along the channel, although sites 1 and 4 showed minimum values (Figure 2b). At sites 1, 3, 4 and 5, polychaetes represented between 60% and 88% of total biomass. In contrast, mollusks

Table 3. Index of geoaccumulation (Igeo) and enrichment factor (EF) for Bahía Blanca estuary sediments, normalized with respect to the iron content. References: 00Igeo01: Uncontaminated to moderately contaminated, EF<1: No enrichment; 1-3=Minor enrichment.

Tabla 3. Índice de geoacumulación (Igeo) y factor de enriquecimiento (FE) para los sedimentos del estuario de Bahía Blanca, normalizados con respecto al contenido de hierro. Referencias: 00Igeo01: De no contaminado a moderadamente contaminado; EF<1: No enriquecido; 1-3=Enriquecimiento mínimo.

Metal	Site 1		Site 2		Site 3		Site 3*		Site 4		Site 5	
	Igeo	EF	Igeo	EF	Igeo	EF	Igeo	EF	Igeo	EF	Igeo	EF
Cu	0.06	0.89	0.06	0.76	0.05	0.65	0.05	0.70	0.06	0.79	0.05	0.64
Pb	0.11	0.40	0.10	0.32	0.09	0.27	0.10	0.36	0.10	0.30	0.09	0.27
Zn	0.04	0.77	0.04	0.72	0.04	0.58	0.04	0.68	0.04	0.67	0.04	0.60
Mn	0.01	0.43	0.01	0.53	0.01	0.56	0.01	0.55	0.01	0.51	0.01	0.52
Ni	0.03	0.79	0.03	0.86	0.03	0.78	0.03	0.86	0.03	0.80	0.03	0.81
Cr	0.03	1.25	0.03	1.33	0.03	1.27	0.03	1.35	0.03	1.35	0.03	1.37
Fe	0.21	1.00	0.21	1.00	0.21	1.00	0.19	1.00	0.23	1.00	0.19	1.00
Cd	-11.83	0.12	-12.81	0.10	-13.84	0.08	-13.94	0.09	-14.69	0.06	-14.49	0.08

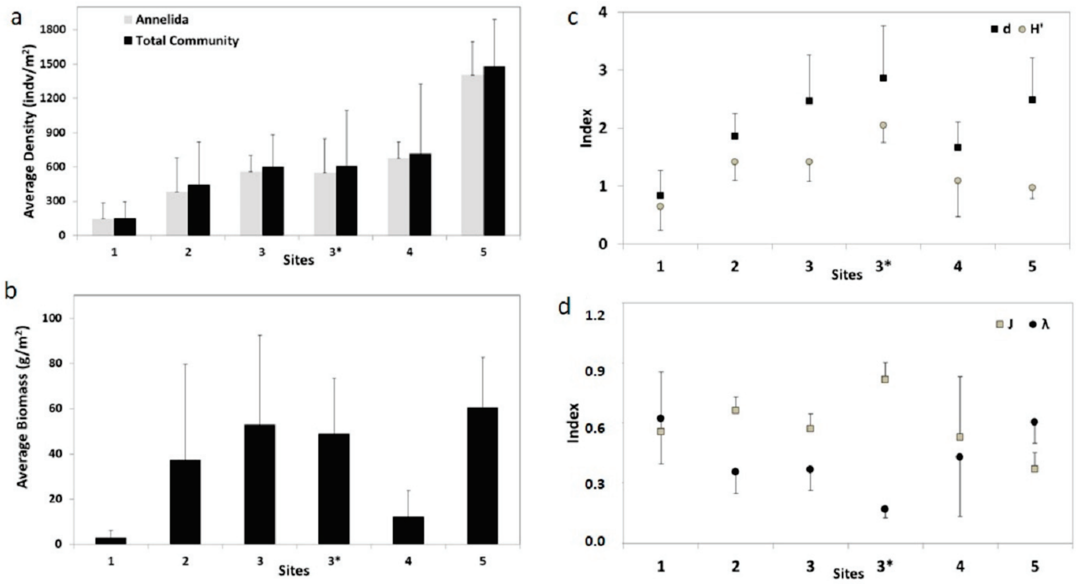

Figure 2. a) Mean density of total assemblage and annelida group; b) Mean total biomass; c) Mean Margalef (d) and Shannon (H´) indices; d) Mean Simpson (λ) and Pielou (J) indices for each site along the sewage channel of Bahía Blanca estuary (bars indicate +/- standard deviation).

