Chemosphere 208 (2018) 257–262

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Mutagenic effects of spent potliner and derivatives on *Allium cepa* L. and *Lactuca sativa* L.: A molecular approach

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HIGHLIGHTS

- Spent Potliner (SPL) is a solid residue from the aluminum industry.
- Fluoride, cyanide and aluminum are the main toxic components of SPL.
- SPL modifies the DNA fingerprints identified from ISSR and SSR markers.
- SPL and its derivatives present mutagenic potential.

ARTICLE INFO

Article history:
Received 15 February 2018
Received in revised form
24 May 2018
Accepted 29 May 2018
Available online 30 May 2018

Handling Editor: A. Gies

Keywords:
Molecular markers
SSR
ISSR
Environmental mutagenesis
Fingerprint

ABSTRACT

Spent potliner (SPL) is a solid residue generated by the aluminum industry. Its composition is variable and complex, containing fluoride and cyanide salts as well as aluminum, which contributes to its toxicity. SPL is sometimes released directly into the soil, where it is prone to leaching and has the potential to cause alterations and damage to DNA. Considering that polymorphism analysis of simple sequence repeat (SSR) and inter-simple sequence repeat (ISSR) DNA markers is an interesting tool to determine the mutagenicity of an environmental pollutant, the present study adopted this approach to verify the mutagenic potential of SPL and its main toxic components (aluminum, fluoride, and cyanide) on root tip cells of *Lactuca sativa* and *Allium cepa*. Alterations in ISSR and SSR regions were identified by DNA fingerprinting (gain and loss of bands and changes in band intensity). The estimated dissimilarities indicated differences between treatments and the negative control. Furthermore, the relationship between the amplification profile of the markers and alterations in cell mitosis was discussed.

1. Introduction

Spent potliner (SPL) is a solid residue generated by mining and bauxite-processing industries in the production of metallic aluminum. It consists of refractory bricks of cryolite that line the electrolytic pots where aluminum reduction takes place (Silveira et al., 2003). Over the years, this process erodes the conducting material, and various substances aggregate into the pot lining, which must then be replaced (Lisbona and Steel, 2008). The material to be discarded is SPL, classified as toxic by the United States Environmental Protection Agency (US EPA), particularly because it contains fluoride and cyanide salts as well as aluminum (US EPA, 1991; Silveira et al., 2003).

At present, most of the SPL produced worldwide is used as raw material in other industries; for instance, in the production of Portland cement (Andrade et al., 2008). For many years, however, more than one million tonnes of SPL produced by the aluminum industry were released into the environment (Lisbona and Steel, 2008). The irregular disposal of this residue is a serious concern, as it is susceptible to leaching and may intersperse in soil layers, contaminating the water table and water bodies (Chandra et al., 2005; Andrade et al., 2008).

Therefore, the importance of studies on the effects of SPL on

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https://doi.org/10.1016/j.chemosphere.2018.05.186
0045-6535/

living organisms becomes evident. Our research group has been pioneering the investigation of the impact of SPL using different biological models, associating its effects with the chemical composition of SPL solutions (see works by Andrade et al., 2008, 2010; Andrade-Vieira et al., 2011, 2012a; Palmieri et al., 2014, 2016a, b).

Chemical analyses of SPL solutions simulating the natural leaching of the residue into the soil have demonstrated that SPL contains heavy metals, such as iron, chromium, zinc, manganese, and aluminum (Andrade et al., 2008). The presence of fluoride-derived compounds has also been identified. All elements detected in the leached solutions were at concentrations above those allowed by the Brazilian environmental legislation (Palmieri et al., 2014). Additionally, the leached SPL solutions were shown to cause DNA damage or alterations (see works by Andrade et al., 2008, 2010; Andrade-Vieira et al., 2011, 2012a; Palmieri et al., 2014, 2016a,b).

In environmental toxicology, the damage caused to DNA by a given pollutant can be evaluated via cell cycle and micronuclei formation assays (Leme and Marin Morales, 2009; Andrade-Vieira, 2012). These assays evidence the final effects or the consequences of primary damage to DNA, which can be detected using molecular markers. In this perspective, sequence repeat (SSR) or inter-simple sequence repeat (ISSR) analyses have shown to be efficient in verifying DNA damage caused by toxic substances (Qari, 2010; Bernardes et al., 2015).

Thus, the present study aimed to evaluate the effects of SPL and its components, aluminum (Al), fluoride (Fl), and cyanide (CN), on DNA via a molecular approach by mapping SSR and ISSR markers in the plant models *Lactuca sativa* and *Allium cepa*, which are suitable for determining the cytogenotoxicity of environmental pollutants (Silveira et al., 2017).

2. Material and methods

Seeds of the plant models *L. sativa* 'Aurélia Manteiga' and *A. cepa* 'Texas Early Grano' were germinated on filter paper moistened with distilled water. Upon reaching a length of approximately 3 mm, the roots of the germinated seeds were treated with 15 mL of the test solutions (SPL, Al, Fl or CN).

The solutions were prepared according to Palmieri et al. (2016a, b) by dissolving solid SPL (granulometry less than 1 mm), potassium cyanide (KCN), sodium fluoride (NaF), or potassium aluminum sulfate ($KAl(SO_4)_2$) in 0.01 M $CaCl_2$. The concentration of SPL used in this study was its IC_{50} (the concentration of SPL that inhibited 50% of *L. sativa* root growth in developing seedlings), as determined by Palmieri et al. (2014). The concentrations of Al, CN, and Fl were determined based on the concentration of each element in SPL at its IC_{50} (Table 1) (Palmieri et al., 2014). Distilled water was used as negative control.

Table 1
Concentrations of the solutions applied in root tip cells of *Allium cepa* and *Lactuca sativa*.

Treatments solutions	½ IC_{50}	IC_{50}[b]	$^3/_2$ IC_{50}
SPL	13.25 g/L	26.5 g/L	39.75 g/L
Aluminum	0.0016 mg/L	0.0046 mg/L	0.0012 mg/L
Fluoride	0.0577 mg/L	0.3938 mg/L	0.435 mg/L
Cyanide	0.0015 mg/L	0.0031 mg/L	0.0045 mg/L
[a]**Refer as concentration**	1	2	3

[a] The concentration of each treatment was codified with the abbreviation of the treatment following for the numbers 1, 2 or 3 to define if it refers to the concentration for ½ IC_{50}, IC_{50} or $^3/_2$ IC_{50}, respectively. For example, SPL1, referring the concentration of SPL found in the ½ IC_{50}, which is 13.25 g/L.

[b] IC_{50}: concentration responsible for the inhibition of 50% of the root growth in developing seedlings according to Palmieri et al. (2014).

The experiment was carried out in a completely randomized design (CRD) with five repetitions (Petri dishes) of each treatment (SPL, Al, F, CN, and distilled water) for each species (*A. cepa* and *L. sativa*). Each dish contained 50 seeds and was maintained in a biochemical oxygen demand (BOD) incubator at 24 °C for 48 h.

After the treatment period, segments of about 1 cm were excised from the root tips for DNA extraction, following the protocol established by Doyle and Doyle (1990) and modified by Bernardes et al. (2015).

Integrity of the genomic DNA was verified in 0.8% agarose gel stained with 1% ethidium bromide (10 mL), whereas quantity and quality were determined via spectrophotometry. For each treatment, two to four DNA samples with an A260/A280 ratio approximate to 1.8 were used.

The following ISSR primers (University of British Columbia - UBC) were used for the amplification: 807, 810, 812, 836, 840, 842, 864, and 878 for *L. sativa* and 807, 808, 809, 818, 834, 884, and 886 for *A. cepa*. Furthermore, ten SSR primers described by Jakse et al. (2005) and Martin et al. (2005) were used for *A. cepa*.

PCR reactions were carried out using the PCR Master Mix kit (Thermo Scientific). Each reaction was performed in a total volume of 18.0 μL, containing 40 ng of DNA, 0.5 μM primer, and 1 U of *Taq* DNA polymerase, under the conditions described by Bernardes et al. (2015).

To analyze the mutagenic effects of the exposure of *L. sativa* and *A. cepa* cells to SPL, Al, F, and CN, the following codes were applied after SSR amplification: 11 and 22 for a homozygous locus, 12 and 13 for a heterozygous locus, and 33 for the absence of a band. For the ISSR data, the code "1" was used for the presence of a band and "0" for the absence.

On the basis of the coded molecular data, the squared Euclidean distance was chosen as measurement for the SSR data, and an index of dissimilarity based on simple matching was used for ISSR data. UPGMA clustering (average linkage) analysis was subsequently performed. Analyses were carried out using the Genes software (Cruz, 2013).

3. Results

Overall, ISSR and SSR analyses evidenced differences between the band patterns of meristematic cells of *A. cepa* and *L. sativa* treated with SPL and its main toxic components and that of cells treated with the control. The modifications observed in DNA fingerprints included gain of bands, loss of bands, and reduction in band intensity (Fig. 1).

In ISSR amplification, the greatest variation in band profiles was observed for cells treated with F and Al (gain of bands), whereas SPL and CN treatments promoted the smallest variations in DNA fingerprints (Fig. 1A). *A. cepa* cells treated with SPL were the only to show gain of bands by SSR analysis. CN treatments generated the smallest number of alterations in the amplification pattern of SSR, the loss of band being more frequent than the reduction in band intensity (Fig. 1B). In the ISSR amplification of *L. sativa* cells (Fig. 1C), Al treatment led to greater band gain, followed by F treatment. Reduction in band intensity was most frequent for cells treated with SPL. Furthermore, CN induced the fewest alterations to the DNA of *L. sativa* cells by the ISSR assay.

Dissimilarities and distances indicate the number of molecular alterations detected in the DNA of treated cells in comparison with the control (Table 2). The greater the dissimilarities or distances, the more alterations were detected in relation to the control. The dissimilarity values of *A. cepa* ISSR bands varied from 0.06 (between control and SPL1) to 0.27 (between F3 and Al1). In *L. sativa*, dissimilarity values varied from 0.01 (between control and SPL2) to