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Cytotoxicity of Spent Pot Liner on *Allium cepa* root tip cells: A comparative analysis in meristematic cell type on toxicity bioassays

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ABSTRACT

Spent Pot Liner (SPL) is a waste generated during the production of aluminum. It is comprised of a mixture of substances most of which, like cyanide, aluminum and fluoride, are toxic. Previous studies indicate the highly toxic nature of SPL. However studies using cells of the differentiation/elongation zone of the root meristem (referred as M_2 cells in this study) after a proper recovery period in water were never considered. Using these cells could be useful to further understanding the toxicity mechanisms of SPL. A comparative approach between the effects on M_2 cells and meristematic cells of the proximal meristem zone (referred as M_1 cells in this study) could lead to understanding how DNA damage caused by SPL behaves on successive generations of cells. *Allium cepa* cells were exposed to 4 different concentrations of SPL (2.5, 5, 7.5 and 10 g L^{-1}) mixed with soil and diluted in a $CaCl_2$ 0.01 M to simulate the ionic forces naturally encountered on the environment. A solution containing only soil diluted on $CaCl_2$ 0.01 M was used as control. M_1 and M_2 cells were evaluated separately, taking into account four different parameters: (1) mitotic alterations (MA); (2) presence of condensed nuclei (CN); (3) mitotic index (MI); (4) presence of micronucleus (MCN). Significant differences were observed between M_1 and M_2 roots tip cells for these four parameters accessed. M_1 cells was more prompt to reveal citogenotoxicity through the higher frequency of MA observed. Meanwhile, for M_2 cells higher frequencies of MCN and CN was noticed, followed by a reduction of MI. Also, it was possible to detect significant differences between the tested treatments and the control on every case. These results indicate SPL toxic effects carries on to future cells generations. This emphasizes the need to properly manage this waste. Joint evaluation of cells from both M_1 and M_2 regions was proven valuable for the evaluation of a series of parameters on all toxicity tests.

1. Introduction

When aluminum is being produced, during the process of bauxite purification and refining, a solid waste composed by a series of substances accumulates in the vats. This waste is called Spent Pot Liner (SPL) and is comprised of various chemicals, amongst them cyanide, fluoride and heavy metals (Silveira et al., 2002). Annually approximately one million tons of this waste is produced worldwide (Lisbona and Steel, 2008).

There are no effective options to deal with the great amounts of this waste produced. It is often deposited in open areas and represents serious environmental risks: SPL may leach trough the action of rainwater and contaminate natural environments like bodies of water, plants and wildlife (Chandra et al., 2005; Andrade-Vieira et al., 2012). Therefore studding the effects of this waste is of great concern in order to develop contingency plans in cases of biological hazards as well as environmental prevention measures.

One way to achieve this goal is through the cell cycle analysis, which is a tried and true cytogenetic assay. When a living organism is exposed to a toxic substance changes in the cell cycle are likely to occur. These changes can be identified through a series of parameters such as variations in cell division rate, presence of chromosomal alterations, micronucleus and condensed nucleus frequencies (Andrade et al., 2008; Klancnik et al., 2011; Kumari et al., 2011). Then, it allows access to important information about the organizational structure of a species' chromosomes, as well as their behavior during the different phases of cell division (Grant, 1994).

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Plant roots meristem are frequently used for cell cycle assays: meristematic cells are in direct contact with the toxic agent and are very easy to manipulate which facilitates its employment for toxicity assays (Fiskesjö, 1985). Plant bioassays are excellent genetic models since they are highly sensible to mutagenic agents and allow the simultaneous evaluation of multiple mechanisms of action of such agents (Leme and Marin-Morales, 2009). Furthermore plant bioassays have been proven to be efficient for environmental monitoring of genotoxic agents and are regarded as valid test systems by United Nations Enviromental Program (UNEP), World Health Organisation (WHO) and US Enviromental Protection Agency (US-EPA) (Grant, 1985, 1999).

There is a variety of plants that are used for toxicity assays, however one of the most well-known and well-established plant used in cytogenetic bioassays is the *Allium cepa* test. Onions germinate easily and the roots collected are simple to store. In addition, both macroscopic and microscopic factors can be evaluated on this species without much effort (Liman, 2013). Finally it is important to observe that there is a relation between results obtained from plant bioassays and other test systems (Fiskesjö, 1985; Çelik and Äslanturk, 2007; Maiti et al., 2016) which further increase their usefulness.

A series of studies were conducted in order to evaluate and understand the toxicity mechanisms of SPL. These prior studies have shown that this waste, as well as most of its main components, is in fact very toxic leading to the formation of chromosome aberrations during cell cycle (Andrade et al., 2008, 2010; Andrade-Vieira et al., 2011, 2012; Palmieri et al., 2014).

