Athens Institute for Education and Research
ATINER

ATINER's Conference Paper Series BIO2015-1697

Potential Mutagenic Activity of Leachate from a Municipal Solid Waste Landfill on Two Higher Plants

Ditika Kopliku
Associate Professor
University of Shkodra Luigj Gurakuqi
Albania

Anila Mesi
Associate Professor
University of Shkodra Luigj Gurakuqi
Albania

An Introduction to ATINER's Conference Paper Series

ATINER started to publish this conference papers series in 2012. It includes only the papers submitted for publication after they were presented at one of the conferences organized by our Institute every year. This paper has been peer reviewed by at least two academic members of ATINER.

Dr. Gregory T. Papanikos
President
Athens Institute for Education and Research

This paper should be cited as follows:

Kopliku, D. and Mesi, A. (2015). **"Potential Mutagenic Activity of Leachate from a Municipal Solid Waste Landfill on Two Higher Plants",** Athens: ATINER'S Conference Paper Series, No: **BIO2015-1697.**

Athens Institute for Education and Research
8 Valaoritou Street, Kolonaki, 10671 Athens, Greece
Tel: + 30 210 3634210 Fax: + 30 210 3634209 Email: info@atiner.gr URL: www.atiner.gr
URL Conference Papers Series: www.atiner.gr/papers.htm
Printed in Athens, Greece by the Athens Institute for Education and Research. All rights reserved. Reproduction is allowed for non-commercial purposes if the source is fully acknowledged.
ISSN: 2241-2891
13/11/2015

Potential Mutagenic Activity of Leachate from a Municipal Solid Waste Landfill on Two Higher Plants

Ditika Kopliku
Associate Professor
University of Shkodra Luigj Gurakuqi
Albania

Anila D Mesi
Associate Professor
University of Shkodra Luigj Gurakuqi
Albania

Abstract

The massive storage of municipal solid waste into landfills undergoes physical, chemical and biological decomposition, generating leachate and biogas. Raw leachate can cause local and far distant toxin accumulation, acute and chronic impact, the reduction of biodiversity and populations of sensitive species and can ultimately affect human health. The purpose of the present study was to assess and compare the potential mutagenic activity of the leachate collected in a municipal solid waste landfill (localized in the North-West part of Albania) by the means of *Allium cepa* and *Vicia faba* root assays. 5, 15, 30 and 60% concentration solutions (in drinking water, v/v) of raw leachate were used to establish the full-scale rhizotoxicity test on onion bulbs and faba seeds. Endpoints as: root length and 50% toxic effective concentration (EC_{50}), mitotic index, frequencies of micronuclei, chromosome aberrations and types (CA) in root tip meristem, were evaluated and compared. All screened endpoints obviously changed in a leachate dose-dependent manner. The leachate significantly induced delay and reduction in growth and mitotic activity of the treated roots along with the concentration increasing. The results revealed substantial increases of physiological (sticky, vagrant and laggard chromosomes, c-mitosis, multipolar anaphases and stars) and clastogenic (bridges and fragments) CA types. The enhanced frequencies of chromosome aberrations and micronuclei in the root cells of both the plants showed that leachate contains genotoxic and mutagenic substances, thus representing an important source of environmental pollution if not handled properly. Despite a certain difference in sensitivity, data confirmed that *A. cepa* and *V. faba* assays are suitable in predicting and monitoring landfill leachate toxicity.

Keywords: *Allium cepa*, genotoxicity, EC_{50}, municipal landfill leachate, mutagenicity, *Vicia faba*

Introduction

The rapid increase of urbanization and industrialization in developing countries like Albania, followed by the absence or incomplete sewage treatment systems due to high cost, have led to uncontrolled disposal of mutagenic wastes into terrestrial and aquatic ecosystems. The most common method of municipal solid waste disposal is storing it in landfill. The massive storage of active and closed municipal solid waste into landfills undergoes physical, chemical and biological decomposition, generating biogas and leachate. Landfill leachate is a liquid complex mixture, presenting fluctuations in composition, as a consequence of spatial and temporal variations. It can be a major source of contamination to groundwaters and surface waters by containing hazardous chemicals derived from: radioactive, hospital, lacquer and dyer wastes, detergents, cosmetics, medicines, solvents, oils and petroleum products, plant protection, motor-service, vehicle cleaning and household preparations (Christensen et al., 1994; Flyhammar, 1997; Kyoseva et al., 2012). Rainfall run off, indiscriminate overflow, land sinking, flooding, etc., may have caused local and far distant toxins accumulation, acute and chronic impact, ultimately reducing the biodiversity and populations of sensitive species. Such contaminants may eventually pass through the food chains to humans and result in a wide range of adverse consequences including cancer induction, acceleration of ageing processes and the appearance of heritable diseases in the offspring and reduction in fertility (Cabrera and Rodriguez, 1999; Majer et al., 2005). However the main risk of leachate pollution is on underground water and hence human health (Loizidou and Kapetanios, 1993). The toxic potential of leachate has been reported to be very strong, depending on various factors, such as: high content of organic and heavy metal compounds, time elapsed after waste disposal, inappropriate projection and construction of landfills, etc. (Schrab et al., 1993; Seco et al., 2003; De Brito-Pelegrini et al., 2007; Sawaittayothin and Polprasert, 2007; Zaharia et al., 2007; Obidoska and Jasińska, 2008; Bakare et al., 2013). However the exposure pathway and toxicity have not been well documented.

