Ecotoxicology and Environmental Safety 124 (2016) 426–434

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Effect of SPL (Spent Pot Liner) and its main components on root growth, mitotic activity and phosphorylation of Histone H3 in *Lactuca sativa* L.

Aline Silva Freitas, Isabela Martinez Fontes Cunha, Larissa Fonseca Andrade-Vieira, Vânia Helena Techio *

Department of Biology, Federal University of Lavras, P.O. Box 3037, 37.200-000 Lavras, Minas Gerais, Brasil

ARTICLE INFO

Article history:
Received 25 May 2015
Received in revised form
2 November 2015
Accepted 10 November 2015
Available online 23 November 2015

Keywords:
H3S10ph
Cellular changes
Fragile sites
Chromosomal alterations
Micronuclei

ABSTRACT

Spent Pot Liner (SPL) is a solid waste from the aluminum industry frequently disposed of in industrial landfills; it can be leached and contaminate the soil, sources of drinking water and plantations, and thus may pose a risk to human health and to ecosystems. Its composition is high variable, including cyanide, fluoride and aluminum salts, which are highly toxic and environmental pollutants. This study evaluated the effect of SPL and its main components on root growth and the mitosis of *Lactuca sativa*, by investigating the mechanisms of cellular and chromosomal alterations with the aid of immunolocalization. To this end, newly emerged roots of *L. sativa* were exposed to SPL and its main components (solutions of cyanide, fluoride and aluminum) and to calcium chloride (control) for 48 h. After this, root length was measured and cell cycle was examined by means of conventional cytogenetics and immunolocalization. Root growth was inhibited in the treatments with SPL and aluminum; chromosomal and nuclear alterations were observed in all treatments. The immunolocalization evidenced normal dividing cells with regular temporal and spatial distribution of histone H3 phosphorylation at serine 10 (H3S10ph). However, SPL and its main components inhibited the phosphorylation of histone H3 at serine 10, inactivated pericentromeric regions and affected the cohesion of sister chromatids, thus affecting the arrangement of chromosomes in the metaphase plate and separation of chromatids in anaphase. In addition, these substances induced breaks in pericentromeric regions, characterized as fragile sites.

1. Introduction

The aluminum mining industry and processing in the world is responsible for the production of more than fifth million tons of aluminum per year (International Aluminum Insttute, 2013). For every 1000 t produced, more than 29,000 t of Spent Pot Liner (SPL) are generated as a solid waste, often disposed of in industrial landfills (Confederação Nacional da Indústira (CNI), 2012).

SPL consists of a mixture of toxic substances, including cyanide, fluoride and aluminum salts, considered highly toxic and environmental pollutants (USEPA-United States Environmental Protection Agency, 1991). When disposed of in landfills without proper treatment, the SPL may be leached and contaminate soil, sources of drinking water and plantations, posing major risks to human health and the various ecosystems (Chandra et al., 2005; Andrade et al., 2008).

In this way, in order to determine the toxic effects of SPL in living organisms, plant bioassays with *Lactuca sativa* L. (lettuce), *Allium cepa* L. (onion) and *Zea mays* L. (maize) have been conducted (Andrade et al., 2008, 2010; Andrade-Vieira et al., 2011; Andrade-Vieira et al., 2012; Palmieri et al., 2014). These bioassays performed with higher plant test systems are widely used in research studies of effects of environmental pollutants since, in addition to being fast, inexpensive and sensitive, they are correlated with animal models (Grant, 1994).

These plant species are highly sensitive when exposed to different SPL treatments. The studies performed showed reduction in germination and root growth rates, reduced mitotic index and induction of chromosomal alterations such as chromosome bridges, c-metaphase, lost chromosomes and sticky chromosomes, and nuclear alterations, including the formation of micronuclei, condensed nuclei and polynucleated cells (Andrade et al., 2010;

Abbreviations: SPL, Spent Pot Liner; H3S10ph, phosphorylation of histone H3 at serine 10; IC_{50}, 50% growth inhibition; MI, mitotic index; CA, chromosomal alterations; NA, nuclear alterations; RG, root growth

* Corresponding author.
E-mail address: vhtechio@gmail.com (V.H. Techio).

http://dx.doi.org/10.1016/j.ecoenv.2015.11.017
0147-6513/

Andrade-Vieira et al., 2011; Palmieri et al., 2014).

However, these studies have not evaluated the effects of SPL at the DNA molecule level and molecular organization of chromosomes. Therefore, it is of great value, the use of new parameters, which provide more subsidies to elucidate the mechanisms of action of this waste and its major constituents in plant cells. Given the above, the immunolocalization is considered an additional methodology to be applied combined with the chromosome alterations tests, for better understanding of the mechanisms of action of SPL on changes related to cell cycle and to progression of mitosis, and could provide answers about the molecular mechanisms of the observed changes. Among these changes, there are problems related to the arrangement of chromosomes in the metaphase plate and separation during anaphase, which can also be explained via immunolocalization of histone H3 phosphorylated at serine 10 (H3S10ph).