However all previous analysis about SPL toxicity in onion cells (Andrade et al., 2008; Andrade-Vieira et al., 2011; 2012) deal only with meristematic cells from the proximal meristem (referred from now on as M_1 cells, Fig. 1). These M_1 cells originate from asymmetrical divisions on the quiescent center (Van den Berg et al., 1997), they are undifferentiated and fast dividing. As they divide, the number of cells on this region rapidly increases and these M_1 cells are pushed to the elongation/differentiation region (referred from now on as M_2 region, Fig. 1). Therefore, M_2 cells derive from proximal meristem cells (M_1) that have gone through multiple cell divisions (Perilli et al., 2012).

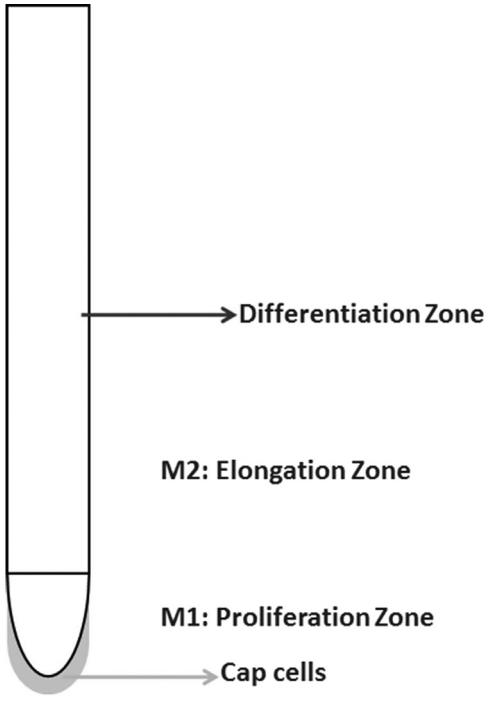

Fig. 1. Graphical model of a root showcasing the location of both meristematic regions studied in this work in relation to the overall root structure.

Studying cells from both the M_1 and M_2 region in a toxicity bioassay after letting the exposed plant go through a proper recovery period in water could prove highly informative, as it could offer insight on how an initial toxic effect observed on the proximal meristem develops and ultimately affects cells on the elongation/differentiation region.

Thus, the objectives of the present study were to compare the behavior of the effects of an environment pollutant on cell cycle of M_1 and M_2 cells from root tips plant model *A. cepa*. These assays could further elucidate the toxicity mechanisms of SPL as well as compare the effectiveness of studding each of these two cells type when conducting a toxicity bioassay.

2. Materials and methods

2.1. Plant materials

A. cepa bulbs L. (2n=2x=16 chromosomes) were used as test systems materials. The bulbs were placed in distilled water to stimulate root emergence. When the root tips reached 0.5 mm in length they were exposed to SPL solutions.

2.2. Matrix Solution

SPL with a granulometry of about 0.1 mm was used to prepare the solutions. One liter of a matrix solution was prepared to be used on the bioassays described below. This solution contained a mixture of 25 g of SPL with soil (on a proportion of 1 g of SPL per 3 g of soil) diluted in 1 L of aqueous $CaCl_2$ 0.01 M. Andrade et al. (2008) analyzed this same solution (named as 25% SPL) for its composition, which contains: cadmium (0.18 mg L^{-1}), copper (0.34 mg L^{-1}), iron (75 mg L^{-1}), lead (0.23 mg L^{-1}), manganese (0.18 mg L^{-1}), sodium (657 mg L^{-1}), zinc (0.34 mg L^{-1}), aluminum (0.7 mg L^{-1}), cyanide (23.4 mg L^{-1}), fluoride (47.8 mg L^{-1}). The solution was shaken at 180 rpm for 12 h, than allowed to rest for 12 h. The procedure was repeated three times, totaling 72 h. Subsequently, the supernatant was collected and filtered. Using aqueous solution of $CaCl_2$ as solvent simulates the ionic forces naturally present in the soil, creating *in vitro* the environmental conditions of SPL adsorption, simulating the natural leaching of this pollutant (Andrade-Vieira et al., 2011).

2.3. Work Solutions and Treatments

Four concentrations of SPL (2.5, 5, 7.5, 10 g L^{-1} – these solution will be referred as SPL2.5, SPL5, SPL7.5 and SPL 10 respectively) were prepared by dissolving the mother solution in an aqueous solution of 0.01 M of $CaCl_2$. Andrade-Vieira et al. (2011) found a cell death rating of over 50% on higher concentrations of SPL (25%). Since the objective of the present study was to evaluate the formation of micronucleus a high ratting of cell death was unwanted and therefore lower concentrations were chosen for the bioassay.

A solution of 100 g of soil dissolved in 0.01 M of $CaCl_2$ was used as control (from now on referred as SPL 0).

The SPL treatments (2.5, 5, 7.5 and 10 g L^{-1}) and the control were applied to eight bulbs (a different set of bulbs was used per treatment, in total 40 bulbs were used) with emergent roots for a period of 6 h. After exposure to SPL solutions or control, the bulbs were transferred to pots containing distilled water and kept there for 46 h for recovery (Ma et al., 1995). After the recovery, the roots from each treatment were collected and fixed in a cold solution of ethanol and acetic acid (3:1 v/v) to be used in the cytogenetic analysis.

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