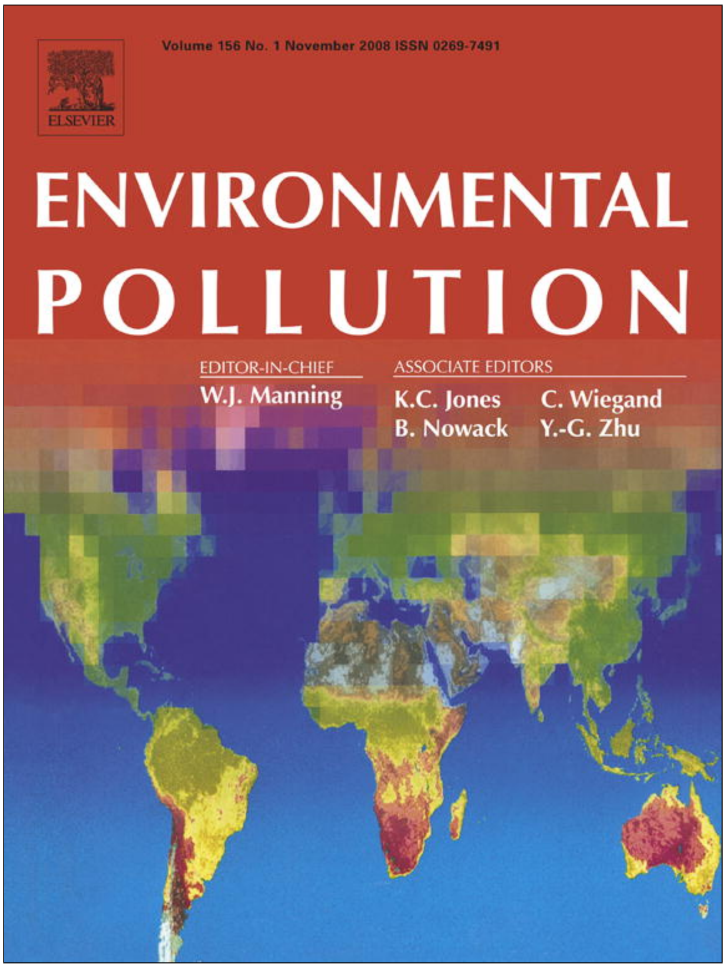
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Evaluation of zoobenthic assemblages and recovery following petroleum spill in a coastal area of Río de la Plata estuarine system, South America

C.S. Ocon [a,b,*], A. Rodrigues Capítulo [a,b], A.C. Paggi [a,b]

[a] *Instituto de Limnología ''Dr. Raúl A. Ringuelet'', UNLP-CONICET CC 712, 1900 La Plata, Argentina*
[b] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

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Oil spill impact was evaluated by zoobenthos response in an estuarine system.

Abstract

The objective of this work was to analyse zoobenthic assemblages in the coastal sector of the Río de La Plata, Argentina, after a petroleum spill. Sampling stations were located in representative sites of various landscapes. Structure, composition, physico-chemical parameters and seasonal changes were recorded in order to assess taxocenosis evolution during the period 1999–2003. Recovery signs were estimated by means of biotic indices and the presence of sensitive species. Tolerant species were dominant in heavily polluted sites, with low diversity and water quality values, according to the biotic indices used. In certain zones, sediment quality remains impoverished, with a visible oil film on the surface. However, during the last sampling, some points showed an increase in biotic indices, pointing to a slight improvement in environmental conditions.

Keywords: Petroleum spill; Zoobenthic macroinvertebrates; Biotic indices; Río de la Plata estuary; South America

1. Introduction

Pollution of the marine environment with oil is potentially one of the most devastating impacts on coastal, estuarine, and inter-tidal regions (Pezeshki et al., 2000). Crude oil represents a complex mixture of both organic and inorganic components which can interfere with oxygen exchange between water and atmosphere, diminishing light penetration and suffocating organisms. Besides, a soluble part in water is very toxic for various forms of aquatic life. In the same way, hydrocarbons are harmful to coastal vegetation causing its deterioration or loss. The coastal marshes are important ecosystems because of their high biological productivity and role as nurseries for coastal fisheries, habitat for wildlife, flood mitigation, shoreline protection from erosion, and water quality enhancement (Lin and Mendelssohn, 1998).

Benthic macroinvertebrates have been extensively used in water quality assessment, since their community structure shows changes in physical and chemical parameters related to anthropogenic disturbances, integrating the environmental factors along time. Several studies (Elmgren et al., 1983; Harrel, 1985; Poulton et al., 1997, 1998; Pontasch and Brusven, 1998; Peterson, 2000; Lytle and Peckarsky, 2001; Colombo et al., 2005a; Gomez Gesteira and Dauvin, 2005; Junoy et al., 2005) have been conducted on the effect of oil spills on zoobenthic organisms.