Figura 2. a) Densidad media de todo el ensamble y del grupo de los anélidos; b) Biomasa total media; c) Valores promedio de los índices de Margalef (d) y Shannon (H´); d) Valores promedio de los índices de Simpson (λ) y Pielou (J) (las barras indican +/- desvío estándar).

contributed more than polychaetes to total biomass at sites 2; the gastropod *Buccinanops deformis* in particular represented 87% of total biomass at this site. Cnidarians and nemerteans only contributed significantly to the assemblage biomass at site 3*, where they represented 30% and 20%, respectively, of the biomass. At this site, polychaetes and mollusks contributed 31% and 19% of total biomass, respectively. At site 1, the richness index (d) showed the lowest value, whereas at sites 3, 3* and 5 there were no significant differences ($F_{(5,18)}$=5.05; P<0.01; Tukey test 5=3=3*>1) (Figure 2c). Diversity (H´) showed significant differences ($F_{(5,18)}$=6.21; P<0.01) between site 3* and sites 1, 4 and 5; site 3* showed the highest mean values of H´ and lowest of dominance (λ). Dominance showed significant differences ($H_{(5,18)}$=14.11; P<0.01) between site 3* and sites 1, 4 and 5; site 3* showed the highest mean values of H´ and lowest of λ. Although no significant differences were found among sites the evenness ($H_{(5,18)}$=10.43; P>0.05) reached the maximum value at site 3* (Figure 2c).

The ANOSIM test found significant differences in benthic assemblage structure among sites (R=0.69; P=0.001). The pairwise test found no significant differences in two pairs of comparisons: sites 1 and 2 (R=0.14; P=0.28) and sites 2 and 4 (R=0.30; P=0.08). The SIMPER analysis identified three species of Polychaeta (*Laeonereis acuta*, *Aphelochaeta* sp. and *Leodamas verax*) as being the main contributors to the differences among sites (70% of dissimilitude). The spatial distribution of *L. acuta* is related and/or restricted to sites closest to the sewage discharge zone (sites 1 and 2), while *Aphelochaeta* sp., although distributed throughout the channel, predominates at site 4. *L. verax* was more abundant in the middle and final section of the sewage cannel (sites 3 and 5). Figure 2 of Supplementary Materials shows the n-MDS plot of these species with samples separated by site (Stress=0.15).

Relationship among biological features, environmental variables and metal distribution

The principal component analyses (PCA) among biological features of the assemblage, environmental variables and sites (Figure 3) showed that the first two principal components (PC1 and PC2) explain 83% (PC1 57%, PC2 26%) of the total variance and reveal significant correlations among the analyzed variables. The first axis (PC1) of the PCA was positively correlated with salinity, turbidity, Zn, Pb and Cu and negatively correlated with biomass, abundance and species richness. The second axis (PC2) was positively correlated with organic matter (TOM), Mn, Cr, Fe and Ni, and negatively correlated with pH and Cd. Significant positive correlations (P<0.05) were

