

MICRONUCLEUS AND CHROMOSOME ABERRATION FREQUENCIES IN *ALLIUM CEPA* CELLS EXPOSED TO COASTAL SEDIMENTS FROM A POLLUTED ESTUARINE SYSTEM

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ABSTRACT

Magdaleno, A., Paz, M., Fabrizio de Iorio, A., Weigandt, C. & Moretton, J. (2021). Micronucleus and chromosome aberration frequencies in *Allium Cepa* cells exposed to coastal sediments from a polluted estuarine system. *Braz. J. Aquat. Sci. Technol.* 25(1). eISSN 1983-9057. DOI: 16461/bjast.v25n1. The genotoxicity and mutagenicity of two compartments of sediment samples (pore water and whole sediment) were evaluated using the *Allium cepa* assay. The samples were collected from thirteen sites on the southern coastal strip of the Río de la Plata (Argentina). Three biological parameters were analyzed in the root cells, the mitotic index (MI), and the chromosome aberrations (CA) and micronuclei (MN) frequencies. Most of the samples were genotoxic in the pore water compartment, probably due to the mixture of bioavailable heavy metal concentrations (Cd = 0.050 to 0.950 mg/Kg, Cu = 0.125 to 7.300 mg/Kg, Cr = 0.019 to 1.850 mg/Kg, Pb = 1.075 to 15.200 mg/Kg, and Zn = 0.925 to 47.225 mg/Kg). However, half of the samples exerted genotoxic and mutagenic effects on the meristematic root cells when they were exposed to the whole sediment. This probably means that some contaminants in this compartment, such as heavy metals, would not be bioavailable in all of the samples. In conclusion, the *A. cepa* assay showed high sensitivity in detecting genotoxicity and mutagenicity in sediment samples from the southern coastal strip of the Río de la Plata.

Key Words: *Allium test*, Chromosome aberration, Micronuclei, Sediment, Pore water, Heavy metals.

INTRODUCTION

Aquatic environments in urban and industrial areas receive discharges of numerous toxic organic and inorganic pollutants daily from municipal, hospital and industrial wastewaters. These contaminants are predominantly associated with suspended particulate material, which is then subsequently incorporated into the bottom sediments (Chen & White, 2004; Wang et al., 2007; Rabodonirina et al., 2015). Thus, sediments represent a reservoir of many hazardous pollutants in aquatic ecosystems. The contaminants associated with sediments can act directly on the benthic biota and indirectly on the pelagic organisms, as they can be reintroduced into the overlying water by different mechanisms, such as resuspension and trophic transfer. Therefore, sediments can be valuable indicators for monitoring pollutants and for evaluating the level of contamination in aquatic ecosystems (Varol, 2011; Pejmana et al., 2015; Roig et al., 2015).

The Río de La Plata system is one of the most important estuarine environments in the American continent, which is located in the extreme north of the Pampas with a mouth of approximately 200 km width and an annual mean discharge of approximately 22,000 m³/s. This microtidal estuary is highly productive and sustains valuable fisheries from

Argentina and Uruguay. However, the southern coast of this estuary is heavily polluted (Kopprio et al., 2015). Colombo et al. (2005, 2006) reported high concentrations of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in many sediment samples. Moreover, many polluted rivers and streams drain into the Río de la Plata with high loads of heavy metal concentrations (Ronco et al., 2001, Magdaleno et al., 2001, 2008). These organic and inorganic contaminants are considered highly persistent and toxic or genotoxic to the biota and they tend to accumulate in sediments and tissues of various organisms (Bryan and Langston, 1990; Zhang et al., 2012; Rabodonirina et al., 2015; Maletić et al., 2019).

In order to evaluate the toxic or genotoxic effects of environmental samples containing complex mixtures of chemical substances, different test systems have been used. In particular, plant test systems have often been employed for environmental monitoring and assessment due to their important role in ecosystems as primary producers (Wang & Freemark, 1995). The use of the *Allium cepa* test (Fiskesjö, 1985; Grant et al., 1994) has increased over recent years due to its sensitivity in detecting genetic damage induced by environmental pollutants (Matsumoto et al., 2006; Leme et al., 2008; Correa Martins et al., 2016; Bollani et al., 2019). One of the advantages of this test is

the presence of a few, large chromosomes ($2n = 16$) which facilitates the evaluation of chromosome damages and/or disturbances in the cell division cycle, including the eventual risk of aneuploidy (Leme & Marin-Morales, 2009).

