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Biomonitors to evaluate the toxic potential of urban solid waste landfill leachate

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ABSTRACT

The accentuated increase in the production of solid urban waste (SUW) and the consequent accumulation of leachate in landfills increase the risk of environmental contamination. Biomonitors are used to assess the toxicity of pollutants on living organisms. In this study, the pollutant potential of leachate from SUW from a deactivated landfill was evaluated by bioassays with *Lactuca sativa* L. and *Lycopersicon esculentum* Mill., and the relationship between chemical characteristics of the effluent and biological parameters was analyzed. The effluent was tested in its raw form and diluted in distilled water at concentrations of 75 and 50%. The percentage of germination, root growth (RG), mitotic index (MI), chromosomal abnormalities index (CAI), and micronuclei frequency (MCN) were analyzed. In the presence of effluent, germination and MI decreased, while RG, CAI and MCN frequencies increased in relation to the negative control (distilled water) for both species. Lead, iron and zinc presented negative relation with seed germination for both species, with RG of *L. sativa* and MI of *L. esculentum*, as well as a positive relation with MCN frequency in the studied species. Because of its larger chromosomes, *L. sativa* is a more suitable biomonitor of SUW leachate toxicity than *L. esculentum*. Even though the landfill is deactivated, it is necessary to treat this effluent, in order to minimize environmental impacts.

Keywords: bioassay, effluent, toxicity.

Biomonitores para avaliação do potencial tóxico do lixiviado de aterro de resíduos sólidos urbanos

RESUMO

O aumento acentuado da produção de resíduos sólidos urbanos (RSU) e o consequente acúmulo de lixiviado em aterros potencializam o risco de contaminação do meio ambiente. Biomonitores são utilizados para avaliação da toxicidade dos poluentes sobre os organismos vivos. Neste estudo, foram avaliados o potencial poluidor do lixiviado de RSU de um aterro desativado por bioensaios com *Lactuca sativa* L. e *Lycopersicon esculentum* Mill. e a relação entre características químicas do efluente e os parâmetros biológicos analisados. O efluente foi testado na sua forma bruta e diluído em água destilada, nas concentrações de 75 e 50%. Foram analisados o percentual de germinação, o crescimento radicular (CR), os índices mitótico (IM) e de anomalias cromossômicas (IAC), e a frequência de micronúcleos (MCN). Na presença do efluente, a germinação e o IM diminuíram, enquanto que o CR, IAC e a frequência de MCN aumentaram em relação ao controle negativo (água destilada) para ambas as espécies. Chumbo, ferro e zinco apresentaram relação negativa com a germinação das sementes de ambas as espécies, com o CR de *L. sativa* e com o IM de *L. esculentum*, além de uma relação positiva com a frequência de MCN nas espécies estudadas. *L. sativa*, por apresentar cromossomos maiores, é uma biomonitora de toxicidade de lixiviado de RSU mais adequada do que *L. esculentum*. Mesmo que o aterro esteja desativado, é necessário o tratamento deste efluente, com vistas a minimizar os impactos ambientais.

Palavras-chave: bioensaio, efluente, toxicidade.

1. INTRODUCTION

Population growth, together with technological development and increased consumption, lead to a rise in solid urban waste (SUW). In Brazil, the generation of SUW in 2016 totaled approximately 78.3 million tons (ABRELPE, 2016). The need for large expanses of land for final waste disposal and the possibility of environmental contamination make SUW management a major challenge for city administration (Rosa *et al.*, 2017).

Landfills remain the most common SUW disposal practices, due to simple execution and relatively low cost (Yang *et al.*, 2014). Landfills can be classified as sanitary, controlled and dumps. Sanitary landfills employ technology to minimize the environmental impact and possible human health risks, using things like composite liners that act as a low-permeable barrier, preventing liquid from leaching. At controlled landfills, the waste is simply covered with earth, with no leachate or biogas collection or processing. At dumps, the waste is left exposed on the ground (IBAM, 2001).

