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Benthic invertebrates structure in wetlands of a tributary of the middle Parana River (Argentina) affected by hydrologic and anthropogenic disturbances

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Abstract: *The present study was aimed at analyzing chromium concentrations in water column and bottom sediments in the main channel of Northern Salado River (tributary of Middle Parana River) and its floodplain. The main changes caused by human activities and hydrological disturbances on benthic invertebrate structure were also analyzed. Sediment concentrations of the reference area varied between 44.2 and 97.1 $\mu g\ Cr\ g^{-1}$ (dw), and in the impacted zone, between 85.5 and 209 $\mu g\ Cr\ g^{-1}$ (dw) reaching the highest values in the wetland floodplain. Alfa, beta and gamma diversities in the reference section have been 33, 9 and 66 species, and in the disturbed section, they have been 37, 8.33 and 74 species, respectively. The species dominant in the disturbed habitats were characterized by a small body size and short life cycles, as the species of oligochaetes Naidinae. The extreme flooding produced a rejuvenation of the area with the consequent physical re-structuration produced by flooding, showing a marked decrease in chromium levels in sediments and in organic matter content, which allowed the colonization of insects (Ephemeroptera and Trichoptera).*

Key words: *Invertebrate assemblages, Chromium, Wetland floodplain, Parana River tributary*
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Introduction

Some nutrients such as phosphorus and several contaminants including heavy metals and pesticides, are transported by rivers and are associated with fine sediments (Allan, 1979; Chukwu and Odunzeh, 2006), which in turn, can be deposited and accumulated in the floodplain during floods but can also re-mobilize and return to the river (Walling *et al*., 2000; Walling and Owens, 2003). However, there is not enough information on contaminant accumulation in floodplains of large rivers, since, generally, only main channels are analyzed, considering them as a source of contaminant discharge. However, wetlands floodplains are important, since these systems, during their floods, inundate large areas and contaminants can be accumulated in these aquatic-terrestrial transitional zones (Villar and Bonetto, 2000; Duran and Suicmez, 2007). The benthic macroinvertebrates are the biological community most frequently used to evaluate water quality in aquatic environments and occupy a variety of trophic levels acting in the nutrients, bottom organic matter and water column dynamics (Rosenberg and Resh, 1993; Traunspurger and Drews, 1996; Reynoldson and Rodriguez, 1999; Marchese and Ezcurra de Drago, 1999; Rodrigues Capitulo *et al.,* 2001, 2002). Moreover, as they have very long life cycles, they act as continuous monitors of the quality of water running over them.

The sediment where benthic invertebrates live is considered a deposit for all types of toxic substances, even for the most volatile ones. The relationship between benthic invertebrates-sediment-toxic substances and their feeding habits determines they are very good indicators of pollution, since these species play an integral role in the effects, rates and circulation of contaminants in the ecosystem (Nalepa and Landrum, 1988; De Pauw and Hawkes, 1993). In addition, benthic invertebrates, together with macrophytes, have the capacity of accumulating toxics in concentrations higher than those found in water, participating, in this way, in pollutant dynamics.

The objectives of this study have been: 1) to analyze the chromium concentrations in water column and bottom sediments in two transversal sections that include the main channel of northern Salado River (tributary of Parana River) and its floodplain; 2) to analyze the benthic invertebrates assemblages in both reference zone and disturbed by human activities zone, and 3) to determine the main changes produced by hydrological disturbances as the extreme floods.

Materials and Methods

Study area: The northern Salado River, in its lower section, drains a predominantly rural basin that covers an area of approximately 29,700 km^2 in the center-northern zone of the Province of Santa Fe, Argentina (29° and 31°30' S - 60°30' and 62° W) (INCyTH, 1986). It is highly disturbed by human activities through infrastructure works (channels, road and railway networks and flood control works) or soil management techniques. This zone receives wastes from numerous industries and from an extensive area which is mainly cultivated with soybean (*Glycine max*), through channels that have been constructed to facilitate the surface drainage and through Las Conchas, San Antonio and Cululu streams. The region of greatest milk, agricultural, cold-storage plants and tanneries productivity of Argentina is located in the northern Salado River basin and it contributes with organic matter and different toxic substances to the system. The system, besides the anthropogenic disturbances, receives the impact produced by the hydrological disturbances, as the extreme flood that occurred in northern Salado River in April 2003 affecting more than 200,000 inhabitants.