Phosphorylation of serine 10 in histone H3 is involved with the cohesion of sister chromatids (Kászas and Cande, 2000; Shibata and Murata, 2004; Paula et al., 2013) and, according to Houben et al. (1999), this post-translational modification is essential for the structure of the centromere/kinetochore, required to ensure mechanical stability to the centromere in the chromosome movement. Analysis of dicentric chromosomes revealed that hyperphosphorylated H3S10 occurs only in the functional centromere (Houben et al., 1999; Han et al., 2006; Fu et al., 2012), indicating that it is an epigenetic mark of active (peri)centromeric regions (Houben et al., 2013).

In this context, this study evaluated the effects of SPL and its main components on root growth and cell cycle of *L. sativa*, by investigating the mechanisms of cellular and chromosomal alterations using immunolocalization of H3S10ph. Among the model species assessed in the presence of SPL, *L. sativa* was highly effective to determine the toxic effect of the waste. Furthermore, this species represents an efficient and advantageous model for presenting large and few chromosomes ($2n=18$) (Campos et al., 2008), rapid germination and high prolificacy.

2. Material and methods

2.1. Plant material and treatment with SPL

Seeds of *L. sativa* var. Lechuga Grandes Lagos, ISLA company, were used as model test system. The SPL used to prepare the test solutions came from an aluminum mining industry and processing located in the southern of Minas Gerais State, Brazil. The SPL was used in solid form, composed of iron oxide present in the form of goethite and aluminum oxide, with an inorganic nature by having thermal stability and ash purport of 71% (Palmieri et al., 2014). Furthermore, the SPL leachate product had basic character with a pH of 9.85.

The concentrations of SPL and its main components used as treatments on this study are shown in Table 1. They were prepared followed the absorption methods based on the IC_{50} (50% growth inhibition) according to Palmieri et al. (2014). The solutions were prepared dissolving the SPL and its main components in 0.01 M $CaCl_2$. $CaCl_2$ was used to simulate the effect of the ionic forces naturally present in the soil during the leaching (Andrade et al., 2008). The solutions were shaken at 180 rpm for 12 h and then allowed to settle for over 12 h. The procedure was repeated three times to complete 72 h, and finally the supernatant was collected and filtered (Palmieri et al. 2014).

2.2. Treatments exposure

Seeds of *L. sativa* germinated in Petri dishes with filter paper moistened with distilled water containing roots with 1-2 mm were subjected to the treatments (Table 1) in germination chamber in the dark at 24 °C, for 48 h.

Table 1
Treatments with SPL and its main components applied to seeds of *Lactuca sativa*.

Treatments	Concentrations
Negative control ($CaCl_2$)	0.01 M
SPL (IC_{50} concentration)	26.5 g/L
Aluminum solution[a]	0.004588 g/L
Cyanide solution[a]	0.0031 g/L
Fluoride solution[a]	0.3938 g/L

[a] Concentrations based on the amount of the element found in the IC_{50} SPL solution (concentration inhibiting 50% root growth), according to Palmieri et al. (2014).

2.3. Analysis of root growth

The experiment was arranged in a completely randomized design with ten Petri dishes for each treatment. Each dish contained thirty seeds of *L. sativa* placed on germination paper with 5 mL of water, totalizing 50 dishes. Root length was measured (total of 1500 roots per treatment), using a digital caliper after germination and root emergence (1 to 2 mm) and after the periods of 48 h after exposure to the treatments. Then it was calculate the average root growth of each dish by the difference in average root length before and after the exposure.

2.4. Root mitotic analysis

For mitotic analysis after the 48 h exposure, roots were collected and fixed in Carnoy (ethanol / acetic acid, 3:1) for at least 24 h. For slide preparation, the fixed roots were washed, hydrolyzed in 1N hydrochloric acid (HCl) at 60 °C for 10 min and squashed in 2% acetic orcein.

It was analyzed 10 slides per treatment and 500 cells per slide, totalizing 5000 cells per treatment. Each slide represented a repetition of a treatment, which contained 5 roots (each from a seed). The slides were examined under light microscope (Zeiss AXIO) and the different stages of mitotic division and possible chromosomal and nuclear alterations were noted. The following parameters were assessed: mitotic index (MI), calculated as the ratio of the number of dividing cells (prophase, metaphase, anaphase and telophase) to the total number of cells in each treatment; frequency of chromosomal (CA) and nuclear (NA) alterations, calculated as the ratio of the number of alterations (chromosomal and nuclear, respectively) to the total number of cells evaluated.

2.5. Statistical analysis

The mean values of growth per dish and the parameters obtained in mitotic analysis (MI, CA and NA) were subjected to analysis of variance (ANOVA) and compared by Kruskal–Wallis test in the software R (R Development Core Team, 2014).

2.6. Immunolocalization of histone H3 phosphorylated at serine 10

For immunolocalization technique, roots were collected after treatments and subjected the protocol described by Manzanero et al. (2000) with some modifications (Paula et al., 2013).

A treated root was fixed for 40 min in 4% paraformaldehyde containing 1 × PBS. Then, enzymatically treated with a cellulase: