Effects of hexavalent chromium on phytoplankton and bacterioplankton of the Río de la Plata estuary: an ex-situ assay

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Abstract We examined the responses of the phytoplankton and the bacterioplankton of the freshwater zone of the Río de la Plata estuary when exposed to an addition of hexavalent chromium (Cr^{+6}). The planktonic community from a coastal site was exposed to a chromium increase of $80 \mu\text{g L}^{-1}$ for 72 h in laboratory conditions. The results showed a decrease in the concentration of Cr^{+6} by 33% in the treatments, along with significant decreases in chlorophyll-a (63%), the chlorophyll-a: pheophytin-a ratio (33%), oxygen production (37%), and in the total density of the phytoplankton (15%). The relative abundance of chlorophytes and diatoms decreased, while the cyanobacteria thrived. Finally, the total bacterial density and the density of viable bacteria decreased. These results show that even small increments in Cr^{+6} can cause significant effects on the phytoplankton and bacterioplankton, which could potentially affect other trophic levels of the community, risking alterations of the entire ecosystem.

Keywords Chlorophyll-a · Pheophytin · Phytoplankton assemblage · Oxygen production · Bacterioplankton · Toxicology

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Introduction

The continuous input of heavy metals from human activities into water bodies constitutes a potential risk for natural ecosystems, due to its effects on productivity and their toxic action on aquatic organisms (Whitton 1984; Crossey and La Point 1988).

Chromium is among the heavy metals that greatly pollute the environment, originated from industries such as tanneries, the steel industry, the wood preservation industry, and even the fertilizer industry (Bagchi et al. 2002), with a recognized toxicity for microorganisms and plants (Ramírez-Díaz et al. 2008).

In polluted environments, the responses of the microbial community to the presence of heavy metals depend on their concentration and availability, the characteristics of the medium, and of the species present in it. Interestingly, nutrient availability (in particular, nitrogen) dramatically increases the ability of algae to accumulate heavy metals (De Rore et al. 1994; Wang and Dei 2001).

References of the effects of chromium on certain species of planktonic algae and bacterial strains are abundant and usually include changes in cellular density, in cell size, in reproduction (sexual-asexual), in pigment concentration, in photosynthetic capacity, and intracellular damages (Wong and Trevors 1988; Wong and Chang 1991; Kusk and Nyholm 1992; Corradi et al. 1995; Cervantes et al. 2001; Rocchetta et al. 2003; Rocchetta and Küpper 2009; Licursi and Gómez 2013). These reported responses involving ecotoxicological studies with algae are usually conducted with

monospecific cultures; while these types of assays provide useful information about the effects of toxic compounds in a cellular or intracellular level, they have a limited application when detecting the effects of environmental pollutants on communities composed of multiple species, each with a different sensitivity to the stressor. Hence, information about some structural changes at the community level can be masked, even when the toxic compound is present at a sub-lethal concentration (Cairns 1983; Margalef 1983; Loez et al. 1995).

The Río de la Plata is a wide, shallow estuary in South America, located between 34°–36° S and 55°–58° W, with a total surface of 35,500 km², composed of 37% freshwater (Fig. 1) (López Laborde and Nagy 1999). The main urban centers of Argentina and Uruguay are set along the shores of the Río de la Plata, where 12.8 million of people live in the city of Buenos Aires and its metropolitan area (INDEC 2010). The high urbanization and industrialization levels on the inner zone of the estuary generate a considerable input of pollutants to the water that poses a threat to both the biota and to human health. These contaminants enter the system through the small streams that run through or

near the city and through the poorly treated effluent sewages. Within the study area, the pollutants in the Luján and Riachuelo rivers, the Sarandí and Santo Domingo channels, and the sewage effluent in Berazategui account for over 80% of the total income of pollutants into the estuary (Fig. 1), which include chromium and other heavy metals from the industrial and urban activities. Concentrations of 80–100 µg L⁻¹ of chromium are common in water samples close to the coastal sites located near the effluents (FREPLATA 2005; INA 2011).