Sample collection and data analysis: The samples were taken in two transects, the reference one (with three sampling sites) upstream San Justo city (SJ) and the disturbed one (with four sampling sites in high waters and only two in low waters because the other two sites were drought) in Esperanza city (E) (Province of Santa Fe, Argentina), during June, August and November 2002 and July 2003. The reference zone has been determined considering the criterion given by Reynoldson *et al*. (1997), who defined reference conditions as those that are representative of a minimally disturbed group of sites, selected by their physical, chemical and biological characteristics.

Two samplings in low waters and two samplings in high waters including seven sampling sites have been carried out in the study, according to the hydrological regime of Salado River (Fig. 1). Benthic samples (three sampling units at each sampling site) were collected using a Tamura grab (322 cm^2) in the main channel and an Ekman grab (325 cm^2) in the wetland floodplains, which were filtered through a 200 µm sieve and preserved in 5% formaldehyde plastic vials. Additional sediment samples for granulometry and organic matter content analysis were taken at the same sites. The organic matter content of sediments has been analyzed by ignition at 500°C for 5 hr; each sample has been weighted with a Mettler M5 balance to determine organic matter by difference of weights. The granometric composition of bottom sediments has been analyzed according to Wentworth´s scale (1932). Depth, pH, dissolved oxygen, conductivity, temperature (measured with a Water Quality Checker Horiba U-10), transparency (with a Secchi disc), BOD_5 and COD (potassium dichromate method) were also measured at each site. Nitrogen as nitrates, nitrites, ammonium, total phosphorus and hardness (titrimetric method with EDTA) were determined in each sample following APHA (2005). Chlorophyll *a* content was determided by extraction with 90% acetone in acordance with the standard methods for the examination of water and wastewater (Strickland and Parsons, 1968).

Subsurface water (obtained with glass vials) and bottom sediment samples (with Tamura grab) have been taken to analyze chromium (with an Analyst 800 atomic absorption spectrophotometer) contents. EPA Protocol 200.2 has been followed for digestion of water and sediment samples (Martin *et al.*, 1991).

Benthic invertebrates were hand-picked from sediments at 10x magnification using a dissection microscope, previously dyed with erythrosin to facilitate observation and then preserved in 70% alcohol. All samples have been analyzed, identifying taxa until the lowest possible level with an optic microscope.

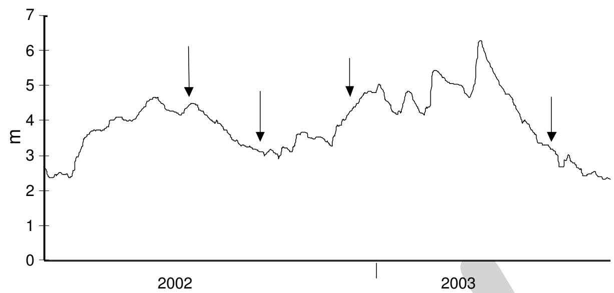

Fig. 1: Water level of the Salado River in 2002-2003. The arrows indicate the sampling data

The Bodenheimer constancy index (Dajoz, 2002) has been used to estimate the frequency of species recorded in the study.

Density (ind.m^{-2}), range-abundance analysis, species richness, species diversity (Shannon-Wiener index) and β and γ diversity (Harrison *et al.,* 1992) have been calculated between the two transects, including the main channel and its floodplain. β diversity has been calculated using the index that allows to compare transects of different sizes with values from 0 (total similarity) to 100 (total dissimilarity). We have used the formula:

$$\beta = [(\gamma/\alpha) - 1]/[N-1] \times 100$$

where γ is the regional diversity (the number of species in a specific section), α is local diversity (the mean species number of the sites) and N is the number of sampled habitats (in this case, sampling stations) within the transect.