The aim of the present work was to analyze the genotoxic and mutagenic effects induced by sediment samples from the southern coast of Río de la Plata estuary, using *A. cepa* root cells as a test system. Two compartments were evaluated, the whole sediment and the pore water.

MATERIAL AND METHODS

Study area and sediment sampling

The Río de la Plata estuary, located between the latitudes 34°S and $36^{\circ}20'\text{S}$ and between the longitudes 55°W and $58^{\circ}30'\text{W}$, flows towards the Atlantic Ocean with an approximate area of $35,000 \text{ km}^2$ (Guerrero et al., 1997). The upper and intermediate sections of the system have typical characteristics of rivers, while the downstream section show gradual increases in salinity (Boschi, 1988). However, as it is an estuarine system influenced by tides, the saline intrusions are frequent in the intermediate and upper sections, mainly during the summer (AGOSBA-OSN-SIHN, 1994). The study area is located in the continental sector that has lotic characteristics, but it may be affected by the oceanic characteristics due to the effects of the tides and winds that can reverse the flow direction of the river and also increase its level. These conditions influence their physical, chemical and biological characteristics (Ocon et al., 2008).

Twelve sampling sites were located along 185 km of the south coast of the Río de la Plata estuary and one site was at the mouth of a highly polluted river, the Matanza-Riachuelo (Figure 1). The sites were named as follows: S1 (San Isidro), S2 (Martínez), S3 (Vicente López), S4 (Riachuelo), S5 (Villa Domínico), S6 (Bernal), S7 (Quilmes), S8 (Berazategui), S9 (Punta Lara), S10 (Berisso), S11



Figure 1 – Localization of the study area.

(Balandra), S12 (Atalaya) and S13 (Punta Indio). The samples were provided by the Naval Hydrography Service, from Argentina. Surface sediments (0 to 10 cm) from the intertidal coastal zone of the river were sampled using a 200 cm^2 stainless steel shovel. Approximately 500 g of sediment were placed in plastic bags, kept cool in the dark and taken to the laboratory as quickly as possible. Two sediment compartments were evaluated, the whole sediment and the pore water. Within 24h of arrival, the samples were homogenised and subjected to separation of the two fractions. The pore water fraction was obtained by centrifugation (5000 rpm, 45 min, 4°C), and collection of the supernatant. Approximately 10 mL of pore water were obtained, which was frozen at -20°C until bioassays (within 2 months). The whole sediment fraction was obtained by drying at room temperature for up to 2 days, protected from light, mechanically broken up, screened through a 2 mm sieve with stainless material and stored immediately at 4°C , protected from light until extraction and bioassays.

Acid extract of sediments and heavy metal quantification

The acidic extracts of sediments were prepared according to Rodrigues da Silva Júnior et al. (2009). Sediment samples (20 g) were stirred (at 115 rpm) at 20°C for 24 h with a solution (40 ml) of 5.7 ml of ultrapure acetic acid and 64.3 ml of ultrapure sodium hydroxide 1.0 M, prepared in 1000 ml of distilled water ($\text{pH } 4.93 \pm 0.05$ — sediment: solvent, 1:2, g/ml) and then centrifuged at $13,000 \times g$ for 15 min at 4°C , filtered ($0.45 \mu\text{m}$ Millipore) and stored for heavy metal quantification. This extraction method using a weakly acid solution is an appropriate method for evaluating the bioavailable soluble fractions of metals (de Souza Pohlen et al., 2012). The quantification of the metals (Cd, Cu, Cr, Pb and Zn) in the acidic extracts was performed by a flame atomic absorption spectrophotometer, in a Perkin Elmer 1100B (Perkin Elmer, Inc. Waltham, MA, U.S.A.), according to APHA et al. (2012). The detection limits (mg/l) are as follows: Cd - 0.001; Cu - 0.003; Cr - 0.004; Pb - 0.01; Zn - 0.003.

Allium cepa test

Organically grown seeds of *A. cepa* ($2n = 16$), variety Valcatorce, with more than 90% germination, from INTA, La Consulta, Mendoza, Argentina, were used for testing. The seeds were genetically and physiologically homogenous. The assays were performed according to a modified version of Grant's protocol (Matsumoto et al., 2006). In this study two treatments were carried out, one with pore water and the second with gross sediment.

One hundred (100) onion seeds were germinated in 90-mm diameter Petri dishes lined with a Whatman® glass microfiber filter grade GF/C, containing 4 mL of the pore water sample previously filtered with a 0.22 µm cellulose acetate membrane. Deionized water (4 ml) was used as a negative control and 4 ml of 4 x10⁻⁴ M of methyl-methanesulfonate (MMS, Sigma–Aldrich, CAS 66-27-3) as a positive control. In order to analyze the sediment directly, onion seeds were germinated (100 seeds in each Petri plate) in several Petri plates containing 15 g of gross sediment from different sites to which 4 ml of deionized water were added. The negative and positive controls were performed in parallel. In the negative and positive controls, the seeds were germinated in the reference area with 4 ml of deionized water and 4 ml of 4.10⁻⁴ M of MMS, respectively. All Petri dishes were kept in darkness in an incubator at 22 ± 2°C for 96 h. After this period, the seeds were removed and the roots fixed in alcohol–acetic acid (3:1) for 24 h. The fixed roots were stored in 70 % ethyl alcohol until microscopic analysis. To prepare the slides, the meristematic regions were covered with coverslips and carefully squashed in a drop of 2% acetic orcein solution. The mitotic index (MI) was calculated by counting all stages of the mitotic cells with respect to the total number of cells. For the chromosome aberration (CA) analyses, several aberrations were analyzed, such as fragments, vagrants, delays and bridges in the anaphase and telophase. All these categories were placed into one category in order to evaluate the CA as a single endpoint, following the criteria used by Hoshina and Marin-Morales (2009). The micronuclei (MN) induction was recorded by observing the interphase cells.

Table 1 - Mitotic Index (MI), and frequency of chromosomal aberrations (CA) and micronucleus (MN) in 5000 cells analyzed (mean ± deviation) of *Allium cepa* meristematic cells after exposure to pore water samples.

Sample	MI	CA	MN
Control	62,27 ± 13,00	0,03 ± 0,07	0,28 ± 0,20
S1	71,44 ± 8,44	0,26 ± 0,31	0,73 ± 0,57 ^a
S2	80,15 ± 2,47	0,06 ± 0,09 ^a	0,77 ± 0,43
S3	74,11 ± 10,02	0,08 ± 0,14 ^a	2,49 ± 2,32
S4	64,90 ± 5,76	0,26 ± 0,23 ^a	1,41 ± 0,91 ^a
S5	59,65 ± 3,67	0,09 ± 0,13 ^a	0,74 ± 0,47
S6	60,89 ± 7,07	0,11 ± 0,09 ^a	0,59 ± 0,18
S7	57,68 ± 4,20	0,16 ± 0,12 ^a	0,88 ± 0,21 ^a
S8	63,87 ± 10,95	0,08 ± 0,10 ^a	0,77 ± 0,30
S9	60,98 ± 4,59	0,11 ± 0,13 ^a	0,53 ± 0,19 ^a
S10	62,22 ± 5,62	0,13 ± 0,09	0,42 ± 0,23 ^a
S11	55,34 ± 6,49	0,03 ± 0,06 ^a	0,70 ± 0,29
S12	51,91 ± 3,85	0,06 ± 0,08 ^a	0,83 ± 0,29
S13	59,57 ± 4,53	0,03 ± 0,06	0,60 ± 0,28
MMS	60,34 ± 6,65	0,55 ± 0,34 ^a	4,21 ± 1,45 ^a

^a Significantly different from negative control (p < 0.05), according to Kruskal-Wallis test.

The analyses were performed by scoring at least 5000 cells per treatment, i.e. a total of 5 slides with 1000 cells per slide. Toxicity was evaluated based on the seed germination index, which was calculated as the ratio of the number of germinated seeds to the total seeds put to germinate. Cytotoxicity was assessed based on MI values, and genotoxicity was evaluated based on the CA and MN frequencies, as frequency = (A/B) x 100; where A is equivalent to the total number of cells with a parameter to be analyzed (CA or MN), and B corresponds to the entire number of analyzed cells (200 telophases and anaphases, and 1000 interphases, respectively).

Data analysis

Kruskal–Wallis test was performed in order to evaluate significant differences between the MI, CA and MN values obtained in each sample and the values obtained in the control. A p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Three biological parameters were analyzed in the *A. cepa* root cells, the MI and the CA and MN frequencies (Tables 1 and 2). Cytotoxicity was evaluated by the MI, but no significant differences were observed in the MI values between the treatments and the negative control in any compartment sample. On the other hand, genotoxicity and mutagenicity were assayed by the analysis of CA and MN frequencies, respectively. As expected, CA and MN frequencies were significantly induced in the positive control MMS when compared to the negative control (Tables 1 and 2).

