



## Toxicological characterization of the landfill leachate prior/after chemical and electrochemical treatment: A study on human and plant cells



Vera Garaj-Vrhovac<sup>a,\*</sup>, Višnja Oreščanin<sup>b</sup>, Goran Gajski<sup>a</sup>, Marko Gerić<sup>a</sup>, Damir Ruk<sup>c</sup>, Robert Kollar<sup>b</sup>, Sandra Radić Brkanac<sup>d</sup>, Petra Cvjetko<sup>d</sup>

<sup>a</sup> Institute for Medical Research and Occupational Health, Mutagenesis Unit, 10000 Zagreb, Croatia

<sup>b</sup> Advanced Energy Ltd., 10000 Zagreb, Croatia

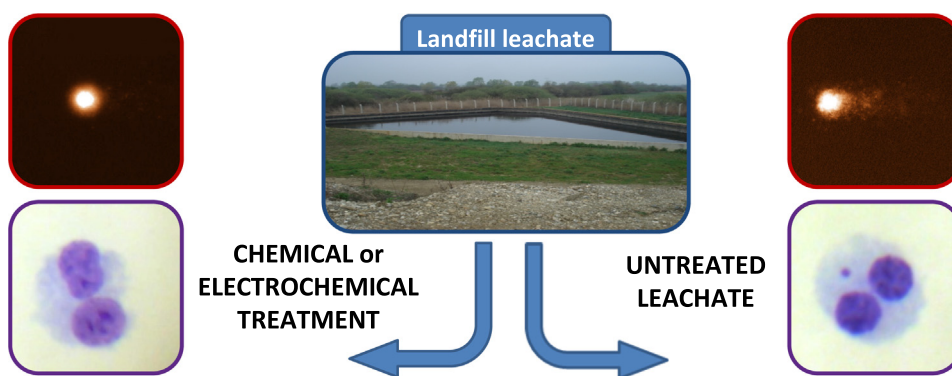
<sup>c</sup> Municipal Company Komunalac, 48000 Koprivnica, Croatia

<sup>d</sup> University of Zagreb, Faculty of Science, Department of Biology, 10000 Zagreb, Croatia

### HIGHLIGHTS

- The efficiency of two methods for landfill leachate purification was investigated.
- Untreated leachate proved to be both cyto- and genotoxic to human or plant cells.
- Treated leachate did not cause increase in cyto- and genotoxic damage.
- Both methods have high removal efficiency and provide toxicological safety.

### GRAPHICAL ABSTRACT



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### ABSTRACT

In this research, toxicological safety of two newly developed methods for the treatment of landfill leachate from the Piškornica (Croatia) sanitary landfill was investigated. Chemical treatment procedure combined chemical precipitation with CaO followed by coagulation with ferric chloride and final adsorption by clinoptilolite. Electrochemical treatment approach included pretreatment with ozone followed by electrooxidation/electrocoagulation and final polishing by microwave irradiation. Cell viability of untreated/treated landfill leachate was examined using fluorescence microscopy. Cytotoxic effect of the original leachate was obtained for both exposure periods (4 and 24 h) while treated samples showed no cytotoxic effect even after prolonged exposure time. The potential DNA damage of the untreated/treated landfill leachate was evaluated by the comet assay and cytokinesis-block micronucleus (CBMN) assay using either human or plant cells. The original leachate exhibited significantly higher comet assay parameters compared to negative control after 24 h exposure. On the contrary, there was no significant difference between negative control and chemically/electrochemically treated leachate for any of the parameters tested. There was also no significant increase in either CBMN assay parameter compared

**Abbreviations:** AO, acridine orange; APDC, ammonium-pyrolidinedithiocarbamate; BOD, biochemical oxygen demand; CBMN, cytokinesis-block micronucleus assay; COD, chemical oxygen demand; EC, electrical conductivity; EtBr, ethidium bromide; HPBL, human peripheral blood lymphocyte; LPO, lipid peroxidation; MAV, maximal allowed values; MN, micronucleus; MNi, micronuclei; NBUD, nuclear bud; NPB, nucleoplasmic bridge; ROS, reactive oxidative species; SCGE, single cell gel electrophoresis assay; SS, suspended solids; TDS, total dissolved solids; TOC, total organic carbon.