Macroinvertebrate sensitive species may be directly or indirectly impaired by lethal or sublethal effects of pollutants. This in turn may alter the species abundance or community composition. Specific toxicant could exert lethal effects over certain species with no visible effects on others. According to Gomez Gesteira and Dauvin (2005), the community's

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* Corresponding author. Instituto de Limnología "Dr. Raúl A. Ringuelet", UNLP-CONICET CC 712, 1900 La Plata, Argentina. Tel.: +54 11 4275 8564; fax: +54 11 4275 7799.
E-mail address: carolina@ilpla.edu.ar (C.S. Ocon).

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response includes very high initial mortalities in sensitive species and their subsequent disappearance. This process is followed by a significant increase in the abundance of opportunistic species.

The Río de la Plata drains the second largest basin of South America, "Del Plata," which has an area of 3,170,000 km^2. Its coastal area is affected by anthropogenic disturbances (Paggi et al., 2006) and natural phenomena related to estuarine characteristics. Benthic fauna distribution is influenced by hydrologic conditions, salinity, and sediment size. According to Rodrigues Capítulo et al. (1997), the more abundant macroinvertebrates are Oligochaeta and Nematoda, mainly in sites with high organic matter concentration. Another numerically important group is Mollusca, but its distribution is more closely related to substratum and physico-chemical characteristics. In January 1999, the collision of two ships (one of them carrying crude oil) led to a spill (approximately 1000 tons) affecting the middle zone of this estuarine system.

In order to assess the Río de la Plata estuary water quality, zoobenthic community structure and composition were analysed. Seasonal changes and taxocenosis evolution were registered through time, in order to evaluate recovery signs. Biotic indices and physico-chemical parameters were also assessed.

2. Material and methods

2.1. Study area

The Río de la Plata is located on the eastern coast of South America, between 34°S, 36°20′S latitude and 55°W, 58°30′W longitudes. The river flows into the Atlantic Ocean with a total average discharge of 20,000–25,000 m^3 s^{-1} (Urien, 1972). It is a funnel shaped (30,000 km^2), 320 km in length, with an open mouth of 230 km along the line Punta Rasa–Punta del Este (Framiñan and Brown, 1996; Guerrero et al., 1997). In this system there are three zones with fluvial-marine characteristics such as salinity, geology, hydrology and biology (Boschi, 1988). The top and middle stretches have characteristics typical of rivers while downstream portions present a gradual increase in salinity. However, being an estuarial system with tidal influence, saline intrusions are frequent in those sections, mainly during summer (AGOSBA-OSN-SIHN, 1994).

According to Urien (1972), the South Coastal Fringe is a morphological unit located in a coastal sector extending from the coastline to the 6–9 m isobath. This unit represents three zones: continental, marginal, and marine environments, according to sediment type and salinity. The study site is located in the continental sector with lotic characteristics; however, it is affected by oceanic phenomenon such as tides. The tides' and winds' effects can invert the flow direction of the river, and also increase its level. These conditions influence its physical, chemical and biological characteristics (Bazan and Arraga, 1993).

Grasses such as Senecio bonariensis, Pontederia cordata, Echinodorus grandiflorus, Typha latifolia, Scyrpus giganteus, Eleochares montana or bonariensis and few arboreal species are the dominant riparian vegetation. On the coastal line Scirpus californicus is the dominant species (Cabrera, 1971). In January 1999, the study area was affected by a petroleum spill extending from Punta Blanca to Punta Piedras (Fig. 1). Eighteen sampling stations were placed in representative sites of different landscapes: (a) open beach (without riparian vegetation): La Balandra (LB), Punta Blanca (PB), Campos de Alberdi (CA), Playa Nueva 1 (PN1), Pearson external and internal (Pe and Pi); (b) stream mouths: Marcelo external (Me), Gauchito Gil external and internal (GGe and GGi), Ricardo (R), Alborada (A), Juan Blanco external and internal (JBe and JBi); and (c) bulrush zones: Juncal 1 (J1), Juncal 2 external and internal (J2i and J2e), Playa Nueva 2 (PN2), Marcelo internal (Mi).

2.2. Macroinvertebrates

Samples were taken seasonally (every 3 months) from 1999 to 2001 and twice in 2002 and 2003 (a single sampling in spring). Benthic replicate samples (three grabs per site) were taken with an Ekman grab (100 cm^2). The total number of analysed samples was 756. Macroinvertebrates were fixed *in situ* with formaldehyde (5%). The organisms were sorted, identified and numbered in the laboratory using a stereomicroscope (Olympus SZ-40) and a compound microscope (Olympus CH-2).

Shannon and Weaver (1963) and Margalef (1955) diversity indexes, species richness, and evenness were estimated. The Biotic Index for Pampean Rivers (IBPamp) by Rodrigues Capítulo et al. (2001) and Macroinvertebrate Index for Pampean Rivers (IMRP) by Rodrigues Capítulo et al. (1995) were applied to assess the water quality.

2.3. Physico-chemical parameters

Conductivity (μS/cm) and pH were measured in the field with portable instruments (Cole Parmer conductimeter and Hanna HI 8633 pH-meter, respectively). The presence of hydrocarbons in sediments was visually determined. NO_2^--N (phenol-disulphonic acid method), NO_3^--N (Zambelli reactive method), NH_4^+ (4500 NH_3C method), and total phosphorus (4500-P) were analysed in the laboratory according to APHA (1992). The macroscopic observation of hydrocarbons in sediments allows for their classification in three quality degrees: 1 = sediment with visible hydrocarbons film, 2 = sediment with diffuse oil slicks, 3 = sediment without observable presence of hydrocarbons.