The pollution level at landfills must be quantified and monitored into the area of deposition and proximity using adequate evaluation methods (Zaharia et al., 2007). The complexity of landfill leachate makes it impossible to carry out the potential toxicity assessment based only on chemical analysis. Over the last decades, the use of bioassays, alone or in combination with chemical analysis, has continually increased both at organism (toxicity) and molecular (genotoxicity) levels by giving comprehensive information about the bio-effects of the hazards present in mixtures such as leachate, which are scarcely identified by chemical analyses (Power and Boumphrey, 2004). Moreover the integral monitoring based on the combination of various assays using organisms from different trophic levels has shown to be efficient in diagnosing their synergic, additive or antagonistic impact (Bernard et al., 1997; Isidori et al., 2003). Plants, which represent a crucial link in food chains, are widely utilized to evaluate the genotoxic and mutagenic effects of risk factors, due to the strongly structural preservation of their genetic material. Plant-based assays have gained notable recognition among the eco-toxicological approaches due to their comparative simplicity, sensitivity related to multiple endpoints, low cost and, ethically speaking, suitability compared to animals (Fiskesjö, 1993; Ma et al., 1995; Grant, 1999; Barbério, 2013). Because of the good correlation with mammalian assays, the reported confident and satisfactory outcomes have helped even in the research of cancer and other diseases. Some plant assays have been validated in international collaborative studies under the United Nations Environment Program, World Health Organization and US Environmental Protection Agency and are recommended for predicting and monitoring the ecotoxicological impact of some different matrices, including water, soils, wastewater and industrial effluent (Grant, 1999; Ma, 1999). Particularly, *Allium cepa* L. and *Vicia faba* L. root assays for chromosome aberrations and micronuclei are suggested to be the most effective tools for the rapid screening of environmental hazards due to large and mostly metacentric chromosomes (Ma *et al.*, 1995).Both *A. cepa* and *V. faba* assays have been recently introduced in Albania to monitor the water quality of the Shkodra region (North-West Albania) and the toxic potential of municipal wastewater (Mesi and Kopliku, 2012; Kopliku and Mesi, 2013; Kopliku and Mesi, 2014; Mesi and Kopliku, 2014).

The purpose of the present study was to assess and compare the potential genotoxic and mutagenic activity of the leachate collected in a municipal solid waste landfill by the means of two higher plant bioassays, *A.cepa* and *V.faba*.

Materials and Methods

Sampling Procedure

Sampling was done in the municipal solid waste landfill of Bushati during May of 2014. The Bushati landfill is localized in the North-West part of Albania, between the cities of Shkodra and Lezha. It opened in 2010, with a maximal capacity of 1,000,000 m^3 (130 tons/day), serving a population of about 200,000 inhabitants. Based on the multi-spot collection criterion (Xi et al., 1996), raw leachate was collected from drainage pipes leading to sumps, by using pre-rinsed plastic containers. The samples were immediately transported on ice to the laboratory and stored at 4°C for a maximum of 48 h. Before the toxicity analysis the same volume of each partial sample was mixed to get the composite sample. Drinking water was used as negative control (NC) and to dilute the raw leachate, obtaining 5, 15, 30 and 60% concentration solutions (v/v), in order to establish the full-scale rhizotoxicity test.

Higher Plant Assays

Healthy-looking *Allium cepa* L. bulbs and *Vicia faba* L. seeds were used as biological material. Phyto- and genotoxicity endpoints, such as: root length (MRL) and 50% toxic effective concentration (EC_{50}), mitotic index (MI), frequencies of cells with micronuclei (FMN), chromosomal aberrations (FAC) and types (CA) in root meristem, were evaluated and compared. All experiments were set up in a completely randomized design with twelve onion bulbs and twelve faba seeds per treatment concentration.

The phyto- and genotoxicity tests for *A. cepa* were performed second Fiskesjö (1993). Onion bulbs were grown and rooted in test tubes filled with drinking water (NC) and the chosen leachate cc solutions. One bulb was put on top of each tube with the root primordia downward in the solution. The test procedure was performed in room temperature (at about +20°C) with a natural light-dark regime and protected from direct sun light. After 96 h the root length of each bulb was measured. Dry *V. faba* seeds of uniform size were sterilized with NaOCl 50%, soaked for 24 h in distilled water and then the seed coats were removed. The seeds were allowed to germinate in 18.5 cm Petri dishes between two layers of moist cotton with NC and the leachate cc solutions. The phytotoxicity test was performed second Fargašová and Lištiaková (2009), by measuring the primary root length of germinated seeds exposed to leachate. Rooting was done under controlled conditions in a thermostat (dark cultivation at 24 °C) for 72 h. For both assays a mean root length (MRL) of each series per treatment was calculated. The EC_{50} value (effective concentration of different chemicals and mixtures, permitting respectively 50% growth of the sample under study compared to control) was statistically evaluated by plotting on graphs the MRL as a percentage to respective NC against leachate concentrations, based on the trend-line equation with the biggest R^2 value.

The cyto- and genotoxicity tests for *V. faba* were done according to Ma et al. (1995) with few modifications. After the removal of the primary roots, the seedlings were aerated in water tanks for 4 days. The newly emerged roots of faba bean (1-2 cm in length) and the roots of onion bulbs were exposed for 48 h to each leachate concentration and then used for microscopic analyzes. From each of the five bulbs and seedlings, randomly chosen in each treated group, one root tip was taken and placed on slides. The terminal part (1-2 mm) was cut off and then used for further preparation. The slides were prepared in accordance with the standard procedure of squashing the orcein-stained material. The root tips were fixed in Carnoy’s solution (glacial acetic acid/ethanol 1:3) at 4 °C overnight and transferred into 70% ethanol for storage. Then they were hydrolyzed in 1N HCl at 60 °C for 7 min and stained with 1% aceto-orcein. The number of dividing cells (NDC) was determined in 2000 examined cells in the field of view and the mitotic index was calculated as a percent ratio of NDC per 1000 cells. The formation of micronuclei was examined in interphase cells and six thousand cells per slide were counted. 300 dividing cells per slide have been observed for the characterization and classification of chromosome aberrations (CA). FMN, FAC and the frequency of CA types were scored as a percent ratio per 1000 cells. A mean value of five slides has been calculated per each endpoint.

