Comparative Sensitivity of *Scenedesmus Acutus* and *Chlorella Pyrenoidosa* as Sentinel Organisms for Aquatic Ecotoxicity Assessment: Studies on a Highly Polluted Urban River

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ABSTRACT: The effects of spatial and temporal differences on the water quality of the urban contaminated Reconquista River (Argentina) were evaluated by means of bioassays based on the growth of two algal populations (*Chlorella pyrenoidosa* and *Scenedesmus acutus*). The effects produced by the addition of cadmium and/or nutrient salts to the samples were also assayed. Noticeable differences in algal biomass were detected among samples from different sites, the highest values corresponding to the most polluted zones. The toxic effect of cadmium was more pronounced in *S. acutus* than in *C. pyrenoidosa*, particularly in the least polluted waters. The addition of nutrient salts to the culture medium attenuated heavy metal toxicity, mainly to *C. pyrenoidosa*, and also revealed nutrient limitation to algal growth in certain sites and dates. Site classifications based both on algal bioassays and on physical and chemical variables showed good agreement. © 2000 by John Wiley & Sons, Inc. Environ Toxicol 15: 14–22, 2000

Keywords: pollution; freshwater; bioassay; algae; *Chlorella pyrenoidosa*; *Scenedesmus acutus*; cadmium; urban river

INTRODUCTION

The water bodies of densely populated and industrialized areas usually receive high and diverse loading of contaminants. Pollution complexity makes the evaluation of its environmental impact difficult if it is exclusively based on physical and chemical analysis (Källqvist, 1984; Bervoets et al., 1996), because this approach does not provide data concerning bioavailability and interactive effects of contaminants (Munawar et al., 1989; Vymazal, 1987). This situation has lead to the development of bioassays aimed at evaluating the integrated effect of pollutants on the biota (Lewis, 1995).

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Among tested organisms, algae have several attributes that make them suitable for pollution bioassays. Bolier (1985) points out that algae are the first organisms to react to changes in nutrient loading, besides they are very sensitive to toxicants (Hörnström, 1990; Lewis, 1995) and have species-specific responses (Wang, 1987; Rachlin et al., 1982). Owing to their simplicity, availability, rapidity, and low cost, algal bioassays have gained great acceptation in water-quality monitoring (Munawar et al., 1989).

The course of Reconquista River (Province of Buenos Aires) extends approximately 55 km, flowing through one of the most densely populated regions of Argentina (ca. 3 million people and 10,000 industries). At present, the river has turned into a collector of domestic sewage and untreated industrial effluents that are disposed intermittently. Numerous toxic discharges generate a pollution gradient, that is reflected in changes of the physical and chemical parameters throughout the watercourse. Headwaters are the zones least affected by anthropogenic activities (Loez and Salibián, 1990; Topalián et al., 1990), whereas high concentrations of phosphorus and nitrogen compounds (mainly ammonia), and also heavy metals have been detected downstream. In addition, heavy metal levels are well over the guide limits for freshwater quality established for the protection of aquatic life in Argentina (García et al., 1996).

Among heavy metals, cadmium (Cd) is considered as one of the most hazardous and prevalent in freshwater environments (Wong et al., 1980; Skowronski and Czernas, 1984); hence its effects on algal populations have particular relevance in rivers receiving this kind of toxic effluents.

The present contribution aims at estimating the degree of deterioration of Reconquista River, by analyzing the effects of its waters on the growth of algal populations under laboratory conditions. The effects of water quality on Cd toxicity to algae were also evaluated. Preliminary results of these bioassays have been published elsewhere (Olguín and Salibián, 1994).

MATERIALS AND METHODS

Water samples were collected in Reconquista River in April, May, and September 1994, from three sites (Fig. 1): Cascallares (near to the headwaters, less polluted), San Martín (downstream from the outflow of Morón Stream, very polluted), and Bancalari (near to the river mouth, heavily polluted). Water samples from each site were filtered through 0.45 μm pore diameter filters and then subjected to the experimental conditions defined in Fig. 2. Culture media were enriched with Detmer salts according to the concentrations indicated in Table I (Accorinti, 1960). Cd was added as chloride in order to get a final nominal concentration of 2 ppm.