A Canonical Correspondence Analysis (CCA) was performed using the mean densities of constant (>50%) and accessory species (25-50%) (obtained by Bodenheimer index) from each sampling site of the reference and disturbed zone using the Multivariate Statistical Package (MVSP 3.2 for Windows, Kovach Computing Services, 2001). Densities data have been standardized through logarithmic transformation ($\log_{10}$ x+1). An analysis of variance (ANOVA) has been carried out using the statistical program Systat 5.0 to determinate the differences (α <0.05) between the benthos density means among sites of the affected zone and the reference ones.

Results and Discussion

The mean discharge of Salado River is 105 m^3s^{-1} and it is characterized by a high conductivity, with a maximum value of 3,100 μScm^{-1} during the studied period.

The main channel has sandy bottom sediment, while the floodplain has silt-clay bottom sediment, with a higher organic matter content, reaching the highest values in the disturbed zone (Table 1). The COD and BOD_5 values have been similar in both areas, remaining the BOD_5 within the limits of clean waters, according to Hynes (1978). The pH has been alkaline and conductivity has been slightly higher in the reference zone. Nutrient concentration has been similar in the main channel and in the floodplain, reaching the highest values of total phosphorus and ammonium in the disturbed

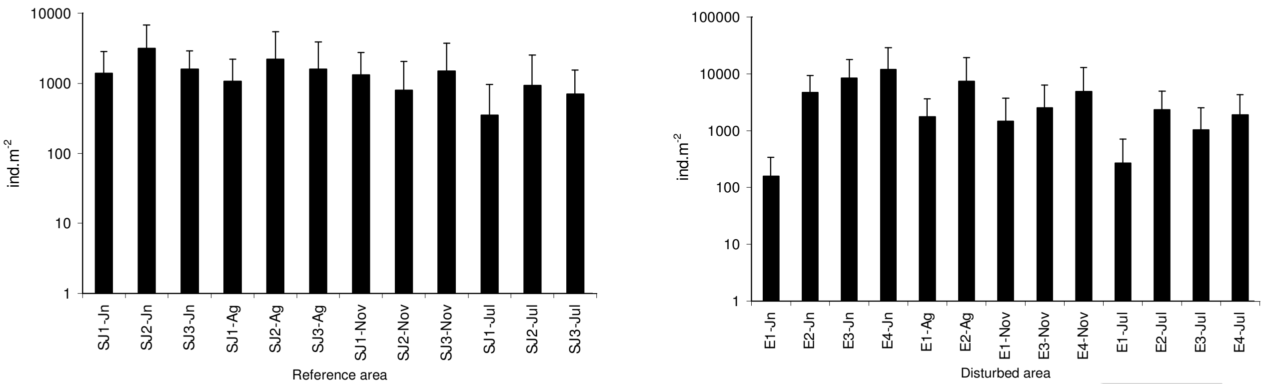

Fig. 2: Density of the benthic invertebrates (mean values and standard deviation) in the main channel of the Salado River and its wetlands floodplain in the reference and disturbed zones

