Accepted Manuscript

Title: Cyto/genotoxicological evaluation of hot spots of soil pollution using *Allium* bioassays in relation to geochemistry

Authors: Asta Stapulionytė, Violeta Kleizaitė, Raimondas Šiukšta, Donatas Žvingila, Ričardas Taraškevičius, Tatjana Čėsnienė

PII: S1383-5718(18)30209-2
DOI: https://doi.org/10.1016/j.mrgentox.2019.01.001
Reference: MUTGEN 403006

To appear in: *Mutation Research*

Received date: 30 June 2018
Revised date: 20 December 2018
Accepted date: 4 January 2019

Please cite this article as: Stapulionytė A, Kleizaitė V, Šiukšta R, Žvingila D, Taraškevičius R, Čėsnienė T, Cyto/genotoxicological evaluation of hot spots of soil pollution using *Allium* bioassays in relation to geochemistry, *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* (2019), https://doi.org/10.1016/j.mrgentox.2019.01.001

Cyto/genotoxicological evaluation of hot spots of soil pollution using *Allium* bioassays in relation to geochemistry

Asta Stapulionytė [a*], Violeta Kleizaitė [a], Raimondas Šiukšta [a], Donatas Žvingila [a], Ričardas Taraškevičius [b], Tatjana Čėsnienė [a]

[a] *Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekio Ave. 7, LT-10257 Vilnius, Lithuania*

[b] *Nature Research Centre, Akademijos 2, LT-08412 Vilnius, Lithuania*

[*] corresponding author. Tel.: +370 5239 8259. *E-mail address*: asta.stapulionyte@gf.vu.lt (A. Stapulionytė http://orcid.org/0000-0001-6840-0041).

Highlights

- Soil from industrial sites is more heavily polluted compared to landfill soil
- Severe cytotoxicity was determined in *Allium* exposed to soils from industrial sites
- Detrimental effects were induced in *Allium* treated with every test-soil
- Combined use of geochemical and cyto/genotoxicity parameters

ABSTRACT

Soil from industrial and landfill sites affected by anthropogenic activity was screened for implicit negative effects in an *Allium* test-system in relation to geochemistry. The concentrations of 15 elements were compared to the ecotoxicologically-based soil guideline values. Admitted geoindices were used to classify test-soils according to risk/hazard categories. Test-soils were screened for the possible deleterious effects in common onion (*Allium cepa* L.) by employing a test battery of cytogenetic bioassays (root growth inhibition, mitotic activity, frequency of chromosome aberrations and micronuclei, and cell death rate) complemented with two assays of molecular DNA markers, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR). Soil from industrial sites was more severely polluted and more cytotoxic for onions compared to soil from landfill sites. However, the cyto/genotoxic outcome of soil exposure in *A. cepa* was the same for all test-soils; the detrimental effects were observed in onions treated with every test-soil. Thus, test-soils could not be classified as non- and genotoxic, although certain of them had permissible contamination levels. The chromosome aberration frequency and cell death rates were consistent with the intensity of soil contamination, contrary to the micronuclei rate, which was independent of the soil risk/hazard level. Despite a relationship between risk (RI) and total soil contamination (*Z*) geoindices, both indices correlated with a different *Allium* cyto/genotoxicity endpoint, although the *Z* index was preferred over the RI index as being more informative in correlation analysis. *Allium* bioassays complemented each other by depicting different aspects of exposure to toxic substances, and determination of cyto/genotoxicity in a battery of different bioassays is important in the risk assessment of ecologically dangerous soils, and an application of a test battery is strongly advised.

Keywords: *Allium*; cyto/genotoxicity; risk assessment; soil contamination.

1. Introduction

The release of toxic waste products has been accelerated by intensive industrialization at orphaned contaminated sites after facility closure [1] along with waste disposal at the landfills [2]. Such contamination hot spots not only reduce environmental quality but are dangerous to human health [1,3,4]. Potentially harmful elements (PHEs), including heavy metals (HM), constitute a persistent environmental hazard risk that is difficult to quantify relying on chemical analysis data [1], but there are studies associating diseases with the specific patterns of the microelemental composition of soils in human environments [5]. Due to significant economic limitations for chemical determinations, it is important to consider a simple screening test of sediment that would reveal whether it may be "contaminated" and qualifies for more

detailed and costly chemical research [4,6]. Total soil contamination may be evaluated through various different geochemical indices [7,8], though the genotoxicity is usually assessed through bioassays that can evaluate the effects of complex mixtures, even without prior knowledge of chemical composition. Cytogenetic *Allium* chromosome assays are standardized for the detection of chemical mutagens in a routine use and enable easy environmental monitoring of complex mixtures present in the surroundings [9–11]. In addition, molecular markers can be applied to detect deleterious HM effects on genetic material integrity by comparing variation in DNA banding profiles between treated and untreated organisms [12,13]. The effects of individual HM can be easily detected in plants by application of highly polymorphic RAPD [14-16] and ISSR markers [12,17] that are considered efficient in the evaluation of the genotoxic activity at least of individual HM [18]; nevertheless, data on the application of DNA markers in analysis of complex mixture of soil pollutants or multi-element HM-induced genotoxicity assessment using *Allium* as a test-system is scarce [18,19].

In this study, the focus was on genotoxicity assessment of contaminated soil from the surroundings of active and abandoned industrial facilities and old landfills within urban areas; these soils give rise to concerns due to their long-term environmental impacts and vicinity to residential sites [20]. Environmental monitoring could be a typical basic physical-chemical analysis of sediments or ecotoxicological classification [6] that, for the first time, was combined with cyto/genotoxicity testing using *Allium* bioassays in this survey. The combination of various methods may offer a powerful analytical technique while studying HM pollution in soil, and our goal was to associate plant bioassay data with soil geochemistry and/or ecotoxicological evaluations using several single- and multi-elemental geoindices for excessive enrichment, contamination and potential ecological risk assessment. Assessment of the cyto/genotoxic risk of contaminated soil exposures was determined by cytogenetic *Allium* assays consisting of a root growth inhibition (RGI) test, modified chromosome aberration (CA) assay and micronucleus (MN) test along with the application of molecular ISSR and RAPD markers. The use of molecular marker systems was grounded on their simplicity, speed and cost, and most important, the relationship between some markers and fitness parameters [21]. This study was intended to compare the effectiveness of *Allium* bioassays for the cyto/genotoxicity evaluation of undisturbed technogenic urban soil enriched with PHEs and to contribute to a broader understanding of the associations among plant biomarkers and variable PHEs content in the soil expressed through summarizing geoindices.

2. Materials and methods

2.1 Soil sampling and geochemistry evaluation according to risk and hazard assessment

Soil samples were collected in Vilnius city from hot spots of soil pollution of anthropogenic origin. The sites in this study were categorised into two groups consisting of industrial facilities and closed landfills (Table 1).

Table 1
Test-soil lithology, sampling site coordinates, period of contamination source activity and expected inorganic contaminants [a,b].

Site code	Coordinates	Contamination source	Period of operation	Lithology	Expected inorganic contaminants
Industrial facilities					
I1	54°40'46.75" N 25°18'7.7" E	Production of electrical engineering products and electrical gauges	1948–2009	Sterile with firebrand	Cu, Zn, Sn, Mo, Cr, Ni, Pb, Co, V
I2	54°40'31.71" N 25°15'47.2" E	Production of drills	1925–1992	Fertile	Mo, Cr, V, Co, Cu, Ni, Sn, Zn, Ba, Pb
I3	54°42'13.66" N 25°18'30.01" E	Production of medical and electrographic equipment	1957–2003	Sandy	Zn, Cu, Pb, Mo, Sn, As
I4	54°43'12.78" N 25°17'17.77" E	Production of motor transport engine fuel equipment	1959–2004	Sterile	Cu, Mo, Zn, Ni, Pb, Cr, Sn
Landfills					
L1	54°42'37.38" N 25°33'8.1" E	Waste dumping site	1972–1990	Technogenic, argillaceous	Pb, Cr, Mo, Zn, Cu, Sn
L2	54°44'37.67" N 25°13'56.14" E	Communal waste dumping site	1962–1987	Technogenic, sandy	Cu, Zn, Mo, Sn, Ba, Pb, Ni
L3	54°41'18.12" N 25°20'51.52" E	Waste dumping site	1946–1979	Technogenic, sandy	Cu, Zn, Sn, Cr, Pb, Mo

[a] Contaminants expected in the vicinity of industrial facilities are given in descending order based on literature data [22].

[b] Contaminants expected at landfill sites are given in descending order based on the municipal monitoring [23] and literature data [24].

Composite topsoil samples were collected using an envelope design. Chemical analysis of the soil was performed by energy-dispersive X-ray fluorescence spectroscopy following methodology from Taraškevičius *et al.* [25] and concentrations of 15 potentially harmful elements (PHEs) were determined. Geochemical soil values of PHEs (As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Se, Sn, V, and Zn) were compared to the ecotoxicologically based soil guideline values (SGV) (Table A.1) and used to calculate existing geoindices: the sum of potential ecological risk indices (RI) and the potential ecological risk index of an individual element E^i_r [7,26,27], excess (C_{SGV}) and element concentration (C_c) coefficients and the total soil contamination index (Z) [28,29], and the geoaccumulation index (I_{geo}) [30]. Geoindices were employed to classify test-soils according to the risk and hazard degrees used to express the single- or multi-elemental contamination (Table 2). We designated only one composite soil sample at each sampling location (from the total of 3-9 samplings) with the most numerous enriched elements as being the most polluted and defined it as "a hot spot", and data for less contaminated samples from each site were excluded from detail analysis in this survey.

Table 2

Comparison of soil classification according to the pollution quantification indices applicable in the incidence of single- ([a, c, e]) or multi-element ([b, d]) excess in the soil.

Limits		Classification	Limits		Classification	Limits	Classification
E^i_r [a]	RI [b]		C_{SGV} [c]	Z [d]		I_{geo} [e]	
<40	<150	Low risk	≤1	<16	Permissible	<0	Unpolluted
40–80	150–300	Moderate risk	1–3	16–32	Average hazard	0–1	Unpolluted to moderately polluted
						1–2	Moderately polluted
80–160	300–600	High risk	3–10	32–128	Hazard	2–3	Moderately to strongly polluted
						3–4	Strongly polluted
160–320	≥600	Very high risk	>10	>128	Extreme hazard	4–5	Strongly to extremely polluted
≥320		Extreme risk				>5	Extremely polluted

[a] E^i_r – potential ecological risk index of an individual element, that considers the pollutant type, concentration, toxicity level and synergistic effects in the environmental response; $E^i_r = \Sigma T^i_r \times C_n / \text{SGV}$, where T^i_r –toxicity response index for each element, which expresses the toxic level, the environmental response and sensitivity to the element (Table A.1), C_n – concentration (mg/kg) of a given element in the examined sample, SGV – soil guideline value (mg/kg) of the element (Table A.1);

[b] RI – potential ecological risk index, that consists of the sum of potential ecological risk indices for all detected PTEs; $\text{RI} = \Sigma E^i_r$;

[c] C_{SGV} – excess coefficient, that shows the magnitude to which the element exceeds the soil guideline value; $C_{SGV} = C_n / \text{SGV}$;

[d] Z – index of the total soil contamination, that accounts only for elements exceeding background level; $Z = \Sigma C_c - (n - 1)$, where C_c – element concentration coefficient; $C_c = C_n / C_{Bn}$, where C_{Bn} – local geochemical background level (mg/kg) of the element determined from the subset of 8 uncontaminated Bn soil samples (Table A.2); n – the sum of elements;

[e] I_{geo} – geoaccumulation index, that considers the effects of natural geological processes and human activities on PHE pollution and is used to quantify the enrichment or pollution degree of PHEs in soils by comparing the current and preindustrial element concentrations; $I_{geo} = log_2 (C_n / 1.5C_{Bn})$

The local background geochemistry was determined from eight presumably uncontaminated soil sub-samples Bn1–Bn8 (Table A.2) that were later combined into one sample to be used as soil control in plant cytogenetic analysis.