A comparison was made between biological data (IMRP, richness and diversity) obtained in this assessment and hydrocarbon data (aromatic and aliphatic compounds) obtained by Colombo et al. (2005a) in the same date and study area. Reference (LB-P) and affected sites (GG-R) were analysed 6, 13, and 42 months after the oil spill.

2.4. Statistical analysis

Canonical Correspondence Analysis (CCA) was used to evaluate relationships between benthic organisms' sensitivity and physico-chemical parameters for each sampling station. Environmental variables were automatically excluded from the analysis if multicolinearity was observed by a variance inflation factor was greater than 10 and were manually excluded when $p > 0.05$. Significant relationships were tested with a Monte Carlo Test using 199 permutations according to Ter Braak (1986).

All biological and physico-chemical variables (except pH) were log transformed prior to the analyses in order to reduce scale variation effects.

Principal Components Analysis (PCA) was applied to evaluate the trends along time for the three different landscapes under consideration. The analysis also included benthic organism's sensitivity, sediment quality and biotic index values.

3. Results

3.1. Macroinvertebrates

The most abundant macroinvertebrates in the study area and their sensitivities are detailed in Table 1. The benthic fauna was dominated by Nematoda, Oligochaeta (Naididae and Tubificidae), Mollusca, and Crustacea. The tolerant taxa were Oligochaeta (Naididae and Tubificidae), Nematoda, and Chironomidae (Insecta, Diptera), generally linked to low DO contents and high organic matter percentages in sediments. The sensitive organisms that were present in sampling sites were *Claudicuma platensis* (Cumacea), *Sinelobus stanfordi* (Tanaidacea), *Dilocarcinus argentinianus* (Decapoda Trichodactylidae), and *Pseudosphaeroma platense* (Isopoda). These

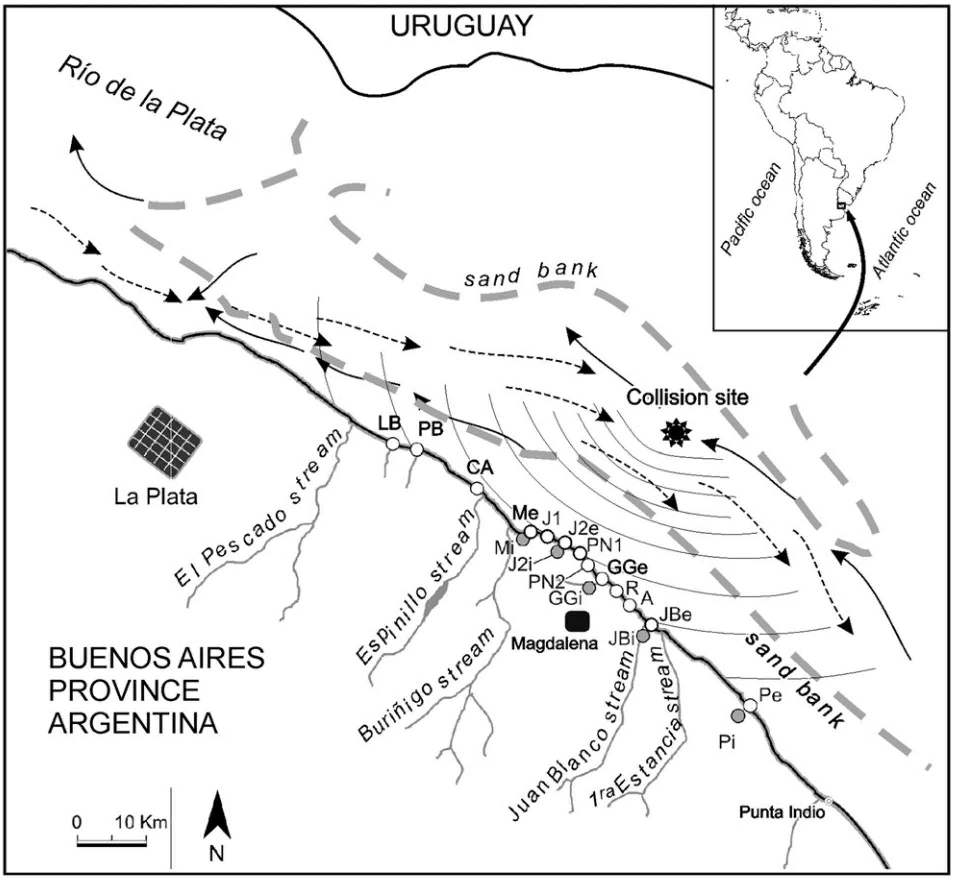

Fig. 1. Map of the study area showing the location of sampling stations (abbreviations in text). The direction of the offshore current was represented by arrows (ascending current represented by entire line and descendent current represented by dashed lines). Collision site and area of the spill spreading can be observed (represented by arcs from the spill point).

taxa were generally found in reference and slightly polluted sites such as PB, LB, PN1 and P1.

