

Environmental toxicity assessment in the Paraná river delta (Argentina): simultaneous evaluation of selected pollutants and mortality rates of *Corbicula fluminea* (Bivalvia) early juveniles

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Received 24 November 1999; accepted 8 May 2000

“Capsule”: Mortality rates of juveniles of *Corbicula fluminea* toxicity corresponded well with sediment pollutant levels in a river delta of Argentina.

Abstract

Water and sediment samples were collected in the lower Paraná delta at four sites with different levels of exposure to pollution to evaluate the anthropogenic impact through chemical analyses and mortality bioassays. Individual polychlorinated biphenyls, chlorinated pesticides, aliphatic hydrocarbons, polycyclic aromatic hydrocarbons, and heavy metals were measured in waters, porewaters and sediments. The same three phases were also subjected to toxicity assays with straight-hinged juveniles of *Corbicula fluminea*. Concentrations of several pollutants were above levels recommended for the protection of aquatic life: in waters, Zn, Cu and Cr were 1.6–4.9 times higher, whereas in the sediments Cr was 1.8–3.6, and benzo(a)pyrene was 2.8–5.6 times higher. Pollutant concentrations followed a clear geographic pattern with highest values in the densely populated area of the Reconquista and Luján rivers, lower levels in the San Antonio, and lowest loadings in the remote Paraná de las Palmas. This gradient was adequately matched by the pattern of mortality rates of *C. fluminea* early juveniles, which were highest in the Reconquista-Luján (40–93%) and lowest (and not significantly different from the control) in the Paraná (3.3–23%). Mortality rates also increased from surface waters (3.3–53%), to porewaters (12–73%), to sediments (23–93%). Although toxicity was probably mainly due to dissolved contaminants, agreement between chemical and biological evidence of pollution was best for the sediment compartment, whereas porewater and surface water showed a higher degree of variability. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Corbicula fluminea*; Toxicity; Contaminants; Paraná river; Molluscs

1. Introduction

Assessment and control of anthropogenic contaminants in freshwater systems is one of the most pressing problems of sustained development. Industrialized nations have long identified major environmental industry-related hazards and have successfully implemented monitoring programs to minimize the risks involved. The fact that Latin American countries lack such control measures or they are lagging behind, is at least partly due to the general paucity of information. In

Argentina, awareness of environmental concerns is increasing fast. In recent years, a growing number of surveys stressed the critical situation of some aquatic environments, including the main waterways of the huge Paraná-Uruguay drainage system. These investigations provided information on the levels, accumulation and distribution of hydrocarbons, heavy metals, and chlorinated compounds in water, sediment and aquatic organisms (Colombo et al., 1989, 1990, 1995, 1997; Topalian et al., 1990; Janiot et al., 1994; Bilos et al., 1998; Villar et al., 1998). However, while very useful as baseline studies, these works do not specifically address the ultimate concern: pollution impacts on the biota. This is particularly relevant in the case of large

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drainage basins, where the multiplicity of waste sources and potentially hazardous substances makes environmental assessments especially complex.

Among the many freshwater organisms that have been used for bioassay purposes, adults and early juveniles of *Corbicula fluminea* have received particular attention. This mollusc, first introduced in Argentina around 1970 (Ituarte, 1994), is widely distributed in the Paraná river delta where it reaches densities up to over 5000 individuals (ind.) m^{-2} (Boltovskoy et al., 1995). In the area of this study, *C. fluminea* has been validated as a useful sentinel organism which effectively concentrates polychlorinated biphenyls (PCBs), chlorinated pesticides (HLPs), dioxins, furans, and trace metals (Colombo et al., 1995, 1997; Bilos et al., 1998). Its very high filtration rates, ease of collection and maintenance in the laboratory, and its environmental sensitivity make it particularly useful for biomonitoring purposes (Clarke et al., 1977; Foster, 1981; Foe and Knight, 1986; Doherty, 1990).

The objective of this work is to evaluate the effects of pollution in the Paraná River delta by means of chemical analyses of some priority pollutants (heavy metals, hydrocarbons and chlorinated compounds) in waters, porewaters and sediments, and mortality rates of juveniles of *C. fluminea* exposed to the same three phases. Results are discussed in terms of the most suitable approach to evaluate the environmental impact in aquatic environments.

2. Materials and methods

2.1. Study area

The Paraná River has the second largest drainage basin in South America ($2.6 \times 10^6 \text{ km}^2$), stretching from approximately 15° S to its mouth into the Río de La Plata estuary at 34° S , where it discharges over 500 km^3 of water per year. Downstream from its confluence with the Paraguay River at approximately 27° S , the Paraná develops a large floodplain which ends in a rapidly prograding delta with a total surface of ca. $15\,000 \text{ km}^2$ (Fig. 1). At the tail of the delta the Paraná branches into several major streams and hundreds of minor waterways; the Paraná de las Palmas is the largest of these major outlets (Fig. 1). The lower stretch of the Paraná river is densely populated, with several major towns (Rosario, San Nicolás, San Pedro, Zárate, Campana) hosting tanneries, petrochemical, medicinal, metallurgic and other plants. Industrial effluents, as well as domiciliary wastes, are flushed into the Paraná with little or no previous treatment. The Luján River, a tributary of the Río de la Plata laterally connected to the Paraná, receives high loads of industrial effluents (mainly from tanneries and meat and wood-pulp processing plants),