Table 2 - Mitotic Index (MI), and frequency of chromosomal aberrations (CA) and micronucleus (MN) in 5000 cells analyzed (mean ± deviation) of *Allium cepa* meristematic cells after exposure to sediment samples.

Sample	MI	CA	MN
Control	62,27 ± 13,00	0,03 ± 0,07	0,28 ± 0,20
S1	62,76 ± 7,09	0,03 ± 0,08	0,40 ± 0,29
S2	63,83 ± 7,11	0,12 ± 0,16	0,47 ± 0,24
S3	53,05 ± 3,97	0,04 ± 0,09	0,83 ± 0,40 ^a
S4	58,28 ± 5,35	0,03 ± 0,07	0,53 ± 0,15
S5	56,06 ± 5,70	0,17 ± 0,16 ^a	0,52 ± 0,43
S6	55,98 ± 4,60	0,11 ± 0,13	0,43 ± 0,17
S7	57,18 ± 2,43	0,08 ± 0,08	0,86 ± 0,35 ^a
S8	61,99 ± 6,40	0,18 ± 0,27	0,39 ± 0,15
S9	60,62 ± 3,81	0,02 ± 0,06	0,70 ± 0,17 ^a
S10	52,45 ± 2,87	0,00 ± 0,00	0,51 ± 0,16
S11	58,80 ± 5,10	0,06 ± 0,17	0,65 ± 0,24 ^a
S12	62,74 ± 2,85	0,04 ± 0,05	0,53 ± 0,13 ^a
S13	49,50 ± 4,53	0,03 ± 0,06	0,64 ± 0,31 ^a
MMS	60,34 ± 6,65	0,55 ± 0,34 ^a	4,21 ± 1,45 ^a

^a Significantly different from negative control (p < 0.05), according to Kruskal-Wallis test.

In the pore water compartment, CA frequencies were statistically different with respect to the control ($p < 0.05$) in all of the samples except for S1, S10 and S13 (Table 1), whereas in the whole sediment only one of the samples (S5) showed this result (Table 2). The variations in the normal CA frequencies are consequences of abnormal segregation of chromosomes, mainly induced by DNA breaks, inhibition of DNA synthesis or replication of altered DNA, which can occur either spontaneously or by the action of aneugenic or clastogenic agents (Morais Leme et al., 2009). Figure 2 shows the chromosomal aberrations observed in the samples (bridges, fragments and delays) in anaphases and telophases and micronuclei in interphase cells.

When assessing the genotoxicity of the

sediment samples, the pore water compartment showed positive results in most of the samples. Many toxic materials are connected to the sediment particles and would be unlikely to resolubilize, whereas others are potentially able to resolubilize in water. According to Tovar-Sanchez et al. (2006), the metals tend to concentrate in silt-clay size particles and this fraction is transported almost entirely by suspension. The transfer of toxic metals from contaminated sediments to the water column occurs via interstitial (pore) waters. Coastal sediments from the Río de la Plata have about 57% of silt, 7.5% of clay and 25% of sand (Colombo et al., 2005). So, it was expected that bioavailable fractions of metals would be found in the pore water. In this study, the bioavailable soluble fractions of metals were evaluated using a weakly

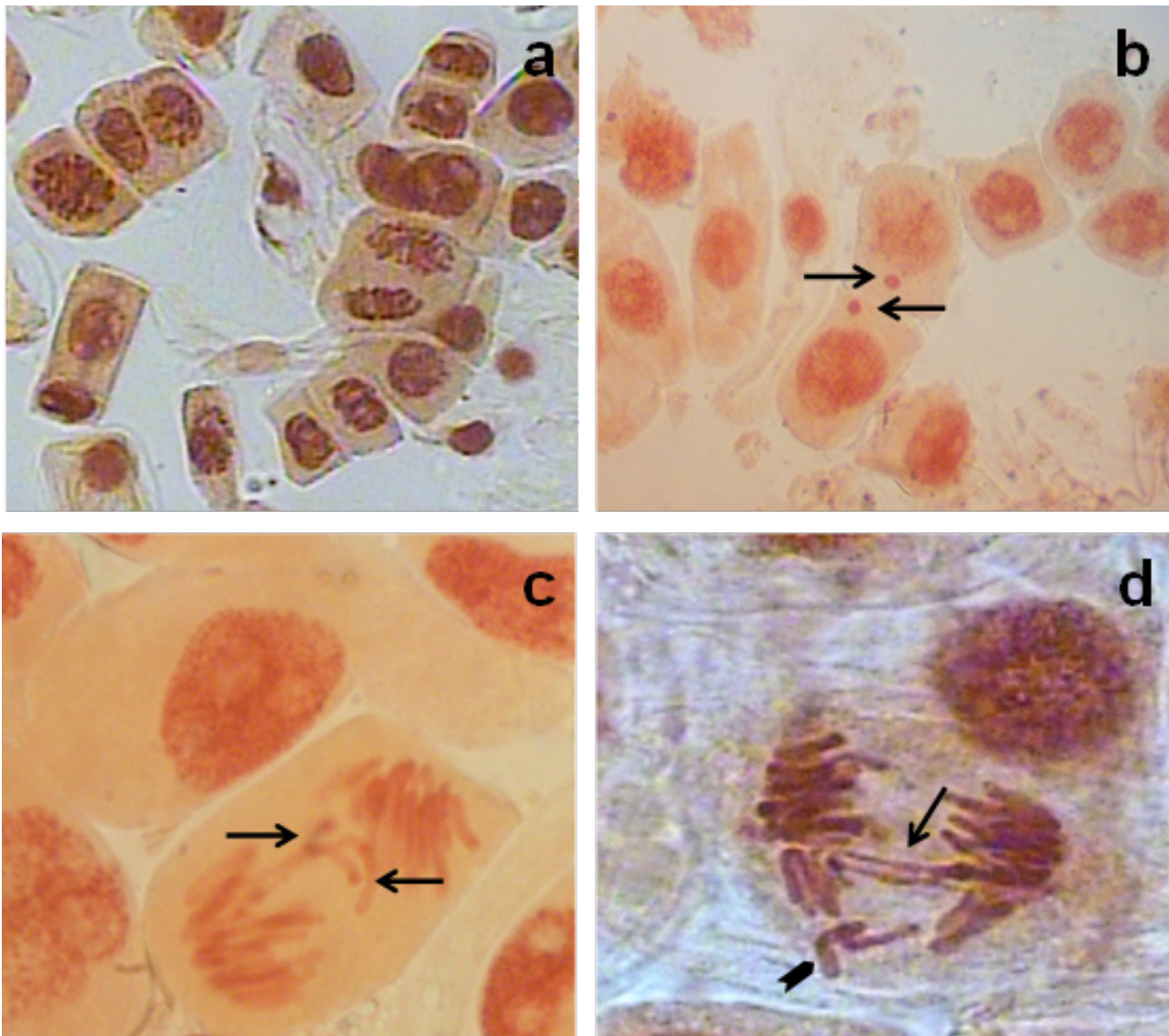


Figure 2 - Chromosome aberration (CA) observed in *A. cepa* meristematic cells exposed to sediment samples from the Río de la Plata. (a) Normal anaphases and telophases; (b) Micronuclei in two interphase cells (arrows); (c) anaphase with two vagrant chromosomes (arrows), and (d) anaphase with two bridges (arrow) and two fragments (arrowhead).

acid solution as an appropriate extraction method (De Souza Pohren et al., 2012). High concentrations of Cd (0.050 to 0.950 mg/Kg), Cu (0.125 to 7.300 mg/Kg), Cr (0.019 to 1.850 mg/Kg), Pb (1.075 to 15.200 mg/L), and Zn (0.925 to 47.225 mg/Kg) were found in the acidic extracts (Table 3). These concentrations were obtained by extracting 20 g of sediment in 40 mL of acidic solution. Hence the concentrations are expressed in mg/Kg equivalent of dry sediment.

Since there are no regulations for sediments in our country, we compared the obtained metal concentrations with the guidance levels of Canadian Standards (Canadian Environmental Quality Guidelines, 2001). According to those levels, the heavy metal concentrations in the acidic extracts did not exceed the Interim Sediment Quality Guideline (ISQG), excepting for Cd at the S4, S5 and S8 sites (Table 3). The most

Table 3 - Concentration of metals (mg/Kg sediment equivalent) in acidic extracts of sediment samples.

Sample	Cd	Cu	Cr	Pb	Zn
S1	0.575	1.925	1.400	3.650	18.600
S2	0.225	2.250	0.019	2.650	36.450
S3	0.500	4.300	0.925	4.475	13.650
S4	0.900	6.325	1.200	15.200	47.225
S5	0.950	3.300	0.350	7.100	33.600
S6	0.350	4.125	0.500	3.500	17.500
S7	0.050	2.225	0.675	2.250	35.900
S8	0.725	7.300	1.025	8.400	4.075
S9	0.075	1.350	0.625	1.075	16.175
S10	0.050	1.975	0.750	2.975	8.150
S11	<0.05	3.900	0.825	1.225	12.100
S12	<0.05	0.125	1.475	3.850	1.750
S13	0.225	0.775	1.850	4.775	0.925
ISQG ^a	0.6	35.7	37.3	35.0	123.0

^a Interim Sediment Quality Guideline (Canadian regulation).

contaminated sites on the southern coast of Río de la Plata estuary are S4 (Riachuelo River mouth) and S8 (the sewage discharge outlet in Berazategui) (AGOSBA-OSN-SIHN, 1994). The highest concentrations of Pb and Zn were found in S4, and the highest concentration of Cu was found in S8 (Table 3). Likewise, higher Cd concentrations than Canadian guidance levels were found in these sites. The Riachuelo is a highly polluted river due to the location of metallurgical, oil and leather industries. In Berazategui there is a major discharge from the sewage outlet of a primary treatment plant. On the other hand, the highest Cd concentration and high concentrations of Pb and Zn were found in S5. This site is located near the mouths of two highly polluted streams, Sarandí and Santo Domingo (Rodríguez Chapítulo et al., 1998). High concentrations of metals in S1 and S2 (Cr and Zn, respectively) could be associated with incorporation of suspended material from the Paraná de las Palmas and Luján rivers (Zorzoli, 2017). The towns of Quilmes (S7) and Atalaya (S12) also receive contributions of sewage discharges. In these two sites, high concentrations of

Zn and Cr were found, respectively (Table 3).

Metals such as Cd, Cu, Cr, Pb and Zn could cause DNA damage, chromosome aberrations, oxidative stress and micronuclei in root cells of *A. cepa* (Borboa & De La Torre, 1996; Inceer et al., 2000; Patnaik et al., 2013; Jiang et al., 2014). However, no relationship was found between the genotoxicity of the pore water and the heavy metal concentrations in the acid extract, probably due to the presence of other toxic compounds not considered in this study. Moreover, most environmental samples are complex mixtures of pollutants since they are often influenced by various sources of pollution. Thus, the biological tests are appropriate tools to evaluate the effects of this mixture.

The MN frequencies enable analysis of the mutagenic effect that chemical products exert when causing damage, not repaired or incorrectly repaired, in the DNA molecule of the parental cells (Ribeiro, 2003). Therefore, micronuclei in both meristematic and F1 root cells arise from the development of some chromosome aberrations, such as breaks and chromosomal losses. In this study, five samples of pore water and six samples of the whole sediment showed MN frequencies that were statistically different ($p < 0.05$) with respect to the control (Tables 2 and 3). Three of the five genotoxic pore water samples were also mutagenic, whereas the six mutagenic sediment samples were not genotoxic. This means that the complex mixture of chemicals present in these sediments may cause non-repairable DNA damage. Several studies have shown that much of the mutagenic activity of complex environmental mixtures is caused by compounds within one or a few classes of chemicals present in the mixture, such as PAHs, pesticides, and heavy metals (White, 2002; Vargas et al., 2008). Previous studies reveal the presence of total PAHs concentrations in sediment samples from Río de la Plata ranging from 0.35 to 2.12 ng/g (Colombo et al., 2006). These compounds are considered as one of the most persistent groups of environmental pollutants in aquatic ecosystems (Rabodonirina et al., 2015) and they tend to accumulate in sediments (Chen & White, 2004). The International Agency for Research on Cancer (IARC) classified several of these compounds as being mutagenic and carcinogenic to humans (IARC, 2010). No persistent organic compound was measured in this study. However, several sediment samples showed genotoxic and mutagenic effects.

CONCLUSION

The *A. cepa* assay showed high sensitivity in detecting genotoxicity and mutagenicity in sediment samples from the southern coastal strip of the Río

de la Plata. The analysis of two compartments of the samples (the pore water and the whole sediment) resulted in an evaluation of the genotoxic and mutagenic effects on sediments. Heavy metals deposited in the sediments could be re-suspended as the bioavailable form in the pore water, exerting genotoxic effects. Genotoxicity and mutagenicity in sediment samples could be largely attributed to heavy metals, whereas further studies will be necessary to establish a possible relationship between mutagenicity and organic compounds such as PAHs.

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