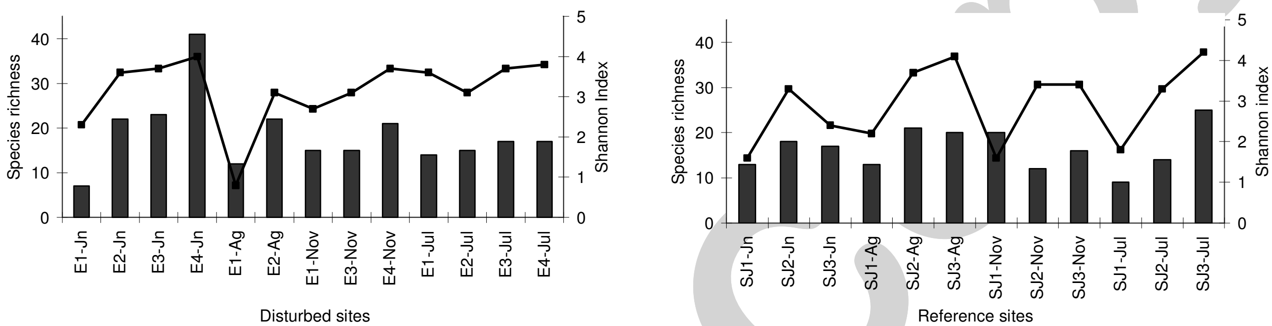

Fig. 3: Richness (bars) and species diversity (line) (Shannon's index) in the main channel of the Salado River and its wetlands floodplain in the reference and disturbed zones

zone. The chlorophyll-a was higher in the main channel than the wetlands (Table 1). Water hardness has been 97-103 mgl^{-1} CO_3Ca_2.

Chromium concentrations in water have been higher in the reference zone than in the disturbed zone, but concentrations in sediment have been much higher in this zone, reaching the highest values in the wetland floodplain (Table 2). Sediment concentrations of the reference zone varied between 44.2 and 97.1 μg Cr g^{-1} (dw), and in the disturbed zone, between 85.5 and 209 μg Cr g^{-1} (dw). Comparing these values with those obtained in the period 1995-96, a marked increase in sediment chromium concentrations was observed, mainly in the reference zone, while concentrations obtained in July 2003, after an extreme flooding were considerably lower. Total water chromium values obtained in this study have been always higher than guidelines given by the Subsecretaria de Recursos Hídricos de Argentina for the aquatic biota (<2.5 μgl^{-1}), but lower than those suggested for hard waters (44 mgl^{-1}), as those characteristic of the Salado River. Therefore, the concentrations obtained in the water were not toxic in coincidence to studies by Bohrer (1995) where the chromium toxicity for invertebrates varied between 0.1 to 20 μgl^{-1}. On the other hand, it can be concluded that the reference area also shows, although at lower levels, signs of contamination exceeding the guidelines given by CEPA (2002) (37.3 μg g^{-1}) according to results obtained on chromium concentrations in sediments, where live the benthic invertebrates.

Density has varied between 156 and 11,796 ind.m^{-2}, generally being higher in the disturbed zone and in the wetland floodplain, and lower in July after the extreme flooding (Fig. 2). Density values in the disturbed zone have showed a wide variation, while in the reference zone the variation has been much lower. The ANOVA applied to density has showed significant differences between the reference zone and the affected area ($p < 0.0001$), between the main channel and the wetland of the reference zone ($p = 0.009$), and between the main channel and the disturbed zone ($p = 0.002$).

Species richness has been higher in the disturbed area (between 7 and 41 species), being always lower in the main channel than in the wetland floodplain. In the reference area (between 9 and 25 species) was similar among the different sampling sites and reaching the highest value after the extreme flooding (Fig. 3). Shannon index in this zone has varied between 1.6 and 4.3, reaching the highest value in the wetland after the extreme flood, and in the disturbed zone between 0.8 and 4.0, reaching similar values in all sampling sites after the extreme flood (Fig. 3). The value obtained in the reference zone has been the highest one obtained in tributaries of Parana river (Marchese and Ezcurra de Drago, 1983; Zilli and Gagneten, 2005; Pave and Marchese, 2005). Alfa, beta and gamma diversities in the reference section have been 33, 9 and 66 species, and in the disturbed section, they have been 37, 8.33 and 74 species, respectively. Thus, β diversity has been similar in both zone, while γ diversity has been higher in the disturbed zone. It could be speculated that a low concentration of toxic materials would increase species diversity if these chemicals remove the competitively superior species or alter their success as a result of the interaction of species, as predicted by the hypothesis of intermediate disturbance (Connell, 1978). In agreement with Newman (2001), it can be inferred that populations chronically exposed to contaminants, as in the disturbed zone, sometimes show a relative increase in their tolerance to toxics. Benthic macroinvertebrates collected from places with moderate levels of heavy metals have been significantly more tolerant to subsequent exposure to Cd, Cu and Zn than those collected from pristine places (Clements, 1999; Courtney and Clements, 2000; Clements and Newman, 2002).