Fig. 2 shows species' sensitivity expressed as relative percentages, in a reference site and three representative sampling stations along time. In the affected sites there were higher densities of tolerant species, while the sensitive organisms were generally absent. However, recovery signs would be considered in M and R stations, where sensitive species were found in the last sampling.

The biotic indices values are shown in Table 2. The sampling points with higher diversity, richness and water quality were PB, LB, PN1, J1, P1 and CA; the minimum values were observed in J2(i) and J2(e), Mi, GGi and GGe, and R. Evenness showed lower values (0.2–0.3) in J2(i) and GG according to the subsistence of few dominant taxa. The highest values were observed in PN and PB.

According to the IBPAMP biotic index, PB, LB, PN1, and CA showed slight pollution (maximum scores 8–9), whereas GGi, R, J2(i), and M were heavily and very heavily polluted. IMRP biotic index results were similar to IBPAMP. However, in certain sampling stations such as Mi, Me, and R, the values of biotic indices increased on the last sampling date, which may indicate an improvement in environmental conditions.

3.2. Physico-chemical parameters

The values for the physico-chemical parameters are summarised in Table 3. pH values in affected sites were akin to those in non-affected ones ($p = 0.06$), whereas conductivity values showed statistical significant variations ($p = 0.002$). The lowest conductivity value was found in LB (749 μS/cm) and the highest corresponded to the R stream (1568 μS/cm).

Table 3 shows sediment classifications for the last sampling, where higher pollution sites, such as GG, are still observed. Loss of coastal vegetation was observed, corresponding to bad sediment quality.

Higher values for NO_3^--N (5.552 mg/L) and NH_4^+-N (3.191 mg/L) were recorded in LB, while higher values for NO_2^--N (0.081 mg/L) and P (0.575 mg/L) were observed in PN.

Fig. 3 shows the comparison between biological and hydrocarbon data. In the reference sites (LB and P) the hydrocarbon values were between 0.02 and 0.22 μg/g (aromatic) and 0.02 and 0.4 μg/g (aliphatic). In the affected sites the average values of aliphatic compounds at 6 and 13 months were 1.8 μg/g for PN and 40 μg/g for GG-R while the aromatic compounds were 0.02 μg/g and 2.2 μg/g, respectively. Hydrocarbon concentrations were significantly reduced at 42 months

Table 1
List of the more frequent taxa registered in the study area, where riverine species and typically estuarine species were observed

Taxa	Very tolerant sp.	Tolerant sp.	Sensitive sp.
COELENTERATA			×
PLATYHELMINTHA		×	
NEMATODA	×		
OLIGOCHAETA			
Tubificidae	×		
Naididae			
Nais sp.		×	
Bratislavia sp.			×
Paranais sp.			×
Pristina sp.			×
Chaetogasler sp.			×
Dero sp.		×	
Pristinella sp.			×
Enchitraeidae	×		
Lumbriculidae		×	
Hirudinea			
Glosiphonidae		×	
MOLLUSCA			
Corbicula fluminea		×	
Limnoperna fortunei		×	
Gundlachia concentrica			×
Biomphalaria peregrine		×	
Drepanotrema kermatoides		×	
Heleobia parchappei		×	
Pomacea canaliculata		×	
ARTHROPODA			
Tardigrada	×		
Harpacticoida		×	
Cyclopoida		×	
Calanoida		×	
Chidoridae		×	
Macrothricidae		×	
Ilyopcryptus sp.		×	
Ostracoda		×	
Tanaidacea			
Sinelobus stanfordi			×
Cumacea			
Claudicuma platensis			×
Amphipoda			
Hyalella sp.			×
Isopoda			
Pseudosphaeroma			×
Decapoda			
Tricodactylidae			×
Palaemonetes sp.			×
Colembolla	×		
Coleoptera			
Hydrophylidae		×	
Dytiscidae		×	
Hemiptera			
Belostomatidae		×	
Belostoma sp.		×	
Corixidae			×
Diptera			
Chironomidae	×		
Ceratopogonidae		×	
Psychodidae	×		
Tipulidae	×		
Ephydridae	×		
Dolicbopodidae		×	
Acari		×	
BRIOZOA			×

Determination was made at different taxonomic levels.

after the spill. Aliphatic compound values were 0.1 μg/g for PN and 1 μg/g for GG-R, decreasing between 80% and 90%. Aromatic compound values were 0.001 for PN and 0.03 μg/g for GG-R, decreasing by approximately 95%. In accordance with these data, the biotic indices, richness and taxa diversity values were stable in reference sites (N: 14–25, H′: 2, IMRP: 12), while PN and GG-R showed a decrease after 13 months and a slow recovery after 42 months (N: 6–8, H′: 1, IMRP: 4). These values were always lower than those at the reference sites (IMRP 40% lower than reference sites).