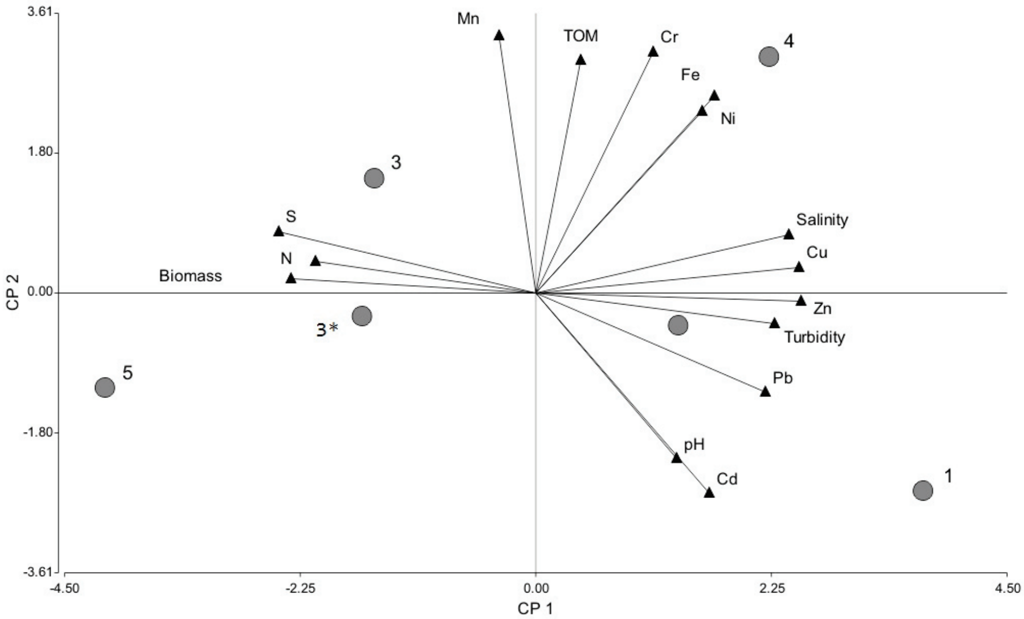

Figure 3. Principal component analysis (PCA) plot of environmental and biological variables, metals and sampling sites along the sewage channel of Bahía Blanca estuary. References: N=Abundance, S=Species richness, Turb=Turbidity, Sal=Salinity, TOM=Sediment total organic matter. Circles represent the centroids of simple sites.

Figura 3. Análisis de componentes principales (ACP) de las variables ambientales y biológicas, metales y sitios de muestreo estudiados a lo largo del canal de aguas residuales del estuario de Bahía Blanca. Referencias: N=Abundancia, S=Riqueza de especies, Turb=Turbidez, Sal=Salinidad, TOM=Materia orgánica total del sedimento. Los círculos representan el centroide de cada sitio de muestreo.

found between sites 1, 2 and 4 and Cu, Pb and Zn and between site 3, 3* and 5 and biomass, abundance and richness (P<0.05).

DISCUSSION

This study is a snapshot of the ecological status of Canal Vieja, a channel influenced by sewage discharges, and represents the first approach to explore the impact of anthropogenic activities over the macrobenthic assemblage of the Bahía Blanca estuary. Most of the environmental variables measured fall within the same range along the entire channel. Only water turbidity shows a heterogeneous behavior, being higher near the point of discharge (site 1) and in the middle zone of the affected channel (site 4). Taking in account that the current study was conducted two years after the deposit of dredging sediment, the pattern of turbidity and the channel's own sedimentological heterogeneity recorded in the site of the dredging sediment deposit could be attributed to this allochthonous material. The characteristic vegetation of coastal areas of Bahía Blanca estuary, like saltmarshes of *Spartina alterniflora*, are absent in site 4 (Figure 1). The lack of erosion control services attributed to these halophyte species could affect the stabilization of fine sediments, increasing the turbidity in the water column of nearby areas (Barbier et al. 2011).

Although unclear pattern was found about metals distribution along the studied channel, some trends were observed. The higher average content of Cd, Pb, Cu and Zn recorded at sites 1 and 2 could be related to their proximity to the area under influence of the sewage discharge mouth. In the site where dredging sediments where deposited, while Cr content was greater than all the sites considered in this study, Cu and Zn concentration was similar to those recorded in site 1. Even though previous studies conducted in Bahía Blanca estuary (Botté et al. 2010) recorded lower values of Cr and similar content of Cu and Zn, the concentration of the above-mentioned metals recorded in this study are within the range recorded in latest studies (Serra et al. 2017). Some metals such as Cr, Cu and Fe showed higher levels than background values, reflecting input from human activities. Given that all of values of metals content where higher than in reference site (Table 2), showing that complete area is influenced by metals input. Cu concentrations recorded at sites 1 and 4 exceed the LEL (Table 2), while Fe exceeds values in all the sites. Metals recorded in the fine sediment fraction do not constitute a threat to aquatic life in line with the SQGs, except for Cu and Fe, whose levels in Bahía Blanca estuary sediments are likely to result in occasionally adverse biological effects on the marine ecosystem. After a constant exposure of these metals, part of the metal concentrations could not be regulated, then basal levels in the individuals could be surpassed affecting to benthic life (Martín-Díaz et al. 2004). A previous study in the 2015-2016 period (CTE 2016) showed that along the inner-middle zone of the estuary, Cd was twice as high as in other areas of the estuary. Given that Cd is one of the most toxic metals measured in this study, it is important to highlight that its concentration was below that of the local background studies, LEL (Table 3), and other similar environments (Kumwimba et al. 2017; Yang et al. 2017). Experimental studies suggest that concentrations of a single isolated metal must exceed current guideline values by a considerable margin in order to elicit a biological effect (Chariton 2005). Since trace metals generally co-occur with other contaminants, the effect of these multiple contaminants is likely additive or synergistic, and should therefore be constantly monitored in case concentrations become hazardous (Beasley and Kneale 2003). The Igeo index indicated a slight sediment contamination by metals in the area. EF showed minor Cr enrichment in sediments but no enrichment with the remaining metals. A value of unity (Fe in this study) denotes neither enrichment nor depletion relative to the Earth's crust (Hasan et al. 2013). EF is a convenient way of measuring geochemical trends and is used for drawing comparisons between areas; in our study it revealed few differences in the impact of metals from urban sewage on sediments along the channel.