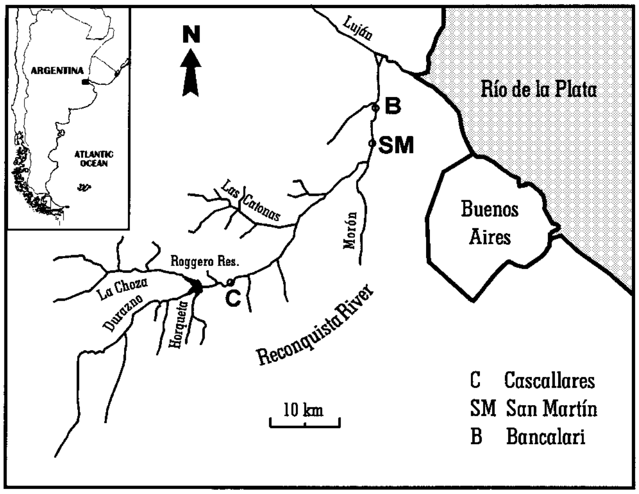

Fig. 1. Location of sampling stations.

In order to analyze possible differences among algal responses at the specific level, bioassays were performed on strains of *Chlorella pyrenoidosa* Chick and *Scenedesmus acutus* Meyen, supplied by Dr. Accorinti (University of Buenos Aires). Liquid cultures in Detmer medium (Table I) of both algal strains were initiated 7 days prior to inoculation, so that they reached the exponential growth phase at the beginning of the assays.

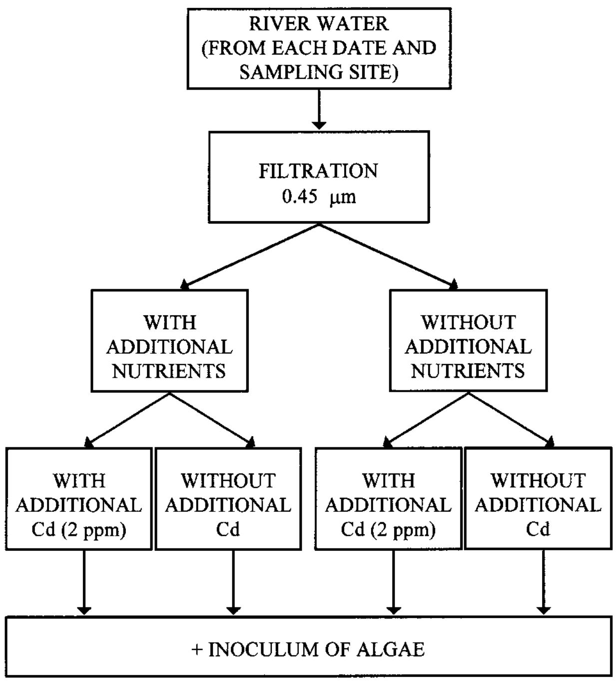

Fig. 2. Experimental design.

TABLE I. Composition of Detmer algal growth medium

Component	g/L
$Ca(NO_3)_2$	8.3×10^{-2}
KPO_4H_2	2.1×10^{-2}
$MgSO_4$	2.1×10^{-2}
KCl	2.1×10^{-2}
$FeCl_3$	4.2×10^{-4}
Tartaric acid	4.2×10^{-4}
H_3BO_3	2.4×10^{-4}
$CuCl_2$	3×10^{-6}
$MnCl_2 \cdot 4H_2O$	1.5×10^{-4}
$ZnCl_2$	1×10^{-5}

Assays were set up in 120 mL Erlenmeyers, plugged with cotton. Thirty mL of each of the assay solutions were placed in separate flasks, and then 2 mL of algal culture were inoculated, obtaining in this way the same initial optical density in each culture condition and algal population. Cultures were incubated at 23°C (±2°C) under continuous light regime (3500 lux), using day-light fluorescent lamps. Flasks were agitated daily. Control cultures in synthetic medium, with and without Cd, were run simultaneously.

The optical density of each culture, considered as an estimation of biomass concentration, was measured as the absorbance at 660 nm, after incubation periods of 96 and 168 hours. Since nonsignificant differences were detected between duplicate assays, subsequent analyses were carried out on mean values.

The characterization of the assay water was based on the following physical and chemical variables: phosphates, ammonia, dissolved oxygen, pH, biological oxygen demand (BOD_5), chemical oxygen demand (COD), chlorides, alkalinity, total hardness, conductivity, phenols, nitrites, nitrates, turbidity, cadmium, zinc, chromium, copper, and arsenic (Castañé et al., 1998).