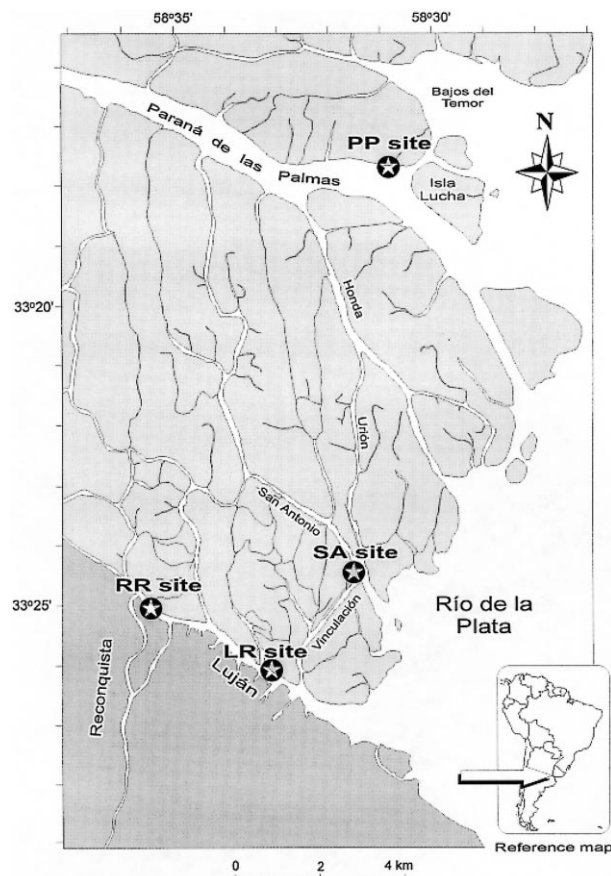


Fig. 1. Map of the area surveyed and location of sampling sites (RR, confluence of the Reconquista and Luján rivers; LR, Luján River, in the vicinity of its confluence with the Vinculación River; SA, San Antonio River, 500 m from its confluence with the Vinculación River; PP, Paraná de las Palmas River, close to its discharge into the Río de la Plata). Dark grey shading indicates densely populated area.

as well as abundant domiciliary wastes. About 3 km from its outlet it receives the Reconquista River (Fig. 1), a small yet extremely polluted stream which concentrates chiefly untreated industrial and domiciliary wastes from a highly industrialized and very densely populated Buenos Aires suburb (Loez and Salibian, 1990). Although the very high discharge rates of the Paraná (around $18\,000 \text{ m}^3$ per second) are assumed to have a strong cleansing effect, studies of contaminant loadings are scarce, and assessments of the associated biological stress even fewer (Colombo et al., 1995, 1997; Boltovskoy et al., 1997).

2.2. Sample collection and initial processing

Samples were collected in December 1996 in the lower Paraná delta at four sites with different levels of exposure to industrial and domiciliary pollution sources (Fig. 1): (1) in the vicinity of the mouth of the Reconquista River (site RR); (2) Luján River, next to its confluence with the Vinculación River (site LR); (3) San Antonio River, 500 m from its confluence with the

Vinculación River (site SA); and (4) Paraná de las Palmas River, close to its discharge into the Río de la Plata Estuary (site PP). Five surface water samples were collected at each station at ~30 cm depth using 4-l glass bottles, and approximately 15 kg of surficial (top 5 cm) sediments were retrieved in five to eight Petersen dredge samples and stored in glass containers. Water and sediment samples from each site were collected within ca. 50 m of each other, and all subsequent analyses and biological tests were carried out using these pooled materials; although this methodology does not provide a measure of within-site variability, it minimizes potential biases associated with single-spot samples. All samples were immediately transported, refrigerated to the laboratory, and stored at 4°C until further processing. Suspended particulate matter (SPM) from surface waters was separated by pressure filtration using acid-extracted Whatman GF/F glass fiber filters (0.7 µm, 4.5 cm). Filtered water samples (~500 ml) were acidified (HNO₃, pH~2) and stored refrigerated (~7°C) in polyethylene bottles. Porewater (~1 l) was extracted from 14 kg of sediments from each station by repeated centrifugation in plastic 250-ml tubes at 8000 rpm (20 min). The samples thus obtained were acidified (HNO₃, pH~2) and stored refrigerated (~7°C). Sediments were homogenized and split in four fractions for the determination of water and organic matter contents (gravimetry after drying at 105 and 450°C), grain size composition (sieve and pipette methods), and for trace metal and organic contaminant analyses.

Two-hundred adult specimens of *C. fluminea* were obtained at site PP (Paraná de las Palmas) with a Petersen-type grab sampler, distributed in plastic containers with abundant water, and transported alive to the laboratory where they were dissected exposing their gill chambers. Straight-hinged juveniles were isolated with a micropipette from gravid individuals and acclimated for 24 h in moderately hard synthetic water (80–100 mg CaCO₃ l⁻¹, pH: 7.9; Weber, 1991). For further work only juveniles which subsequently showed normal displacement activity were used.

2.3. Chemical analyses

Dissolved organic carbon (DOC) was determined by wet digestion (H₂SO₄ and Cr₂O₇K₂) of filtered surface water and porewater samples (50 and 10 ml, respectively) and subsequent titration of residual dichromate (Golterman, 1969). For the assessment of trace metals acidified water samples (250 ml) were gently concentrated to 25 ml in a clean hood at ~70°C. Filters with SPM and air-dried mortar-crushed sediments (~1 g) were extracted using a mixture of concentrated HNO₃:H₂O₂:HCl (4:1:1; Kimbrough and Wakakuwa, 1992) at 90–105°C. The extracts were centrifuged (3500 rpm, 20 min), diluted to 25 ml with deionized water, and

quantified by atomic absorption spectrometry using a Perkin–Elmer 3110 spectrophotometer equipped with deuterium background correction, air-acetylene flame and Perkin–Elmer or ISTC hollow cathode lamps. Surface water and sediment samples were analyzed in duplicate. Analytic blanks were processed following the same techniques. Multi-element working standard solutions (Johnson Matthey PLC, USA) were prepared for Cd, Cu, Mn, Ni, Pb and Zn; and single metal solutions for Cr and Fe. The instrument detection limits were (in µg ml⁻¹), Cd: 0.020, Cu: 0.025, Mn: 0.035, Ni: 0.055, Pb: 0.065, Zn: 0.020, Cr: 0.035, and Fe: 0.055. Non detectable levels in the samples are indicated as < quantitation limit in the tables. The accuracy of trace metal determinations was tested analyzing certified reference sediments BCSS-1 (National Research Council, Canada). The concentrations measured were within 69–106% of the values reported.