The toxicity of chromium varies according to its oxidation state, to temperature, and pH. In an oxygen-rich environment, hexavalent forms are favored (AGOSBA-OSN-SHN 1994). The study area shows slightly alkaline waters with dissolved oxygen (DO) concentrations close to saturation (Gómez et al. 2012), which constitutes an adequate environment for the oxidation of chromium to its hexavalent form.

In this study, we examine the responses of the planktonic community from a coastal site of the freshwater area of the estuary with chromium concentrations lower than 2 µg L⁻¹ (the guideline value for aquatic life preservation) when exposed to a chromium increase

Fig. 1 Map of the “Río de la Plata” estuary showing the sampling site and its location in reference to the main urban centers and the main effluent discharges on the coast. The shaded areas mark the total chromium concentration isolines (modified from INA 2011)

up to concentrations found in heavy polluted sites of the study area (INA 2011). For this purpose, a laboratory assay was conducted exposing the planktonic community to a concentration of $80 \mu\text{g L}^{-1}$ of Cr^{+6} . This information will contribute to understand and alert about the effects of chromium on the lower trophic levels in estuary ecosystems.

Materials and methods

For the ex-situ assay, water was collected in a coastal site with chromium concentrations lower than $2 \mu\text{g L}^{-1}$ (INA 2011) and transported to the laboratory in the dark and in coolers (4°C). Six 500-mL Erlenmeyers were filled with 400 mL of unfiltered estuarine water, and Cr^{+6} (from a potassium dichromate solution (1 mg Cr mL^{-1})) was added to three of them at $80\text{-}\mu\text{g L}^{-1}$ final concentration (*treatments*), while the other three remained unamended (*controls*).

The chromium exposure, which lasted 72 h, was conducted in an incubator at 24°C (SEMEDIC I-500PF), and light was provided by 5050 red LED lights with an intensity of $120 \mu\text{E}$ of photosynthetically active radiation with a photoperiod of 14-h light–10-h dark. The Erlenmeyers were placed on a shaker at a speed of 150 mot min^{-1} , to ensure the homogeneous mixture of the metal and a proper gas exchange.

DO, pH, conductivity, and water turbidity were measured using a Horiba-U50 multiparametric sensor. Soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN) were analyzed at the beginning and end of the assay, to ensure nutrient availability throughout the experiment. All nutrient determinations were conducted by colorimetric standard protocols (Clesceri et al. 1998). The concentration of Cr^{+6} was determined before and after the initial addition of chromium, using a Macherey-Nagel PF12 photometer. First Cr^{+6} oxidizes diphenylcarbazide to diphenylcarbazone, being itself reduced to Cr^{+3} . Combined with the enol form of the carbazone, these Cr^{+3} ions form an intensively red colored complex (Visocolor® ECO kit; detection limit of $2 \mu\text{g L}^{-1}$).

The biological variables analyzed were as follows: potential oxygen production (POP) and consumption, chlorophyll-a and pheophytin-a concentration, total density, and specific composition of the phytoplankton. All variables were measured at the beginning and end of the exposure period. Out of the 400 mL of each

experimental unit, 5 mL was used for the algal density counts, 1 mL was used for the bacterial counts, and 300 mL for the POP measurement. After the POP was measured, chlorophyll-a, pheophytin-a, and nutrient analyses were conducted with the water from the same bottles.

Chlorophyll-a and pheophytin-a were measured after Clesceri et al. (1998) and their concentrations were calculated according to Lorenzen (1967).

Phytoplankton counts were conducted using an Olympus IX50 inverted microscope at $\times 400\text{--}\times 600$ magnification, following the proposed methodology by Utermöhl (1958). Algal density was expressed as cells mL^{-1} (Lund et al. 1958; Clesceri et al. 1998), and the determination of each taxa was conducted using specific keys.