The similarity among algal responses under 16 different treatments, considering data on three sites and three sampling dates ($n = 9$), was evaluated through the Pearson product-moment correlation coefficient. The same analysis was performed on physical and chemical variables. The similarity among water samples (sites and sampling dates) was determined through the Manhattan distance coefficient. The classification of algal responses and water samples was accomplished by cluster analysis. Dendrograms were built on distance matrices through the weighted pair-group method using arithmetic averages (WPGMA). In the case of correlation matrices the weighted pair-group method using Spearman's average (WPGMS) was applied. Physical and chemical data were standardized to reduce all the variables to a common scale prior to statistical analysis. The statistical program NTSYS was used for multivariate analysis.

RESULTS

Estimated algal biomass depended on treatment, bioassay test species, and sampling site and date. As evidenced by the high correlations among the results, similar trends were observed in the response of the different treatments to spatial and temporal variations in river water quality. Although very similar, cluster analysis showed that *C. pyrenoidosa* and *S. acutus* form separate response groups, which may reflect differences in growth between both populations. After 168 h, the growth of both assay algae tended to behave differently, as reflected by a decrease of the correlation coefficients to 0.4 (Fig. 3).

(a) Effect of treatment on algal population growth (Figs. 4–6)

Bioassay duration: After 96 and 168 h, similar trends were observed in the response of algae to the different treatments, except those with added Cd.

River water: Depending on sampling site and date, significant differences were detected in the growth of both algal populations. The highest biomass values were attained in the assays of water from San Martín and Bancalari, and from April and September samples.

River water enriched with Detmer salts: The addition of Detmer salts produced a homogeneous raise of algal biomasses in the assays of waters from all of the study sites. The increase was more pronounced in the assays of water from Cascallares in April and September, and from all of the study sites in May. These results revealed the existence

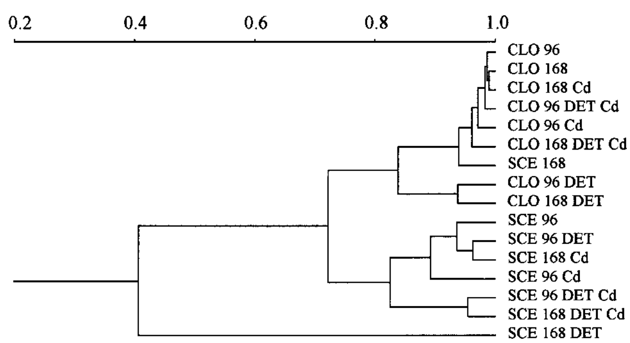

Fig. 3. Dendrogram showing correlations among algal biomasses measured under the culture conditions indicated in Fig. 2. CLO: *Chlorella pyrenoidosa*; SCE: *Scenedesmus acutus*; DET: Detmer medium; 96 and 168: assay time.