Trace organics were solvent-extracted and subsequently quantified by high-resolution gas chromatography. Water samples (2.8 l for surface waters and 0.5 l for porewaters) were extracted by liquid–liquid partitioning with dichloromethane. Sediment samples (20 g) were extracted with dichloromethane and sonication. Organic extracts were concentrated under N₂ and subsequently purified on silica gel microcolumns eluted with petroleum ether (F₁ = aliphatic hydrocarbons and PCBs) and 1:3 dichloromethane:petroleum ether (F₂ = aromatic hydrocarbons and CHLPs). Quantification of individual components was carried out by high-resolution gas chromatography using a KONIK-3000-HRGC gas chromatograph equipped with a 0.25 mm×30 m DB-5 silica column, a split-splitless injector heated at 250°C, a flame ionization and an electron capture detector, both operated at 320°C. The column temperature was programmed from 50 (2-min hold) to 135°C (2-min hold) at 15°C per min and then to 295°C (10-min hold) at 3°C per min. Procedural blanks and authentic standards (*n*-C₁₁ to *n*-C₃₀ *n*-alkanes, polycyclic aromatic hydrocarbons [PAHs], Aroclor, and CHLPs) were run periodically. The individual response factors of PCB congeners were calculated from a 1:1:1 Aroclor 1242:1254:1260 mixture, the published complete composition of the formulations and individual congener solutions (CLB1 mixtures from the Canadian National Research Council; Colombo et al., 1995). The recovery yield of spiked water samples averaged 65% for C₁₁ to C₃₀ *n*-alkanes, and 82% for PCBs. The reproducibility of the analyses ranged from 8 to 25% (relative standard deviation).

2.4. Bioassays

Assays of toxicity of water and porewater were performed in semi-static laboratory tests under controlled conditions at 25±1°C. For each sampling site, four dilutions (25, 50, 75 and 100%) and a control (synthetic

water according to Weber, 1991) were used. Water was oxygenated for 24 h prior to exposure, and renewed at 24-h intervals. Test vessels were 20 ml tissue culture plate wells, each containing 20 ml of medium. Twenty straight-hinged juveniles 170–200 μm in length (Kraemer and Galloway, 1986) were pipetted into each well, using three replicates for each media; the hardness of test waters varied between 25 and 30 $\text{mg CaCO}_3 \text{ l}^{-1}$, and that of the porewaters 97–314 $\text{mg CaCO}_3 \text{ l}^{-1}$; pH was around 7.3–7.8 in both cases. Organisms were not fed during the experiments. All chambers were examined under the inverted microscope at 24-h intervals and percentages of dead individuals were assessed on the basis of lack of muscular and ciliar activity (Doherty and Cherry, 1988).

For the solid phase, acute lethality was evaluated following the method proposed by Phelps and Warner (1990). Three milliliters of sediment previously sieved through a 100- μm mesh stainless-steel screen and 6 ml of moderately hard synthetic water were pipetted into 10-ml wells of a tissue culture plate. Twenty juveniles were then placed in each well, using three replicates for each media. Other experimental conditions were similar to those described above. At the end of each exposure period (24, 48, 72, 96 and 144 h) the contents of each well was pipetted out and again gently sieved through a 100- μm mesh stainless-steel screen. The early juveniles retained on the screen were transferred to clean synthetic water and examined under the inverted microscope assessing the percentages of dead individuals.

In all cases duration of the experiments was 144 h (6 days); however, for comparative purposes 96 h mortalities (the standard for acute toxicity tests with aquatic biota) were used. Statistical significance of the results was assessed by means of repeated measures analysis of variance (ANOVA; Winer, 1971), using arcsinus transformation of the raw data.

3. Results

3.1. Water

Table 1 and Fig. 2A illustrate the levels of SPM, DOC, and the pollutants measured at the four sampling sites, along with the corresponding mortality rates of *C. fluminea*. DOC values were highest at RR (9.1 mg l^{-1}), decreasing gradually towards PP. The concentrations of CHLPs in water differed little between sites (3.9–5.1 ng l^{-1}); Lindane (γBHC) accounted for 45–63% of total CHLPs, reflecting the relatively high water solubility of this chemical (7 ppm). Comparable concentrations: 1–5 ng l^{-1} , and the dominance of lindane have been previously reported for Río de la Plata coastal waters (Colombo et al., 1995). Total aliphatic hydrocarbons (ALIHCS) were low at RR and PP (0.32–

3.44 $\mu\text{g l}^{-1}$), with noticeably higher values at the two intermediate sites, LR and SA (6.62–9.09 $\mu\text{g l}^{-1}$).