3.3. Statistical analysis

Fig. 4 shows sampling stations classification according to the taxa sensitivity and the physico-chemical parameters obtained by means of CCA. The correlation matrix consists of 7 environmental variables, 10 samples, and 3 cases (species). Three environmental variables were excluded from the analysis because of their high inflation factor or their high p values. Eigenvalues obtained for the first two axes were 0.125 and 0.057 respectively. When four main axes were considered, species–environment correlation was greater than 0.874. Monte Carlo permutation test indicated that all axes were significant (F-ratio 3.907 $p < 0.035$). Axes 1 and 2 explain 75.8% of the cumulative variance for the species data and 100% for the species–environment relation. The sampling stations' distribution was strongly determined by conductivity and sediment quality.

Axis 1 shows a positive association with conductivity and was also related to pH and NO_3^--N (pointed out in decreasing order of explanatory importance). Axis 2 was mainly related to sediment quality.

Sensitive species were correlated with minimum conductivity values and high nutrient concentrations, the stations related to those conditions were LB, CA, J1 and P. Stations PN and M were more connected with the presence of tolerant species and pH, while GG, J2(i), and R were related to the presence of very tolerant species, high conductivity and bad sediment quality. The PCA graphics (Figs. 5–7) show the performance over time of three representative sampling stations: PN 1 (open beach), GGi (stream mouth) and J2(i) (zones with bulrush) in contrast with a reference point (LB). The reference station was positively related to the Shannon index (H′), high sediment quality, and sensitive species. J2(i) was associated with very tolerant species, high densities of macroinvertebrates (individuals/m^2), and low values in diversity and sediment quality.

PN1 was primarily associated with sensitive species although it also showed low diversity values. It can be observed that the performance of this station during the last sampling date changed, showing a high number of very tolerant species. This does not necessarily imply increased pollution or damages in environmental conditions, and it could be due to the fluctuating system dynamics. GGi station was mainly related to the presence of very tolerant and tolerant species and negatively correlated to each one of the quality parameters considered. Eigenvalues obtained for the first two axes were 0.386

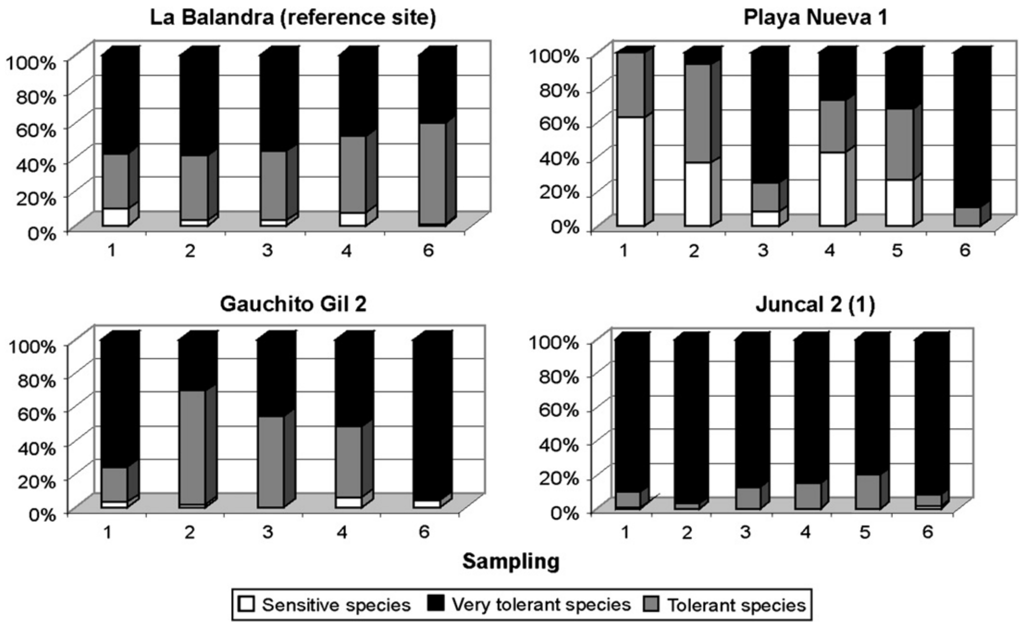

Fig. 2. Tolerance of macroinvertebrates to disturbances (expressed as relative percentage) for each sampling in reference (La Balandra) and affected sites (Playa Nueva 1, Gauchito Gil and Juncal 2i).

and 0.222 respectively. Axes 1 and 2 explain 60.8% of the cumulative variance of species data.

4. Discussion

Sensitive species such as *Claudicuma platensis*, *Sinelobus stanfordi*, *Dilocarcinus argentinianus*, and *Pseudosphaeroma platense* were not found in highly polluted sites, despite their presence of stable populations in similar but non-affected zones. Similar results were obtained by Poulton et al. (1998) who observed four dominant species of benthic community at upstream sites, which were virtually eliminated below the spill. In the same vein Peterson (2000) observed that the densities of clams were reduced directly and the abundance of several toxin-sensitive amphipods declined dramatically.

