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# Landfill leachate sludge use as soil additive prior and after electrocoagulation treatment: A cytological assessment using CHO-k1 cells

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## HIGHLIGHTS

- Electrocoagulation process efficiency was investigated in landfill leachate.
- Electrocoagulation process have high removal efficiency of effluent characteristics.
- Despite electrocoagulation treatment both sludges presented toxic effects.
- Sludge from landfill leachate proved to be genotoxic and mutagenic to cells.
- Landfill leachate sludge probably induce polyploidization process in cells.

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## ABSTRACT

Electrocoagulation has recently attracted attention as a potential technique for treating toxic effluents due to its versatility and environmental compatibility, generating a residue chemically suitable to be used as a soil additive. In the present study, landfill leachate sludge hazardous effects were investigated prior and after electrocoagulation process using *in vitro* assays with the mammalian cells CHO-k1. An integrated strategy for risk assessment was used to correctly estimate the possible adverse landfill leachate sludge effects on human health and ecosystem. Electrocoagulation process proved to be an effective treatment due to possibility to improve effluent adverse characteristics and produce sludge with potential to be used as soil additive. Despite low cytoxicity, the residue presented genotoxic and mutagenic effects, indicating a capacity to induce genetic damages, probably due to induction of polyploidization process in cells. The observed effects demand an improvement of waste management methods for reduce negative risks of landfill leachate sludge application.



## 1. Introduction

Landfill leachate is recognized as an important environmental problem considering its potentially toxic properties which may cause harmful effects on the ecosystem (Atmaca, 2009; Baderna et al., 2011; Li et al., 2016). Thus, this residue requires a proper treatment to minimize the contaminants to an acceptable level prior to discharge into the environment (Aziz et al., 2010; Wang et al., 2016).

Electrocoagulation process (EP) is a simple and efficient method used for treatment of many types of wastewaters, successfully used for removal of pollutants and pathogens (Aziz et al., 2013; Kobya et al., 2006; Amor et al., 2015). EP is performed by applying an electrical current across two electrodes immersed in effluent which destabilizes the solution and promote the coagulation of colloidal

*Abbreviations:* ULL, Untreated landfill leachate; TLL, Treated landfill leachate.

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contaminants (Silva et al., 2015). This treatment generates a sludge which can be used as a soil additive (Khandegar and Saroha, 2013), but the toxic potential of this residue must be investigated through several assessments to ensure a safety application.

Ecotoxicological methodologies are widely used in order to assess environmental risks of pollutants and complex mixtures (Aziz et al., 2004). Among the different techniques that can be applied, cytogenetic evaluation is a useful tool to detect cellular and DNA damages caused by exposure to contaminants (Fenech et al., 2003; Lemos and Erdtmann, 2000).

Micronucleus test is a sensitive DNA damage indicators and it has been applied to several organisms and tissues for the evaluation of environmental contaminants (Lemos et al., 2012). In addition, mammalian cell culture provides quick responses with a high sensibility to chemical and physical agents (Hartmann et al., 2001) that can but used in monitoring environmental risks (Lemos and Erdtmann, 2000).

Thereby, bioassays can effectively assess the toxic potential of a complex leachate when considering different factors such as physical and chemical characteristics of residue, providing an accurate interpretation of exposure effects (Bhargav et al., 2008; Geiszinger et al., 2009). Thus, the present study investigates leachate composition and its effects prior and after electrocoagulation process through cytotoxic, genotoxic and mutagenic assays in mammalian culture using CHO-K1 cell type.

## 2. Materials and methods

### 2.1. Sampling

Landfill leachate was collected in Center of Waste Treatment in Espírito Santo, Brazil, from homogenization tank that receives a liquid generated by landfill operation cells. Samples were transferred into clean plastic bottles and kept in a refrigerator at 4 °C.

### 2.2. Sample processing

A fraction of landfill leachate was decanted and the semi solid part was collected and identify as untreated landfill leachate (ULL) and the remainder was subjected to electrocoagulation process.

#### 2.2.1. Process of leachate electrocoagulation

Electrocoagulation (EC) process was conducted using a 4 L becker (as reactor), a stainless steel electrode and a mechanical shaker. Direct current power supply (ICEL, PS-6000) was used to provide current (current density: 207 A/$m^2$; *I*: 9 A; reaction time: 1 h). Voltage values were monitored using digital multimeter (Minipa, ET-2076). After samples were decanted for 12 h and then three distinct phases were obtained: supernatant, clarified effluent and sludge. Finally, sludge were collected and dry at room temperature and identified as treated landfill leachate (TLL).

#### 2.2.2. Solubilization process

Solubilized fraction were prepared from ULL and TLL sludges using 250 g of dry material in 1 L of distilled water for the preparation of base solutions, according to ABNT guidelines (ABNT, 2004). Thus, was obtained a solubilized TLL sludge at 250 g $L^{-1}$ and three dilutions were prepared (125 g $L^{-1}$, 62.5 g $L^{-1}$ and 31.25 g $L^{-1}$).

### 2.3. Physical and chemical characterization

Turbidity, conductivity, pH, color, total solids, fixed total solids, volatile total solids and chemical oxygen demand (COD) were analyzed in landfill leachate prior and after the electrocoagulation process. Methods used for characterization are described in Standard Methods for Examination of Water and Wastewater (APHA, 2005).

As, Ba, Cd, Pb, Cu, Ni, Se, Al, Zn, Cr were analyzed in TLL sludge samples with a Mass Spectrometer Inductively Coupled Plasma (ICP-MS), Agilent 7500cx, USA, equipped with an ASX-100 autosampler (CETAC Technologies, Omaha, NE). All samples were digested in triplicate. Concentrations of elements were determined in triplicate. The repeatability of ICP-MS measurements was generally 97%.

### 2.4. In vitro assays

#### 2.4.1. CHO-k1 cell culture conditions

Chinese hamster ovarian - CHO-k1 (wild-type), a nondrug-metabolizing cell line, was provided by Rio de Janeiro Cell Bank, Brazil. Cells were grown in 5.0 mL DMEM/F12 culture medium (GIBCO®, Paisley, Scotland, UK), supplemented with 10% fetal bovine serum (GIBCO®, Paisley, Scotland, UK). Cells were cultivated as monolayers in 25 $cm^2$ flasks at an initial concentration of $1.0 \times 10^6$ cells per flask in a BOD type incubator at 37 °C.

#### 2.4.2. Cell treatment

CHO-K1 cells were first grown for a complete cell cycle (12 h), and then 15.0 mL of serum-free medium and 5.0 mL of the test substances were added for a 1 cycle period treatment. The following treatments were performed: (I) Negative Control (NC), (II) Positive Control (PC) with DNA damage inducing agent – Methyl methanesulfonate $4 \times 10^{-4}$ M (MMS) (Sigma, St. Louis, MO, USA) (4 h treatment), (III) untreated leachate sludge at 250 g $L^{-1}$ (ULL), (IV) electrocoagulation treated landfill leachate sludge at 250 g $L^{-1}$ (TLL), and three TLL dilutions: (V) 125 g $L^{-1}$, (VI) 62.5 g $L^{-1}$ and (VII) 31.25 g $L^{-1}$.

#### 2.4.3. MTT cell proliferation assay

MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was performed as described by Mosmann (1983). CHO-k1 cells were cultivated in 96-well plate with F12/DMEN culture medium (GIBCO®, Paisley, Scotland, UK), supplemented with 10% fetal bovine serum (FBS) (GIBCO®, Paisley, Scotland, UK) in a $2 \times 10^4$ cell/well concentration. Plate was maintained in a BOD type incubator at 37 °C. After stabilization, cells were exposed to treatments for a cycle period (12 h). At the end of this period, cells were incubated with MTT salt for 4 h. After incubation, 100 μL of DMSO was added to each well in order to dissolve the precipitated formazan. The solution was mixed-by-pipetting and the absorbance of each well was read in spectrophotometer (Epoch Microplate®, Biotek) at 540 nm.

#### 2.4.4. Cell viability assay (trypan Blue® exclusion test)

The dye exclusion test was performed according Strober (2001). After treatments, cells were detached and 50 μL of the remaining cell suspension was mixed with 50 μL of trypan blue solution (GIBCO®, Paisley, Scotland, UK). Experiments were performed three times and in duplicate per treatment.

#### 2.4.5. Nuclear division index

Nuclear division index was calculated in at least 100 cells per slide scored according to the criteria of Fenech et al. (2003), based on ratio of mononucleated, binucleated and multinucleated cells.

#### 2.4.6. Cytokinesis-block micronucleus assay

After exposure, cells were washed twice with PBS and 5.0 ml of complete medium were added with 3 μg $mL^{-1}$ cytochalasin-B (Sigma®, St. Louis, MO, USA). Cells were then incubated an