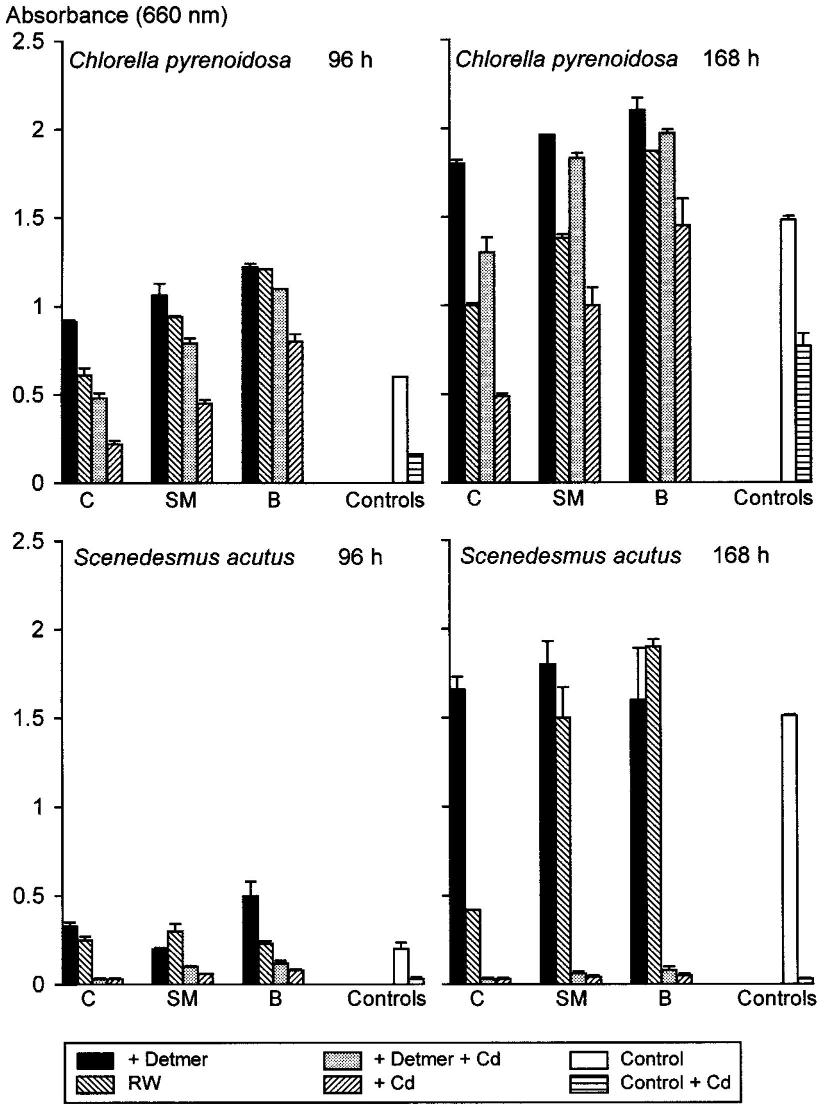

Fig. 4. Mean absorbances of cultures in samples from each site under the culture conditions indicated in Fig. 2. April bioassays. Vertical lines are 1 SD. C: Cascallares; SM: San Martín; B: Bancalari; and RW: plain river water.

of nutrient limitation in Cascallares zone and also of temporal variation in the nutrient levels in all of the study sites.

River water with added Cd: Both algal populations were sensitive to the addition of 2 ppm Cd; however, their response degree varied considerably. The effects on *C. pyrenoidosa* depended on sampling site, and to a lesser extent on sampling month. In April and September, the toxic effect was higher in the assays of water from Cascallares, where algal biomasses were on average 50% lower than in assays without added Cd. The protective effect of waters from San Martín and Bancalari was not observed in May. The effect of the toxicant was more marked after a 96 h exposure, since after 168 h algal growth was less inhibited than in assays without added metal. Contrasting with the response of *C. pyrenoidosa*, *S. acutus* growth was totally inhibited by Cd.

River water with added Detmer salts and Cd: A significant reduction of the toxic effect of Cd, in relation to assays without added Detmer salts, was observed in *C. pyrenoidosa*, mainly in the assays of waters from San Martín and Bancalari. Like in the previous treatment, the toxic effect of the metal was more pronounced after a 96 h exposure. The reduc-