In the Galician coast, de la Huz et al. (2005) registered changes in the macrofaunal composition and a decrease in the species richness related to the *Prestige* oil spill. Lytle and Peckarsky (2001) concluded that a diesel fuel spill reduced the density of invertebrates and taxonomic richness, but the density recovered within a year. According to Crunkilton and Duchrow (1990) the visible appearance of oil in the stream substrate is a simple predictor of the status of the benthic invertebrate community. This conclusion is valid for GG, R, and J2(i) sites, where there was an observable hydrocarbon film in connection with an impoverished community.

Table 2
Average values of biotic indices

Sampling station	Biological indices					
	S	R1	H′	E1	IMRP	IBPAMP
LB	17	1.64	1.97	0.70	9.0	6
PB	10	0.93	1.94	0.92	5.8	5
CA	21	1.64	1.74	0.58	12.1	6
Mi	7	0.56	1.28	0.69	2.4	3
Me	8	0.76	1.15	0.57	3.4	4
J1	14	1.35	1.99	0.76	7.0	5
J2(i)	9	0.66	1.12	0.61	3.2	3
J2(e)	10	0.95	1.42	0.65	5.8	5
PN1	10	1	1.56	0.67	5.5	6
PN2	9	0.94	1.47	0.71	5.9	5
GGi	7	0.56	0.95	0.53	3.2	6
GGe	9	0.81	1.25	0.64	5.0	5
R	8	0.7	1.14	0.57	3.1	3
A	8	0.77	1.69	0.84	4.2	4
JBi	9	0.75	1.37	0.65	2.7	3
JBe	5	0.45	1.38	0.86	3.5	6
Pi	11	1.16	1.72	0.71	7.1	6
Pe	9	0.94	1.51	0.72	6.7	5

N, species richness; R1, diversity index (Margalef, 1955); H′ = Shannon diversity index (Shannon and Weaver, 1963); E1, evenness, IMRP, Macroinvertebrate Index for Pampean Rivers; IBPAMP, Biotic Index for Pampean Rivers and Streams.

Table 3
Average values of physicochemical data

Sampling stations	pH	Conductivity (μS/cm)	Sediment quality	N-NO$_3$ (mg/L)	N-NO$_2$ (mg/L)	N-NH$_4$ (mg/L)
LB	6.80	749	3	5.552	0.045	3.191
CA	6.92	796	2	5.083	0.003	0.076
M	7.13	955	2	3.941	0.039	2.261
J1	6.94	998	2	4.945	0.035	1.641
J2(i)	6.66	1510	1	3.663	0.053	1.697
PN	7.14	1048	2	3.347	0.081	0.226
GG	6.59	1380	1	1.969	0.036	0.365
R	6.67	1568	2	1.490	0.017	0.710
JB	6.65	1429	2	2.285	0.010	0.393
P	6.92	917	3	4.478	0.004	0.434

The sediment quality data correspond to the last sampling.