Bacterial counts of both live and dead cells were conducted using a bacterial LIVE/DEAD® BacLight™ kit, a dual staining kit that enables a two-color fluorescent staining on both live bacteria (green) and dead bacteria (red) using SYTO®9 and propidium iodide. The former is a green-fluorescent dye which stains both live and dead bacteria with intact and damaged cell membranes, while the red-fluorescing propidium iodide only stains dead bacteria with damaged cell membranes. Each sample was filtered through a black polycarbonate filter ($0.2\text{-}\mu\text{m}$ pore-size, 25-mm diameter; Gamafil). Filters were observed under at $\times 1000$ magnification in an Olympus BX50 epifluorescence microscope (excitation filter BP 460–490; emission filter BA 520IF; dichromatic filter DM 500 (Olympus U-MWB2) (Boulos et al. 1999). Twenty fields per filter were counted at random from photographs captured with an Olympus Q-Color 5 camera (Romaní and Sabater 2001).

The POP was conducted following the protocol by Knöpp (1968). 300 mL of water from each experimental unit were incubated at 24°C in sealed BOD bottles. DO was measured with an YSI-52 sensor in the bottles after they were exposed to continuous white light ($400 \mu\text{E}$) for 4 h. The potential oxygen consumption of the assemblage was measured after the bottles were kept in the dark for 4 h.

Statistical analysis

To measure the effects of the Cr^{+6} on the different biological variables, analyses of variance (ANOVA) were conducted between controls and treatments. As a measure of the effect size, η^2 , partial was calculated, and

the guide values suggested by Cohen (1988) were used to distinguish between a strong effect ($\eta^2 > 0.8$), a moderate effect ($0.8 < \eta^2 > 0.25$), and a small biological effect ($\eta^2 < 0.25$). The difference between the specific composition of the phytoplankton between the controls and treatments was analyzed using an analysis of similarities (ANOSIM) between both assemblages, followed by a similarity percentage analysis (SIMPER) to establish which species contributed most to the differences between controls and treatments (Clarke 1993; Clarke and Gorley 2001). The percentage of inhibition of algal growth (%) was also calculated (USEPA 1989).

Results

Initial conditions

At the beginning of the assay, the estuarine water had a mean pH of 8.7, a DO concentration of 9.8 mg L^{-1} , a conductivity of $337 \text{ } \mu\text{S cm}^{-1}$ (salinity 0.16 PSU), and a turbidity of 119 NTU. The mean concentrations of nutrients were 0.21 mg L^{-1} of SRP and 4.45 mg L^{-1} of DIN. The total mean density of the phytoplankton was $22,862 \text{ cells mL}^{-1}$, where the most abundant group included the cyanobacteria (67.2%), followed by chlorophytes (20.5%), and diatoms (12.1%), while euglenophytes and dinoflagellates only represented 0.2% of the total community. The initial chlorophyll-a concentration was $67.2 \text{ } \mu\text{g L}^{-1}$ and the initial pheophytin-a concentration was $25 \text{ } \mu\text{g L}^{-1}$. The total initial bacterial density was $21,000 \text{ cells mL}^{-1}$, from which 80% were viable bacteria (with an intact membrane).

Final conditions

After 72 h of exposure to chromium, the SRP concentrations in the controls were 0.24 ± 0.04 and $0.32 \pm 0.01 \text{ mg L}^{-1}$ in the treatments. Mean DIN concentration in the controls were 2.23 ± 1.2 and $3.48 \pm 0.47 \text{ mg L}^{-1}$ in the treatments. Final concentration of Cr^{+6} in the treatments was $54 \pm 12 \text{ } \mu\text{g L}^{-1}$.

Among the biological variables, chlorophyll-a, POP and consumption, and the proportion of higher taxa in the phytoplanktonic assemblage were significantly affected by the exposure to Cr^{+6} .

Chlorophyll-a values in the controls had a mean of $321.7 \pm 32.7 \text{ } \mu\text{g L}^{-1}$ and in the treatments of $119.1 \pm$

$8.7 \text{ } \mu\text{g L}^{-1}$. While the ratio of chlorophyll-a:pheophytin-a was near 3 in the controls, in the treatment bottles it was closer to a value of 2.