Only eight species from the 66 and 74 ones recorded in the reference and disturbed zones, respectively, have been constant (> 50%), being ostracods Cytheridae the most representative group in the first one, and *Dero sawayai, Slavina isochaeta* and nematods Mermithidae in the second one. On the other hand, there have been 14 accessory species (25-50%), *Campsurus* cfr. *notatus, Polypedilum* sp and *Cordylophora caspia* have been dominant in the reference area and *Dero nivea, Pristina leidyi* and *Aulodrilus pigueti* have been dominant in the disturbed zone.

In the Canonical Correspondence Analysis (CCA), which has analyzed constant and accessory species and physico-chemical variables, the first two axes explained 72.85% (axis I, 57.46%, eigenvalue: 0.031, species-environment correlation: 0.96, and

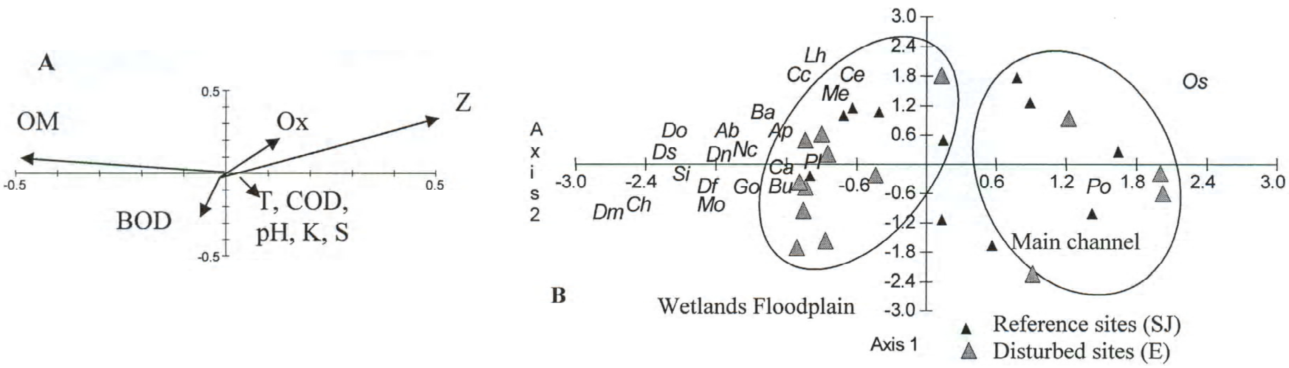

Fig. 4: Ordination of the sampling sites and benthic invertebrate data with environmental variables by Canonical Correspondence Analysis. A = Environmental variables that explain axis 1 and 2. OM = Organic matter, Ox = Oxygen, BOD_5 = Biological oxygen demand, COD = Chemical oxygen demand, T = Temperature, pH, K = Conductivity, S = Secchi disc, Z = Depth. B = Ordination of the species. Os = Ostracods Cytheridae, Ce = Ceratopogonidae, Me = Mermithidae, Lh = *Limnodrilus hoffmeisteri*, Cc = *Cordylophora caspia*, Ba = *Botrhioneurum americana*, Ap = *Aulodrilus pigueti*, Pl = *Polypedilum* sp, Ca = *Campsurus* cfr. *notatus*, Bu = *Bratislavia unidentata*, Ab = *Ablabesmyia* sp, Nc = *Nais communis*, Dn = *Dero nivea*, Go = *Goeldichironomus* sp, Df = *Dero furcatus*, Mo = *Pomacea* sp, Do = *Dero obtusa*, Ds = *Dero sawayai*, Si = *Slavina isochaeta*, Ch = *Chironomus* gr. *decorus*, Dm = *Dero multibranchiata*