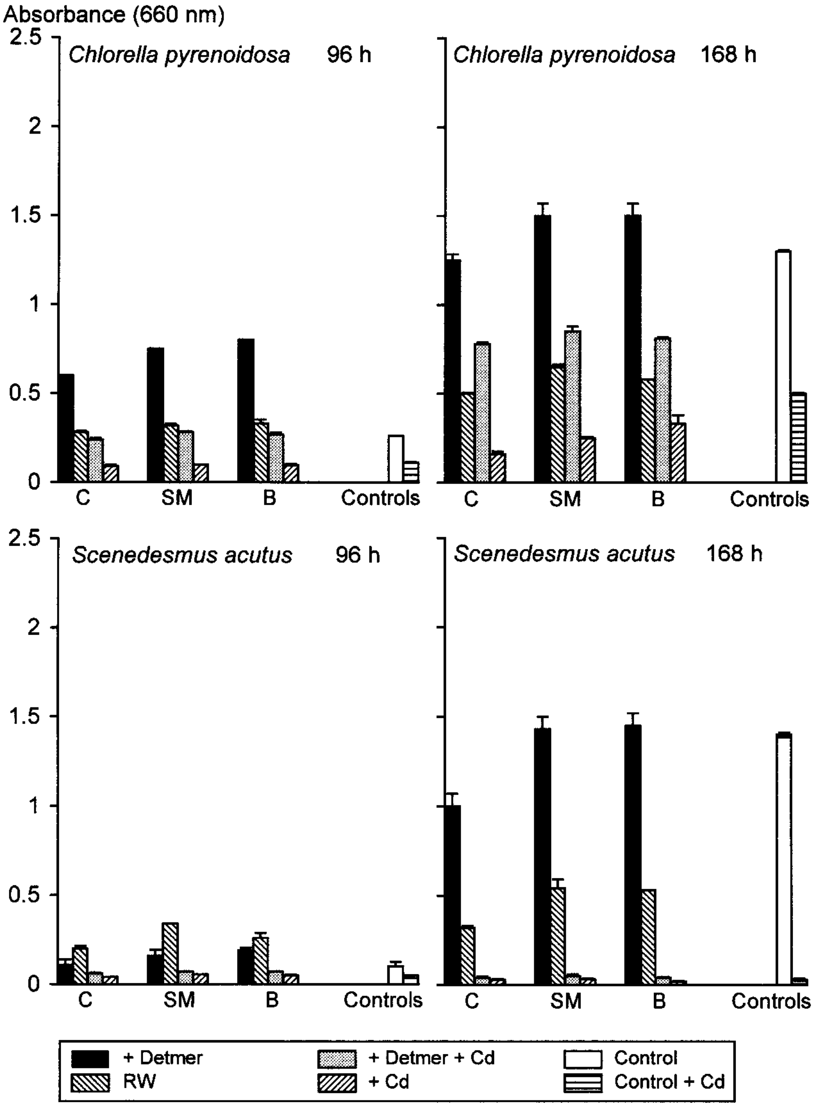

Fig. 5. Mean absorbances of cultures in samples from each site under the culture conditions indicated in Fig. 2. May bioassays.

tion of the toxic effect of Cd was less noticeable in *S. acutus*. The protective effect of water was not detected in the assays corresponding to May.

Detmer culture medium: Both algal populations exhibited similar growths in all three assays; however, some differences were found when they were compared to the growths attained in river water from different sites and dates. April and September assays produced higher algal biomasses than the assays of water from Cascallares, but they yielded lower biomasses than waters from San Martín and Bancalari. May assays yielded higher biomasses than river water without added salts.

Detmer culture medium with added Cd: Compared to synthetic medium without Cd, the addition of heavy metal completely inhibited *S. acutus* growth, as well as reducing *C. pyrenoidosa* growth about 50%. Comparing this treatment to the assays of river water with added Cd, it was evident that the effect of the toxicant was remarkably lower in the assays of waters from San Martín and Bancalari.

(b) Physical and chemical characterization of assay water

Marked spatial and temporal differences were detected in the physical and chemical variables considered (Table II). Orthophosphates, ammonia, chlorides, conductivity, total hardness, BOD_5, and alkalinity increased their values from Cascallares to Bancalari, and were all highly intercorrelated (Fig. 7). Although COD, turbidity, and phenols tended to increase in the same direction, they were poorly correlated with the above-mentioned variables (Fig. 7). Varying in the opposite direction, two groups of highly correlated variables were also detected: pH

