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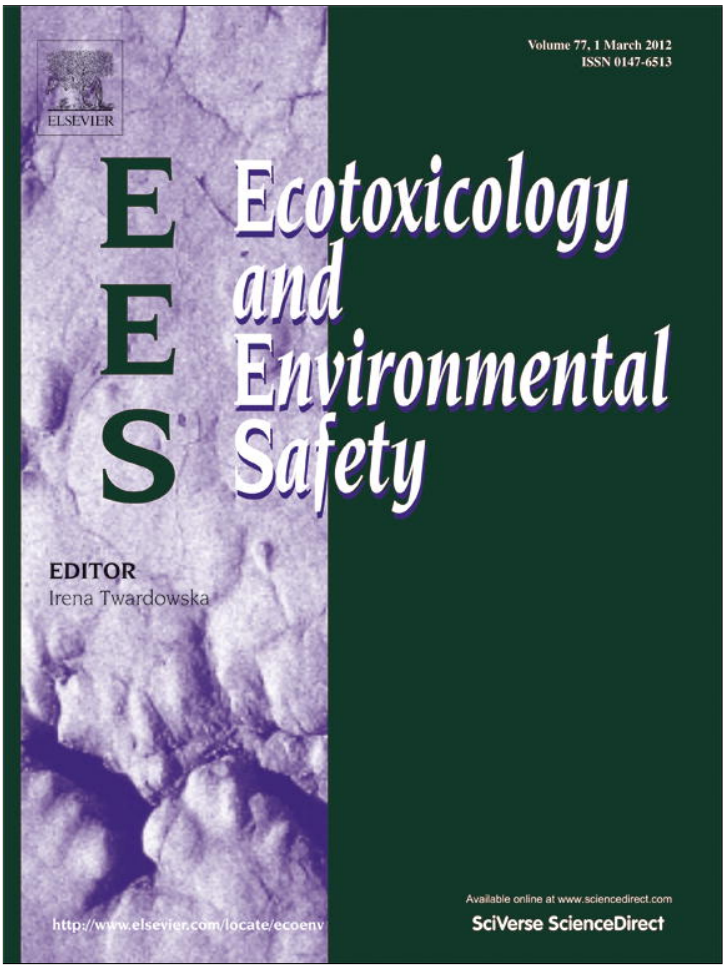


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# An *in situ* test to explore the responses of *Scenedesmus acutus* and *Lepocinclis acus* as indicators of the changes in water quality in lowland streams

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ABSTRACT

This bioassay was designed with the aim of exploring the responses of two wild planktonic microalgae exposed *in situ* (72 h) as indicators of the changes in water quality. Monocultures of both strains within dialysis membrane bags were placed at two sites in a small lowland stream. Site 1 is located at a suburban area with low horticultural activity and Site 2 is impacted by toxic industrial discharges and urban land use.

There was a decrease in population growth of both species at Site 2 compared with Site 1. The comparison of the algae exposed *in situ* with the normal specimens cultured at the laboratory indicated a significant increase in the cellular volume for both species at both sites. Abnormal shape was recorded at both sites, the percentage being significantly greater for *Scenedesmus acutus* at Site 2. Significant changes in pyrenoids size were observed in *S. acutus* and in the percentage of fragmented nuclei in *Lepocinclis acus*. Also in the latter abnormal paramylon grains were observed. These responses were accentuated at Site 2.

This bioassay was sensitive, short term, low cost, and therefore is a suitable tool to contribute with the monitoring and ecological risk assessment of lowland streams.

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## 1. Introduction

Human activities have profoundly altered the chemistry, and particularly the nutrient levels, in many of the world's surface bodies of water from both agricultural and urban sources (Paul and Meyer, 2001). Physical, chemical, and biotic conditions have changed, especially in urban landscapes (Meyer et al., 2005), where runoff from the associated surfaces along with municipal and industrial discharges result in an increased loading of nutrients, metals, pesticides, and other contaminants into streams. These inputs lead to a degradation of the fluvial systems and the aquatic biota associated with those habitats (Paul and Meyer, 2008).

Aquatic organisms respond to biotic and abiotic parameters that influence their habitat and, in doing so, provide a continuous record of environmental quality. Various elaborate systems for deducing water quality on the basis of observations of indicator organisms have been developed, improved, and diversified both in the field and in the laboratory as bioassays (Chapman, 1996). Toxicity tests traditionally use a single standard species under standardized laboratory conditions. Such a procedure, albeit useful and practical, makes the extrapolation of results from the laboratory to cumulative site-specific conditions difficult. Some authors have advocated the use of multispecies tests in mesocosms in field experiments, and other types of *in situ* assays, in order to add some greater realism to the effects of pollution upon the indicator systems (Twist et al., 1998; Graça et al., 2002; Moreira-Santos et al., 2004a, 2004b; Nayar et al., 2004).