Statistical Analysis

An analysis of Variance One-way ANOVA and a post-hoc SNK test were used to test for significant differences of all evaluated parameters, plants and treatment concentrations. All the results were expressed as the mean of three replicates per sample ± standard deviation (SD). Parameter differences against corresponding control and between plants were considered statistically significant at level of 5%.

Results

Table 1 and figures 1-4 represent all data related to the morphological and cyto-genetic analyzes of *A. cepa* and *V. faba* roots, exposed to 5%, 15%, 30% and 60% concentrations of raw leachate collected from an Albanian landfill. A remarkable leachate dose-dependence and a certain variation between plant assays through all screened parameters as compared to negative control were detected by using ANOVA and SNK tests.

Phyto- and Cytotoxic Effects of Landfill Leachate on A. cepa and V. faba Roots

The roots of onion bulbs and the primary roots of faba seedlings grown in NC had a mean length (MRL) of 4.82 and 2.91 cm and a mitotic index (MI) of 14.82 and 11.09%, respectively. As shown in table 1 and figures 1 and 2, a rapid and significantly changing reduction of both root length and mitotic activity with an increasing leachate concentration was observed particularly in the treated groups of both plants with 15-60% concentration solutions, as compared to respective NC ($p<0.05$, using SNK test). Compared with the corresponding NC-s MRL and MI values were decreased as high as: 39.6-83.2% and 44.5-89.8% in *A. cepa* and 22.8-84.8% and 33.1-91.6% in *V. faba*, respectively, along with 15-60% cc. On the other hand, only the root length of faba seedlings resulted not significantly reduced under the lowest treatment cc of 5%.

Table 1. *Phytotoxic Effect of Analyzed Municipal Landfill Leachate on A. cepa and V. faba Root Length*

Plant assay	MRL ± SD (in cm)				
	Landfill leachate concentration (%)				
	NC	5	15	30	60
A. cepa	4.82±0.41	3.98±0.53[a]	2.93±0.33[b]/*	1.95±0.26[c]	0.81±0.19[d]
V. faba	2.91±0.69	2.59±0.46	2.25±0.49[b]	1.33±0.21[c]	0.44±0.08[d]

MRL-mean root length; SD-standard deviation; NC-negative control. Means labeled with letters and asterisks are significantly different from corresponding NC-s according to SNK and One-Way ANOVA tests: a, b $p<0.05$ and * $P<0.05$ across landfill leachate concentrations and plant assays, respectively.

Based on the polynomial equations that had the maximum R^2, the EC_{50} values of analyzed leachate resulted in 23.1% for *A. cepa* and 28.4% for *V. faba* (Figure 1).

Figure 1. *Statistical Evaluation of EC_{50} Value of Analyzed Municipal Landfill Leachate Using a Growth Inhibition Test of A. cepa and V. faba Roots*
NC-negative control

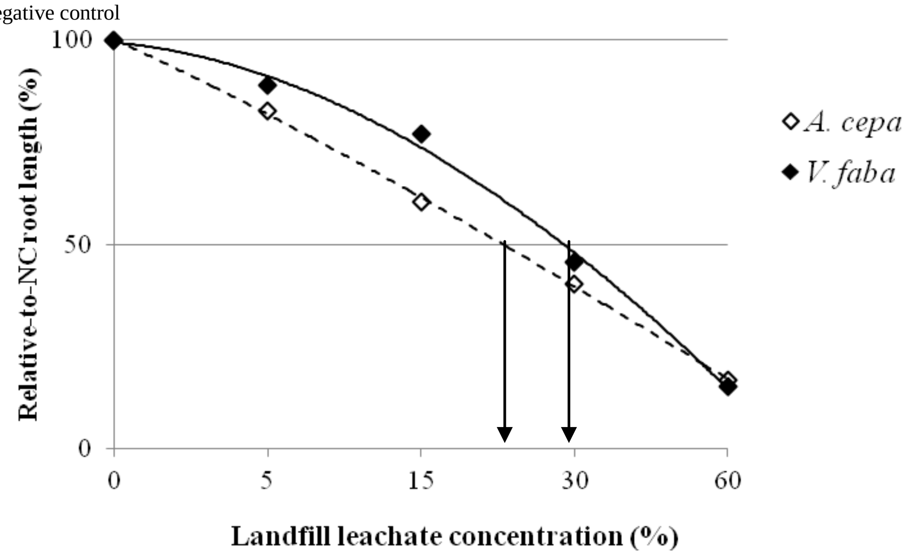
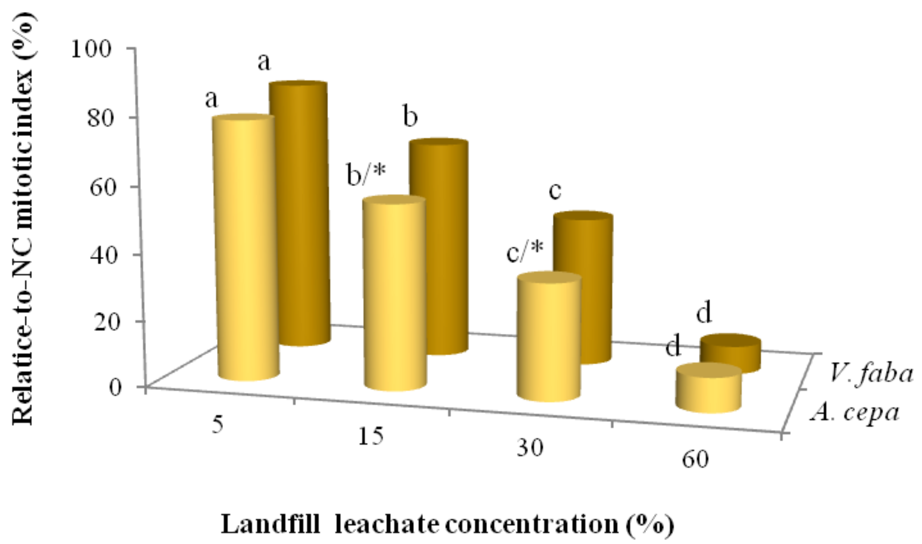