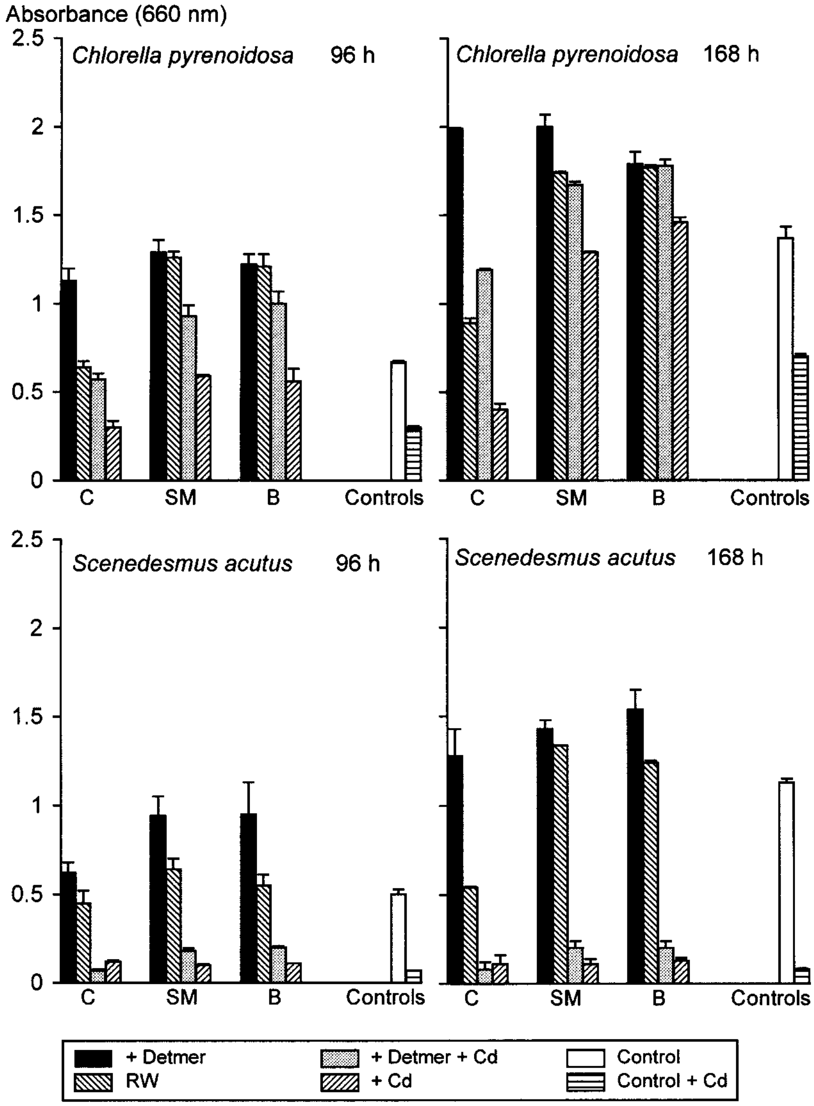

Fig. 6. Mean absorbances of cultures in samples from each site under the culture conditions indicated in Fig. 2. September bioassays.

and oxygen, and nitrite and nitrate. Variations in the levels of all of the parameters considered reflected the deterioration of water quality towards Bancalari. Variations in heavy metal concentrations were neither associated with space nor with time. This fact was interpreted as an evidence of intermittent pollution process (de la Torre et al., 1997). The highest heavy metal levels detected corresponded to chrome, zinc, and copper (Table II).

(c) Similarity among sampling sites as shown by biological, physical, and chemical information

Noticeable spatial and temporal differences in the water quality of Reconquista River were evidenced both by physical and chemical information (Fig. 8) and by algal growth data (Fig. 9). Either analytical method produced similar characterizations of river water. Gradual variations among the three sampling sites were observed in April; whereas in September, San Martín and Bancalari showed the same degree of deterioration. In May all of the study sites showed similar water qualities, denoting homogeneous conditions.

DISCUSSION

Differing degrees of increment in the growth of algal populations in response to the addition of nutrient salts to a water sample, are indicative of nutrient limitation, given the absence of substances that inhibit algal growth. The maximum concentrations of heavy metals (Cd: 10 ppb, Zn: 200 ppb, and Cr: 300 ppb), phenols, and pesticides determined in Reconquista River, were always too low to inhibit algal growth. Instead, the present investigation revealed the existence of nutrient limitation to algal growth in Cascallares during the whole period analyzed, and also in all of the study sites in May. These results are in agreement with the high correlations observed between nutrient values and algal growth, as well as with variations in the concentration of phosphorus and nitrogen compounds estimated in river water. Nutrients together with BOD_5 levels, are a good reflection of the organic loading of the river, and therefore of the bioavailability of nutrients to algae.

In relation to Cd, it was evident that (a) its toxic effect is species specific, (b) its toxicity shows marked differences depending on space and time, (c) the cul-

TABLE II. Mean values (in parentheses) and ranges of physical and chemical parameters of the assayed water samples[a]