Microalgae have ecological significance as a result of their position at the base of the aquatic food webs; an approach using algal testing is clearly justified in terms of environmental protection (McCormick and Cairns, 1994). Because of their small size, they have a high surface-to-volume ratio, and this characteristic has the advantage of allowing a rapid uptake of pollutants. These organisms also possess high reproductive rates and are easily maintained under culture conditions (Geoffroy et al., 2004; Dewez et al., 2005). Those attributes have allowed the microalgae to be used as test organisms in environmental studies in order to evaluate the toxicity of various chemicals or pollution discharges, and particularly inputs of metals (Munawar and Munawar, 1987; Geoffroy et al., 2004; Gómez de Barreda Ferraz et al., 2004; Torricelli et al., 2004; Dewez et al., 2005; Le Faucheur et al., 2006; Wei et al., 2006; Labra et al., 2007; Liebig et al., 2008; Rodríguez et al., 2008).

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Algal *in situ* assays have been less investigated than algal laboratory assays. In freshwater environments, there are some *in situ* studies using alginate immobilized algae placed in contact with the aquatic environment, including laboratory strains (Faafeng et al., 1994; Moreira-Santos et al., 2004a, 2004b; Correa et al., 2009) and local phytoplankton (Moreira-Santos et al., 2005, 2011). However, the use of monocultures of wild strains isolated from the systems under study is scarce (Twist et al., 1998) and should be further investigated. For site-specific risk assessment is relevant the use of species adapted to and tested under the specific natural conditions of the region (Janssen and Heijerick, 2003; Burton et al., 2005; Baird et al., 2007). The major difficulties using field experiments in populated areas are the damage or stolen of the bioassay units; also, the bioassay units must be adequately protected from predators.

International entities such as the International Organization of Standardization (ISO), Organization for Economic Cooperation and Development (OECD), and the United States Environmental Protection Agency (US EPA) recommend the use of chlorophytes chlorococcales because of their wide distribution and their sensitivity with respect to the environmental evaluation of freshwater ecosystems. In this study, in addition to the chlorococcal *Scenedesmus acutus*, we have explored the euglenophyte *Lepocinclis acus* as a potential indicator of environmental status. These species are of widespread distribution in lowland streams, where the low current velocity allows longer water residence times adequate for the development of phytoplankton species (Conforti et al., 1995, 2009; Bauer, 2009).

The aim of this study was to explore the potential of the responses of two wild planktonic microalgae, exposed in a polluted stream, to contribute with the monitoring and ecological risk assessment of lowland streams. Changes in population growth, modifications in cellular morphology, and alterations in subcellular structures, such as the pyrenoids, the paramylon grains, and the nucleus, upon exposure to different degrees of environmental stress, were investigated.

## 2. Materials and methods

### 2.1. Study area

We selected two sites in order to test the responses of two wild planktonic microalgae to industrial impacts, upstream, and downstream of a reach impacted by industrial discharges in the Don Carlos Stream (Fig. 1). This small lowland stream, within the vicinity of the La Plata City (Argentina), is naturally rich in nutrients, suspended solids, and humic compounds. Site 1 was situated within a suburban area with low horticultural activity, while Site 2 was located in an urban zone downstream from the input of effluents from a textile and a metallurgic plant; furthermore, at Site 2 the watercourse is modified by canalization (Table 1). No site had canopy cover. The sites were selected on the basis of an available background of biological and chemical information (Graça et al., 2002; Tolcach and Gómez, 2002; Gómez and Licursi, 2003; Gómez et al., 2008; Bauer, 2009; Sierra, 2009; Cortelezzi, 2010; Sierra and Gómez, 2010).

### 2.2. Test organisms and culture conditions at the laboratory

The planktonic microalgae *S. acutus* Meyen (Chlorophyta) and *L. acus* (O.F. Müller) B. Marin and Melkonian (Euglenophyta) were the species selected to perform the *in situ* bioassay because of their widespread distribution and documented sensitivity to contaminants in laboratory conditions (Conforti, 1998; Olguín et al., 2000; Gómez de Barreda Ferraz et al., 2004; Cetin and Mert, 2006). Both native strains were isolated from natural plankton samples: *S. acutus* from the headwaters of the Don Carlos Stream and *L. acus* from the Matanza–Riachuelo River.

In the laboratory, both species were cultured axenically in 125-mL Erlenmeyer flasks with 50 mL of culture solution (Clesceri et al., 1998), either modified Detmer (Acorinti, 1960) medium (*S. acutus*) or in soil-water supernatant (*L. acus*; Pringsheim, 1946). The cultures were incubated in an orbital shaker at 100 rpm with a continuous incident photon flux density of 100 μmol $m^{-2}$ $s^{-1}$ of cool-white fluorescent light (Twist et al., 1998) at 24 ± 1 °C and pH 7.5 (ISO, 1989).