In the controls, the mean POP was $1.1 \pm 0.3 \text{ mg L}^{-1} \text{ h}^{-1}$ and the mean potential oxygen consumption $0.33 \pm 0.01 \text{ mg L}^{-1} \text{ h}^{-1}$. In the treatments, the mean POP was $0.7 \pm 0.3 \text{ mg L}^{-1} \text{ h}^{-1}$ and the mean potential oxygen consumption was $0.30 \pm 0.02 \text{ mg L}^{-1} \text{ h}^{-1}$ (Fig. 2). The effect size measure indicates that the treatment had a strong effect on the phytoplanktonic descriptors (Table 1).

Changes in the structure of the phytoplanktonic community included a decrease in total density and in the density of certain higher taxa, such as cyanobacteria, diatoms, and chlorophytes (Table 1).

The total density of the phytoplankton in the controls was $111,959 \text{ cells mL}^{-1}$ and in the treatments was $95,485 \text{ cells mL}^{-1}$. Regarding the proportion of higher taxa, the mean relative abundance of cyanobacteria in the controls was 79.5%, while chlorophytes represented 14% and diatoms only 5.6%. In the treatments, while the total density of each phytoplanktonic group was lower, the cyanobacteria had a relative abundance of 81.8%, chlorophytes represented 12.6%, and diatoms 5.5% of the overall composition.

The bacterial community was also significantly affected by the chromium addition. The total density decreased in the treatments ($28,700 \text{ cells mL}^{-1}$) compared to the controls ($30,400 \text{ cells mL}^{-1}$) and so did the mean density of live bacteria ($19,100 \text{ cells mL}^{-1}$ in the controls and $11,300 \text{ cells mL}^{-1}$ in the treatments), while the mean density of dead bacteria increased from $11,200 \text{ cells mL}^{-1}$ in the controls to the $17,400 \text{ cells mL}^{-1}$ in the treatments (Fig. 2) (Table 1).

There were no significant differences between the specific composition of the assemblage in the controls and treatments at the end of the experiment ($p = 0.8$) nor in the differences within-groups (ANOSIM Rglobal = -0.148).

The SIMPER results showed an overall dissimilarity between the controls and treatments of 24%, mainly expressed by the 13 species listed in Table 2. Species such as *Aphanocapsa delicatissima*, *Merismopedia punctata*, *Merismopedia tenuissima*, *Microcystis aeruginosa*, *Diplochlois decussata*, *Dictyosphaerium pulchellum*, *Actinocyclus normanii*, *Cyclotella atomus*, and *C. meneghiniana* were negatively affected by the exposure to chromium,

Fig. 2 Biological variables measured in the controls and treatments after 72 h of the addition of Cr^{+6}

decreasing their relative abundance, while species such as *Anabaenopsis circularis*, *Aphanocapsa incerta*, *Merismopedia convoluta*, and *Planktothrix agardhii* increased their relative abundance in the treatments. The difference between controls and treatments in relative abundances was significant in most cases, except species such as *A. incerta*, *M. convoluta*, *M. punctata*, *M. aeruginosa*, and *D. decussata* (Table 2).

Discussion

The results obtained in this experiment indicate that after an addition of $80 \mu\text{g L}^{-1}$ of Cr^{+6} , there were significant changes in the planktonic community, both in structural (density, proportion of higher taxonomic groups and some species, chlorophyll-a, density of bacteria, and changes in the rate of live/dead bacteria) and functional (POP and respiration) descriptors.

Table 1 ANOVA results for the biological variables between the controls and treatments and the effect size (η^2)

Variable	<i>p</i>	η^2
Chlorophyll-a ($\mu\text{g L}^{-1}$)	0.001	0.994
Chlorophyll-a:pheophytin-a	0.001	0.986
Total phytoplanktonic density (cells mL^{-1})	0.001	0.981
Cyanobacteria (cells mL^{-1})	0.001	0.968
Chlorophytes (cells mL^{-1})	0.001	0.987
Diatoms (cells mL^{-1})	0.001	0.981
Euglenophytes (cells mL^{-1})	0.178	0.578
Dinoflagellates (cells mL^{-1})	0.198	0.556
Total bacteria (cells mL^{-1})	0.002	0.960
Bacteria (live) (cells mL^{-1})	0.025	0.843
Bacteria (dead) (cells mL^{-1})	0.02	0.960
Potential oxygen production ($\text{mg L}^{-1} \text{h}^{-1}$)	0.001	0.998
Oxygen consumption ($\text{mg L}^{-1} \text{h}^{-1}$)	0.001	0.997

