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Toxicity and genotoxicity assessment in sediments from the Matanza-Riachuelo river basin (Argentina) under the influence of heavy metals and organic contaminants

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ABSTRACT

The aim of this study was to investigate the parameters of chemical extraction associated with the detection of toxicity and genotoxicity in sediment sample extracts. Quantitative analysis of metals and polycyclic aromatic hydrocarbons (PAHs), together with a battery of four bioassays, was performed in order to evaluate the extraction efficiency of inorganic and organic toxicants. The extracts were carried out using two inorganic solvents, two organic solvents and two extraction methodologies, making a total of five extracts. Two toxicity tests, the algal growth inhibition of *Pseudokirchneriella subcapitata* and the root elongation inhibition of *Lactuca sativa*, and two genotoxicity tests, the analysis of revertants of *Salmonella typhimurium* and the analysis of micronuclei and chromosomal aberrations in *Allium cepa*, were performed. According to the chemical analysis, the acidic solution extracted more heavy metal concentrations than distilled water, and dichloromethane extracted more but fewer concentrations of PAH compounds than methanol. Shaker extracts with distilled water were non-toxic to *P. subcapitata*, but were toxic to L. *sativa*. The acidic extracts were more toxic to *P. subcapitata* than to L. *sativa*. The methanolic organic extracts were more toxic to the alga than those obtained with dichloromethane. None of these extracts resulted toxic to L. *sativa*. Mutagenic effects were only detected in the organic dichloromethane extracts in the presence of metabolic activation. All the inorganic and organic extracts were genotoxic to *A. cepa*. This study showed that the implementation of different extraction methods together with a battery of bioassays could be suitable tools for detecting toxicity and genotoxicity in sediment samples.

1. Introduction

Many areas of Latin America have been affected by industrial development and the intensive use of natural resources by agriculture and livestock. These activities release a wide variety of pollutants into the environment that reach surface waters through industrial and domestic effluents, runoff and atmospheric deposition. Both water-soluble and hydrophobic contaminants can be persistent and maintain their physical and chemical characteristics while they are transported and distributed throughout the aquatic environment. These non-degradable pollutants may accumulate in different compartments or undergo transformations resulting in compounds with more or less bioavailability. The use of stream sediments for assessing aquatic pollution in environmental studies is mostly due to the ability of this compartment to concentrate pollutants, acting either as a sink or as a secondary source of contaminants in the water column and aquatic biota (Minissi et al., 1998; White et al., 1998; Vargas et al., 2001; Chen and White, 2004). Most of these chemicals are toxic, genotoxic or carcinogenic, and they become part of complex environmental mixtures which can have adverse health effects on humans and indigenous biota (Ohe et al., 2004; Vargas et al., 2008; Cappi da Costa et al., 2012).

Tools such as biological tests are useful for integrating the effects of all bioavailable contaminants and their interactions in the ecosystems (White, 2002; Chen and White, 2004; Klamer et al., 2005; Magdaleno et al., 2008; Cappi da Costa et al., 2012). These assays evaluate possible synergistic or antagonistic effects of the contaminants, broadening the

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study of the physical and chemical parameters commonly used in evaluating complex mixtures. Toxicity and genotoxicity assessment of sediment can be conducted on whole sediments, interstitial water, sediment elutriates, or sediment extracts. Many extraction techniques have been used to detect the mutagenicity potential of chemical compounds using organic, aqueous and acidic solvents, as well as other methods, such as shaking, sonication, soxhlet and accelerated solvent extraction (Di Giorgio et al., 2011; Cappi da Costa et al., 2012; de Souza Pohren et al., 2012). A selection of the appropriate solvent and extraction method depends on the physical–chemical properties of the sediment and putative contaminants (Chen and White, 2004).

Ecotoxicological test methods on small-scale, based on cellular components, cells, organs, small animals and plants, have the advantage of being highly sensitive, rapid and reproducible and they only require minute amounts of sample material. One of the most common aquatic toxicological tests is the algal growth inhibition test (USEPA, 2002; ISO, 2009). This test utilises the in vivo phytotoxic effects of sample matrixes such as pore water and organic extracts of sediments and water (Källqvist et al., 2008). Algae are used in test batteries for environmental hazard assessment due to their importance as dominant primary producers in most aquatic ecosystems (Blaise et al., 1998; Franklin et al., 2002; Vendrell et al., 2009). In particular, the green microalga *Pseudokirchneriella subcapitata* is one of the most widely used species in toxicity tests due to its sensitivity to different pollutants, its easy maintenance in laboratory cultures, and its relatively short life cycle (Lewis, 1995; USEPA, 2002; Magdaleno et al., 2014). Additionally, many tests on higher plant species have been shown to be highly sensitive to environmental stress (Dutka, 1989; Wang and Freemark, 1995; Bowers et al., 1997; Charles et al., 2011; Abreu et al., 2014). *Lactuca sativa* present several advantages among the plant species recommended by the environmental agencies and organizations for standard toxicity tests (USEPA, 1996; OECD, 2006): the test is simple, quick, reliable, inexpensive, and does not require major equipment.

The *Salmonella*/microsome assay (Ames test) is a widely accepted short-term assay for identifying substances that can cause genetic damage (Mortelmans and Zeiger, 2000). It is used worldwide for detecting the mutagenicity of samples from different environmental matrices, such as water, sediment, soil and atmosphere, as well as pure chemicals (Ducatti and Vargas, 2003; Ohe et al., 2004; White and Claxton, 2004; Magdaleno et al., 2008; Umbuzeiro et al., 2008). On the other hand, aspects ranging from gene mutations to chromosome damage and aneuploidies can be identified by the analysis of eukaryotes. Higher plants present characteristics that can be used in genetic models to assess environmental pollutants, and they are often used in monitoring studies (Leme and Marin-Morales, 2009). Various tests have been performed with a variety of plant species, e.g. *Tradescantia pallida* (Ma, 1981), *Vicia faba* (Kanaya et al., 1994), *Zea mays* (Grant