According to the most recent outlook on solid waste in Brazil, from 2016, conducted by the Brazilian Association of Public Cleaning and Special Waste Companies (ABRELPE), of all the waste collected in Brazilian municipalities, 58.4% is sent to sanitary landfills and 41.6% to controlled landfills and dumps (ABRELPE, 2016). This is despite Law 12,305/2010, which instituted the National Policy on Solid Waste, determining an end to controlled landfill and dump activities, as these means of disposal are considered incompatible with environmental protection. A maximum period of four years from the date of sanctioning was established for replacement with sanitary landfills.

Leachate, the byproduct resulting from the breakdown of solid waste, calls for closer attention due to its variability and the complexity in treating it, not to mention the myriad environmental impacts it can cause. Leachate can contain high levels of metals, suspended particles and organic matter. Leaching occurs in operating landfills, but also once they have been deactivated, as the organic matter continues to degrade and breakdown (Rosa *et al.*, 2017).

Characterizing the leachate is an important factor in establishing a strategy to effectively manage it (El-Fadel *et al.*, 2002), as the chemical composition tends to vary greatly (Budi *et al.*, 2016). The characteristics depend on the type of deposited waste, the means of disposal, management and length of time the landfill has existed. They are also greatly influenced by meteorological factors, among which rainfall volumes and air temperature are important (El-Fadel *et al.*, 2002). Due to the highly soluble substances and toxic composites, beside soil contamination, leaching may lead to the contamination of groundwater and aquifers in areas surrounding landfills, negatively impacting the environment and public health (Rosa *et al.*, 2017). CONAMA Resolution 397/2008 of Brazil's National Council on the Environment (CONAMA, 2008) establishes the conditions and standards for disposing of this class of effluents.

Leachate toxicity tests can be conducted by means of bioassays using plant species sensitive to toxicity of complex composites, although studies are scarce (Silva *et al.*, 2015; Budi *et al.*, 2016). Among the leading species for conducting toxicity bioassays evaluating seed germination, root growth, cell division and chromosome behavior and structure organization are *Lactuca sativa* L. (lettuce) and *Lycopersicon esculentum* Mill. (tomato), as they constitute species sensitive to toxic agents and germinate rapidly, while are also easily found in stores throughout the year at a low cost (Silva *et al.*, 2015).

Within this context, the study proposed the following objectives: (a) to assess the toxic potential of SUW leachate in a deactivated controlled landfill by means of bioassays using *L. sativa* and *L. esculentum*; (b) to identify the biomonitoring organism and biological parameter most suited to detecting effluent toxicity; (c) to verify the relation between the physical and chemical properties of the leachate and the data obtained for biological parameters.

2. MATERIALS AND METHODS

2.1. Deactivated controlled landfill

The SUW landfill, occupying an area of 4,750 m^2, is located in a municipality with a population of 22,514 inhabitants (IBGE, 2017), located in the Metropolitan Region of Porto Alegre, the capital of Rio Grande do Sul state, in the south of Brazil. The landfill remained activated for 13 years until 2005.

2.2. Collection and analysis of leachate physical and chemical parameters

A 5-liter sample of leachate was collected at the landfill in January 2017. Collection, storage, preservation and transport of the leachate sample were in full accord with criteria established by the Standard Methods for the Examination of Water and Wastewater (APHA *et al.*, 2012). The following parameters were noted: pH, temperature, electric conductivity (EC) and dissolved oxygen (DO) with the aid of the AK88-ASKO® multi-parameter meter; biochemical oxygen demand (BOD_5), by manometry (SM 5210); chemical oxygen demand (COD), by titration (SM 5220); ammoniacal nitrogen (NH_3-N), by titration (4500 NH_3); nitrite (NO_2), through ultraviolet–visible spectroscopy (SM 4500 NO_{2-}); nitrate (NO_3), through ultraviolet–visible spectroscopy (SM 4500 NO_{3-}) and concentrations of iron (Fe), zinc (Zn), lead (Pb), cadmium (Cd), nickel (Ni) and copper (Cu), by flame atomic absorption spectroscopy (SM 3111).

2.3. Bioassays

Bioassays were prepared in a laminar flow chamber in the laboratory. Leachate was used in its raw form (100% original concentration) and diluted in distilled water, at concentrations of 75% and 50%. Distilled water was used as the negative control, and for positive control, the minimum inhibitory concentration of copper (3 mg L^{-1} of $CuSO_4$) was used, equal to the lowest concentration of the metal able to inhibit root growth (Di Salvatore *et al.*, 2008). *Lactuca sativa* and *L. esculentum* seeds were germinated in Petri dishes with a diameter of 9 cm, containing a sheet of quantitative filter paper (GE Healthcare™) sterilized and moistened with 5 mL solution of the specified solutions (leachate concentrations and controls). Three dishes were prepared for each treatment and biomonitor, each containing fifteen seeds and sealed with plastic film. The dishes were kept at a temperature of 25±1°C, for a 16-hour photoperiod.

2.4. Germination

Germinated seeds in each dish were counted after two days of exposure to the treatments, according to Aguiar *et al.* (2016). Seeds showing evidence of root protrusion, visible without the aid of instruments, were considered germinated. The data were used to calculate the average of germinated seeds for each biomonitor in each treatment.

2.5. Root growth

Root growth was assessed after seven days of exposure to the treatments (Carvalho *et al.*, 2014) with the aid of a millimeter rule. Root lengths of five random seedlings per dish were measured, totaling 15 seedlings per treatment and biomonitor. Root length was measured from the hypocotyl of the seedling to the apical meristem of the root system, according to Gatti *et al.* (2004).

2.6. Mitotic index, chromosomal abnormality index and micronuclei frequency

Root tips were randomly removed from five seedlings from each Petri dish after two days of exposure, totaling 15 roots per treatment and organism. The tips were fixed in an ethanol: acetic acid (3:1 v/v) solution for 24 hours at room temperature and then transferred to 70% ethanol at 4°C. Of the 15 roots removed per treatment, the meristem region of only 10 random roots were used. The remaining material was kept in reserve. To prepare the microscopic slides, the root tips were sequentially treated for 2 minutes in distilled water, then hydrolyzed for 6 minutes in HC11N and washed again for 2 minutes in distilled water. Microscopic slides were prepared according to Cuchiara *et al.* (2012). The hydrolyzed root tips were squashed on microscopic slides and stained with 2% aceto-orcein. The number of cells in mitosis and cells with chromosomal abnormalities, and the number of micronuclei (MCN) were counted at 500 cells per root through the scanning technique, using an optic microscope (Nikon Eclipse E200) at 400x magnification. The mitotic index (MI) was calculated using the formula MI = [(number of mitotic cells/total cells)100]. The chromosomal abnormalities index (CAI) was calculated by CAI = [(number of cells with abnormality/total cells in division)100]. Micronuclei frequency was expressed as MCN/100 cells.

2.7. Statistical analysis

Data were subjected to the Shapiro-Wilk normality test and as they complied with assumption of normality, differences between the averages were analyzed by ANOVA, followed by the Duncan test. The relation between biological parameters and the metals Pb, Fe and Zn was verified using the Pearson correlation coefficient. All statistical analysis was conducted using the SPSS program, version 20.0, at 5% probability.

3. RESULTS AND DISCUSSION

The COD value was three times greater than the BOD_5 in the raw leachate sample (Table 1). BOD_5 refers to the biodegradable organic mass, while COD refers to all the oxidizable matter in the effluent (CETESB, 2016). The BOD_5/COD calculated for the raw leachate was 0.29. Due to the BOD_5/COD ratio, the landfill in the present study would be classified as moderately stable, according to the Solid Waste Association of North America (SWANA, 1997) classification. This ratio could be considered an indicator of the level of organic matter biodegradability, which diminishes over time (El-Fadel *et al.*, 2002).

The leachate presented pH levels higher than 7 (Table 1). The pH variation over the years is linked to the COD and BOD_5 values, with the pH starting at around 5.5 and increasing to around 8 within two years of the landfill initiating operations. An alkaline pH is usually found in landfills after depositing waste for 10 years (Farquhar, 1989), which would corroborate the findings of this study.

The electric conductivity (EC) value of the raw leachate sample was 1,562 $\mu S\ cm^{-1}$ (Table 1), double the average found by Riguetti *et al*. (2015), which, when analyzing the toxicity of leachate from a sanitary landfill located in the state of Minas Gerais, Brazil, recorded an EC average of approximately 630 $\mu S\ cm^{-1}$ in a period of one year. High EC indicates a large volume of ions present in the effluent. In general, levels above 100 $\mu S\ cm^{-1}$ indicate environmental impacts (CETESB, 2016).