Metal absorption rates by the phytoplanktonic community depend on the species present in each bioassay, as well as on the concentration of nutrients and the salinity of the medium (Wong and Trevors 1988; Wang and Dei 2001). In our study with a salinity of 0.16 PSU and after 72 h of incubation, the concentration of Cr^{+6} in the treatment decreased by 33%; while at the end of the experiment, the DIN and SRP concentrations in the treatments was higher than in the controls (36% and SRP 23%, respectively). Frey et al. (1983) found that the inhibiting effect of Cr^{+6} on some of the most common phytoplanktonic species in estuaries was

greater at lower salinities (0.04 PSU), and these negative effects diminished at higher salinities until no observable effects were measured in the high salinity zone (32.5 PSU); it is therefore likely that in freshwater areas, such as the one studied here, they are more vulnerable to Cr^{+6} additions.

In our study, the treatments evidenced a decrease of 63% in chlorophyll-a, a 15% drop in total density and in some higher taxonomic groups, such as chlorophytes and diatoms, while the relative abundance of cyanobacteria increased. The POP and respiration also exhibited differences related to the chromium addition after 72 h. Also, significant detriments were measured in the total bacterial density and in the density of viable bacteria, while dead bacteria increased.

This high toxicity at a cellular level is expected from Cr^{+6} , since it quickly penetrates the cytoplasm causing toxic effects (Wong and Trevors 1988; Katz and Salem 1993). Oxidative damage in DNA is reported as the possible responsible of genotoxic effects (Kawanishi et al. 1986; Aiyar et al. 1991; Kadiiska et al. 1994), since once inside the cell the Cr^{+3} binds to the phosphates in the DNA strand (Bridgewater et al. 1994; Plaper et al. 2002). The presence of chromium decreases the growth of microbial population and affects microbial respiration as well as various other physiological processes (Mishra and Bharagava 2016). In this experiment, the increase in dead bacteria (by 55% in the treatments) within the 72 h of the exposure could be an indication that these oxidative mechanisms are taking place. However, microbes have diverse resistance

Table 2 Mean relative abundance of the species that contributed most to the differences in the assemblages of controls and treatments, their growth inhibition percentage in the treatments, and the results of the ANOVA between the relative abundance of each species

Species	Control	Treatment	% growth inhibition	<i>P</i>
<i>Merismopedia punctata</i>	2.2	1.2	−58.3	0.16
<i>Aphanocapsa delicatissima</i>	14.3	9.1	−50.4	0.03
<i>Merismopedia tenuissima</i>	39	25.3	−43.9	0.001
<i>Cyclotella atomus</i>	1.7	1.1	−43	0.001
<i>Diplochloris decussata</i>	2.4	1.5	−39.9	0.06
<i>Microcystis aeruginosa</i>	3.8	3.4	−31.9	0.25
<i>Cyclotella meneghiniana</i>	1.5	1.3	−25.9	0.01
<i>Actinocyclus normanii</i>	2.9	2.7	−14.6	0.01
<i>Dictyosphaerium pulchellum</i>	2.1	2.1	−14.4	0.002
<i>Planktothrix agardhii</i>	1.9	9.6	323.5	0.03
<i>Anabaenopsis circularis</i>	1.6	4.9	183.2	0.01
<i>Aphanocapsa incerta</i>	3.8	7.6	61.7	0.12
<i>Merismopedia convoluta</i>	9.5	15.1	50	0.09

mechanisms to cope with chromate toxicity that enable them to survive in such harsh environmental conditions (Cervantes and Campos-Gracia 2007); there are several reports of bacteria capable for the effective reduction/detoxification of hexavalent chromium from contaminated environments and hence can be used as a potential agent for the effective bioremediation of metal contaminated sites (Bharagava and Mishra 2018).