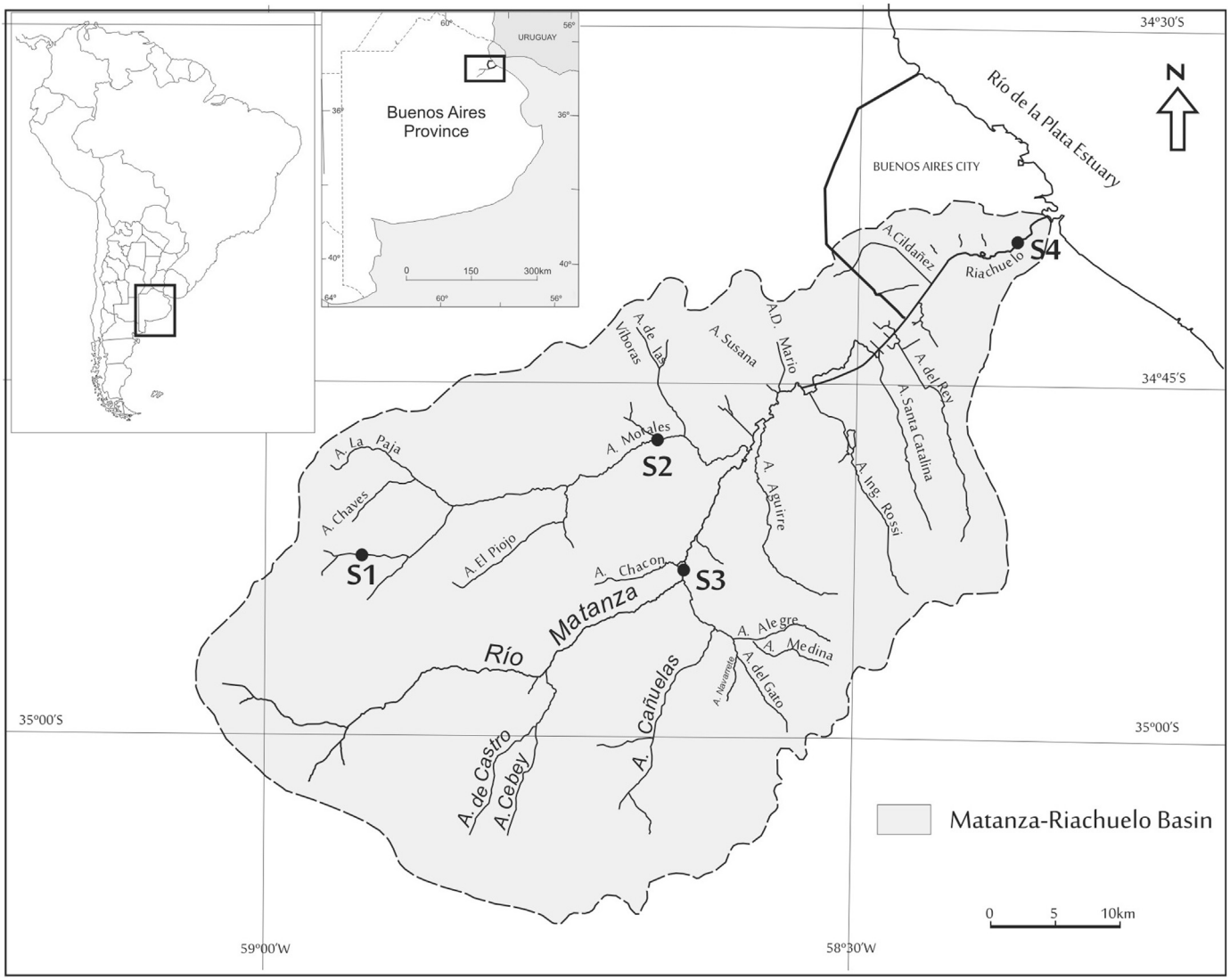

Fig. 1. Location of sampling sites in the Matanza-Riachuelo River (Buenos Aires, Argentina).

and Owens, 2006), and *Allium cepa* (Grant, 1982). The *A. cepa* test is an excellent genetic assay due to its high sensitivity, good correlation with other test-systems, easy handling and low cost. Additionally, this species has the advantage of having a low number ($2n$=16) of large chromosomes (Fiskesjö 1985; Rank and Nielsen, 1997).

So the use of pre-concentration techniques and extraction of environmental samples in combination with testing in small-scale bioassays has become a versatile tool in the initial screening of environmental samples for toxicity (Klamer et al., 2005; Källqvist et al., 2008). Therefore, the aim of this study was to assess the toxicity and genotoxicity of sediments from a highly polluted river (the Matanza-Riachuelo River, Argentina), using different sediment extraction techniques. For this, a total of five extracts were obtained: three inorganic extracts (aqueous and acidic) and two organic extracts (using two different solvents). Samples from areas with different land uses (urban, rural and industrial) were selected. Toxicity and genotoxicity of the extracts were assessed using the *P. subcapitata* and L. *sativa* tests, and the *Salmonella*/microsome and *A. cepa* tests, respectively.

2. Materials and methods

2.1. Sediment sampling

Sediment samples were collected from four sites located in the Matanza-Riachuelo river basin, Buenos Aires, Argentina (Fig. 1). Watercourses in this basin are potentially subjected to different types of contaminants from agricultural and urban runoff, industrial effluents, sewage treatment plants and leaching from domestic garbage dump (Magdaleno et al., 2008). Sites 1 and 2 were situated approximately 8.3 km and 13.5 km from the headwaters of the Morales stream, respectively, with a population density of approximately 20 habitants per km^2. Site 3 was located on the Matanza river at 40.9 km (1500 habitants per km^2), and site 4 on the Riachuelo river at 79.5 km from the headwaters (8500 habitants per km^2). The main economic activity around the first two sites involves cattle feedlots, poultry and pig production, and agricultural crops (mainly soybean, corn and sunflower). The corresponding activities around site 3 are similar to those at sites 1 and 2, but with more emphasis on extensive agriculture. Site 4 is located in the most highly urbanized region surrounded by industrial areas.

The upper 20 cm of the river sediments (between 500 g and 1 kg) were collected using a piston corer and transferred to plastic bags, protected from light. Three samples were collected for each site and stored immediately at 4 °C. The samples were frozen in the laboratory at −20 °C until analysis (within 2 months). Whole sediments were freeze-dried, and sediment fractions of less than 85 μm in size were isolated by dry sieving. The < 85 μm fractions of sediments were stored in the dark until extraction of organic and inorganic chemicals.

2.2. Inorganic extraction

2.2.1. Shaking

Sediment samples (20 g) were stirred (115 rpm) at 20 °C for 24 h with a solution (40 ml) of 5.7 ml of acetic acid ultrapure (purchased from Sintogram, ≥99.7% purity) and 64.3 ml of 1.0 M sodium hydroxide ultrapure (purchased from Merck, ≥99% purity), prepared in 1000 ml of distilled water (acidic extract: pH 4.93 ± 0.05 — sediment: solvent, 1:2, g/ml) or distilled water (aqueous extract: pH 5.50 ± 0.05 — soil: solvent, 1:2, g/ml), according to Rodrigues da Silva Júnior et al. (2009). The processed samples were then centrifuged at 13,000×*g* for 15 min at +4 °C, filtered (0.22 μm Millipore), divided into aliquots and stored at −20 °C until the toxicity and genotoxicity assessment.

2.2.2. Sonication

Sediment samples (25 g) were extracted by sonication using a solution (50 ml) of 5.7 ml of acetic acid ultrapure and 64.3 ml of 1.0 M sodium hydroxide ultrapure, prepared in 1000 ml of distilled water (acidic extract: pH 4.93 ± 0.05 — sediment: solvent, 1:2, g/ml), 25 ml per 10 min (2 cycles, 1000 W power), according to Rodrigues da Silva Júnior et al. (2009). The pre-filtered extracts were passed through glass–wool and then centrifuged at 13,000g for 15 min at 4 °C, filtered (0.22 μm Millipore), divided into aliquots and stored at −20 °C until the toxicity and genotoxicity assessment.