Figure 2. *Cytotoxic Effect of Analyzed Municipal Landfill Leachate on Mitotic Activity of A. cepa and V. faba Root Tip Meristem*

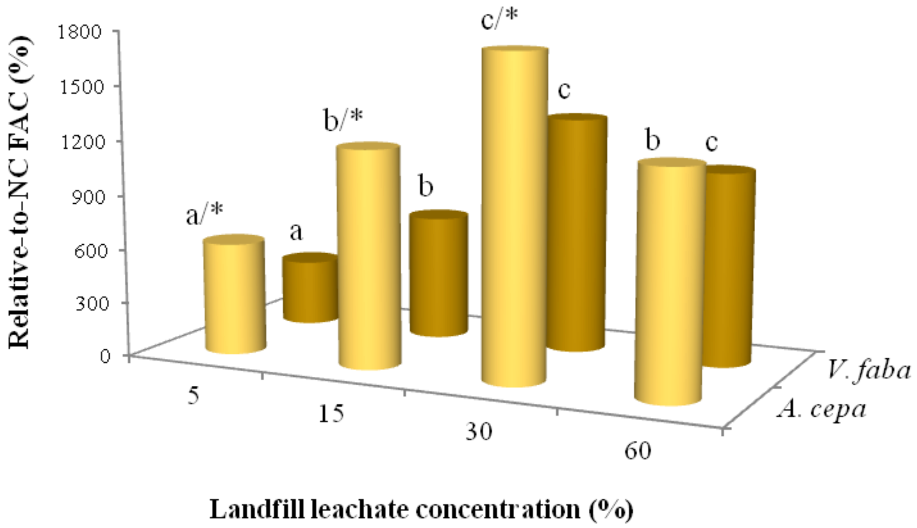


NC-negative control. Means labeled with letters and asterisks are significantly different from corresponding NC-s according to SNK and One-Way ANOVA tests: a, b $p<0.05$ and * $P<0.05$, across landfill leachate concentrations and plant assays, respectively.

Genotoxic and Mutagenic Effects of Landfill Leachate on A. cepa and V. faba Roots

The recorded frequencies of abnormal cells with chromosome aberrations (FAC) and micronuclei (FMN) in root tip meristem of groups of each tested plant grown in NC were respectively as follow: 2.47 and 0.09% for *A. cepa* and 1.94 and 0.22% for *V. faba*. Data on leachate-induced chromosome aberrations and micronuclei are illustrated in Figures 3 and 4.

Figure 3.*Genotoxic Effect of Analyzed Municipal Landfill Leachate Measured by the Frequency of Chromosome Aberrations in A. cepa and V. faba Root Tip Meristem*

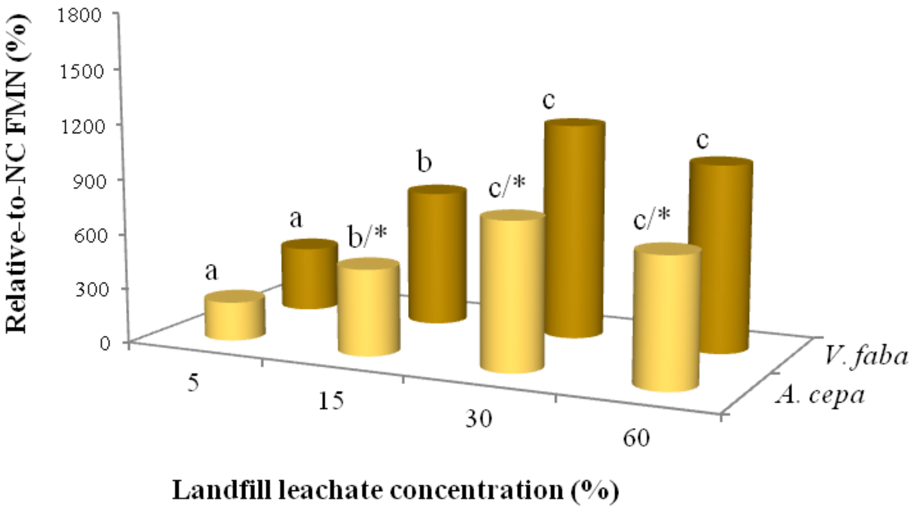


NC-negative control. FAC-frencuency of abnormal dividing cells with chromosome aberrations. Means labeled with letters and asterisks are significantly different from corresponding NC-s according to SNK and One-Way ANOVA tests: a, b $p<0.05$ and * $P<0.05$, across landfill leachate concentrations and plant assays, respectively.