Parameter	Cascallares	San Martín	Bancalari
Conductivity (μs/cm)	357–1037 (734.8)	609–1222 (916.3)	628–1507 (1184)
pH	7.8–9.2 (8.4)	7.7–7.9 (7.8)	7.6–7.7 (7.7)
Alkalinity (mg $CaCO_3$/L)	400–900 (806.7)	560–1140 (856.7)	560–1140 (933.3)
Dissolved oxygen (mg/L)	6.7–9.9 (8.5)	0–2.3 (0.97)	0–1 (0.33)
Hardness (mg $CaCO_3$/L)	80–130 (113.3)	120–180 (148.3)	120–180 (166.7)
BOD_5 (mg O_2/L)	2.6–15.7 (7.6)	10.9–81 (38.3)	8.4–77 (46.1)
COD (mg O_2/L)	18–64.5 (48)	73.5–245 (136.4)	64–288 (131.8)
Phenols (mg/L)	0.4–0.9 (0.6)	0.3–0.9 (0.63)	0.15–1.7 (0.98)
Orthophosphates (mg PO_4^{3-}/L)	0.3–1.8 (1.2)	2.2–6 (4.2)	2.4–7.5 (5.6)
Nitrites (mg N–NO_2^-/L)	0.03–0.21 (0.1)	0.03–0.21 (0.09)	0.04–0.5 (0.2)
Nitrates (mg N–NO_3^-/L)	0.15–2.03 (0.95)	0.64–1.85 (1.12)	0.45–1.69 (1.26)
Chlorides (mg/L)	2.05–70.5 (50.8)	48–110 (78.7)	50–168 (124.3)
Ammonia (mg N–NH_4^+/L)	0.15–1.7 (0.75)	6.5–13.7 (10.3)	6.3–17.1 (13.4)
Turbidity (UNF)	66–130 (89)	64–116.5 (87.5)	63–203 (114)
Heavy metals (ppb):			
Zn	70–200	80–110	30–100
Cd	1–6	1–2	1–10
As	nd–30	nd–20	nd–20
Cu	0.02–70	nd–15	nd–40
Cr	4–20	20–50	150–300

[a] nd: not detected. Data taken from Castañé et al., 1998.

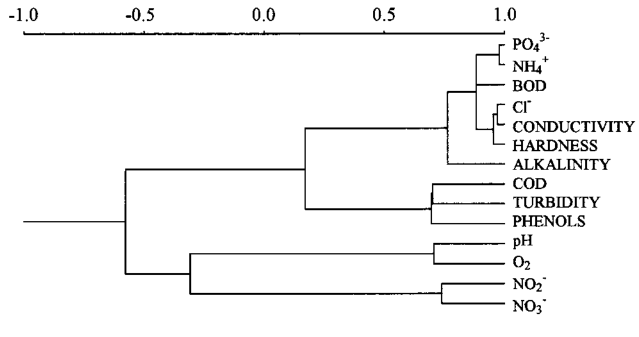

Fig. 7. Dendrogram showing correlations among the physical and chemical parameters.

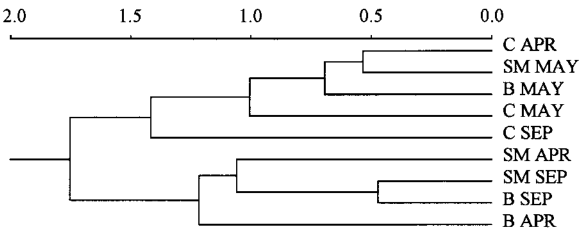

Fig. 8. Dendrogram showing distances (Manhattan index) among sites and sampling dates, based on physical and chemical parameters.

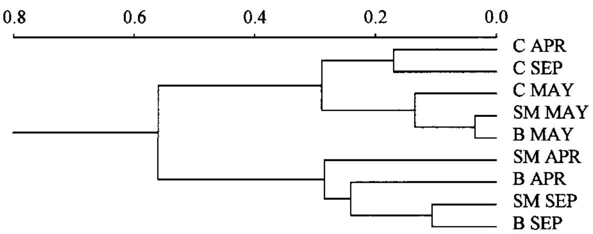

Fig. 9. Dendrogram showing distances (Manhattan index) among sites and sampling dates, based on algal biomass.

ture medium has a protective effect against its toxicity, and (d) its adverse effect is more pronounced after a 96 h exposure.