Growth inhibition and chlorosis are common symptoms of metal phytotoxicity in several algae, in which photosynthesis is probably the most affected metabolic process (Ali et al. 2006). The negative effects of chromium on chlorophyll-a and pheophytin-a are varied. For instance, in *Chlamydomonas reinhardtii* (Chlorophyceae), it was observed that 3 mg L^{-1} of Cr^{+6} was critical for algal viability, causing a complete pheophytinization of the chlorophyll content (Rodríguez et al. 2007). Also, chromium inhibited photosynthesis in species of *Chlorella* sp. (Wong and Trevors 1988) and *Scenedesmus* sp. (Corradi et al. 1995) and has been reported to affect algal growth in general. For instance, with concentrations of $50\text{--}100 \text{ } \mu\text{g L}^{-1}$ of chromium, Petria (1978) reported a total inhibition of the photosynthetic process; while with 10 mg L^{-1} of the metal, the algal growth was inhibited in species of *Chlorella*. On the other hand, Schroll (1978) reported that the effects of chromium were stronger in the growth rate of *Chlorella pyrenoidosa* than in the photosynthesis of the same species.

In our study, $80 \text{ } \mu\text{g L}^{-1}$ of Cr^{+6} caused a diminish in the proportion of chlorophyll-a:pheophytin-a (from 3:1 in the controls to 2:1 in the treatments). This result was also consistent with a decrease of 37% in oxygen production in the treatments.

The presence of large concentrations of chromium ions in the environment has an inhibiting action for most microorganisms (Zibilske and Wagner 1982; Ajmal et al. 1984), generating in some cases the selection of variants resistant to such concentrations (Summers and Jacoby 1978; Luli et al. 1983; Wong and Trevors 1988). This action causes effects not only at a physiological level but also at a community level, altering planktonic diversity. For instance, Patrick (1978) found that an addition of 0.4 mg L^{-1} of Cr^{+6} caused a change in the specific composition of the phytoplanktonic assemblage, from a community dominated by diatoms to one dominated by green and blue-green algae. In bioassay studies with water from lakes where Cr^{+6} was added at concentrations of 1 to 40 mg L^{-1} , growth was only

measured in species of *Oscillatoria*, while other algal species that were present in the controls (*Scenedesmus* sp., *Chlorella* sp., *Microcystis* sp.) were absent in the treatments (Filip et al. 1979). But studies such as the one conducted by Sudhakar et al. (1991) highlighted the fact that some species are not affected by high chromium concentrations, such as species of *Oscillatoria*, *Phormidium*, *Scenedesmus*, and *Pandorina*, that even after an exposure of $200\text{--}800 \text{ } \mu\text{g L}^{-1}$ of total chromium that originated from a paper factory were tolerant and even reported growth. It has to be mentioned that all those results are the product of assays conducted with single-species cultures; while in our study, the entire community was exposed to the Cr^{+6} addition. Our results also show that some species were negatively affected by the treatment, such as *A. delicatissima*, *M. punctata*, *M. tenuissima*, *M. aeruginosa*, *D. decussata*, *D. pulchellum*, *A. normanii*, *C. atomus*, and *C. meneghiniana*, decreasing their abundance between 14 and 58% when compared to the controls. But at the same time, some species were not negatively affected by the chromium addition, increasing their relative abundance between 50 and 320%, such as *A. circularis*, *A. incerta*, *Merismopedia convoluta*, and *P. agardhii*, all cyanobacteria.

The effects of Cr^{+6} in the Río de la Plata estuary were also studied by Licursi and Gómez (2013) on episammic biofilms, where changes in the structure of the diatom assemblage were observed after 96 h of exposure to Cr^{+6} in laboratory conditions.

While the balance between realism and replicability of bioassays conducted ex-situ and their comparison with field studies are greatly discussed in the literature (e.g., Guasch and Serra 2009), the results obtained in this study are evidence of the effects that Cr^{+6} has on the phytoplankton and bacterioplankton, which might lead to consequences in the structure and functioning of the coastal plankton of the Río de la Plata.

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