FAC and FMN values started being significant at the lowest concentration (5%), exciding the corresponding NC-s respectively as follow: 6.2 and 2.1 times in *A. cepa* sets and 3.7 and 3.6 times in *V. faba* ones ($p<0.05$). At 15-30% concentrations both parameter values distinctly increased, respectively varying: for *A. cepa* 12-17.5 and 4.7-8 folds and for *V. faba* 6.9-13 and 7.5-11.9 folds higher than the corresponding NC-s ($p<0.05$). A certain decrease of induced genotoxic and mutagenic effects on both assays exposed to the highest leachate cc (60%), as compared to the treatment of 30%, was noted. Unequal-sized nuclei and binucleated cells were increasingly found under 5-30% cc treatments, while almost lacked at 60% cc one. Additionally an increased frequency of dead cells was detected in roots exposed to the highest leachate cc solutions (30-60%).

Concerning the spectrum of the chromosomal aberrations (CA) induced by the tested leachate solutions of 5-60% in onion and soy bean root tip meristem, the most observed types were due to chromatin dysfunction (stickiness, bridges and fragments) or spindle failure (c-mitosis, vagrant and laggard chromosomes and multipolar anaphases). The frequencies of the CA types changed in a certain leachate concentration-dependent manner, but they were similar through the plant tests. A drastic increase and predomination of chromosome bridges, sticky and laggard chromosomes could be noted across the highest leachate concentrations of 15-60%. Meanwhile the frequencies of clastogenic aberrations as fragments did not increase, even present since at the exposure to 15% cc. Lower frequency of c-mitosis was observed during whole treatment and even a significant decrease occurred under the treatment of 30 and 60% cc solutions.

Figure 4. *Mutagenic Effect of Analyzed Municipal Landfill Leachate Measured by the Frequency of Micronucleated Cells in A. cepa and V. faba Root Tip Meristem*



NC-negative control. FMN-frencuency of micronucleated interphase cells. Means labeled with letters and asterisks are significantly different from corresponding NC-s according to SNK and One-Way ANOVA tests: a, b $p<0.05$ and * $P<0.05$, across landfill leachate concentrations and plant assays, respectively.

Discussion

In the current study *A. cepa* and *V. faba* root assays were applied to evaluate the potential toxicity of raw leachate, collected from an Albanian municipal solid waste landfill, through the induced growth inhibition (phytotoxicity) and cytogenetic damage (cyto- and genotoxicity).

The phytotoxic effects of pollutants are usually determined based on abnormal morphology and growth inhibition of roots. Related to root growth as the combination of cell division and elongation, the reduction over 55% is a strong index of rhizotoxicity, being a general phenomenon caused by most chemical hazards in plants (Fiskesjö, 1993). Additionally, the mitotic index (MI) of root tip meristem reflects the frequency of cell division, being a confident endpoint in identifying the presence of cytotoxic pollutants in the environment. The reported data have considered MI reduction of 50% (of control) as a threshold value: a decrease below 50% indicates a sublethal effect

(Panda and Sahu, 1985), while below 22% denotes a lethal effect on the test organism (Antonise-Wiez, 1990). Glińska et al. (2007) suggested that the recorded mito-depressive action in the treated roots is probably due to either disturbances in the cell cycle or chromatin dysfunction induced by pollutant-DNA interactions. In the present investigation root length and mitotic activity of both onion bulbs and faba seedlings were significantly and increasingly reduced across 15-60% concentrations, reflecting the highly and cumulatively toxic effects of leachate on related metabolic processes. The restriction of root elongation over 55% occurred at 30% cc. As illustrated in figure 2, the analyzed diluted leachate could obviously induce sublethal and lethal effects in both plants at the highest cc treatments of 30% (with MI of 40.4 and 45.6% as compared to NC) and 60% (16.8 and 15.2% of NC), respectively, thus indicating high toxicity. In this context, even the undiluted leachate (100% cc) is consequently expected to be lethal. Additionally, the mitotic index in both assays could be positively correlated to root length, proving that the inhibition of root growth was a consequence of mito-depressive activity along with 5-60% leachate cc treatments. On the other hand, the rhizotoxic effect induced by leachate was more severe in *A. cepa* than in *V. faba*, resulting even significantly different ($P<0.05$) under the treatments of 5-15% cc and 15-30% cc, for MRL and MI, respectively (Figures 1 and 2). Based on the recorded values of the EC_{50} endpoint as a phytotoxicity threshold, the analyzed landfill leachate strongly affected the root growth of onion bulbs and faba seedlings at concentrations much lower (respectively, 23.1 and 28.4%) than the undiluted leachate (100% cc), which directly percolates the soil and can potentially contaminate surface and ground waters at the area of the Bushati landfill. The EC_{50} values of leachate on roots implyed a certain difference between the two plants related to the effect on root growth, mentioned above as well, revealing *A. cepa* as more sensitive to the analyzed samples of landfill leachate.

The toxic effects of the environmental contaminants, mainly during chronic exposure, are not visible immediately hence ecotoxicological studies also request the assessment of genotoxicity. The genotoxicological effect of pollutants is generally developed in lower concentration than the toxicological one. The use of the root tip meristem of higher plants makes it possible for the detection of genotoxic and clastogenic potency of pollutants. The suppression of its mitotic activity is usually accompanied by an increase of the chromosome aberrations frequency in dividing cells (Power and Boumphrey, 2004). The micronuclei frequency is another effective and simple biomarker of genetic damage accumulated during the cell cycle. MNC often results from the acentric fragments or laggard chromosomes that fail to incorporate into the daughter nuclei during the telophase stage (Ma et al., 1995). Genotoxicity and mutagenicity assays in the present investigation showed high inducement of chromosomal aberrations and micronuclei in the root meristem of both treated plants, positively depending on landfill leachate concentration (Figures 3 and 4). Data gave us an insight into the strong genotoxic and mutagenic potential of the chemicals present in this mixture of leachate and/or possibly of their interactions. The observed decrease of FAC and even FMN values under the highest cc treatment (60%) corresponded to the drastic decline of mitotic index, sustaining again the high physiological toxicity of leachate against the roots of both plants. This fact confirmed that EC_{50} is an appropriate toxicity endpoint to select the concentrations for genotoxicity assays, because there are enough mitotic cells present to permit the microscopic investigation of chromosome aberrations. The increased frequency of dead cells scored at the highest leachate concentrations (30-60%) demonstrated their lethal effect, as well. It might be due to the deletion of primary genes, leading to cellular death. Concerning the comparison between the two plant assays (Figures 3 and 4), it may be noted that *A. cepa* was more responsive than *V. faba* related to FAC endpoint. Meanwhile the feedback of *V. faba* was stronger in the formation of micronuclei. The recorded values of these endpoints were statistically different between plant assays particularly under 15-30% cc treatments. The lack of unequal-sized nuclei and binucleated cells in the roots of the groups treated with 60% cc solution was most probably due to high cytotoxicity.

The induction of: aberrant metaphase, anaphase and telophase (with bridges, loss and stickiness of chromosomes), polyploidy, irregular nuclei and nuclear buds, are indicators of genotoxicity, while the micronuclei and chromosome breaks are used to assess the mutagenicity effects (Ma et al., 1995; Leme and Marin-Morales, 2009). In the present investigation the spectrum of chromosomal aberrations (CA) induced by the tested leachate solutions of 5-60% in the onion and soy bean root tip meristem included mostly stickiness, bridges, fragments, laggard chromosomes and c-mitosis. Stickiness is considered a common sign of highly toxic effects on chromosomes, an irreversible type eventually leading to cell death (Fiskesjö, 1993). It is caused probably through the immediate reaction of chemicals with DNA during its inhibition period, inducing DNA-DNA or DNA-protein cross-linking or through altering the physicochemical properties of nucleic acids and/or nucleoproteins. Chromosome bridges result from chromosome and/or chromatid breakage and fusion. However the molecular mechanism of DNA breakage is not yet clearly understood. Concerning c-mitosis, data have suggested that the excess of some heavy metals may decrease the tissue distribution of Ca^{2+} ions by displacing them from the exchange sites, preventing as a consequence calmodulin (CaM) to activate the key enzymes of the mitotic spindle which in turn leads to disturbance or inhibition of mitosis (Liu et al., 1995). The chromosome lagging is induced by a weak c-mitotic effect and may indicate a risk of aneuploidy (Leme and Marin-Morales, 2009).

A possible mechanism for leachate-induced chromosomal aberrations and micronuclei involve the formation of free radicals, either through auto-oxidation or by enzyme-catalyzed oxidation of organic compounds present in leachate, such as chlorinated and non-chlorinated hydrocarbons (Li et al., 2004 Li and Sang, 2005; 2008; Bakare et al., 2013). The reported data have suggested that the free radicals may affect DNA by causing base substitution and sites base modification or DNA single-strand and double strand breakage, eventually inducing mutation (Kurtis et al., 1988; Sang and Li, 2004). Another proposed mechanism of induced-DNA damage by leachate in living cells may be due to the cross-linking of some of the present metals with DNA and/or proteins. It could be through the inhibition of DNA repair processes inside the cells. Metals have been reported to induce unique mechanism(s) of repair inhibition (Hartwig and Schwerdtle, 2002). On the other hand, some heavy metals have shown to affect, even indirectly, DNA by producing reactive oxygen species (ROS) that cause DNA, protein, and lipid damage. Heavy metals, such as: Cd, Fe, Pb, As, Zn, Hg, Cu, Ni, and Mn, have been found in leachate samples at concentrations higher than the limits set by international regulatory authorities (Bakare et al., 2013). Some reported data have sustained the idea of the induced-genotoxicity of leachate in eukaryotes via free radical generation in treated cells (Ferrarri et al., 1999; Ali et al., 2004; Radetski et al., 2004; Li et al., 2006).

Our results from both toxicity assays highlighted the strong pollution potential of leachate collected from the Bushati landfill. The impacts caused by the leachate are usually due to synergic interactions between the various pollutants present in this type of matrices. The data were in compatibility with findings from other studies with plant assays following treatment with raw leachate from solid wastes (Sang and Li, 2004; Sang et al., 2006; De Brito-Pelegrini et al., 2007; Li et al., 2008; Obidoska and Jasińska, 2008; Žaltauskaitė and Čypaitė, 2008; Bialowiec and Randerson, 2010; Antonio et al., 2013; Bakare et al., 2013; Mesi and Kopliku, 2014). Although, the microbial bioassays have been widely used in the detection of mutagens present in landfill leachate, studies have however shown that they have a low sensitivity for heavy metals (Schrab et al., 1993; Gatehouse et al., 1994). For this reason most recent studies are successfully using battery of assays including plants and animals in order to confidentially assess the adverse effects of leachates (Bernard et al., 1997; Ferrarri et al., 1999; Isidori et al., 2003; Radetski et al., 2004; De Brito-Pelegrini et al., 2007).