\* Corresponding author. Address: Institute for Medical Research and Occupational Health, Mutagenesis Unit, Ksaverska cesta 2, 10000 Zagreb, Croatia. Tel.: +385 1 4673 188; fax: +385 1 4673 303.

E-mail address: [vgaraj@imi.hr](mailto:vgaraj@imi.hr) (V. Garaj-Vrhovac).

to the negative control following the exposure of the lymphocytes to the chemically or electrochemically treated landfill leachate for both exposure periods while the original sample showed significantly higher number of micronuclei, nucleoplasmic bridges and nuclear buds for both exposure times. Results suggest that both methods are suitable for the treatment of such complex waste effluent due to high removal efficiency of all measured parameters and toxicological safety of the treated effluent.

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## 1. Introduction

United Nations (UN) predicts that the production of solid (including hazardous) waste could rise from 2–4.9 billion tons annually in 2006 to 2.4–5.9 billion tons annually in 2025. These negative trends in waste production demand consideration of several issues. Among them there are three endpoints that should be highlighted: public health, impact on environment, and waste management (UN, 2010).

The aim of this research was toxicological characterization of the landfill leachate prior/after the purification treatment using two different approaches. Landfill leachate was taken from the Piškornica (Koprivnica, Croatia) sanitary landfill which is situated approximately 9 km north of Koprivnica city (Fig. 1). The municipal as well as industrial waste was deposited since 1982 at the Piškornica landfill. Until 2005 the waste material was loaded directly on the ground without: preliminary foundation of the basic leak proof layer, daily cover of the deposited waste, and leachate collection and treatment. Today, the estimated production of the leachate is  $25 \text{ m}^3 \text{ d}^{-1}$ . As the result of the completed fermentation and waste aging processes, landfill leachate is characterized by very low biochemical oxygen demand/chemical oxygen demand ( $\text{BOD}_5/\text{COD}$ ) ratio and high amount of bio-refractory compounds (Oreščanin et al., 2011).

The first attempts of the treatment of landfill leachate from the Piškornica landfill were done by Oreščanin et al. (2011,2012) and resulted in the removal efficiencies of color, turbidity, suspended solids (SS), ammonia, COD, Fe and Zn for up to 99.48%. Although, treatment methods gave satisfactory results concerning the chemical composition of the final effluent, suitability and safety of the methods prior to their wide range applications must be confirmed by toxicological characterization using various test systems.

Consequently, in this work we examined the cytotoxic and genotoxic potential of treated leachates as well as untreated leachate on human and plant cells. As each test organism/cell type can be sensitive to different toxic substances, in toxicity assessment of environmental samples, it is generally recommended to use at least two species belonging to different trophic groups (George et al., 1995; Wenzel et al., 1997). Application of biotests with different model systems gives the opportunity to treat data from the tests as the information about the whole ecosystem, which afterwards makes it easier to assess a real hazard in the environment (Wenzel et al., 1997). Conventionally, *Allium cepa* as a plant model system has been used to evaluate DNA damage in terms of chromosome aberrations and disturbances in the mitotic cycle. However, in the present study, *A. cepa* was used in the comet assay in order to obtain better comparison with the results of the comet assay performed on human cells.

## 2. Materials and methods

### 2.1. Sampling and sample handling

A 100 L of landfill leachate was collected from the lagoon of Piškornica sanitary landfill in five polyethylene containers and transported to the laboratory. In order to obtain homogeneous

sample the effluent was combined into the single tank and mixed for 10 min (600 rpm) before analysis or each purification experiment.

### 2.2. Purification experiments

#### 2.2.1. Chemical treatment

Chemical treatment of landfill leachate consisted of three steps procedure as described in our previous paper (Oreščanin et al., 2011). Briefly, 1 L of landfill leachate was mixed with 25 g of calcium oxide on a magnetic stirrer for 30 min and subjected to a further treatment by  $0.570 \text{ mg Fe}^{3+} \text{ L}^{-1}$  added in the form of



Fig. 1. The position of Piškornica landfill situated approximately 10 km north from Koprivnica city which is the capital of Koprivnica-Križevci County in the north-east of Croatia.

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