2.3. Organic extraction

Sediment samples (25 g) were extracted by sonication using dichloromethane (DCM) (purchased from J.T. Baker, ≥99.9% purity) or methanol (purchased from Biopack, ≥99.9% purity), 50 ml for 10 min (2 cycles, totalling 100 ml, 1000 W power). The pre-filtered extracts were passed through a chromatography column with a filter plate containing sodium sulphate and celite, concentrated in rotavapor at +40 °C (organic extract: sediment: solvent, 1:4, g/ml). This final extract was used for the analysis of the organic compounds. The same procedure was carried out for the toxicity and genotoxicity assays, but the final extract was totally evaporated and the residue was re-suspended in 10 ml dimethylsulfoxide (DMSO) (purchased from Sintogram, ≥99.9% purity) (Chen and White, 2004). So, this extract was concentrated 10 times. As the initial sediment concentration in the extraction procedure was 250 g/L, the final extract concentration in 100% DMSO represented 2500 g/L. The extract obtained was then filtered through a 0.22 μm nylon membrane and stored at −20 °C until further use.

2.4. Quantification of metals and PAHs

The quantification of metals (Zn, Cr, Pb and Cu) in the aqueous and acidic extracts was performed by a flame atomic absorption spectrophotometer, in an Analyst 200 (Perkin Elmer), Inc. Waltham, MA, U.S.A.), according to APHA et al. (2012). The detection limits (mg/l), calculated as 3x the standard deviation for 10 measurements of the blank, were the follows: Zn-0.003; Cr-0.003; Pb-0.01; Cu-0.005. In the organic extracts, concentrations of 14 polycyclic aromatic hydrocarbons (PAHs) were determined that are included in the list of 16 PAHs as priority compounds according to the United States Environmental Protection Agency (USEPA). The analytical determinations were performed by gas chromatography and mass spectrometry on a model Agilent 7890 A GC with “auto sampler” model 7693, coupled to 5975 C inert MSD model. The terms of use were: helium gas, HP-5MS column length (30 m) internal diameter (0.25 mm), initial and final temperatures (120–300 °C), start and end time (0–5 min), gradient (5 °C/min). Injector temperature (300 °C), constant flow (1 ml/min), “Split”: the ratio (5:1). Detector MSD: Source of MS (230 C), Quad MS: 150 °C, detection limits (50–300 amu). The analytical reference standards were purchased from AccuSandard and the percentages of compound recoveries were between 76% and 103%.

2.5. Algal growth inhibition test

The microalga *Pseudokirchneriella subcapitata* (Korshikov) Hindak (previously known as *Selenastrum capricornutum* Printz), obtained from the Culture Collection of Algae and Protozoa, U.K. (CCAP No. 278/4), is currently kept in the Culture Collection of the laboratory of Protists Biology, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. The axenic stock alga was cultivated in 125 ml Erlenmeyer flasks, containing 50 ml sterilized Bold´s Basal Medium (BBM, Archibald and Bold, 1970) and agitated on a shaker at 80 rpm, under continuous cool-white fluorescent light (80 μmol photons m^{-2} s^{-1}). The flasks were maintained at 22 ± 2 °C for 7 days to obtain the inoculum in the exponential growth phase (approximately 2.5×10^6 cells ml^{-1}). The experimental treatments were prepared

according to algal growth inhibition test standards using sterile 96-well microplates (Environmental Canada, 2007). The bioassays were conducted in four replicate wells containing a total volume of 200 µL and an initial cell density of 2.5×10^4 cells ml^{-1}. Equivalent amounts of the BBM stock solutions were added to the extracts before each test in order to provide the amount of nutrients needed for algal growth. In order to prevent toxicity of the acidic solution and DMSO, preliminary test was performed to assess the pH toxicity and toxic concentrations of DMSO. Those solutions that showed no statistically significant differences (ANOVA; α=0.05) with respect to BBM were selected as controls. Then, eight replicates of BBM culture medium, acidic solution with adjusted pH =6.5, and BBM with 1% DMSO were used as controls. A solution of NaOH 1N was used to adjust the pH of acidic solution. Serial dilutions of the inorganic extracts were then prepared using BBM: aqueous extracts (500, 250, 125 and 50g/L), acidic extracts (250, 125, 50, 25 and g/L), and one dilution of the organic extracts (25g/L). The selection of all these extract dilutions were previously tested by screening tests. The acidic extracts were used with adjusted pH =6.5 to prevent algal growth inhibition. All extracts were tested using at least four replicates. The microplates were incubated under the same conditions as the inoculum cultures. Cell densities were estimated by absorbance at 620 nm after 96 h culture. The percentages of algal growth inhibition (*%I*) with respect to the control at each extract concentration were estimated by the following equation:

$$\%I=\frac{C-S}{C}\times100 \quad (1)$$

where C is the absorbance measured in the controls, and S is the absorbance measured in each extract concentration. In those extracts in which the EC50 could be determined, the classical sigmoidal equation was used:

$$Y=A_2+\frac{A_1-A_2}{1+\left(\frac{x}{x0}\right)^p} \quad (2)$$

where p is the slope parameter, x_O is the centre point of the curve, and A_1 and A_2 are the upper and lower asymptotes, respectively. Graphics and equations were obtained using the OrigenPro 8 program. One-way analysis of variance (ANOVA) followed by a Dunnett's post hoc test were performed to evaluate significant differences between each extract concentration and the control. A p value less than 0.05 was considered statistically significant.

2.6. Lactuca sativa test

Organically grown seeds of L. *sativa* (variety Gallega) with 97% germination, from INTA, La Consulta, Mendoza, Argentina, were used for testing. In order to prevent toxicity of the acidic solution and DMSO, a preliminary test was performed to assess pH toxicity and toxic concentrations of the acidic solution and DMSO. The concentrations that showed no statistically significant differences (ANOVA; α=0.05) with respect to distilled water were selected as controls. Then, distilled water was used as the control of aqueous extracts, 10% acidic solution with adjusted pH =6.5, diluted in distilled water was used as the control of acidic extracts, and 1% DMSO diluted in distilled water was used as the control of the organic extracts. One concentration of each extract was evaluated: 100% of aqueous (500 g/L), 10% of acidic (50 g/L), and 1% of organic extracts (25 g/L). A seed was considered germinated when visible appearance of the radicle was detected. Tests were carried out in 90-mm diameter Petri dishes lined with filter paper, with 20 seeds each, containing 4 ml of the extract concentration. Three replicates were performed for each concentration and control. The Petri dishes were kept in darkness in an incubator at 22 ± 2 °C for 120 h. The assay was considered valid when the mean control survival was at least 90% germination and the coefficient of variation for root elongation was below 30% (OECD, 2006). The radicle lengths were measured using a digital caliper and elongation data was used to calculate the relative growth index (RGI) and the germination index (GI%), according to Young et al. (2012). Calculation of these phytotoxicity indexes is shown in the following equations:

$$RGI=\frac{RLI}{RLC} \quad (3)$$

$$GI(\%)=\frac{RLI\ x\ GSI\ x100}{RLC\ x\ GSC} \quad (4)$$

where RLI is the radicle length of the extract concentration treated seeds, RLC is the radicle length of the control seeds, GSI is the number of germinated seeds in the extract concentration and GSC is the number of germinated seeds in the control. The RGI values were differentiated into three categories according to the toxic effects observed:

1. Inhibition of root elongation (I): $0 < RGI < 0.8$
2. No significant effects (NSE): $0.8 \leq RGI \leq 1.2$
3. Stimulation of root elongation (S): $RGI > 1.2$

Statistical analysis of the data was performed using the analysis of variance (ANOVA). When the F values of the ANOVA were significant ($p < 0.05$), the means of the treatments were compared by Tukey's test.