Table 1. Physical and chemical characteristics of different leachate concentrations, with their respective detection limit.

Parameters	Leachate			Detection limit	CONAMA 397/2008
	100%	75%	50%		
pH	7.22	7.26	7.35	2.00	n.i.
Temperature (°C)	17.8	16.8	16.5	0.0	n.i.
BOD_5 (mg O_2 L^{-1})	39	40	44	5.0	n.i.
COD (mg O_2 L^{-1})	131	92	62	3.10	n.i.
EC (µS cm^{-1})	1,562	1,200	852	200	n.i.
DO (mg L^{-1})	3.2	5.9	7.4	0.0	n.i.
Ammoniacal nitrogen (mg L^{-1})	36.3	28.2	18.1	5.0	20.0
Nitrite (mg L^{-1} N in NO_2)	0.289	0.206	0.225	0.002	n.i.
Nitrate (mg L^{-1} N in NO_3)	0.2516	0.2418	3.1063	0.0785	n.i.
Fe (mg L^{-1})	1.181	0.541	0.277	0.1414	15.0
Zn (mg L^{-1})	0.017	0.020	0.018	0.0095	5.0
Pb (mg L^{-1})	0.056	0.041	0.037	0.0112	0.5
Cd (mg L^{-1})	n.d.	n.d.	n.d.	0.0075	0.2
Ni (mg L^{-1})	n.d.	n.d.	n.d.	0.0643	2.0
Cu (mg L^{-1})	n.d.	n.d.	n.d.	0.0316	1.0

n.i. = not informed by CONAMA Resolution 397/2008.

The concentration of ammoniacal nitrogen was 36.3 mg L^{-1}, considered high, according to CONAMA Resolution 397/2008 (20.0 mg L^{-1}), with this constituting the only parameter found over the limit permitted in legislation (CONAMA, 2008). To the contrary, the concentrations of DO and metals detected were low (Table 1). In the early years, young landfills produce a leachate with greater pollutant potential, due to the presence of metals in higher concentrations. The older the landfill, the higher the index of ammoniacal nitrogen due to hydrolysis and fermentation, and the lower the concentrations of organic matter and metal ions (Renou *et al.*, 2008). In the raw leachate, these ions are associated with organic and inorganic colloidal particles and in the form of complexes. The reductive conditions of the effluent and the alkaline pH lead to metal ions being complexed in the form of sulfides, sulfates, carbonates and oxyhydroxides and also precipitated in insoluble composites considerably reducing bioavailability of the metals (Riguetti *et al.*, 2015).

At its maximum concentration, leachate reduced the germination capacity of *L. sativa*, with 86.6% of the seeds germinating. Comparably, the raw leachate from a deactivated sanitary landfill located in the region of Vale dos Sinos, in the state of Rio Grande do Sul, Brazil, also presented toxicity for this species, with 90% of the seeds germinating (Klauck *et al.*, 2015). *Lycopersicon esculentum* seeds appeared to be more sensitive than the *L. sativa* seeds, with the leachate reducing the number of germinated seeds with an increase in concentration and completely inhibited germination at a 100% concentration (Table 2).

This study recorded a significant increase in root length in the presence of leachate when compared to the controls in both plant species (Table 2). The lower concentration of leachate (50%) led to a higher average in root growth in *L. sativa* (5.70 cm) and *L. esculentum* (8.28 cm). Rodrigues *et al.* (2013) also noted root growth stimulation for *L. sativa* when analyzing the water from an urban stream located in the municipality of Alfenas, in the state of Minas Gerais, Brazil, and attributed this result to a greater offer of nutrients provided by the organic load in water.

Table 2. Values (average ± standard deviation) of germination percentage (GM), root growth (RG in cm), mitotic index (MI), chromosomal abnormality index (CAI) and micronuclei frequency (MCN) in *Lactuca sativa* and *Lycopersicon esculentum* exposed to leachate and control treatments.