In general, the macrobenthic assemblages of the studied channel showed lower levels of richness and diversity than other sites in the Bahía Blanca estuary where these indices reach mean values of 4 and 2.3 respectively (CTE 2016). Polychaeta, the most abundant and diverse biological group found in this work, is known to be poorly sensitive to anthropogenic disturbances (Warwick and Clarke 1993). Indeed, its abundance commonly increases under stressful conditions in comparison to more sensitive taxa such as the Crustacea and Mollusca (Warwick and Clarke 1993; Wildsmith et al. 2009). Three species of polychaetes significantly changed their dominance along the study channel: *Laeonereis acuta*, *Aphelochaeta* sp. and *Leodamas verax*, all of which are deposit-feeding species. These species, that ingest sediments with organic matter and microorganisms associated, could have an advantage over other species in this channel since urban effluents providing them with a continuous source of food. *L. acuta* belongs to the Nereidida family of Polychaeta, characterized by tolerate well organically contaminated environments. This species was the only taxa found in significantly large numbers in the area closest to the sewage discharge (site 1). Early studies indicated opportunistic behavior of *L. acuta* because of its relatively short life cycle and rapid sediment recolonization strategies (Netto and Lana 1994; Omena and Amaral 2000). Other studies highlighted the species as pertaining to a tolerant assemblage in Brazilian urbanized estuaries (Pagliosa and Barbosa 2006). Under experimental conditions, *L. acuta* is able to accumulate cadmium in the body (Sandrini et al. 2006), which could explain its tolerance to the high concentration of metals in the most impacted zone. Using biomarkers, Weis et al. (2016) reported that the main response of *L. acuta* to polluted environments is an increase in individual length and weight, but with concomitant molecular damage. The allocation of energy by *L. acuta* to body enlargement in polluted environments and the consequent reduction in population turnover is contrary to expectations for an opportunistic species (Weis et al. 2016). On the other hand, the site-specific abundance of the cirratulid *Aphelochaeta* sp. could indicate tolerance of certain metals or combination of contaminants, since its major abundance occurs near site 4, characterized by high concentrations of Cu, Cr, Fe and Ni. It has been postulated that certain species belonging to the Cirratulidae family could regulate the metabolism of metals (Pocklington and Wells 1992; Reish and Gerlinger 1997). Cirratulids are also considered to be indicators of moderate or intermediate organic pollution (Elías and Rivero 2009). Finally, *L. verax* was the dominant species in the middle and final section of the sewage channel (sites 3 and 5), where there is lower metal concentration and turbidity. Abundance or dominance of *L. verax* has been reported in many areas of the Bahía Blanca estuary (CTE 2016). A similar species, *Leodamas uncinata*, was found to be characteristic of non-impacted sites in other Argentinian coastal areas (Sánchez et al. 2013).

Total biomass was, in general, dominated by polychaetes. At site 2, mollusks were the main group due to the presence of the snail *Buccinanops deformis* in one of the samples of this site. This gasteropod is an intertidal and subtidal species occurring in soft substrates (mud or sand) along the Atlantic coast of South America (34° S to 48° S). Given that the shells of mollusks were not eliminated to estimate total biomass, the contribution of *B. deformis* could be considered as overrepresented. Another important species of mollusks, relatively abundant along the channel but with a restricted distribution, was *Nucula semiornata*. This is a small clam with thin valves, frequently found in the Bahía Blanca estuary (Forcelli and Naroski 2015) and described as being very sensitive to disturbances. In this study *N. semiornata* was only found at sites more distant from the areas affected by the disturbances (sites 5 and 3*).

Due to the limitations of the sampling design, which only considers spatial variability, the conclusions of the study are limited, especially with regard to the association between assemblages and environmental variables whose temporal variability is expected to be high (e.g., salinity, temperature, turbidity, pH). However, considering the spatial component the analyzed disturbances generated a marked difference in assemblage structure along the studied channel, favoring the colonization of a few opportunistic species of polychaetes. Previous studies conducted on the sewage plume showed that the plankton displayed the same response that macrobenthic biota, low abundance of all assemblage and dominance of certain taxa such as the Larvae of the detritivorous crab *Neohelice granulata* or small non-siliceous algae which favorably to adapt to these conditions (Biancalana 2012; Dutto et al. 2012; Barría de Cao et al. 2003). This study was conducted two years after the deposition of dredging material in the area but its effect on the structure of the biological assemblage was still evident.

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