2.7. Salmonella mutagenicity test

The tester strains TA98 and TA100 were obtained from the University of California, Berkeley, USA. These two strains allow the detection of reading frame shift and base substitution mutations, respectively. The assay was conducted using the procedure of pre-incubation (30 min at 35 °C) followed by plate incorporation described by Maron and Ames (1983). All the extracts were tested at 100% concentration, with and without the hepatic S9 fraction. Sodium azide (SAZ: 5 µg $plate^{-1}$) and 2-aminoflurene (2AF: 10 µg $plate^{-1}$) were used as a positive control for TA100 without the S9 fraction, and TA98 and TA100 with the S9 fraction, respectively. All the plates were run in triplicate. Sterile distilled water, acidic solution and DMSO were used as negative controls. Plates were then inverted and placed in a dark incubator at 37 °C for 72 h. We considered the results as positive when the number of revertants obtained in the plates exposed to the extract were two times or greater than the spontaneous reversion rates in the negative control, according to Mortelmans and Zeiger (2000).

2.8. Allium cepa test

Organically grown seeds of *A. cepa* (2n =16), variety Valcatorce, with more than 80% 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). One hundred (100) onion seeds were germinated in 90-mm diameter Petri dishes lined with filter paper containing 4 ml of the extract. Distilled water, acidic solution with adjusted pH =6.5 (10%) and DMSO (1%) were used as negative controls, and with 12 mg/L methyl methanesulfonate (MMS) as positive controls (Cappi da Costa et al., 2012). The Petri dishes were kept in the dark in an incubator at 22 ± 2 °C for 96 h and the seeds were collected after this period. The roots were fixed in alcohol–acetic acid (3:1) for 24 h and then stored in 70% ethyl alcohol until the 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 mitotic cells with respect to the total number of cells. For the chromosome aberration (CA) analyses, several aberrations such as fragments, vagrants and bridges in the anaphase and telophase were analyzed. 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. The analyses were performed by scoring 5000 cells per treatment, i.e. 1000 cells per slide and a total of 5 slides. Toxicity was based on the seed germination index, which was calculated as the ratio of the number of germinated seeds to the total seeds allowed to germinate. Cytotoxicity was based on the MI values, and genotoxicity was 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). Statistical analysis was performed using the Kruskal–Wallis test at a significance level of 0.05.

2.9. Statistical analysis

Correlation analysis between all the biological variables measured at each assay and chemical determinations in the extracts (concentrations of heavy metals and PAHs) were performed using the Pearson correlation matrix ($p < 0.05$) and the program InfoStat 2014.

3. Results

3.1. Inorganic and organic extracts

Sediment samples from rural and urban areas were submitted to different extraction processes and some metals were identified from these extracts (Table 1). The comparison between the methods employed (sonication versus shaker and acidic extract versus aqueous extract) shows great differences in metal concentrations. The acid method extracted more concentrations of metals than the aqueous method. On the other hand, sonication extracted more total metal concentrations than shaking, in S2, S3 and S4. As expected, the most highly urbanized and industrialized site (S4) showed the highest concentrations of metals. However, site S2 with rural characteristics also showed high concentrations of Zn, Pb and Cu (Table 1).

Quantitative analyses of PAHs present in the extracts submitted to sonication and the organic solvents, methanol or DCM, were performed to assess the efficiency of the organic extraction. Fourteen compounds were quantified. As expected, site S4 showed the highest concentrations of PAHs in the sediments, also reaching the highest levels of carcinogenic PAHs in both extraction solvents (3.71 mg/kg in methanol extract, and 5.24 mg/kg in DCM extract) (Table 2). At this site, DCM extracted more PAH compounds (eleven) than methanol (five), although the latter extracted a greater concentration with respect to DCM. At the other sites with crop and livestock characteristics, concentrations of PAHs were also detected. In S2, concentrations of carcinogenic and total PAHs were higher in methanol extract (1.31 and 3.23 mg/kg, respectively) than in DCM extract (0.11 and 0.23 mg/kg, respectively). However, at S1 and S3, the highest concentrations of total PAHs were found in the DCM extract. At S1, located downstream from a feedlot, carcinogenic compounds were not found.

According to the guidance levels of contaminants for the quality of sediments proposed by the Canadian standard (Canadian Council of Ministers of the Environment, 2001), the heavy metal concentrations in the extracts did not exceed the Interim Sediment Quality Guideline (ISQG) or the Probable Effect Level (PEL) (Table 1). However, the organic extracts showed high levels of some PAH compounds compared with the Canadian standard (Table 2). Those compounds that exceeded PEL values were: fluorene and anthracene in the S1 and S3 sediments, pyrene and chrysene in S4 (DCM extract), and anthracene in S2, benzo(a)anthracene and benzo(b)pyrene in S4 (methanol extract).

3.2. Toxicity bioassays

All concentrations of the aqueous extracts showed stimulation of algal growth at all the sites. Therefore, no concentration-dependent inhibition of the *P. subcapitata* growth was found. In the contrary, the acidic extracts caused concentration-dependent inhibition of the algal growth at the concentrations tested: 250, 125, 50 and 25 g/L (Table 3). All the acidic extracts were toxic to the alga, obtaining EC20 and EC50 values. The extracts obtained by shaking were more toxic than those obtained by sonication. The EC20 values for shaker varied from < 25–66.5 g/L and for sonication varied from 32.5 to 177.5 g/L, whereas the EC50 values varied from 37.5 to > 125 g/L, and from 78.5 to > 250 g/L, respectively. According to these values, S1 and S2 were the most toxic sites (Table 3). However, no statistically significant correlations between concentrations of metals measured in each extract and those values were observed (Table S1).

As the organic extracts were obtained in DMSO and this compound is toxic to the alga, the bioassays were performed using a dilution (1% of the extract) of the extract in BBM. These extracts were concentrated 10 times after the evaporation of DCM and methanol, therefore the final concentration test was 25 g/L. The final point obtained was the *%I* of algal growth in the extract compared with the growth in the control (1% DMSO). All the sites showed toxicity (inhibition between 10% and 48% in DCM extracts, and 21% and 86% in methanol extracts). Therefore, the methanol extracts were more toxic than those in DCM, except for S4 (Table 3). This is probably related to the extraction of compounds in higher concentration in methanol with respect to DCM (Table 2). On the other hand, the extracts obtained from S1 were less toxic. The toxicity of PAH compounds depends on the number of aromatic rings in the molecule. Therefore, the correlation analysis was performed between *%I* and compounds with 3, 4, 5 and 6 rings separately. According to this analysis, no statistically significant correlations were observed between those parameters (Table S2).

To analyse the effects of different extracts on the germination and root elongation in L. *sativa*, one concentration of extract was tested: aqueous extracts (100% or 500 g/L), acidic extracts (10% or 50 g/L), and organic extracts (1% of 10 times concentrated or 25 g/L). The number of germinated seeds in the extracts was similar to the controls, showing no significant differences between the percentage germination and the control (Table 4). Therefore, the EC50 value could not be estimated. However, the phytotoxicity analysis using the RGI para-

Table 1
Concentration of four metals (mg/Kg sediment equivalent) in acidic and aqueous extracts of sediment samples from the Matanza-Riachuelo basin following two extraction procedures.

Inorganic Extract	Site	Zn	Cr	Pb	Cu	Total concentration
Aqueous shaker	S1	0.080	< 0.006	< 0.020	< 0.010	0.080
	S2	0.100	< 0.006	0.160	< 0.010	0.260
	S3	–	–	–	–	
	S4	0.160	< 0.006	< 0.020	< 0.010	0.160
Acidic shaker	S1	0.340	0.040	< 0.020	0.080	0.460
	S2	0.120	< 0.006	0.300	< 0.010	0.420
	S3	0.020	< 0.006	0.200	< 0.010	0.220
	S4	20.200	1.780	0.120	1.620	23.720
Acidic sonication	S1	0.180	< 0.006	< 0.020	< 0.010	0.180
	S2	0.700	< 0.006	0.260	0.200	1.160
	S3	0.080	< 0.006	0.180	0.080	0.340
	S4	21.880	1.980	0.180	3.300	27.340
ISQG[a]		123.0	37.3	35.0	35.7	
PEL[b]		315.0	90.0	91.3	197.0	

[a] Interim Sediment Quality Guideline, concentration below which no adverse biological effect is observed (Canadian Council of Ministers of the Environment, 2001)
[b] Probable Effect Level concentration above which an adverse biological effect is observed (Canadian Council of Ministers of the Environment, 2001)

Table 2
Concentration of fourteen PAHs (mg/Kg sediment equivalent) in the organic extracts (dichloromethane and methanol) of sediment samples from the Matanza-Riachuelo basin following sonication extraction procedure.

	DCM				Methanol					
Compound	S1	S2	S3	S4	S1	S2	S3	S4	ISQG[a]	PEL[b]
Fluorene	1.27	< 0.04	0.86	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	0.021	0.144
Phenanthrene	0.04	< 0.04	< 0.04	0.05	< 0.04	0.23	0.17	0.24	0.042	0.515
Anthracene	0.62	< 0.04	0.25	0.10	0.05	0.79	< 0.04	< 0.04	0.047	0.245
Carbazole	< 0.04	< 0.04	< 0.04	0.05	< 0.04	< 0.04	< 0.04	< 0.04	–	–
Fluoranthene	0.09	0.06	< 0.04	0.23	0.10	0.90	0.40	< 0.04	0.111	2.355
Pyrene	0.48	0.06	< 0.04	0.91	< 0.04	< 0.04	< 0.04	< 0.04	0.053	0.875
Benzo(a)anthracene[d]	< 0.04	< 0.04	< 0.04	0.25	< 0.04	< 0.04	< 0.04	0.47	0.032	0.385
Chrysene[d]	< 0.04	0.11	< 0.04	1.30	< 0.04	0.26	< 0.04	< 0.04	0.057	0.862
Benzo(b)fluoranthene[d]	< 0.16	< 0.16	0.26	0.47	< 0.16	0.57	0.40	0.78	–	–
Benzo(k)fluoranthene[d]	< 0.16	< 0.16	< 0.16	0.20	< 0.16	0.48	< 0.16	2.52	–	–
Benzo(a)pyrene[d]	< 0.16	< 0.16	< 0.16	0.56	< 0.16	< 0.16	< 0.16	1.47	0.032	0.782
Indeno(1,2,3-cd)pyrene[d]	< 0.40	< 0.40	< 0.40	0.93	< 0.40	< 0.40	< 0.40	< 0.40	–	–
Dibenzo(a,h)anthracene[d]	< 0.40	< 0.40	< 0.40	< 0.40	< 0.40	< 0,40	< 0.40	< 0.40	0.006	0.135
Benzo(g,*h*, i)perilene	< 0.40	< 0.40	< 0.40	0.94	< 0.40	< 0.40	< 0.40	< 0.40	–	–
Carcinogenics	nd[c]	0.11	0.26	3.71	nd[c]	1.31	0.40	5.24	–	–
Total	2.50	0.23	1.37	5.99	0.15	3.23	0.97	5.48	–	–

[a] Interim Sediment Quality Guideline, concentration below which no adverse biological effect is observed (Canadian Council of Ministers of the Environment, 2001)
[b] Probable Effect Level concentration above which an adverse biological effect is observed (Canadian Council of Ministers of the Environment, 2001)
[c] nd — not detected
[d] Carcinogenic compound

Table 3
Inhibition of the *Pseudokirchneriella subcapitata* growth rate after 96 h exposure to sediment extracts from the Matanza-Riachuelo basin.

Sediment extract Concentrations (g/L)	Site	Maximum inhibition (*%I*)	EC20 (g/L)	EC50 (g/L)
Aqueous shaker	S1	nd[a]	–	–
(500, 250, 125, 50)	S2	nd[a]	–	–
	S3	nd[a]	–	–
	S4	nd[a]	–	–
Acidic shaker	S1	–	66.5 (43.5–90)	> 125
(250, 125, 50, 25)	S2	–	< 25	37.5 (35–39)
	S3	–	41.5 (40–44)	67.5 (50–85.5)
	S4	–	37 (31–42.5)	54 (35–60)
Acidic sonication	S1	–	55.5 (51–60)	112.5 (86–140)
(250, 125, 50, 25)	S2	–	35 (5–66)	146 (66–225)
	S3	–	177.5 (141–215)	> 250
	S4	–	32.5 (4.5–60)	78.5 (56–102.5)
DCM sonication	S1	10	–	–
(25)	S2	22	–	–
	S3	24	–	–
	S4	48	–	–
Methanol sonication	S1	21	–	–
(25)	S2	51	–	–
	S3	86	–	–
	S4	42	–	–

[a] nd — not determined, stimulation effect

Table 4
Phytoxicity parameters tested in *Lactuca sativa*: (percentage germination, Germination Index (GI) and Relative Growth Index (RGI).

Sediment extract Concentrations (g/L)	Site	Percentage Germination	Germination index (GI %)	Relative growth index (RGI)
Control distilled water		75.00 ± 15.00	100	1
Aqueous shaker	S1	85.00 ± 13.23	93.02 ± 34.11	0.80 ± 0.20
(500)	S2	65.00 ± 13.23	55.07 ± 14.56[a]	0.63 ± 0.07[a]
	S3	67.50 ± 10.61	37.34 ± 15.21[a]	0.41 ± 0.10[a]
	S4	72.50 ± 10.61	30.77 ± 12.67[a]	0.33 ± 0.18[a]
Control acidic solution		73.33 ± 7.64	100	1
Acidic shaker	S1	75.00 ± 13.23	73.19 ± 12.24[a]	0.73 ± 0.10[a]
(50)	S2	81.67 ± 7.64	113.11 ± 21.28	1.13 ± 0.19
	S3	81.67 ± 14.43	75.23 ± 14.80[a]	0.7 ± 0.14[a]
	S4	81.67 ± 7.64	77.02 ± 17.46[a]	0.79 ± 0.14[a]
Acidic sonication	S1	58.33 ± 20.82	81.63 ± 5.85[a]	0.76 ± 0.14[a]
(50)	S2	68.33 ± 17.56	84.70 ± 5.22[a]	0.85 ± 0.09
	S3	65.00 ± 15.00	93.91 ± 6.96	0.81 ± 0.08
	S4	65.00 ± 13.00	73.95 ± 12.24[a]	0.73 ± 0.09[a]
Control DMSO		68.33 ± 10.41	100	1
DCM sonication	S1	65.67 ± 11.55	83.31 ± 18.70	0.92 ± 0.06
(25)	S2	71.67 ± 2.89	95.30 ± 5 .16	0.91 ± 0.01
	S3	66.6 14.43	84.38 ± 27.38	0.85 ± 0.11
	S4	66.67 ± 10.41	98.09 ± 22.59	0.99 ± 0.12
Methanol sonication	S1	76.67 ± 7.64	105.45 ± 33.63	0.97 ± 0.04
(25)	S2	78.33 ± 7.64	87.70 ± 27.38	0.87 ± 0.13
	S3	73.33 ± 10.41	66.17 ± 5.48[a]	0.82 ± 0.05
	S4	76.67 ± 2.89	91.56 ± 9.18	0.96 ± 0.03

[a] Significant differences with respect to the control ($p < 0.05$), according to Tukey's test

meter indicated that all the inorganic extracts significantly inhibited root elongation. The RGI values between 0 and 0.8 indicate an inhibition effect, and the values obtained were between 0.33 ± 0.18 and 0.79 ± 0.14 (Table 4). These results were reinforced by the significant reduction of the GI% in the inorganic extracts with respect to the control. On the other hand, the aqueous extracts (RGI values between 0.33 ± 0.18 and 0.63 ± 0.07) showed more inhibition effect than the acidic extracts (RGI values between 0.73 ± 0.09 and 0.79 ± 0.14). Similarly the GI% parameter showed the same inhibition effect (values from 30.77 ± 12.67 to 55.07 ± 14.56 in the aqueous extracts and values from 73.19 ± 12.24 to 113.11 ± 21.28 in the inorganic extracts). These differences could be due to a higher concentration of sediment in the aqueous extract (500 g/L) than in the acid extract (50 g/L) in the bioassay. However, the metal concentrations measured in those extracts were very low or undetectable (Table 1). According to the correlation analysis, no statistically significant correlations between RGI or GI% values and metals were observed (Table S3). On the other hand, the organic extracts were not toxic to L. *sativa*, except in the methanol extract from S3 (Table 4). As these extracts were obtained in

DMSO and this compound is toxic to plants, bioassays were performed using 1% dilution of extract concentrated 10 times (25 g/L). Therefore, concentrations of PAHs were forty times lower than those indicated in Table 2 and so probably these concentrations were not toxic for L. *sativa*.

3.3. Genotoxicity tests

Two short-term mutagenicity bioassays, the *S. typhimurium* and *A. cepa* assays, were selected in order to seek the optimal extraction parameters for the detection of substances with a mutagenic potential. Table 5 shows the mutagenicity results using the inorganic and organic extracts, in the presence and absence of S9 mix. None of the inorganics extracts was genotoxic. Only DCM organic extracts were mutagenic to a TA100+ S9 mix at the four sites. Taking into account the concentrations of PAH compounds measured in those extracts, only benzo(a) anthracene and benzo(a)pyrene exceeded ISQG and PEL values from site S4 (Table 2). However, the mutagenic response was similar for the four sites. Moreover, in the methanolic extracts, in which no mutagenic response was observed, PAH concentrations exceeded those guide values at the four sites (fluorene and anthracene in S1, pyrene and chrysene in S2, fluorene and anthracene in S3, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene and benzo(a) pyrene in S4 (Table 2). According to the correlation analysis, the PAH compounds (grouped into 3, 4, 5 and 6 rings, carcinogenic and total PAHs) present in DCM extracts were not significantly correlated with the number of revertants/plate in TA100+ S9 mix (Table S4). These results could indicate potential synergistic and/or antagonistic effects between PAH compounds or in combinations with other organic contaminants not analyzed in this study.

The *A. cepa* test enables the assessment of different genetic endpoints, including CA and MN. Besides, this assay provides information about the cytotoxicity of several agents by the analysis of MI. The four sites showed cytotoxicity in some of the inorganic and organic extracts (Table 6), with maximum values of aqueous shaker, acidic shaker, acidic sonication, DCM sonication, and methanol sonication, being significantly higher than their respective controls. These high IM are

Table 6
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 inorganic and organic extracts.

Sediment extract Concentrations (g/L)	Site	MI	CA	MN
Control distilled water		39.77 ± 4.41	0.55 ± 1.36	0.07 ± 0.09
MMS		55.54 ± 1.42	32.57 ± 0,30[a]	5.90 ± 1.77[a]
Aqueous shaker	S1	45.78 ± 7.20	1.31 ± 2.14	0.35 ± 0.22[a]
(500)	S2	49.74 ± 3.14[a]	2.60 ± 3.97	0.45 ± 0.35[a]
	S3	60.14 ± 6.79[a]	11.49 ± 6.67[a]	2.88 ± 1.95[a]
	S4	50.58 ± 6.26[a]	5.20 ± 3.58[a]	1.03 ± 0.32[a]
Control acidic solution		45.09 ± 7.89	0.00 ± 0.00	0.04 ± 0.09
MMS		62.21 ± 1.87[a]	18.38 ± 5.93[a]	5.38 ± 1.40[a]
Acidic shaker	S1	66.88 ± 11.35[a]	7.77 ± 7.53[a]	3.51 ± 2.09[a]
(50)	S2	53.41 ± 7.29	7.80 ± 4.84[a]	2.39 ± 1.49[a]
	S3	58.97 ± 5.21[a]	9.33 ± 9.99[a]	4.19 ± 2.55[a]
	S4	51.19 ± 8.74	8.89 ± 6.31[a]	1.71 ± 1.03[a]
Acidic sonication	S1	55.50 ± 6.07[a]	3.63 ± 1.34[a]	2.71 ± 1.52[a]
(50)	S2	60.27 ± 5.43[a]	3.17 ± 4.81[a]	1.05 ± 0.39[a]
	S3	49.69 ± 5.39	5.06 ± 4.24[a]	1.34 ± 1.55[a]
	S4	52.58 ± 8.48	3.91 ± 5.43[a]	1.61 ± 0.57[a]
Control DMSO		46.59 ± 3.44	0.00 ± 0.00	0.21 ± 0.14
MMS		46.91 ± 11.433	35.10 ± 2.50[a]	1.30 ± 0.40[a]
DCM sonication	S1	54.79 ± 5.03[a]	7.06 ± 5.91[a]	3.50 ± 0.97[a]
(25)	S2	54.14 ± 3.15[a]	6.69 ± 3.94[a]	4.05 ± 1.48[a]
	S3	55.32 ± 3.59[a]	16.52 ± 10.81[a]	3.96 ± 1.23[a]
	S4	54.82 ± 6.40[a]	0.38 ± 0.99	0.68 ± 0.38[a]
Methanol sonication	S1	42.73 ± 2.78[a]	4.18 ± 4.22[a]	0.58 ± 0.27[a]
(25)	S2	53.69 ± 1.91[a]	2.29 ± 3.58[a]	0.87 ± 0.27[a]
	S3	48.40 ± 2.88	1.72 ± 2.16[a]	0.51 ± 0.14[a]
	S4	46.28 ± 5.71	3.41 ± 3.52[a]	0.85 ± 0.36[a]

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

Table 5
Analysis of *Salmonella* mutagenicity assay in the absence (−S9) and presence (+S9) of metabolic activation of the inorganic and organic extracts. Mean of number of revertants/plate and standard deviation (S.D.).

Sediment extract Concentrations (g/L)	Site	TA 98 (-S9)	TA 98 (+S9)	TA 100 (-S9)	TA 100 (+S9)
Control distilled water		45 ± 5	23 ± 6	151 ± 15	156 ± 12
Aqueous shaker	S1	36 ± 5	19 ± 2	138 ± 7	131 ± 17
(500)	S2	37 ± 2	19 ± 3	159 ± 15	160 ± 17
	S3	32 ± 1	26 ± 5	132 ± 9	122 ± 6
	S4	36 ± 5	25 ± 9	142 ± 6	177 ± 17
Control acidic solution		27 ± 2	46 ± 5	217 ± 1	258 ± 3
Acidic shaker	S1	25 ± 2	44 ± 8	177 ± 9	203 ± 26
(500)	S2	26 ± 2	46 ± 8	158 ± 21	197 ± 52
	S3	25 ± 3	45 ± 10	197 ± 11	212 ± 56
	S4	26 ± 5	34 ± 7	215 ± 44	235 ± 15
Control acidic solution		40 ± 4	46 ± 5	103 ± 4	126 ± 12
Acidic sonication	S1	34	44 ± 5	105 ± 7	125 ± 15
(500)	S2	30 ± 12	51 ± 5	107 ± 4	124 ± 1
	S3	42 ± 11	31 ± 6	93 ± 12	116 ± 8
	S4	19 ± 7	47 ± 2	101 ± 12	119 ± 15
Control DMSO		33 ± 7	23 ± 3	157 ± 7	237 ± 22
DCM sonication	S1	24 ± 2	19 ± 2	143 ± 18	467 ± 15[a]
(2500)	S2	27 ± 1	31 ± 1	175 ± 7	454 ± 12[a]
	S3	18 ± 8	19 ± 4	135 ± 6	447 ± 23[a]
	S4	26 ± 5	22 ± 7	67 ± 8	423 ± 28[a]
Control DMSO		29 ± 9	23 ± 3	120 ± 6	237 ± 22
Methanol sonication	S1	30 ± 1	24 ± 1	126 ± 8	211 ± 22
(2500)	S2	27 ± 9	25 ± 3	99 ± 11	207 ± 16
	S3	30 ± 4	25 ± 2	87 ± 7	91 ± 30
	S4	41 ± 4	22 ± 2	120 ± 14	240 ± 30
2AF (10 µg per plate)[b]		–	320 ± 14[a]	–	798 ± 13[a]
SAZ (5 µg per plate)[c]		–	–	1200 ± 110[a]	–

[a] Positive results: the number of revertants/plate in sample is two times or greater than the spontaneous revertants/plate in the control (Mortelmans and Zeiger, 2000).
[b] Positive control: 2-aminofluorene
[c] Positive control: sodium azide

results of an increase in cell division, which can be harmful to the cells, leading to a disordered cell proliferation and even to the formation of tumor tissues.

On the other hand, all the extracts from the four sites were genotoxic (Table 6). The acidic extracts were generally more genotoxic than the aqueous, and the organic DCM were more genotoxic than methanol extracts. Comparing the acidic extracts, shaker was more genotoxic than sonication: CA =7.77 ± 7.53–9.33 ± 9.99, and 3.17 ± 4.81–5.06 ± 4.24, respectively; MN =1.71 ± 1.03–4.19 ± 2.55, and 1.05 ± 0.39–2.71 ± 1.52, respectively (Table 6). However, no positive statistical differences between genotoxicity parameters and metal concentrations were observed (Table S5). The correlation analysis showed that some PAHs were negatively correlated with MN frequencies in DCM extract (Table S6): fluoranthene (R=−0.95, p=0.04), benzo(a)anthracene (R=−0.99, p=0.01), chrysene (R=−0.97, p=0.03), benzo(k)fluoranthene (R=−0.99, p=0.01), benzo(a)pyrene (R=−0.99, p=0.01), indeno(1,2,3-cd)pyrene (R=−0.99, p=0.01), and benzo(g, *h*, i) perylene (R=−0.99, p=0.01).

4. Discussion

4.1. Inorganic extracts

In the inorganic extracts of the sediment samples, heavy metals are a class of priority compounds in the definition of probable contaminants of anthropogenic origin. Some trace metals are considered to be essential for living organisms, e.g. they facilitate electron transfer reactions, catalyze enzymatic reactions and take part in the structural functions of nucleic acid metabolism, but they may be toxic or mutagenic in excessive amounts (Borboa and La Torre, 1996; López et al., 1998; Fedorova et al., 2007; Nagajyoti et al., 2010). The comparison of methodologies for inorganic compound extraction in the present study identified the largest total concentration of metals in acidic extracts submitted to extraction by ultrasound, followed by acidic extracts prepared by shaking. These results were different from those obtained by Rodrigues da Silva Júnior et al. (2009) in soil extracts, in which the acidic extracts followed by the aqueous extracts in shaker identified more total concentrations of metals than the acidic sonication extraction. The high concentration of total metals is due to the high concentration of lead and zinc.