Zn was the most abundant metal in the dissolved phase, reaching 146 $\mu\text{g l}^{-1}$ at RR and dropping gradually to 72.7 $\mu\text{g l}^{-1}$ at PP. Cr was somewhat more abundant at RR (dissolved phase), or at RR and LR (particulated phase), than elsewhere; whereas Cu showed comparable concentrations at all sites sampled in both phases. Particulated Pb followed a spatial trend similar to that of dissolved Zn, with highest values at RR (Table 1, Fig. 2A).

In total, 300 straight-hinged juveniles were used for bioassays with surface water (Table 1, Fig. 2A). After 96 h of exposure, mortality rates in Paraná de las Palmas water were below 4% (Table 1), not differing significantly from the control ($P=0.798$). On the other hand, mortality in waters from the other three sites (RR, LR, SA) was always higher and very significantly different ($P<0.000$) from the control. Highest inferred toxicity levels were recorded in Luján-Reconquista (site RR) waters, yielding mortalities of 53% after 96 h, and 57% after 144 h (Fig. 2A). Differences in mortality rates between the two intermediate sites, LR and SA, were non significant ($P=0.416$), reaching 40–42% after 96 h.

3.2. Porewater

Data for this compartment are illustrated in Fig. 2B and listed in Table 1. The concentration of DOC in porewaters followed roughly the same trend of surficial waters, with values decreasing stepwise from RR–LR to SA and PP. As expected from the higher dissolved organic levels of porewaters (20–35 vs. 6.8–9.1 mg l^{-1} DOC in surface water) and strong hydrophobicity of the organic contaminants, the concentrations were usually higher in porewaters. For CHLPs, only site SA exhibited total values below 3 ng l^{-1} , whereas at RR, LR and PP concentrations varied between >8 and 10 ng l^{-1} . Total ALIHC concentrations ranged from 1.7 to 26.4 $\mu\text{g l}^{-1}$ in porewaters, as compared to 0.3–9.1 $\mu\text{g l}^{-1}$ in surface waters. This hydrophobic-related partition behavior was also reflected by the composition of ALIHCS and CHLPs: porewaters presented a higher contribution of more hydrophobic higher-molecular weight compounds than surface waters, i.e. ALIHCS > *n*-C₂₀, chlordane, and DDTs (Table 1). Cr was the most abundant metal, followed by Zn and Pb, with Cu and Ni exhibiting lower concentrations (Fig. 2B, Table 1). Porewater concentrations of Cr, Cu, Ni, and Pb were significantly ($P<0.000$, ANOVA) higher than those of surficial waters, whereas Zn showed the opposite trend.

Clam mortality rates in porewater from the four sites surveyed showed a trend similar to that described above for surficial water but, in agreement with the higher contaminant load in this phase (Fig. 2B), all porewater figures were higher. Mortality in the control was below 2% until the end of experiment, and mortality in Paraná

Table 1

Concentration of suspended particulate matter (SPM), dissolved organic carbon (DOC), total contaminants with major components and mortality assays in surface waters and porewaters of the Paraná Delta

	Surface water ^a				Porewater ^a				
	RR	LR	SA	PP	RR	LR	SA	PP	
SPM (mg l ⁻¹)	30.8	49.7	41.9	31.6					
DOC (mg l ⁻¹)	9.1	8.2	7.4	6.8	34.6	34.6	27.9	19.8	
<i>Chlorinated pesticides (ng l⁻¹)</i>									
γBHC	2.31	2.44	2.41	NA	3.31	3.16	2.62	5.05	
Heptachlor	1.62	0.87	1.04	NA	—	—	—	—	
<i>trans</i> -Chlordane	0.62	0.59	—	NA	5.41	2.58	—	—	
<i>cis</i> -Chlordane	0.56	—	—	NA	—	—	—	—	
DDE	—	—	0.63	NA	—	—	—	—	
DDT	—	—	—	NA	—	4.06	—	3.25	
Total	5.11	3.90	4.08	NA	8.72	9.80	2.62	8.30	
<i>Aliphatic hydrocarbons (μg l⁻¹)^b</i>									
<i>n</i> -C15	—	1.34	0.60	0.15	0.48	0.91	0.38	—	
<i>n</i> -C16	—	1.43	0.71	0.54	0.48	1.39	0.39	1.48	
NorPristane	—	0.54	0.36	0.18	—	—	—	—	
<i>n</i> -C17	—	1.07	0.71	—	—	—	0.44	—	
Pristane	—	0.38	0.19	—	1.68	4.27	—	—	
<i>n</i> -C18	—	0.67	0.45	—	—	—	—	0.87	
Phytane	—	0.22	—	—	2.31	3.85	—	0.44	
<i>n</i> -C19	—	0.21	0.21	—	—	—	0.48	0.91	
<i>n</i> -C20	—	0.22	—	—	—	0.56	—	0.45	
<i>n</i> -C24	—	0.67	1.00	1.00	—	—	—	—	
<i>n</i> -C25	—	0.38	0.77	0.77	1.32	1.26	—	—	
<i>n</i> -C26	—	—	—	—	—	1.10	—	—	
<i>n</i> -C27	—	—	—	—	2.31	3.30	—	—	
<i>n</i> -C28	—	—	—	—	3.36	3.21	—	—	
<i>n</i> -C29	—	—	—	—	4.33	4.14	—	—	
Total	0.32	9.09	6.62	3.44	16.69	26.45	1.69	4.58	
<i>Metals</i>									
<i>Dissolved (μg l⁻¹)</i>									
Cr	4.97	3.53	3.16	3.96	59.90	33.90	27.90	31.30	
Zn	146.00	130.00	90.00	72.70	49.70	29.30	12.60	17.80	
Cu	3.48	3.54	3.79	3.87	19.20	13.20	11.50	19.00	
Ni	< 5.50	< 5.50	< 5.50	< 5.50	12.80	12.10	12.40	12.10	
Pb	< 6.50	< 6.50	< 6.50	< 6.50	25.60	21.90	16.30	15.70	
Cd	< 1.40	< 1.40	< 1.40	< 1.40	< 1.94	< 1.94	< 1.94	< 1.94	
<i>Particulated (μg g⁻¹ dry wt.)</i>									
Cr	367.00	368.00	280.00	303.00					
Zn	NA	NA	NA	NA					
Cu	60.40	54.80	52.50	55.90					
Ni	< 94.2	< 58.4	< 69.2	< 91.8					
Pb	263.00	119.00	113.00	115.00					
Cd	< 22.2	< 14.0	< 17.0	< 22.0					
<i>Corbicula fluminea</i> assay (% mortality)									
	Control								
24 h	0.0	21.7	6.7	3.3	0.0	38.3	25.0	16.7	5.0
48 h	1.7	41.7	26.7	18.3	1.7	53.3	28.3	21.7	6.7
72 h	1.7	46.7	36.7	36.7	3.3	58.3	41.7	33.3	8.3
96 h	1.7	53.3	41.7	40.0	3.3	73.3	46.7	45.0	11.7
144 h	1.7	56.7	48.3	45.0	3.3	76.7	56.7	48.3	11.7