Wang (1987) and Ivorra et al. (1995) indicate that the species of a bioassay organism is an important variable to be considered when evaluating the effects of heavy metals. For example, there is a great variability in the outcomes of bioassays between *Chlorella* and *Scenedesmus* species. From the investigation by Wong et al. (1979), it can be concluded that *Chlorella* species are more sensitive to Cd than *Scenedesmus* species, and also that among the members of the genus *Chlorella*, *C. pyrenoidosa* is much more sensitive than *C. vulgaris*. Conversely, Burnison et al. (1975) inform that *Scenedesmus* species are much more sensitive to Cd than *Chlorella* species, and that as little as 0.1 mg L^{-1} Cd can have toxic effects on *C. pyrenoidosa*, whereas in *C. vulgaris* the threshold concentration is 1 mg L^{-1} Cd. On the other hand, Wong et al. (1982) point out that the primary productions of *S. quadricauda* and *C. pyrenoidosa* are equally affected by Cd.

By means of multispecific bioassays, Loez et al. (1995) who evaluated the effect of zinc on the phytoplankton assemblage of Reconquista River, detected that *C. vulgaris* was the population most resistant to the metal. It is interesting to note that in agreement with the results of our bioassays, blooms of the above-mentioned alga have been recorded in the most polluted sites of river (Loez et al., 1994).

Temporal and spatial fluctuations of the toxicity level of a heavy metal, would be related to variations in its bioavailability, probably owing to changes in the physical and chemical profile of water. It has been demonstrated that in natural waters, heavy metals exist as free metallic ions and also combined into several different chemical forms (Skowronski et al., 1992); their toxicity to a given organism depends on the chemical form available (Allen et al., 1980; Wang, 1987; Bervoets et al., 1996). Although free metallic ions are toxic to phytoplankton, some compounds are able to reduce their activity as well as their toxicity (Tubbing et al., 1994). Cd toxicity is a function of free metallic ion concentration, which in turn depends on many other factors. Inorganic ligands, such as Cl^- and to a lesser extent OH^- and PO_4^{3-}, form complex compounds with metallic ions (Bervoets et al., 1996). Kulaev (1979) demonstrated that when the external availability of PO_4^{3-} exceeds algal requirements, it accumulates inside the cells in the form of polyphosphate. The latter has functional groups with affinity for metallic cations, which are potential sites for the captation of toxic metallic ions, and therefore can reduce the bioavailability of the metal (Twiss and Nalewajko, 1992). Garnham et al. (1992) found that different *Chlorella* species possess a common system for the incorporation of zinc, manganese, and Cd. This system has higher affinity for those metals that are present in lower concentrations in the environment; on the other hand, metals also compete to join this system.

The presence of organic ligands also affects the availability of metallic ions. It has been demonstrated that natural waters contain several kinds of soluble organic compounds, partially originated by phytoplankton, that are capable of forming complex compounds with metals through a chelation reaction, which as a result reduce the bioavailability of the toxicant (Vymazal, 1987; Ogiwara and Kodaira, 1989; Verweij et al., 1992).

Wang (1987), who analyzed the effect of hardness and alkalinity on metal toxicity, postulates two possible mechanisms to explain its decrease: complexation of metallic ions with carbonates or competition between metallic ions and calcium and/or magnesium ions.

Lewis (1995) also emphasizes the protective effect of culture media on metal toxicity.

Multiple are the factors that may be acting concurrently to reduce the activity of free Cd ion in situations of complex contamination, such as Reconquista River. Among the involved factors we may mention: high phosphate concentrations, enhanced alkalinity and hardness in the most contaminated sites, competition with other metals, high organic loading, and episodes of massive blooms of *C. vulgaris* and *Microcystis aeruginosa* (Loez et al., 1994). The latter factor may increase the amount of metallic ions captured by metabolic products of algae.

The attenuation of the toxic effects of Cd after exposure times exceeding 168 h, may be related to a decrease in the bioavailability of the metal, owing to the formation of complex compounds with products of algal degradation, to changes in the pH of the test solution, and to the adsorption of the metal to algae and/or to the walls of flasks (Hörnström, 1990; Lewis, 1995).

Our results illustrate the convenience of using algal bioassays to evaluate the ecotoxicological quality of waters receiving effluents of industrial and domestic origin. In the particular case of algae as sentinel organisms, we have demonstrated their usefulness as bioindicators of the toxicity of complex mixtures integrated by substances and interacting elements. Owing to the varied susceptibility to toxicants of different algal species, multispecific bioassays are preferable to single-species tests, in order to achieve a higher degree of confidence when evaluating the impact of contaminant emissions on the biota. The correspondence between biological, physical, and chemical information confirms the usefulness of algae as reliable bioindicators of water-quality changes.

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