The aqueous extracts inhibited root elongation in L. *sativa*, except in S1, but they did not inhibit algal growth. The differences observed in both tests were probably due to the addition of algal nutrients in the extracts, as indicated by the algal protocol, whereas no nutrients were added in the L. *sativa* test, so the algal nutrients probably superimposed the toxic effect of metals. On the contrary, *P. sucapitata* showed more sensitivity than the lettuce in the acidic extract tests. One explanation could be that the extracts were evaluated in a dilute concentration (10%) in the latter bioassay due to the toxicity of the acidic extraction solution and so the metals were also diluted in the test. Thus, the maximum concentrations of Zn, Cr, Pb and Cu in the algal assay were 5.470, 0.495, 0.075, and 0.825 mg/L, respectively, whereas in the L. *sativa* test they were 2.188, 0.198, 0.030, and 0.330 mg/L, respectively. According to data in the literature, some metal concentrations in the extract were higher than the EC50 values for *P. subcapitata*: Zn =0.05–0.31 mg/L, Cr =0.04–0.90 mg/L, Pb =0.26–0.59 mg/L, and Cu =0.02–0.94 mg/L (Blaise et al., 1998; Magdaleno et al., 2014). On the other hand, Dutka (1996) reported Zn–EC50=11.36 mg/L for L. *sativa*. The inhibition of root elongation in lettuce could be due to the synergistic effects between the metals in the aqueous and acidic extracts, as was observed in experiments on soil samples.

None of the inorganic extracts were mutagenic for *S. typhimurium*. According to Codina et al. (1995) and, the metals Zn, Pb and Cu are not mutagenic to TA98 and TA100 strains, whereas Cr hexavalent is genotoxic at 0.020 mg/plate concentration. In this study the highest Cr concentration (0.099 mg/plate) was obtained in the acidic extracts of S4. However, no revertant colonies were observed (Table 5). On the contrary, *A. cepa* showed genotoxicity in all the inorganic extracts. In general the acidic shaking extracts were the most genotoxic. The metals Zn, Cr, Pb and Cu could cause breaks in the chromosomes, delays and bridges, and increases in the frequency of MN (Borboa and De La Torre, 1996; Inceer et al., 2000; Matsumoto et al., 2006). However, no correlations were found between genotoxic parameters and metals. Other metals not analyzed in this study, such as Hg, Cd and Ni, could also lead to induce aneuploidy (Fiskesjo, 1988; Borboa and De La Torre, 1996). The elevated frequencies of CA indicate aneuploidy as a consequence of abnormal segregation of chromosomes, which can occur either spontaneously or by the action of aneugenic agents (Leme and Marin-Morales, 2009). According to ACUMAR (2012), Ni and Hg were found in high concentrations in the sediments of the Matanza-Riachuelo basin (Ni =21.7–99.7 mg/kg and Hg =1.3–6.5 mg/kg). So, the presence of Ni and Hg in sediment extracts and also the synergistics effects between metals inducing genotoxicity should not be discarded.

The battery of bioassays demonstrated sensitivity in detecting toxic and genotoxic effects of sediment inorganic extracts. Some changes in the environment, such as the pH variations, could mobilize some metals retained in the sediment into the water column. Then, the extraction techniques in combination with bioassays may serve to assess the potential risk that these contaminants exert in the environment.

4.2. Organic extracts

The greatest number of compounds identified was measured in the most polluted site S4 as expected. At this site, DCM extracted more PAH compounds (eleven) than methanol (five), although the latter extracted greater concentrations with respect to DCM. When comparing the total concentration of the PAHs in S4, few differences between the two solvents were observed. On the other hand, concentrations of carcinogenic and total PAHs were higher in the methanol extract than in the DCM extract in S2. These results indicate that both solvents may be used complementarily as extraction parameters, although the possibility of using a mixture of both solvents could not be excluded.

Two organic extracts showed inhibition of algal growth and, as expected, the *%I* values increased from S1 to S4, except in S4 in methanolic extract. On the other hand, the methanolic extracts were more toxic than DCM extracts, probably due to the highest concentrations of PAHs. When the two toxicity bioassays were compared, *P. subcapitata* was more sensitive than L. *sativa*. Only 8 compounds on the list of the 16 PAH compounds as priorities by the EPA are carcinogenic (IARC, 2011) and require the presence of an activation system so that the resulting metabolites show their effect (Courty et al., 2008; Watanabe et al., 2005, 2008). Several authors have reported the mutagenic effects of PAHs using an Ames test with the addition of the microsomal fraction. The strains TA98 and TA100 are capable of detecting metabolites of pro-mutagens by frame shifts and base pair substitution, respectively (Ames et al., 1973; Isono and Yourno, 1974; Mortelmans and Zeiger, 2000). Thus, the positive mutagenic response in the organic extracts with the TA100 strain indicates the presence of mutagens whose mechanism of action is the replacement of base pairs."

On the other hand, the *A. cepa* test is sensitive to the presence of PAHs in environmental samples, such as industrial effluents (Odeigah et al., 1997), river surface water contaminated by petroleum hydrocarbons (Leme and Marin-Morales, 2008), and complex mixtures of hydrocarbons (Leme et al., 2008). In the present study, the two organic extracts were genotoxic to *A. cepa* in all the samples, the DCM extracts being generally more genotoxic than the methanol extracts. However, the correlation analysis showed that some PAHs were negatively correlated with MN frequencies in the DCM extract. Although there are few studies about the synergistic and/or antagonistic effects of

PAHs on the mutagenicity, Cherng et al. (1996) reported antagonistic effects of nitropireno in binary mixtures with coronene, benzo(g, h, i perylene, benzo(e)pyrene, dibenzo(a,h)pyrene, benzo(a)pyrene, pyrene, naphthalene and chrysene. In the present study, genotoxicity is clearly observed and so synergistic effects probably occurred.

The extraction of various organic contaminants, such as PAHs, from sediments in combination with the detection of toxic and genotoxic effects, may serve to assess the potential risk that these contaminants exert in biotic populations and human health.

5. Conclusion

In this study different extraction techniques were used to assess the toxicity and genotoxicity of sediments from a highly polluted river by obtaining five extracts (three inorganic and two organic) and using two toxicity bioassays and two genotoxicity tests. Extraction with acidic solution using the shaker method is more effective in detecting toxicity in algae and genotoxicity in *A. cepa*, but the sonicated method is more effective according to the metal concentrations. The two organic solvents are also complementary. Methanolic extracts are more effective in detecting toxicity in *P. subcapitata*, whereas DCM extracts are more effective in detecting genotoxicity in the Ames and *A. cepa* tests. When considering the four tests used in this study, *P. subcapitata* and *A. cepa* were the most sensitive assays. However, the application of parameters, such as type of solvent and extraction procedures, together with different bioassays offers an attractive approach to the problem of evaluating pollution in the Matanza-Riachuelo River. This study showed that the implementation of different extraction methods together with a battery of bioassays could be suitable tools for detecting toxicity and genotoxicity in sediment samples.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2016.09.024.

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