^a RR, Reconquista River; LR, Luján river; SA, San Antonio River; PP, Paraná de las Palmas.

^b *n*-C15 to *n*-C29, *n*-alkanes with that chain length; –, not detected.

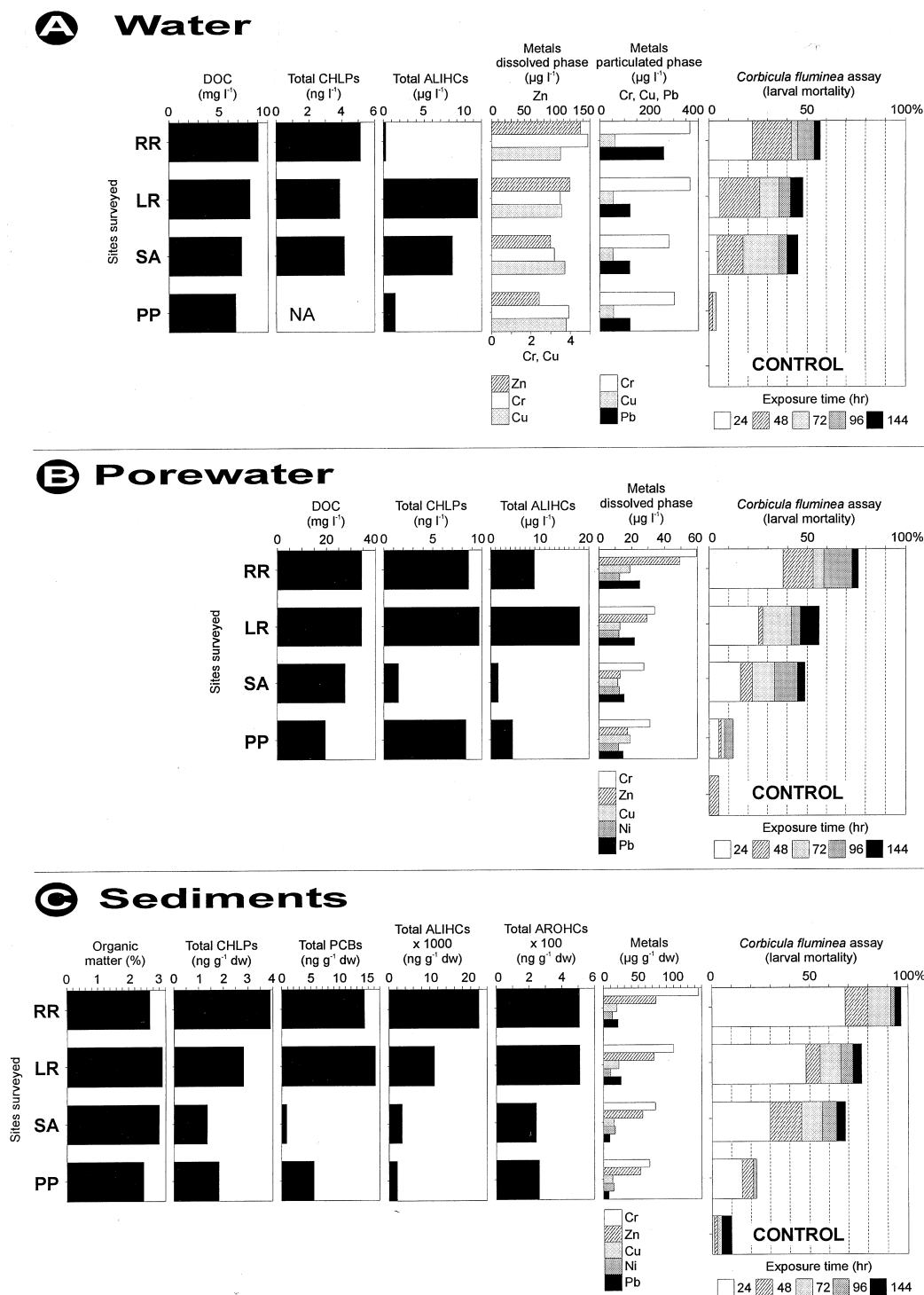


Fig. 2. Measured pollutant loadings and toxicity to *Corbicula fluminea* juveniles of surface water, porewater and sediments from four sites of the Paraná delta.

de las Palmas porewater again did not differ significantly from the blank ($P=0.068$). Porewater from site RR yielded highest mortalities, with 73% dead clams after 96 h ($P<0.000$). Differences between control and sites LR and SA were also significant ($P<0.000$), however, as opposed to results with water, site SA proved slightly less fit than LR (Fig. 2B).