Parameters		Treatments				
		Leachate			Controls	
		100%	75%	50%	H_2O	$CuSO_4$
L. sativa	GM	86.66 ± 6.66 c	93.33 ± 0.00 abc	95.55 ± 3.84 ab	100.00 ± 0.00 a	88.88 ± 3.84 bc
	RG	4.24 ± 0.68 c	4.99 ± 0.85 b	5.70 ± 0.92 a	4.11 ± 1.08 c	2.00 ± 0.98 d
	MI	4.98 ± 1.13 b	5.36 ± 1.41 b	5.14 ± 1.29 b	9.58 ± 1.79 a	4.58 ± 0.52 b
	CAI	28.04 ± 11.48 b	25.68 ± 7.69 b	13.94 ± 5.72 b	13.94 ± 5.72 a	30.93 ± 8.05 b
	MCN	4.64 ± 1.23 c	3.56 ± 1.35 b	3.06 ± 0.83 ab	2.38 ± 0.51 a	4.70 ± 1.40 c
L. esculentum	GM	0	24.44 ± 16.77 c	51.11 ± 13.87 b	88.88 ± 10.18 a	66.66 ± 6.66 ab
	RG	n.a.	7.94 ± 1.44 ab	8.28 ± 1.15 a	6.83 ± 1.41 b	5.67 ± 1.95 c
	MI	n.a.	2.74 ± 0.66 c	4.24 ± 0.54 b	4.82 ± 0.45 a	2.10 ± 0.31 d
	CAI	n.a.	12.96 ± 5.95 a	7.29 ± 5.16 a	7.04 ± 2.82 a	21.63 ± 13.77 b
	MCN	n.a.	0.14 ± 0.16 b	0.04 ± 0.08 a	0.00 ± 0.00	0.06 ± 0.09 ab

Averages followed by the same letter on the line do not differ significantly in accord with the Duncan test at 5% probability / n.a. = not analyzed.

For both species studied, the MI was significantly lower in the different leachate concentrations and in the positive control than in the negative control (Table 2). In *Allium cepa*, besides recording negative effects from the leachate on the MI in five of the six municipal solid waste landfill sites analyzed in Southern Poland, a negative relation was noted between the physical and chemical quality of each leachate and their genotoxicity (Kwasniewska *et al.*, 2012), which corroborates with the results obtained in this study with *L. sativa* and *L. esculentum.* Alterations of the mitotic index are considered good indicators of environmental pollution, especially in the assessment of substances with cytotoxic potential (Leme and Marin-Morales, 2009).

In *L. esculentum*, CAI values in the leachate concentrations and in the negative control did not differ and were lower than those of the positive control. *Lycopersicon esculentum* chromosomes are smaller, as such, this characteristic complicated the analysis of chromosomal abnormalities for this plant species. Further, in the raw leachate treatment (100%), the *L. esculentum* seeds did not germinate, as such, the comparison with other plant species is jeopardized. *Lactuca sativa*, on the other hand, showed significantly higher CAI values in leachate concentrations and in the positive control, when compared to the negative control (Table 2). Chromosomal abnormalities (CA) are changes that occur in the chromosome structure through the action of chemical and physical agents or can even occur spontaneously (Leme and Marin-Morales, 2009).

The quantified CA were constituted by C-metaphase, isolated chromosome, or anaphase and telophase with bridges (Figure 1). A break down in the spindle apparatus is responsible for aneugenic alterations resulting from disturbances in spindle activity. The chromosomes are not correctly linked to the spindle and do not take on the correct position in the cell and, thus, they are spread throughout it internally instead. The chromosomal bridges are clastogenic alterations observed in anaphase and telophase and occur due to a loss of telomeres, the region responsible for guaranteeing chromosome protection and stability, preventing erroneous fusions and pairings (Leme and Marin-Morales, 2009).

The occurrence of MCN in *L. esculentum* was low, with the negative control not presenting them. In *L. sativa*, greater MCN frequency was noted, even registering MCN in the negative control (2.38 MCN). The highest frequencies were found when plants were exposed to raw leachate (4.64 MCN) and the positive control (4.70 MCN). We may infer that the MCNs found in the negative control form spontaneously. For other treatments, the higher MCN frequency in relation to the control shows the toxic effect of leachate and $CuSO_4$. The MCNs are tiny cell structures formed through chromosomal loss or chromosomal fragmentation caused by CA and also due to the structural alteration in the spindle apparatus fibers, which can be induced by genotoxic agents or even may be a result of spontaneous mutation (Kieling-Rubio *et al.*, 2015).