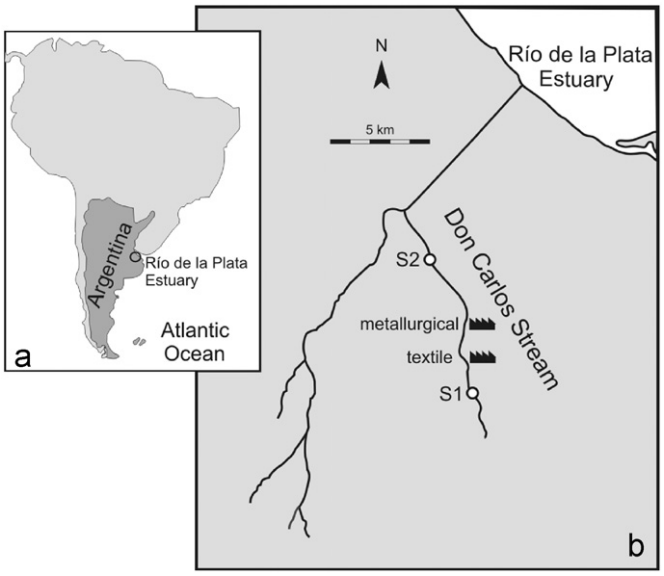


**Fig. 1.** (a) Location of the Don Carlos Stream in South America (circle), (b) detail of the stream showing the two sites where the bioassay was performed and the industries located in its vicinity.

**Table 1**
Location, morphometric, and hydraulic characteristics of the selected sites at Don Carlos Stream.

| | S1 | S2 |
|---|---|---|
| Latitude (S) | 34°55′30″ | 34°50′50″ |
| Longitude (W) | 58°01′23″ | 58°03′30″ |
| Order number | 1 | 1 |
| Depth (m) | 0.50 | 0.25 |
| Width (m) | 1.5 | 5.0 |
| Current velocity (m $s^{-1}$) | 0.11 | 0.46 |
| Natural watercourse | x | |
| Modified watercourse | | x |

### 2.3. Laboratory and field work

New cultures were started 6 day before the beginning of the experiment in order to obtain an inoculum in exponential growth for each species (Olguín et al., 2000; Rocchetta et al., 2003). Cultures for the bioassay were initiated with 1 mL of inoculum in 50 mL of nutrient solution to give a final concentration of $10^4$ cells $mL^{-1}$ for *S. acutus* (ISO, 1989) and one of $10^2$ cells $mL^{-1}$ for *L. acus*.

For the *in situ* bioassay we employed dialysis membrane bags enabling the exchange with the aquatic environment of substances of molecular weight lower than 12/14 kDa. Each of the bags was filled with 50 mL of one of the algal cultures in the laboratory before transporting to the stream in plastic containers filled with the culture medium. There, each bag was placed inside a plastic hair curler covered with a mesh, to protect them from macroinvertebrate and fish predators (Fig. 2). The units were submerged to a depth of 10 cm and weighted down to prevent displacement by the current (Fig. 3). Simultaneously, cultures of the two algal strains were incubated under standardized laboratory conditions as described above. The bioassay lasted 72 h and was performed in quadruplicate (ISO, 1989). At the end of the assay the bags were transported to the laboratory in plastic containers filled with water from each site. At the arrival to the laboratory the content of the bags and that of the Erlenmeyers maintained there was fixed with Lugol's solution for posterior observation.

Width, depth, and current velocity were recorded at each site; these parameters remained constant throughout the experiment since no rainfall occurred (Table 1). Temperature and pH (Hanna HI 8424), turbidity (ESD-800), conductivity (Lutron CD-4303), and dissolved-oxygen concentration (ESD-600) were recorded at the field at the beginning of the assay and in the successive three days. Water samples to be analyzed for the dissolved inorganic nutrients were also collected daily and were filtered immediately through glass fiber filters (Whatman GF/C) and, together with samples for biochemical oxygen demand ($BOD_5$) and chemical oxygen demand (COD), were stored at 4 °C until arrival at the laboratory. Soluble reactive phosphorus, nitrite, and ammoniacal nitrogen were determined colorimetrically; nitrate was reduced to nitrite before colorimetric measurement (Mackereth et al., 1978). $BOD_5$ was determined after 5 day incubation at 20 °C

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**Fig. 2.** Dialysis membrane bag containing the culture.

**Fig. 3.** Dialysis membrane bag placed in the stream.

and COD by oxidation with potassium dichromate after acidification (Clesceri et al., 1998). At the beginning of the assay and in the successive three days water samples from the two sites were also removed for determination of metals, these samples were taken and stored according to Clesceri et al. (1998). The concentrations of Cd, Cr, Cu, Zn, and Pb, both ionized and nonionized, in the water were analyzed in a combined sample from the four days (including Day 0). The determination of the metals was performed in accordance with the US Environmental Protection Agency (1986): Cd (3050A M7130-EAA), Cr (SW 846 M7190), Cu (SW 846 M7210), Zn (SW 846 M7950), and Pb (SW 846 M7420).