3.3. Sediments

Sediments from all four sites sampled hosted high proportions of silt and clay (only at LR sand exceeded 50%, Table 2), and moderate amounts of organic matter (OM). As expected for hydrophobic substances, all sediment samples were enriched in organic contaminants

Table 2

Fines and organic mater (OM) content, concentration of total contaminants with major components, and mortality assays in sediments from the Paraná Delta^a

	RR	LR	SA	PP
Fines (%)	77.8	42.8	84.8	92.3
OM (%)	2.7	3.1	3.0	2.5
<i>Chlorinated pesticides (ng g⁻¹)</i>				
γBHC	0.10	0.20	0.43	0.44
Heptachlor	0.17	0.10	0.11	0.14
<i>trans</i> -Chlordane	1.13	0.87	0.20	0.15
<i>cis</i> -Chlordane	0.20	0.19	0.06	0.06
DDE	0.39	0.35	0.15	0.29
TDE	0.59	0.42	0.23	0.17
DDT	0.33	0.13	0.18	0.12
Total	3.90	2.82	1.35	1.83
<i>PCBs</i>				
101–90	1.33	1.48	0.21	0.92
110	0.89	1.17	0.00	0.35
138	1.57	1.87	0.24	1.02
149–123	1.60	1.79	0.00	0.41
153	2.15	2.03	0.30	1.12
170	0.53	0.67	0.00	0.00
180	0.84	0.94	0.13	0.00
Total	14.37	16.24	0.88	5.62
<i>Aliphatic hydrocarbons (ng g⁻¹)^b</i>				
<i>n</i> -C17	341.7	200.0	50.0	33.3
Pristane	393.3	219.5	27.4	–
<i>n</i> -C18	344.6	182.4	30.4	–
Phytane	375.0	171.4	42.9	–
<i>n</i> -C19	352.3	295.5	45.5	–
<i>n</i> -C25	1593.8	906.3	312.5	187.5
<i>n</i> -C26	1248.2	401.8	160.7	80.4
<i>n</i> -C27	2365.4	1384.6	576.9	288.5
<i>n</i> -C28	1687.5	500.0	187.5	156.3
<i>n</i> -C29	3369.1	2021.5	673.8	527.3
<i>n</i> -C30	1201.2	380.9	117.2	117.2
<i>n</i> -C31	2812.5	1406.3	468.8	351.6
Total	22056.0	11162.2	3200.4	2037.7
<i>PAHs (ng g⁻¹)</i>				
Fluoranthene	37.50	46.88	18.75	–
Pyrene	51.14	51.14	25.57	–
Bz(a)Fluorene	30.41	40.54	30.41	20.27
Bz(a)Anthracene	72.92	83.33	20.83	20.83
Chrysene	66.18	55.15	22.06	22.06
Bz(e)Pyrene	37.50	56.25	37.50	75.00
Bz(a)Pyrene	178.57	125.00	89.29	125.00
Total	509.90	511.85	244.40	263.16
<i>Metals (μg g⁻¹)</i>				
Cr	136.00	100.00	74.00	66.00
Zn	75.00	72.00	56.00	53.00
Cu	19.00	22.00	15.00	13.00
Ni	13.00	10.00	17.00	15.00
Pb	21.00	25.00	9.00	8.00
Total	264.00	229.00	171.00	155.00

Table 2 (continued)

	RR	LR	SA	PP
<i>Corbicula fluminea</i> assay (% mortality)				
Control				
24 h	1.7	68.3	48.3	30.0
48 h	3.3	80.0	56.7	46.7
72 h	5.0	91.7	66.7	56.7
96 h	10.0	93.3	71.7	63.3
144 h	10.0	96.7	76.7	68.3

^a RR, Reconquista River; LR: Luján river; SA, San Antonio River; PP, Paraná de las Palmas.

^b *n*-C17 to *n*-C31, *n*-alkanes with that chain length; –, not detected.

relative to water or porewater samples. Highest total values were recorded in the Reconquista and Luján rivers (sites RR and LR; Fig. 2C, Table 2). CHLPs ranged from 2.8–3.9 at RR–LR to 1.3–1.8 at SA–PP. As noticed previously for Río de la Plata sediments (Colombo et al., 1990), the abundance of TDE (dichlorodiphenyldichloroethane = DDD) and DDE (dichlorodiphenyldichloroethylene), relative to the parent compound, indicates that DDT (dichlorodiphenyltrichloroethane) is readily metabolized in the sediments. Concentrations of total PCBs varied between ca. 14–16 ng g⁻¹ dry weight at RR–LR and 1–6 ng g⁻¹ dry weight at SA and PP. These compounds were undetectable in water and porewater.

Concentrations of ALIHCs in sediments of the Paraná delta ranged between 2000 and 22 000 ng g⁻¹ dry weight with a strong proportion of *n*-alkanes with more than 20 carbon atoms (average: 85%). The geographic distribution of ALIHCs in the sediments follows the general decreasing pattern from RR to PP (11 200–22 100 μg g⁻¹ dry wt. at RR–LR to 2000–3200 ng g⁻¹ dry wt. at SA–PP). Aromatic hydrocarbons, which were undetectable in water and porewater, were particularly abundant at RR and LR sediments (over 500 ng g⁻¹ dry wt.), and about half as concentrated at the two more remote sites SA and PP (below 264 ng g⁻¹ dry wt.). Four, out of a total of six metals measured in the sediments, showed decreasing loadings from RR to PP. Ni, on the other hand, was most abundant at SA and PP, with somewhat lower values at RR and LR (Table 2). The sharp decreasing trend of sediment-associated contaminants from RR to PP reflects the major input sources: industrial plants along the upper Reconquista and Luján rivers, as well as port-related activities in the lower Luján River.