Conclusions

In the present study all screened phyto-, cyto- and genotoxic endpoints of *A.cepa* and *V. faba* roots obviously changed in a leachate dose-dependent manner. Leachate significantly induced delay and reduction in growth and mitotic activity of treated roots along with the concentration increasing. The enhanced frequencies of chromosome aberrations and micronuclei in the root cells of both plants highlighted the strongly toxic potential of analyzed landfill leachate, thus representing an important source of environmental pollution if not handled properly. Data suggested that components present in leachate may be highly toxic in plant cells, implying that long-term exposure at low concentrations in both aquatic and terrestrial environments may pose a potential genotoxic and mutagenic risk to organisms and humans. Differences in the response among the bioassays were found, confirming that even for leachates it would be useful and advisable to perform toxicity tests with a battery of organisms. However our findings confirmed that *A. cepa* and *V. faba* root assays are suitable model systems with multiple macro- and microscopic endpoints to predict and monitor the landfill leachate toxicity.

References

Ali, M., Parvez, S., Pandey, S., Atif, F., Kaur, M., Rehman, H. and Raisuddin, S. 2004. Fly ash leachate induces oxidative stress in freshwater fish *Channa punctata* (Bloch). *Environ International* 30, 933-938.

Antonio, M., Rodrigues, S. and da Silva, L. B.2013. Toxicological evaluation of landfill leachate using plant (*Allium cepa*) and fish (*Leporinus obtusidens*) bioassays. *Waste Manag Res* 31(11), 1148-1153. DOI=10.1177/0734242X13502 388

Antonise-Wiez, D. 1990. Analysis of the cell cycle in the root meristem of *Allium cepa* under the influence of Ledakrin. *Folia Histochemica et Cytobiologica* 26, 79-96.

Bakare, A. A., Alimba, C. G. and Alabi,O. A. 2013. Genotoxicity and mutagenicity of solid waste leachates: A review. *African J of Biotechnology* 12(27), 4206-4220.

Barbério, A. 2013. Bioassays with plants in the monitoring of water quality. In: *Water treatment*, Elshorbagy, W. and Chowdhury, R. K., Eds. InTech 2013, Rijeka, Croatia. DOI= http://dx.doi.org/10.5772/2883

Bernard, C., Colin, J. R. and Anne, L. D. D. 1997. Estimation of the hazard of landfills trough toxicity testing of landfills. *Chemosphere* 35(11), 2783-2796.

Bialowiec, A. and Randerson, P. F. 2010. Phytotoxicityof landfill leachate on willow *Salix amygdalina* L. *Waste Management* 30(8-9), 1587-1593.

Cabrera, G.L., Rodriguez, D.M. G. and Maruri, A. B. 1999. Genotoxicity of the extract from the compost of the organic and the total municipal garbage using three plant bioassays. *Mut Res* 426, 201-206.

Christensen, H., Kjeldsen, P., Albrechtsen, H., Heron, G., Nielsen, P. H., Bjerg, P.L. and Holm, P. E. 1994. Attenuation of landfill leachate pollutants in ground water. *Crit Rev Environ Sci Technol* 24, 119-202.

De Brito-Pelegrini, N. N., Pelegrini, R. T. and Paterniani, J. E. S. 2007. Ecotoxicological evaluation of leachate from Limeira sanitary landfill with a view to identifying acute toxicity. *Revista Ambiente e Ágμa* 2(3), 34-43.

Fargašová, A. and Lištiaková J. 2009. Cr and Ni simultaneous phytotoxicity and mutagenicity assay. *Nova Biotechnologica* 9(2), 107-112.

Ferrari, B., Radetski, C. M., Veber, A. M. and Ferard, J. F. 1999. Ecotoxicological assessment of solid wastes: A combined liquid- and solid-phase testing approach using a battery of bioassays and biomarkers. *Environ Toxicol Chem* 18, 1195-1202.

Fiskesjö,G. 1993. The *Allium* test in wastewater monitoring. *Environ Toxicol Water Quality* 8 (3), 291-298.

Flyhammar, P. 1997. Estimation of heavy metal information in municipal solid waste. *Sci Total Environ* 198(2), 123-133.

Gatehouse, D., Haworth, S., Cebula, T., Gocke, E. Kier, L., Matsushima, T., Melcion, C., Nohmi, T., Ohta, T., Venitt, S. and Zeiger, E. 1994. Recommendations for the performance of bacterial mutation assay. *Mut Res* 312, 217-233.

Glińska, S., Bartczak, M., Oleksiak, S., Wolska, A., Gabara, B., Posmyk, M. and Janas, K. 2007. Effects of anthocyanin-rich extract from red cabbage leaves on meristematic cells of *Allium cepa* L. roots treated with heavy metals. *Ecotox*