Counts were done with an Olympus CK2 inverted microscope at a magnification of 400x using 5 mL Utermöhl sedimentation chambers. Sufficient cells were counted to give an error lower than 10% (Lund et al., 1958).

To determine the morphological variables, 25 cells of each replicate were observed under an Olympus BX50 light microscope with phase contrast and Normarski DIC optics at 1,500x and registered their lengths and widths. From these measurements, the length-to-width ratio and the cell volume were calculated. For *S. acutus*, the diameter of the pyrenoids was measured and the number of normal and deformed cells was recorded, for the latter of which observations at least 250 cells of each replicate were registered. For *L. acus* the number of paramylon grains was recorded and scrutinized the morphology of the nuclei in 25 cells of each replicate.

### 2.4. Data analysis

The bioassay was performed in quadruplicate. We used the one-way Analysis of Variance (ANOVA) to determine statistically significant differences among the morphometric variables of the cultures in the laboratory and in the two sampling sites of the stream. The multiple comparisons *a posteriori* were performed by the Student–Newman–Keuls Method. Statistical significance was established at a level of $p < 0.05$ (Zar, 1996).

## 3. Results

### 3.1. Water quality

The temperature, the pH, and the concentration of $N\text{–}NO_2^-$ were similar at the two sampling sites. In contrast, the turbidity, the concentrations of dissolved oxygen and $PO_4^{3-}$, and the COD and $BOD_5$ were lower at Site 2, whereas the conductivity and the concentrations of $N\text{–}NH_4^+$ and $N\text{–}NO_3^-$ were higher (Table 2). The hardness of the water varied between 80 and 110 $mgL^{-1}$ of $CaCO_3$ at both sites.

The concentrations of Cd, Cr, and Cu at Site 1 were 2.5, 4.5, and 14.5 times higher, respectively, than the maximum levels prescribed for the protection of aquatic life by the Argentine regulations (Decreto reglamentario de la Ley Nacional 24051 de Residuos Peligrosos, 1993), while at Site 2 the concentrations of Cd, Cr, Cu, Zn, and Pb were 2.5, 3.5, 19.5, 2.5, and 215 times higher, respectively, than their corresponding prescribed levels (Table 3).

### 3.2. In situ test with S. acutus

After a 72 h exposure of the bioassay cultures to the ambient conditions at both sites of the river, the cell density of *S. acutus* increased above its initial concentration only at Site 1, and less than 2-fold (Fig. 4). The cell density in the laboratory controls increased 16-fold after 72 h, in accordance with ISO validity criteria (ISO, 1989).

At the end of the bioassay, all of the variables analyzed were sigificantly different ($p < 0.001$) among the cultures in the

**Table 2**
Mean values ( ± standard deviation) of physical and chemical variables during the course of the bioassay in the two sites at Don Carlos stream ($n=4$).

| | S1 | S2 |
|---|---|---|
| Temperature (°C) | 23.1 ( ± 0.6) | 23.1 ( ± 1.2) |
| pH | 8.0 ( ± 0.1) | 7.9 ( ± 0.1) |
| Turbidity (NTU) | 31 ( ± 10) | 6 ( ± 3) |
| Conductivity ($\mu S\ cm^{-1}$) | 632 ( ± 147) | 1018 ( ± 128) |
| [DO] ($mg\ L^{-1}$) | 9.3 ( ± 1.7) | 5.0 ( ± 1.7) |
| COD ($mg\ L^{-1}$) | 28 ( ± 3) | 17 ( ± 6) |
| $BOD_5$ ($mg\ L^{-1}$) | 21 ( ± 5) | 12 ( ± 5) |
| $[PO_4^{3-}\text{–P}]$ ($mg\ L^{-1}$) | 1.01 ( ± 0.10) | 0.19 ( ± 0.12) |
| $[NH_4^+\text{–N}]$ ($mg\ L^{-1}$) | 0.05 ( ± 0.04) | 0.34 ( ± 0.13) |
| $[NO_2^-\text{–N}]$ ($mg\ L^{-1}$) | 0.11 ( ± 0.07) | 0.12 ( ± 0.07) |
| $[NO_3^-\text{–N}]$ ($mg\ L^{-1}$) | 1.62 ( ± 0.58) | 2.45 ( ± 1.27) |

**Table 3**
Heavy-metal concentrations at the two sites of the stream and guidance levels for protection of freshwater aquatic life. All concentrations are expressed in $\mu g\ L^{-1}$.