Mortality rates of *C. fluminea* exposed to sediments from the four sites compared (Fig. 2C) were higher than those with water and porewater, yet the contrasts between site PP, on one hand, and sites RR, LR and SA, on the other, generally paralleled those described above. Again, mortality rates in PP sediments did not differ significantly from the control after 96 h ($P=0.966$). Comparable figures for the other three sites

were, RR: 93%, LR: 72%, and SA: 63% ($P < 0.000$ in all cases; Table 2).

4. Discussion

In the chemical and biological assessments performed each site was represented by only one sample; therefore no data on within-site variability are available. Because this circumstance hinders comparisons between sites, subsequent discussions are chiefly aimed at exploring associations between chemically measured contamination and biologically assessed toxicity. However, albeit not conclusive, our evidences on between-site pollution gradients are supported by the very high overall coherence of the chemical and biological database presented (which in turn agrees well with the known geographic distribution of the major sources of contaminants in the area, Section 2.1), as well as with previous information on pollution levels in the lower Paraná delta (see below). In addition, the fact that each site was represented by a pool of five to eight individual samples collected within a 50-m radius (see Section 2) minimizes the risk of having retrieved 'atypical' water or sediments. These considerations do not prove that between-site differences are greater than within-site variability, but they support the assumption that the results obtained are a fair indicator of actual geographic gradients of pollution and toxicity.

Our chemical analyses complement the few previous measurements of pollution levels in the lower Paraná delta and neighboring Río de la Plata waters (Topalian et al., 1990; Verrengia Guerrero and Kesten, 1993, 1994; Colombo et al., 1995). Several of the pollutants measured are well above levels considered as hazardous for aquatic life. For example, in water, levels exceeding the guidelines of the Argentine Law of Dangerous Wastes ("*Ley de Residuos Peligrosos*") were registered for Zn (threshold value: $30 \mu\text{g l}^{-1}$, our measurements yielded concentrations 2.4–4.9 times higher), Cu (threshold value: $2 \mu\text{g l}^{-1}$, our measurements yielded concentrations 1.7–1.9 times higher) and Cr (threshold value: $2 \mu\text{g l}^{-1}$, our measurements yielded concentrations 1.6–2.5 times higher). In the sediments, some PAHs and metals exceed the risk levels defined by the Canadian Waters Quality Guidelines (Argentine regulations do not stipulate guideline values for sediments). Concentrations of benzo(a)pyrene were in excess up to 2.0 times (threshold value for freshwater sediments: $88.8 \mu\text{g g}^{-1}$ dry wt.), while the loadings of Cr (66–136 $\mu\text{g g}^{-1}$ dry wt.) exceeded 1.8–3.6 times the levels for protection of aquatic biota (37.3 $\mu\text{g g}^{-1}$ dry wt.). PCB concentrations in sediments of the Luján River (RR and LR sites, 14.37–16.24 ng g^{-1} dry wt.) were significantly higher than those recorded along the Río de la Plata coastline (0.05 to 2.42 ng g^{-1} dry wt., cf. Colombo et al., 1995). Hazardous levels of these compounds in the sediments

are still a matter of controversy; thus, while Dutch regulations set an upper limit of 24 ng g^{-1} dry weight and Canadian regulations recommend 34.1 ng g^{-1} dry weight, Battelle (1986) suggested that 6 ng g^{-1} dry weight is the maximum loading that can be tolerated by 95% of freshwater organisms, a value which is two to three times lower than those recorded at sites RR and LR (Fig. 2).

Despite some variability between substances and compartments, our results clearly indicate a general trend in pollution levels. As expected, highest loadings of pollutants occur in areas located closer to the most urbanized area (sites RR and LR), decreasing toward the more remote sites (SA and PP). This general trend in pollution levels was adequately matched by gradients in mortality rates of *C. fluminea* early juveniles. Environmental conditions at RR proved the most harmful to the organisms; sites LR and SA followed with roughly similar toxic effects, and Paraná River (PP) conditions did not differ significantly from the control. All three compartments biologically tested (water, porewater, sediments) followed the geographic trend indicated, but strongest responses were obtained with sediments (63–93% mortality, excluding site PP), in agreement with their highest pollution levels. Porewaters yielded somewhat lower mortality rates (45–73%), followed by waters (40–53%; Table 1 and Fig. 2).

These results furnish clear evidence of the deleterious biological effects of pollution in the area and support previously reported indirect evidences of pollution-related clam mortality and abnormal growth patterns. On the basis of monthly samplings at SA (November 1991 through June 1993) and PP (March and April 1995), Boltovskoy et al. (1997) observed that clam lengths at SA were monotonous throughout the 19-months period covered, over 90% of the molluscs varying between 16 and 22.5 mm in length. No discernible cohorts were recorded. At PP, on the other hand, juveniles below 5 mm were largely dominant, and shells above 25–30 mm in length were common. Cataldo and Boltovskoy (1998) collected benthic individuals and drifting planktonic juveniles of *C. fluminea* at PP at monthly intervals between October 1995 and October 1996. Whereas densities of settled clams above 1 mm varied little throughout the year (around 300–1000 ind. m^{-2}), below 1 mm benthic juveniles showed a single conspicuous abundance peak in October–November (up to 1722 ind. m^{-2}), and were practically absent during the rest of the year. Drifting juveniles showed one major peak in December 1995 (160 ind. m^{-3}), and a minor one at the end of March (24 ind. m^{-3}). Length-frequency analyses of the monthly field data clearly suggested a highly structured population with a single well-defined reproduction period centered on October–November. Thus, adverse environmental conditions at SA seem not only to affect survival of the newborns, but also to slow down clam growth. Boltovskoy et al. (1997) observed

that the first internal growth-related mark in clams from SA was closer to the umbo (mean: 14.2 mm), than that in clams from PP (mean: 24 mm), indicating considerably slower growth rates at the former. These results were subsequently confirmed with in situ growth experiments with caged individuals, which showed very significantly lower growth rates at SA than at PP (unpublished).