Table - 1: Physico-chemical characteristics of the sampling sites. Mean (±SD) values of N = 4 samples. Abbreviation for the sites: Salado River in the reference zone (SJ) and disturbed zone (E)

	Main channel SJ1	Wetland SJ2	Wetland SJ3	Main channel E1	Wetland E2	Wetland E3	Wetland E4
Nitrate (mgl^{-1})	1.32 (0.67)	1.08 (0.57)	1.25 (0.71)	1.22 (0.85)	1.05 (0.57)	1.8 (0.98)	1.13 (0.85)
Nitrite (mgl^{-1})	0.01 (0.01)	0.009 (0.01)	0.007 (0.009)	0.005 (0.003)	0.006 (0.002)	0.002 (0.002)	0.01 (0.01)
Ammonium (mgl^{-1})	0.10 (0.07)	0.10 (0.05)	0.10 (0.07)	0.23 (0.13)	0.19 (0.11)	0.17 (0.11)	0.18 (0.11)
Phosphate (mgl^{-1})	0.83 (0.10)	0.85 (0.22)	0.83 (0.18)	0.89 (0.14)	0.93 (0.18)	0.99 (0)	0.91 (0.09)
Chlorophyll *a* (mgm-^{3})	2.54 (2.28)	0.86 (0.42)	0.94 (0.45)	1.15 (0.49)	0.61 (0.30)	0.75 (0.19)	0.65 (0.62)
COD	43 (10.24)	46 (32.58)	40 (20.56)	51 (5.03)	51 (5.50)	45 (5.77)	48 (5.56)
BOD_5	1.39 (1.32)	2.56 (1.40)	1.17 (0.33)	1.95 (0.99)	2.35 (1.45)	2.11 (1.43)	1.70 (0.50)
pH	7.8 (0.25)	7.65 (0.28)	7.9 (0.27)	8.1 (0.42)	7.9 (0.51)	7.8 (0.55)	7.9 (0.51)
Conductivity (μScm^{-1})	2525 (377.49)	2100 (559.76)	2350 (238.04)	2300 (653.19)	2466 (305.50)	1933 (472.58)	1833 (737.11)
Oxygen (mgl-1)	7.3 (2.0)	4.2 (3.86)	6.9 (2.12)	7.1 (2.56)	7.6 (2.76)	7.0 (2.40)	7.0 (2.80)
Temperatura (ºC)	17.5 (5.80)	18 (4.83)	18.5 (7.23)	17.5 (6.60)	13.3 (3.05)	17.3 (8.08)	17.6 (8.32)
Secchi disc (cm)	14 (2.87)	25 (4.99)	2 (9.12)	21 (6.35)	17 (4.16)	26 (10.14)	21 (6.55)
Depth (m)	5.4 (0.65)	0.60 (0.29)	1.29 (0.67)	7.86 (0.84)	0.61 (0.34)	1.30 (0.78)	0.7 (0.21)
Organic matter (%)	2.0 (1.93)	11.5 (1.25)	4.6 (4.66)	0.6 (1.11)	3.0 (4.16)	18.5 (0.01)	14.1 (0.01)
Sand (%)	75.1 (15.40)	9.2 (9.27)	26.9 (17.73)	85.8 (19.34)	19.6 (3.70)	3.9 (1.81)	23.2 (1.68)
Silt (%)	14.1 (9.14)	26.0 (15.74)	30.2 (9.55)	7.0 (10.85)	36.0 (10.48)	20.4 (2.00)	16.7 (1.25)
Clay (%)	10.7 (6.90)	64.7 (24.96)	42.7 (23.69)	7.1 (8.50)	44.3 (14.15)	75.6 (3.77)	60.1 (2.92)

Table - 2: Total chromiun concentrations in water and bottom sediment samples in the reference (SJ) and disturbed area (E). Sites 1 = main channel of Salado River, sites 2-4 = wetlands floodplain