Environ Safety 68, 343-350.
Grant, W. F. 1999. Higher plant assays for the detection of chromosomal aberrations and gene mutations - A brief historical background on their use for screening and monitoring environmental chemicals. *Mut Res* 426(2), 107-112.
Hartwig, A. and Schwerdtle, T. 2002. Interactions by carcinogenic metal compounds with DNA repair processes: toxicological implications. *Toxicol Letter* 127, 47-54.
Isidori, M., Lavorgna, M. Nardelli, A. and Parrella, A. 2003. Toxicity identification evaluation of leachates from municipal solid waste landfills: a multispecies approach. *Chemosphere* 52, 85-94.
Kopliku, D. and Mesi, A. D. 2013. Assessment of cytotoxic and genotoxic potency of Cr(VI)-doped river water of Nen-Shkodra lowland, Albania, on *Allium cepa* L. *J Environ Res Develop* 7(4), 1325-1332.
Kopliku, D. and Mesi, A. 2014. Capability of *Vicia faba* L. in evaluating surface and waste waters toxicity of Shkodra Lake, Albania. In *Proceedings of the Ninth Annual International Symposium on Environment* Athens, Greece, May 12-15, 2014). ATINER'S Conference Paper Series, No: ENV2014-0949.
Kurtis, J. F., Hughes, M. F., Mason, R. P., Eling, T. E. 1988. Peroxidase catalyzed oxidation of (bi) sulfite: reaction of free radical metabolites of (bi) sulfite with 7,8-dihydroxy-7,8-dihydro-benzo[α]-pyrene. *Carcinogenesis* 9, 2015-2021.
Kyoseva, V., Todorovab, E., Dombalova, I. and Pelovskya, Y. 2012. Solid waste of Sofia municipality as raw material and energy resource. *J Environmental Protection and Ecology* 13(3), 1442-1449.
Leme, D. M. and Marin-Morales, M. A. 2009. *Allium cepa* test in environmental monitoring: A review on its application. *Mut Res* 682, 71-81.
Li, G., Sang, N. and Zhao, Y. 2004. Micronuclei induced by municipal landfill leachate in mouse bone marrow cells in vivo. *Environ Res* 95, 77-81.
Li, G., Sang, N. and Guo, D. 2006. Oxidative damage induced in hearts, kidneys and spleens of mice by landfill leachate. *Chemosphere* 65, 1058-1063.
Li, G., Yun, Y., Li, H. and Sang, N. 2008. Effect of landfill leachate oncell cycle, micronucleus, and sister chromatid exchange in *Triticum aestivum*. *J Hazard Mater* 155, 10-16.
Liu, D. H., Jiang, W. S., Wang, W. and Zhai, L. 1995. Evaluation of metal ion toxicity on root tip cells by the *Allium* test. *Israel J. Plant Sci* 43, 125-133.
Loizidou, M. and Kapetanios, E. G. 1993. Effect of leachate from landfills on underground water quality. *Sci Total Environ* 128, 69-81.
Ma, T. H. 1999.The international program on plant bioassays and the report of the follow-up study after the hands-on workshop in China.*Mut Res* 426, 103-106.
Ma, T. H., Xu, Z., Xu, C., McConnell, H., Rabago, V. E., Arreola,A. G. and Zhang, H. 1995. The improved *Allium*/*Vicia* root tip micronucleus assay for clastogenicity of environmental pollutants. *Mut Res* 334(2), 185-195.
Majer, B. J. Grummt, T., UhlM. And Knasmüller, S. 2005. Use of plant bioassays for the detection of genotoxins in the aquatic environment. *Acta Hydrochimica Hydrobiologica* 33, 45-55.
Mesi, A. and Kopliku, D. 2012. Toxicity evaluation of leachates from some urban wastewater collectors of Shkodracity using *Allium cepa* L. test. *J International Environmental Application & Scienc*e 7(5), 907-912.
Mesi, A. and Kopliku, D. 2014. Seasonal phyto- and genotoxicity monitoring of municipal landfill leachate on *Allium cepa* roots. *J. Environmental Protection and Ecology* 15(4), 1671-1679.
Obidoska, G. and Jasińska, D. 2008. Phytotoxicity and potential genotoxicity of

radiowo municipal landfill leachate. *Ann. Warsaw Univ Life Sci* 40, 39-44.

Panda, B. B. and Sahu,U. K. 1985. Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of *Allium cepa* by the organophosphorus insecticides fensulfothion. *Cytobios* 42, 147-155.

Power, E. A. and Boumphrey, R. S. 2004. International trends in bioassay use for effluent management. *Ecotoxicology* 13(5), 377-398.

Radetski, C. M., Ferrari, B., Cotelle, S., Masfaraud, J. F. and Ferard, J. F. 2004. Evaluation of the genotoxic, mutagenic and oxidant stress potentials of municipal solid waste incinerator bottom ash leachates. *Sci Total Environ* 333, 209-216.

Sang, N. and Li, G. 2004.Genotoxicity of municipal landfill leachate on root tips of *Vicia faba. Mut Res* 560, 159-165.

Sang, N. and Li, G. 2005. Chromosomal aberrations induced in mouse bone marrow cells by municipal landfill leachate. *Environ Toxicol Pharmacol* 20, 219-224.

Sang, N., Li, G. and Xin, X. 2006. Municipal landfill leachate induces cytogenetic damage in root tips of *Hordeum vulgare. Ecotox Environ Safety* 63, 469-473. DOI=www.ncbi.nlm.nih.gov/pubmed/16406595

Sawaittayothin, V. and Polprasert, C. 2007. Nitrogen mass balance and microbial analysis of constructed wetlands treating municipal landfill leachate. *Biores Technol* 98, 565-570.

Schrab, G. E., Brown, K. W. and Donnelly, K. C. 1993. Acute and genetic toxicity of municipal landfill leachate. *Water Air Soil Pollut* 69, 99-112.

Seco, J. I., Fernández-Pereira, C. and Vale, J. 2003. A study of the leachate toxicity of metal-containing solid wastes using *Daphna magna*. *Ecotoxicol Environ Safety* 56(3), 339-350.

Xi, D. L., Sun, Y. S. and Liu, X. Y. 1996. *Environment Monitoring*. Beijing, China: Higher Education Press.

Zaharia, C., Surpateanu, M. and Macoveanu, M. 2007. Assessment of environmental impact generated by municipal waste deposition into a Romanian landfill. *J Environmental Protection and Ecology* 8(2), 332-345.

Žaltauskaitė, J. and Čypaitė, A. 2008. Assessment of landfill leachate toxicity using higher plants. *Engineering* 4(4), 42-47.