| | Ionized | | Nonionized | | Totals | | Guidance levels |
|---|---|---|---|---|---|---|---|
| | S1 | S2 | S1 | S2 | S1 | S2 | Decree 831/93[a] |
| Cadmium | 1 | 1 | 1 | 1 | 2 | 2 | 0.8[b] |
| Chromium | 4 | 3 | 5 | 4 | 9 | 7 | 2 |
| Copper | 14 | 18 | 15 | 21 | 29 | 39 | 2[b] |
| Zinc | 21 | 31 | 5 | 43 | 26 | 74 | 30 |
| Lead | < 2 | 95 | < 2 | 334 | < 2 | 429 | 2[b] |

[a] Implementing Decree of National Law 24,051 of Hazardous Wastes (Decreto reglamentario de la Ley Nacional 24051 de Residuos Peligrosos, 1993).
[b] Hardness of 60–120 $mg\ L^{-1}$ $CaCO_3$.

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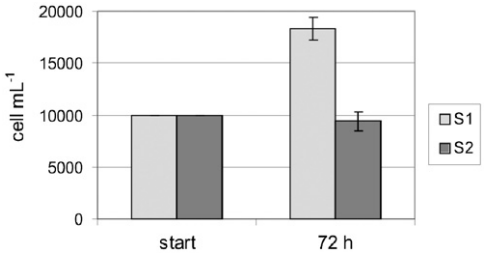


**Fig. 4.** *Scenedesmus acutus* population growth at the two sampling sites.

**Table 4**
Mean values (± standard deviations) of cellular morphometric variables and percentage of morphologically altered cells of *Scenedesmus acutus* at the end of the bioassay (morphometric variables: $n=100$, morphologically altered cells $n=1{,}000$).

| | Laboratory culture | S1 | S2 |
|---|---|---|---|
| Cellular length (μm) | 10.4 (±1.1) | 13.7 (±1.8) | 12.8 (±2.4) |
| Cellular width (μm) | 3.0 (±0.8) | 3.0 (±0.5) | 4.5 (±1.9) |
| Length/width | 3.7 (±0.7) | 4.6 (±0.8) | 3.2 (±1.2) |
| Cellular volume ($\mu m^3$) | 91 (±66) | 117 (±54) | 330 (±311) |
| Pyrenoids diameter (μm) | 1.6 (±0.3) | 2.0 (±0.2) | 2.4 (±0.6) |
| Morphologically altered cells (%) | 0 | 2.8 (±0.9) | 28.5 (±4.1) |

**Table 5**
Results of the *a-posteriori* multiple comparisons (*: $p<0.05$) of morphometric variables and morphologically altered cells of *Scenedesmus acutus* from the laboratory culture (lab) and the two sites on the Don Carlos Stream at the end of the bioassay; n.s.=not significant.

| | *A-posteriori* multiple comparisons | | |
|---|---|---|---|
| | S1 *vs.* lab | S2 *vs.* lab | S2 *vs.* S1 |
| Cellular length | * | * | * |
| Cellular width | n.s. | * | * |
| Length/width | * | * | * |
| Cellular volume | * | * | * |
| Pyrenoids diameter | * | * | * |
| Morphologically altered cells | n.s. | * | * |

laboratory and those situated at Sites 1 and 2 (Tables 4 and 5). A comparison of the algae from the *in situ* bioassay with the specimens in the laboratory, whose data were considered as representative of normal for each species, indicated that the length and volume of the cells along with the diameter of the pyrenoids were significantly greater in the algae incubated at both Sites 1 and 2. In addition, the length-to-width ratio was significantly greater at Site 1, while the cellular width was significantly greater at Site 2.

A comparison of the morphometric features between the algae exposed at the two field sites showed that the cell length and the length-to-width ratio were significantly greater at Site 1, whereas the cell width, the diameter of the pyrenoids, and the cellular volume were significantly greater at Site 2.

At both sites we observed globose cells, which morphology differs from the characteristic fusiform shape of the species (Fig. 5), and the percentage of the abnormal shapes was significantly greater in the algae at Site 2. Moreover, exudates were present surrounding the cells and were particularly abundant at Site 2.

### 3.3. *In situ test with L. acus*

At the end of the 72 h of exposure of the algae to the environmental conditions of the river, the cell density increased

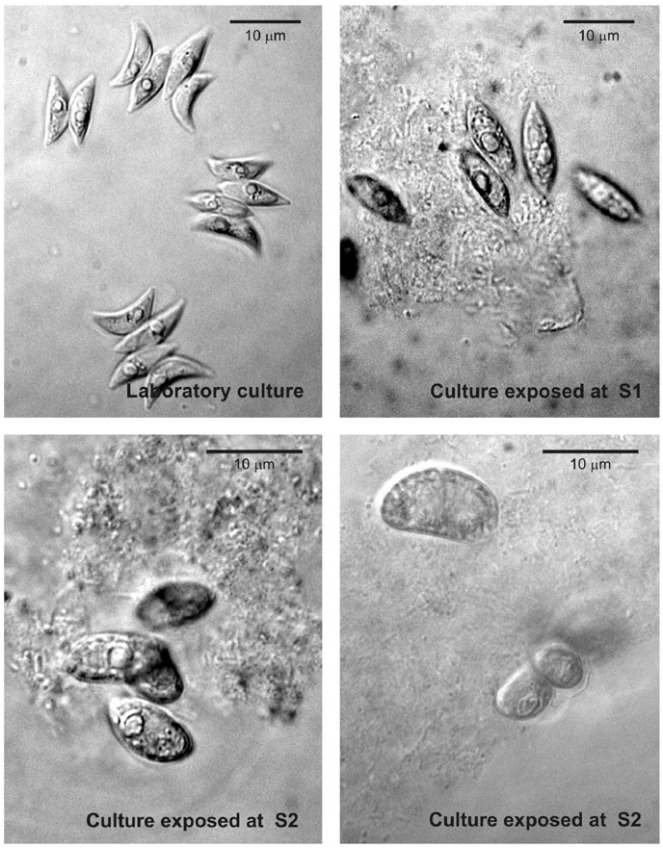


**Fig. 5.** *Scenedesmus acutus* photographs obtained by light microscopy with interferential contrast at the end of the bioassay.

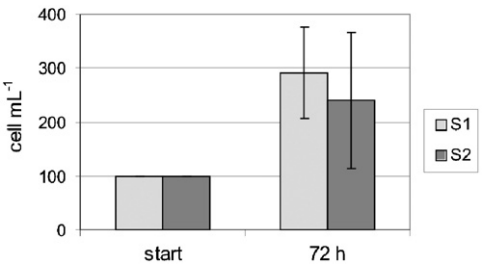


**Fig. 6.** *Lepocinclis acus* population growth at the two sampling sites.

more than 2-fold at both sites, comparing the two sites the increase was less at Site 2 (Fig. 6). The use of *L. acus* for laboratory assays is not standardized. In our assay the cell density in the laboratory controls increased 2-fold after 72 h, with a specific growth rate of 0.26 μ.

The variables analyzed differed significantly ($p<0.001$) at both Sites 1 and 2 and in the laboratory cultures (Tables 6 and 7). The increment in cell length was similar at both sites, while the increase in the cell width and volume was significantly greater at Site 1; furthermore, the length-to-width ratio was reduced at both sites, with the formation of globose cells, remaining flattened from the side view. In addition, the number of paramylon grains was slightly lower in the algae at Site 2, with the grains in those cells appearing clearly disorganized (Fig. 7). The percentage of fragmented nuclei was significantly greater in the algae exposed at both sites.

A comparison of the morphometric features between the algae exposed at the two field sites showed that the cell length was the

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**Table 6**
Mean values ( ± standard deviation) of cellular morphometric variables and percentage of nucleus alteration of *Lepocinclis acus* from the laboratory culture and the two sites at Don Carlos Stream at the end of the bioassay ($n=100$).

| | Laboratory culture | S1 | S2 |
|---|---|---|---|
| Cellular length (μm) | 170.5 ( ± 1.8) | 213.4 ( ± 15.7) | 214.1 ( ± 13.8) |
| Cellular width (μm) | 8.8 ( ± 0.5) | 21.7 ( ± 6.2) | 19.5 ( ± 4.3) |
| Length/width | 19.4 ( ± 1.1) | 10.7 ( ± 3.1) | 11.8 ( ± 4.9) |
| Cellular volume ($\mu m^3$) | 9450 ( ± 1177) | 75,260 ( ± 48564) | 59,237 ( ± 25,613) |
| No of paramylon grains | 7.8 ( ± 1.0) | 7.4 ( ± 1.9) | 6.7 ( ± 1.3) |
| Nucleus alteration (%) | 0 | 17.9 ( ± 1.6) | 85.8 ( ± 8.8) |

**Table 7**
Results of the *a posteriori* multiple comparisons (*: $p<0.05$) of morphometric variables and nucleus alteration of *Lepocinclis acus* from the laboratory culture (lab) and the two sites on the Don Carlos Stream at the end of the bioassay. n.s.: not significant.

| | *A posteriori* multiple comparisons | | |
|---|---|---|---|
| | S1 *vs.* lab | S2 *vs.* lab | S2 *vs.* S1 |
| Cellular length | * | * | n.s. |
| Cellular width | * | * | * |
| Length/width | * | * | * |
| Cellular volume | * | * | * |
| No of paramylon grains | n.s. | * | * |
| Nucleus alteration | * | * | * |

only variable with no significant differences among Site 1 and Site 2. Moreover, the cell width and volume and the number of paramylon grains were significantly greater in the cells exposed at Site 1, while the length-to-width ratio and percentage of fragmented nuclei was greater in the algae exposed at Site 2.

One other qualitative feature that deserves mention was a discoloration of both the chloroplasts and the stigma, particularly in the cells at Site 2.

## 4. Discussion

Algae are abundant, common, and ubiquitous; because of their trophic position at the bottom of the aquatic food webs they provide data with ecological significance and have a rapid response to ecosystem changes due to their short generation times (McCormick and Cairns, 1994). In our study we employed two phytoplanktonic algae frequent in Pampean Plain running waters. *L. acus* and *S. acutus* showed a significant increase in cell volume at both sites in the stream compared to the laboratory cultures. At these sites the content of Cd, Cr, and Cu was above the maximum levels prescribed for the protection of aquatic life by the Argentine regulations. The increase in cell volume is recognized to be a symptom of phytotoxicity (e. g. exposure to metals). According to de Filippis and Pallaghy (1994), algae continuously exposed to high concentrations of metals increase in volume because of a reduction in the export of certain products of photoassimilation into the medium. Those authors furthermore indicated that the increase in volume resulting from growth under such conditions is not accompanied by cell division, so that enlarged cells are formed. Greater cell volume and a significantly higher percentage of morphologically altered cells were recorded in this study for *S. acutus* at Site 2, where Zn and Pb were also above the prescribed levels, and the concentration of the latter was especially high.

The exposure to metals also resulted in a greater production of exudates by *S. acutus* at Site 2. This response is recognized as a detoxification mechanism since the exudates have organic ligands that bind metals and form complexes, reducing the concentration of free ionic metal, and interact with the cell surface, with a decrease in metal uptake (de Filippis and Pallaghy, 1994; Marsálek and Rojicková, 1996; Le Faucheur et al., 2006; Koukal et al., 2007).

The significant increases of cell volume and in the length/width relation of *L. acus* exposed in the stream was enhanced at Site 1, with greater organic matter content, a factor that accentuate these responses, according to the results reported by Conforti (1998) from laboratory assays. It has been also reported that chromium treated cells of *Euglena gracilis* changed their shape from elongated to rounded (Rocchetta et al., 2003, 2006).

Among the subcellular level responses, the pyrenoids diameter in *S. acutus* increased significantly at both sites in the stream, being greater at Site 2. The increase in size and quantities of starch granules inside the cells upon exposure to metals was observed in *Scenedesmus quadricauda*, *Chlamydomonas reindhardtii*, and *Chlorella pyrenoidosa* at the laboratory (Morlon et al., 2005; Liu and Xiong, 2009; Xu et al., 2011). Meanwhile, *L. acus* showed a disorganization of the paramylon grains especially at Site 2. This effect has been associated with an inhibition of the enzyme ß-1.3-glucanohydrolase, giving rise to a rupture of the paramylon (Falchuck et al., 1975). In previous studies this species evinced a marked increase in the number of paramylon grains in response to environmental deterioration (Conforti et al., 1995; Conforti, 1998). The percentage of fragmented nuclei of *L. acus* was significantly higher at Site 2, a response previously reported in *E. gracilis* upon exposure to high concentrations of chromium in laboratory assays (Rocchetta et al., 2007).

Regarding the population growth, our findings for *S. acutus* agree with the results of Moreira-Santos et al. (2011) who observed in green algae lower growth in the field than in the laboratory, both in reference and impacted sites. According to these authors this could be due to the physico-chemical field conditions especially temperature, changing light climate, and nutrient supply. With regard to the latter, in both sites at Don Carlos Stream the dissolved nutrients were above the limiting concentrations proposed for phytoplankton by Reynolds (2006), so this factor was no detrimental for the algae. Otherwise, in our study the population growth of *L. acus* was higher in the field than in the laboratory, it could be favored by the organic matter content of the field water. However, a decrease of the growth of both species was recorded at the most impacted Site 2 compared with Site 1. The decrease in growth of *L. acus* observed in our study with increasing metal concentration is coincident with the observations of Rocchetta et al. (2003, 2006) and Rocchetta and Küpper (2009) in *E. gracilis* under laboratory conditions.

The effects of environmental stress on species analyzed in the field experiment were similar to those observed by other authors in the laboratory, with the exception of the lower growth in the field of the green algae, as detailed above. The *in situ* tests offer the possibility of assessing contaminant effects under fluctuating natural environmental conditions, thus monitoring site-specific physical, chemical, and biological processes with a minimum degree of manipulation (Twist et al., 1998; Chapie and Burton, 2000; Burton et al., 2005; Crane et al., 2007).