Although the fact that pollution is responsible for the differences in the mortality rates recorded seems beyond doubt, identification of the compounds that affect the mollusc's survival most is seriously complicated by the wealth of polluting substances associated with the multiple industrial and domiciliary sources involved. In an attempt to address this problem we compared, by means of Spearman's rank correlations, between-site gradients of pollutant and organic matter concentrations in water, porewater and sediments with the corresponding trends in *Corbicula's* mortality rates. As shown in Table 3, between-site differences in mortality rates are best paralleled by the corresponding trends of pollutant loads in the sediments: for seven of the nine pollutants compared, between-site gradients in concentration and in mortality rates were either identical (correlation coefficient = 1), or very similar (0.8). Porewater and water yielded fewer positive results (three out of seven, and two out of five, respectively). Of the substances for which data for all three compartments are available, most consistent agreements with mortality rates were shown by Zn; however, only in water did the loadings of this metal reach levels considered as harmful for freshwater organisms; in the sediments it was present at levels below 50% critical values (Table 2). Thus, although these data may suggest that Zn plays an important role in the pollution-related biological stress involved, the evidence available is inconclusive. Among the other pollutants which showed high positive correlations with mortality rates (Table 3), only PAHs in the

sediments were present at levels suggested as critical for aquatic life.

Within the constraints given by the lack of intra-site replicates (see above), it seems noteworthy that albeit most of the compounds identified are present in highest concentrations in the vicinity of the Reconquista–Luján confluence, several showed peaks at other locations, including the Paraná River. Paraná River samples (where mortality rates of early juveniles did not differ significantly from the control), yielded the highest levels for Cu in water, Bz(e)pyrene in sediments, and lindane in porewater and sediments, and were above norm for a range of others (e.g. Cr, Zn and Cu in water). Disagreements between chemical and biological evidences of the impact of pollution may respond to several factors. Biological availability of the pollutants depends not only on their concentration in the medium, but also on several other factors, including pH, redox potential, salinity, temperature, organic matter contents, sediment particle size, etc. (Long et al., 1990; Burton and Scott, 1992; Schubauer-Berigan et al., 1993). Synergic and antagonistic relationships between contaminants complicate interpretations further (e.g. Ferm and Carpenter, 1967; Marking and Mauck, 1975; Simonin and Skea, 1977; Weinstein and Anderson, 1978; Chengelis et al., 1986; Walkes et al., 1992).

Aside from pollutants, *Corbicula's* survival is known to be affected by sediment composition (grain size), OM loadings, and pH (Belanger, 1991); these clams dwell best in clean, well-oxygenated sediments with high proportions of sand. However, in our study highest proportions of sand in the sediments were recorded at LR, where overall pollution was the second highest and mortality was massive, and lowest ones at PP, with low contamination levels and no mortality (Table 2). Spatial differences in DOC (water and porewater, Table 1) and OM (sediments, Table 2) between the four sites analyzed seem too small (Table 1) to account for such dissimilar biological responses. Phelps (1993) found that mortality rates of *C. fluminea* juveniles in riverine sediments were more closely associated with the presence of unionized ammonia, than with the levels of metals, pesticides and PCBs. Similar results were also reported by Wade (1992), using the bivalve *Anodonta imbecillis* as monitor of porewater toxicity. Ammonia, however, was not measured in our samples.

The investment in time, effort, and resources needed to pinpoint exactly which pollutants are responsible for the mortalities observed, and to produce a scaling of the relative importance of the substances/combinations involved, is prohibitive for routine investigations. Thus, most surveys using simultaneous chemical and biological assessments of pollution in aquatic environments handled this question in a probabilistic manner, rather than with specific ad hoc experiments (e.g. Long et al., 1990; Wade, 1992; Phelps, 1993, 1994; Reynoldson,

Table 3

Spearman's Rank correlation indices for between-site gradients of the concentrations of selected pollutants in three of the compartments analyzed (surface water, porewater, sediments) with the corresponding trends in *Corbicula's* mortality rates^a

	Sediment	Porewater	Water
PCBs	0.600	–	–
ALIHCS	1.000	0.600	–0.200
CHLPs	0.800	0.600	0.800
PAHs	1.000	–	–
Cu	0.800	0.400	–1.000
Zn	1.000	0.800	1.000
Ni	–0.600	0.632	–
Pb	0.800	1.000	–
Cr	1.000	0.800	0.400

^a PCBs, polychlorinated biphenyls; ALIHCS, aliphatic hydrocarbons; CHLPs, chlorinated pesticides; PAHs, polycyclic aromatic hydrocarbons.

1994). Uncertainties associated with this approach curtail the value of the results because effective control of pollution requires knowledge of the toxic agents involved. On the other hand, precise identification of the effects of isolated compounds is also of limited interest since such situations seldom occur in nature. In this context, our survey suggests that mortality of juveniles of *C. fluminea* constitutes a reliable indicator of overall levels of pollution in this deltaic environment.

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