Sites		**Chromiun**			
		Dec-95 (data from INALI)	**Set-96 (data from INALI)**	**Nov-02 (this study)**	**Jul-03**
SJ1	Water ($\mu g l^{-1}$)			7.22	2.6
	Bottom sediment ($\mu g\ g^{-1}$ dw)	1.01	5.5	44.2	5.4
SJ2	Water ($\mu g l^{-1}$)			6.81	9.6
	Bottom sediment ($\mu g\ g^{-1}$ dw)			97.1	20.4
SJ3	Water ($\mu g l^{-1}$)			2.75	1.6
	Bottom sediment ($\mu g\ g^{-1}$ dw)			72.1	10.9
E1	Water ($\mu g l^{-1}$)			3.36	2
	Bottom sediment ($\mu g\ g^{-1}$ dw)	55.1	46.9	85.5	5.9
E2	Water ($\mu g l^{-1}$)				1.9
	Bottom sediment ($\mu g\ g^{-1}$ dw)				17.4
E3	Water ($\mu g l^{-1}$)			2.73	2.4
	Bottom sediment ($\mu g\ g^{-1}$ dw)			209	11.7
E4	Water ($\mu g l^{-1}$)			4.02	2.9
	Bottom sediment ($\mu g\ g^{-1}$ dw)			142	25.2

axis II, 15.38%, eigenvalue: 0.008 species-environment correlation: 0.92). The highest depth in the main channel and the greater content of bottom organic matter in the wetlands floodplain, associated to a sandy sediment in the river and a clay-silty sediment in the floodplain, have been the variables that mainly explained the differences. In the wetlands, the sampling sites from the reference zone are separated from those of the disturbed zone because of the higher values of BOD_5 and organic matter content of sediments. Most representative taxa have been Ostracoda and *Polypedilum* sp in the main channel, *Limnodrilus hoffmeisteri*, *Cordylophora caspia* and nematods Mermithidae and Ceratopogonidae in the reference zone of the floodplain and *Dero multibranchiata, D. sawayai, D. obtusa, Slavina isochaeta* and *Chironomus* gr. *decorus* in the impacted area (Fig. 4).

In the results obtained in this study, the most species were not very abundant and only a very few species reached a higher density in the wetlands of the disturbed zone, as can be expected in the early successional stages in which *r*-strategists dominate, or in a community of a severe environment where one or a few factors determine the success of the species. Pollution-tolerant species generally include organisms with a high intrinsic growth rate, a rapid colonization and morphological and physiological adaptations that allow them to resist the exposure to toxics or the habitat alteration. Moreover, species that initially colonize disturbed habitats are characterized by a small body size and short life cycles, as the species of Oligochaetes Naidinae that have dominated in the impacted area of the northern Salado River. In general, communities from environments affected by anthropogenic disturbances would be initially dominated by small species with a short life cycle and a high reproductive rate and its recovery would be greatly influenced by the dispersion ability of species. This could be observed after the flooding impact, where this natural disturbance, when decreasing the contaminant effect, had been colonized by Ephemeroptera (*Campsurus* cfr. *notatus*) and, in a lower density, by Trichoptera (*Smicridea* sp).

Although there is not much consensus among the ecologists about which are the most appropriate key characteristics of the community as indicators of biological integrity, Karr and Dudley (1981), suggested species diversity and composition as endpoints. However, in this study, the greatest difference between the reference area and the disturbed area has been marked by invertebrate density, being higher in this last one.

We conclude that the wetlands floodplain acting as a contaminant accumulation area and the sediments shows better the differences between the reference and disturbed zone.

The extreme flooding in the Salado River produced a rejuvenation of the area with the consequent physical re-structuration produced by flooding, which produced a re-mobilization of sediments showing a marked decrease in chromium levels in sediments and in organic matter content, which allowed the colonization of insects (Ephemeroptera and Trichoptera), considered as indicators of a better water quality (Rosenberg and Resh, 1993).

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