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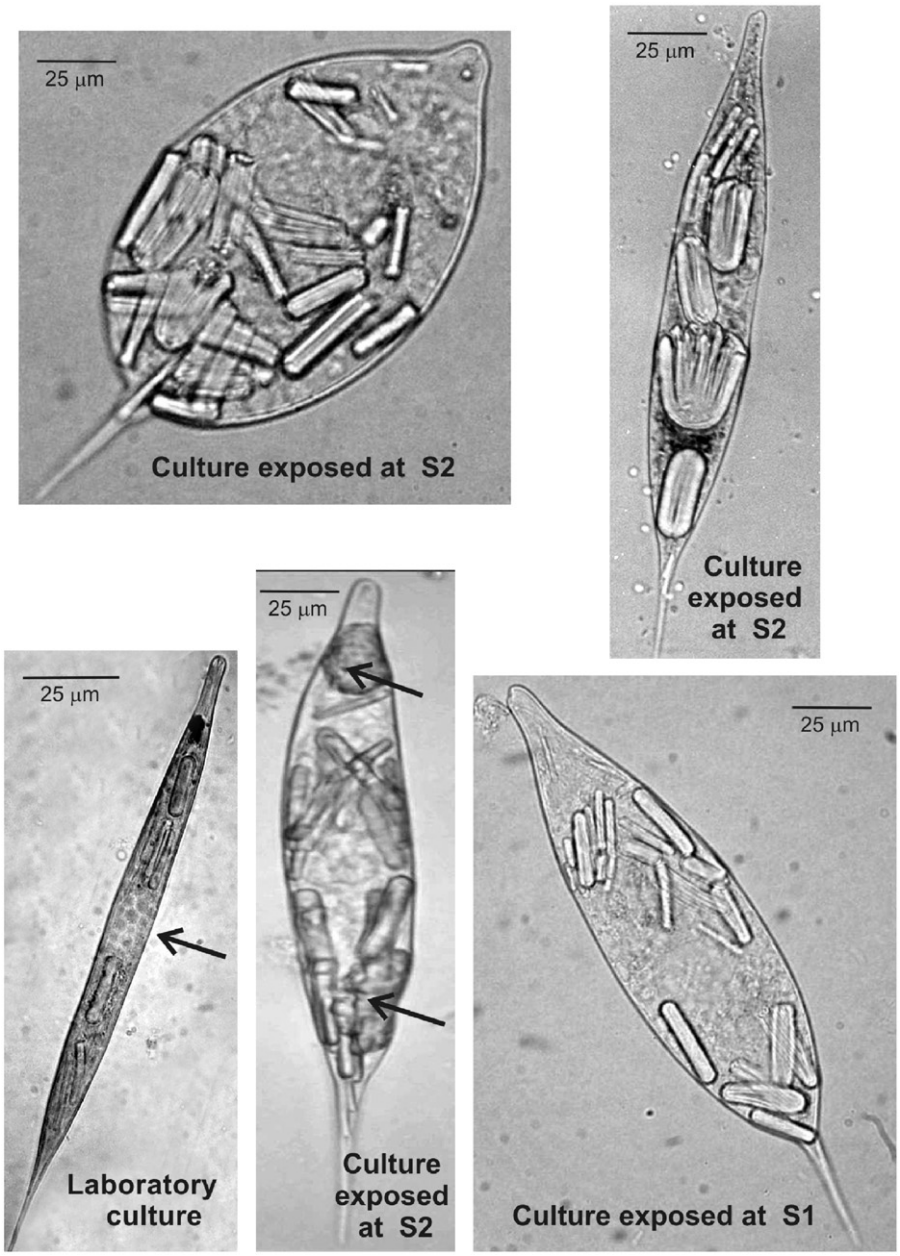


**Fig. 7.** *Lepociclis acus* photographs obtained by light microscopy at the end of the bioassay. Arrows indicate the location of the nuclei in the specimen cultured in the laboratory and the fragmented nuclei in the specimen exposed to the environmental conditions at Site 2.

The responses documented in this study for both species and at both sampling sites; show the sensitivity of this *in situ* assay. The nucleus alteration of *L. acus* and more marked responses of *S. acutus* seen at Site 2 clearly illustrate the stress-producing impact of toxic industrial discharges at this site. Moreover, other responses of *L. acus* reflect greater organic enrichment at Site 1.

These results furthermore permit the inference that both species are tolerant to stress that allow them to survive under adverse conditions, being an advantageous attribute for ecological risk assessment because it allows the demonstration of morphological alterations.

## 5. Conclusions

The responses at various levels of biological organization, population, cellular, and subcellular of *S. acutus* and *L. acus* to site-specific conditions showed that the algae were sensitive to pollution in a short time of exposure to different water quality. Furthermore, these species survive under adverse conditions favoring the expression of cellular abnormalities. The bioassay with both local wild strains exposed in the stream within the dialysis membrane bags is a suitable tool to identify polluted sites, taking into account all cumulative environmental variables, in addition, this is a relatively simple tool for rapid implementation and with low cost. This bioassay could be included in the integrative assessment of ecotoxicity of freshwater systems along with other ecotoxicological tools such as physico-chemical parameters, laboratory assays, and biological monitoring surveys.

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