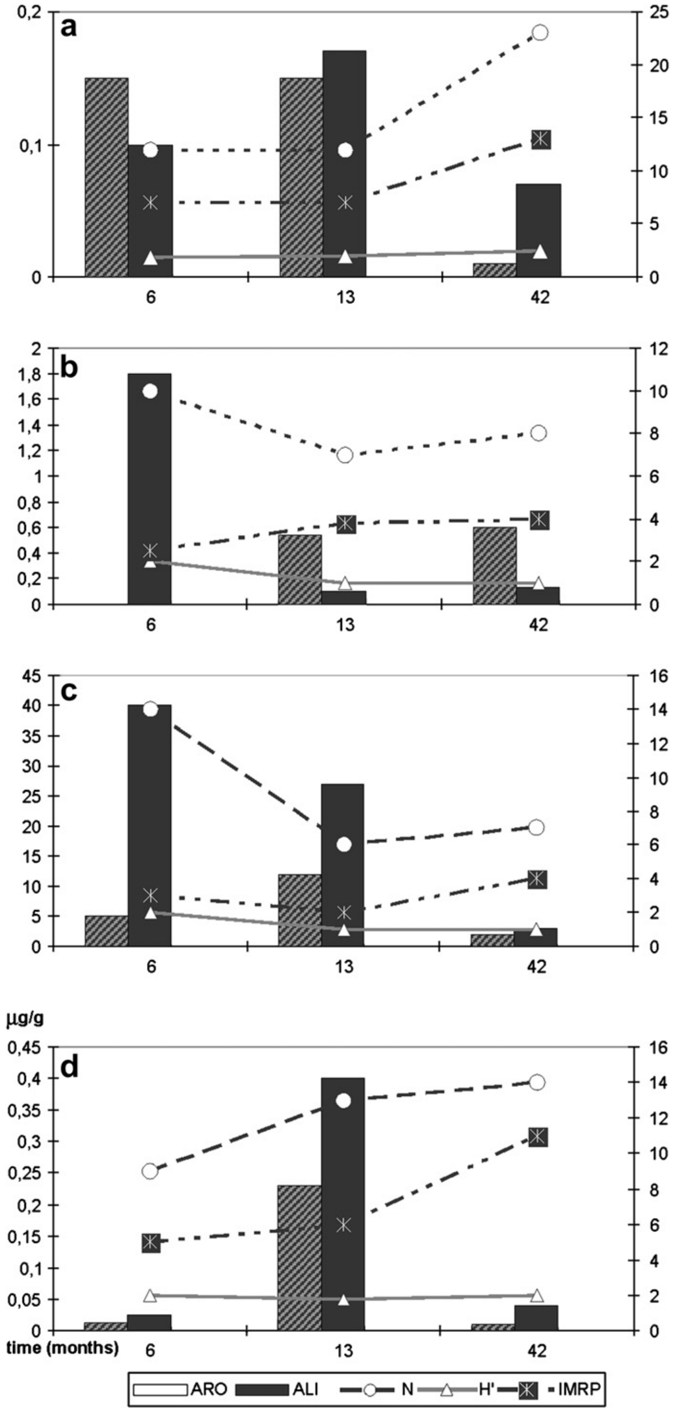

Fig. 3. Comparison between biological and hydrocarbon data in the reference and the affected sites. (a) LB (north reference site); (b) PN (affected site); (c) GG-R (affected site); (d) P (south reference site). Aro, aromatic compounds; Ali, aliphatic compounds; N, taxa richness; H′, Shannon diversity index; IMRP, Macroinvertebrate Index for Pampean Rivers.

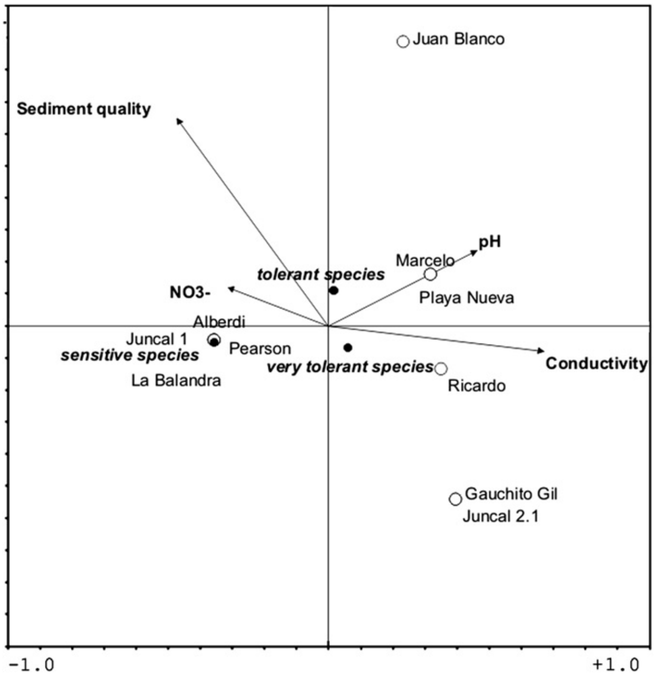

Fig. 4. Triplot of sampling sites, species, and environmental variables in relation to the first two ordination axes of CCA.

The IBPAMP and IMRP biotic indices were useful to assess water quality and temporal changes in the study area. Likewise, Pontasch and Brusven (1998) applied different biotic indices and concluded that some of them (e.g. Bray–Curtis and average χ^2 community comparison indices) were effective in quantifying differences in macroinvertebrate composition between gas-impacted and reference areas of Wolf Lodge Creek following a spill.

The affected sampling stations that showed recovery signs were the ones located in zones with a higher influence of tides. The stations that did not show evident improvement exhibited

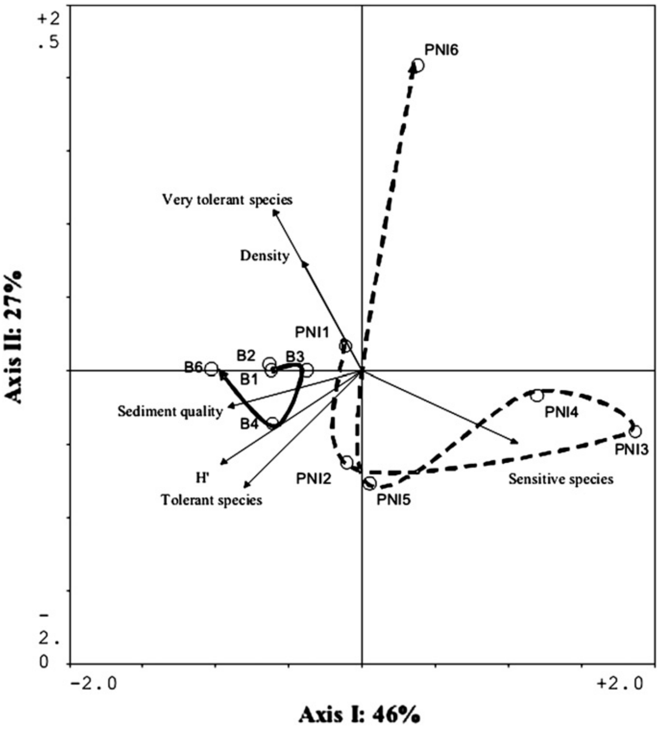

Fig. 5. PCA graphic that shows the evolution over time in PN (open beach) showing good recovery compared with the reference site (LB).