The $CuSO_4$ was shown to be a good positive control, as it was able to damage both plant species, observed by means of reduced germination and root growth, by the reduced number of cells in mitosis and also by the stimulation and increase of CA and MCN frequency (Table 2). Despite copper figuring as an essential micronutrient in plant development, in elevated concentrations it becomes toxic, inhibiting several plant metabolism processes (Kabata-Pendias, 2011).

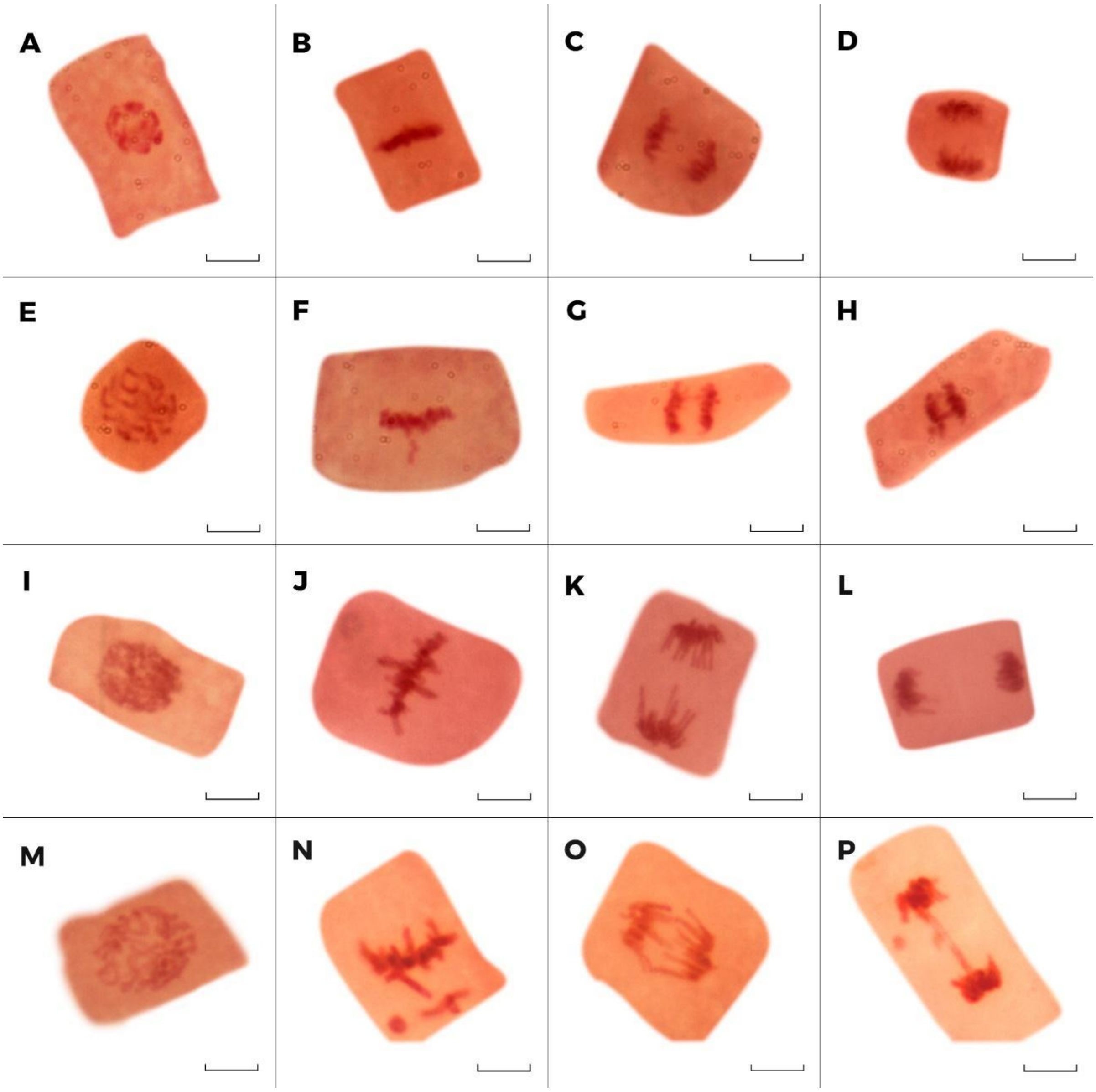

Figure 1. Chromosomes with and without abnormalities observed in *Lycopersicon esculentum* (A - H) and *Lactuca sativa* (I – P) root meristematic cells exposed to different leachate concentrations. (A and I) prophase; (B and J) metaphase; (C and K) anaphase; (D and L) telophase; (E and M) C-metaphase; (F and N) metaphase with isolated chromosome; (G and O) anaphase with bridge; (H and P) telophase with bridge and MCN. Bar: 10 µm

L. esculentum germination and MI related negatively with Pb, Fe and Zn ($r=-0.728$; $p<0.001$), ($r=-0,757$; $p<0.001$), respectively, while the frequency of MCN was positively related to the same metals ($r=0.449$; $p=0.013$). In *L. sativa*, germination was also negatively related to Pb and Fe ($r=-0.720$; $p<0.001$). Root growth showed a negative relation with Pb ($r=-0.571$; $p<0.001$) and Fe ($r=-0.585$; $p<0.001$), while the frequency of MCN related positively to Pb ($r=0.570$; $p<0.001$) and Fe ($r=0.582$; $p<0.001$). Despite being considered essential micronutrients in plant metabolism (Kabata-Pendias, 2011), when Fe and Zn are present in elevated concentration in the environment, they may induce the formation of micronuclei (Kieling-Rubio *et al.*, 2015) and reduce germination and root growth (Wang *et al.*, 2011). Lead does not play an essential role in plant metabolism and it is also considered one of the most toxic metals to them. Its toxic effects are chiefly related to fundamental biological process damage, such as photosynthesis, growth and mitosis (Kabata-Pendias, 2011). This metal may delay or inhibit plant germination, as described for wheat (Lamhamdi *et al.*, 2011), while also interfering in root growth (Moraes *et al.*, 2014). The relation found between the formation of MCN and Pb was also observed in *Vicia faba* root cells, with MCN frequency used as one of the parameters to determine the toxic potential of Pb (Pourrut *et al.*, 2011). The reduction in cell division induced by Pb may be associated to this metal's link to the cell wall and membranes, increasing rigidity and defects in microtubule organization. As a result, malformation of the microtubules can lead to an increase in the number of chromosomal abnormalities (Jiang *et al*., 2014).

4. CONCLUSIONS

The physical and chemical results indicate that the controlled landfill is at the methanogenic stage. Based on the analysis of biological parameters in different leachate concentrations, one may conclude that the effluent presents potential toxicity for *L. sativa* and *L. esculentum,* as it reduced the germination percentage and the number of mitotic divisions in both plant species, while also promoting the formation of chromosomal abnormalities and micronuclei, especially in *L. sativa.* The rise in root growth may be associated with the volume of organic matter present in the effluent. Biological parameters that best responded to the effluent toxicity were seed germination and the number of mitotic divisions, along with MCN frequency for *L. sativa.* The reduced index of CA and MCN in *L. esculentum* may be associated with the small size of chromosomes in comparison with *L. sativa,* thus jeopardizing visibility. It is also possible to infer that both plant species demonstrated being efficient biomonitors of environmental quality by showing evidence of sensitivity to the analyzed variables. However, *L. sativa* showed a more significant treatment x response relation in all analyzed biological parameters, which allowed for a unique interpretation of the results from specific leachate treatments. The relation noted between germination, root growth, MI and MCN frequency indicated a negative influence on the species studied from Pb, Fe and Zn present in the effluent. This relation reiterates the importance of associating physical and chemical analysis with an assessment of biological parameters, by means of assays using environmental quality biomonitors. Based on these findings, one may suggest that, even with a controlled SUW landfill deactivated for over a decade, there is a need to treat this effluent, with the goal of minimizing possible impacts on the environment.

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