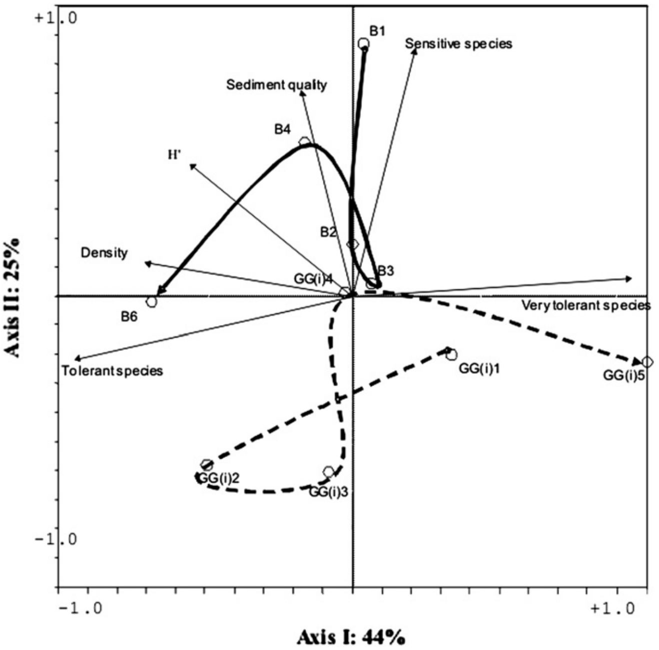

Fig. 6. PCA graphic that shows the evolution over time in J2i (bulrush zone) with low recovery compared with the reference site (LB).

lower average depth and lower tidal influence. This coincides with Colombo et al. (2005a), who concluded that the coast in this study area was severely oiled and persisted 6 months after the spill with very high hydrocarbon levels in the coastal waters, sediments, and soils, exceeding baseline concentrations by 1–3 orders of magnitude, especially in low-energy stream embouchures and bays, which acted as efficient oil traps. According to Colombo et al. (2005b), apart from some low-energy, heavily polluted sites such as GG and R, most stations have reverted to previous levels of hydrocarbon concentrations at the time of the last sampling. These authors concludes that, at the time of the last sampling, all hydrocarbon fractions, including the UCM (Unresolved Complex Mixture), were consistently reduced, reflecting the combined effects of biodegradation and physical removal.

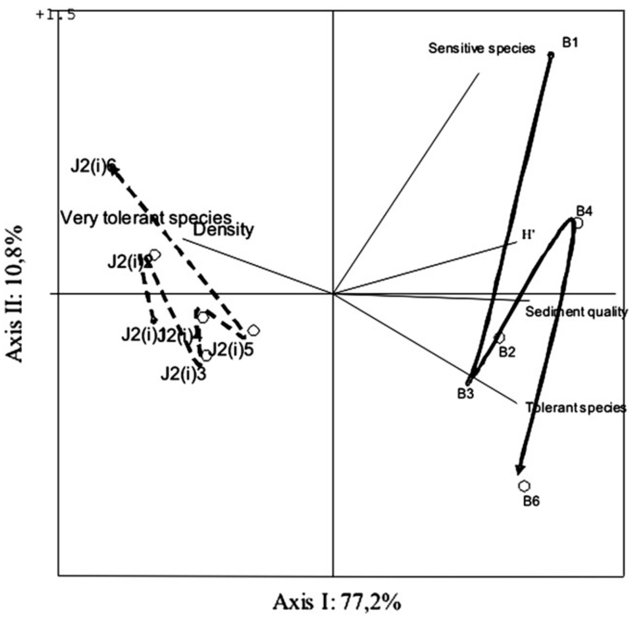

Fig. 7. PCA graphic that shows the evolution over time in GGi (stream mouth zone) without recovery compared with the reference site (LB).

AGOSBA-OSN-SIHN (1994) and Crunkilton and Duchrow (1990) considered that the most apparent factors controlling the recovery were the total volume of water passing through the contaminated area and the occurrence of scouring flood in contaminated rivers. They concluded that certain physico-chemical parameters such as DO, pH, and conductivity were not affected. Accordingly, pH and nutrients recorded in this study did not show significant differences between affected and non-affected sites. Conversely, conductivity showed differences between highly polluted and slightly or non-polluted sampling stations.

According to Margalef (1983), the velocity at which a system returns to its previous status after a disturbance is proportional to its stress magnitude. In our study area, it is still difficult to determine the amount of time it may be required for the system to return to the previous equilibrium position. This agrees with the caution of Dauvin (1998) that it is necessary to survey the affected communities for a long period of time (>10 years) to identify the real ecological impact of an oil spill.

5. Conclusions

- Macroinvertebrate community and biotic indices were useful tools to assess water quality after an oil spill in the study area.
- Heavily polluted sites showed higher hydrocarbons levels and an impoverished macroinvertebrate community.
- In the last sampling, the affected sites located in zones with higher influence of tides showed recovery signs such as presence of sensitive organisms.

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