2.2 Cytogenetic analyses

Common onion *Allium cepa* bulbs were grown in 50 g of sieved dry weight soil dampened with distilled water following Rank and Nielsen [31,32] methodology. Tap water was used as negative control (C_N), a solution of 10 mg/l of methyl methanesulfonate (MMS) (Sigma) – as a positive control (C_P), and uncontaminated background soil – as a soil control (C_{Bn}) (Table A.2). A set of 6 onions was used for each treatment (controls and soil samples), and all experiments were performed in triplicate.

A set of onions was grown in soil for 96 hours for the root growth inhibition (RGI) test. The average length of the root bundle for each bulb was measured and expressed as the RGI % of growth compared to the negative control. Another set of onions was grown in soil for 24 h for a modified *Allium* chromosome aberration (CA) assay and determination of exposure mitotic index (MI_{24}), and then recovered in tap water for additional 24 h to determine the frequency of micronuclei (MN) induction, cell death rate (CDR) and recovery MI_{48}. CA and MI indices were calculated

according to standardized methodology [31,33,34], and the MN and CDR rates were determined for 1000 non-dividing interphase cells per bulb.

2.3 DNA extraction and PCR procedures

A set of 6 onions was grown in sieved soil for 96 hours for every test-soil dampened with distilled water. Total DNA from 100 mg of root tissue was extracted using the Genomic DNA Purification Kit according to the manufacturer's instructions (ThermoFisher Scientific, Lithuania). Concentrations of the extracted DNA samples were measured at 260 nm, and the DNA purity was estimated by measuring the 260/280 m absorbance ratio by NanoDrop® 2000. Diluted DNA samples (50 ng/µL) from the same onion set per treatment were pooled into a composite DNA sample.

Nine primers (Metabion, Germany) were used in RAPD analysis: B389 (5′-CGCCCGCAGT-3′), BC374 (5′-GGTCAACCCT-3′), OPA01 (5′-CAGGCCCTTC-3′), OPA03 (5′-AGTCAGCCAC-3′), OPA07 (5′-GAAACCGGTG-3′), OPA13 (5′-CAGCACCCAC-3′), OPB05 (5′-TGCGCCCTTC-3′), OPB07 (5′-GGTGACGCAG-3′) and OPB10 (5′-CTGCTGGGAC-3′). PCR was performed in 8 µL with a final volume containing 60 ng DNA, 0.2 mM dNTP, 0.5 µM primer, 0.15 U DreamTaq DNA polymerase (Thermo Fisher Scientific, Lithuania). PCR was performed as follows: 2 min initial denaturation at 95°C; 45 cycles of 1 min at 95°C, 1 min at 36°C and 2 min at 72°C; and a final extension for 8 min at 72°C in a Mastercycler® Gradient PCR machine (Eppendorf, Germany).

Five primers were used in ISSR analysis: UBC811 (5′-$(GA)_8$C-3′), UBC823 (5′-$(TC)_8$C-3′), UBC825 (5′-$(AC)_8$T-3′), UBC844 (5′-$(CT)_8$TRC-3′) and UBC886 (5′-VDV$(CT)_7$-3′), where R = (A, G) and V = (A, C, G). PCR was performed in 10 µL with a final volume containing 60 ng DNA, 0.2 mM dNTP, 0.4 µM primer, 0.5 U DreamTaq DNA polymerase. PCR was performed as follows: 3 min initial denaturation at 95°C; 38 cycles of 30 s at 95°C, 30 s of primer annealing and 100 s at 72°C; and final extension for 10 min at 72°C in Mastercycler® Gradient PCR machine (Eppendorf, Germany).

All amplifications were duplicated, and only reproducible polymorphic bands were analysed after separation with 1.5% (w/v) agarose gels using TAE buffer, run at 8 V/cm for 3 h, stained with ethidium bromide, aligned to GeneRuler™ DNA Ladder Mix SM0331 (Thermo Fisher Scientific, Lithuania) and visualized under UV light (BioDocAnalyze, Biometra).

2.3 Statistical analysis

Prior to the data analysis, the Kolmogorov-Smirnov test was performed to verify the distribution of the data. Raw data on PHE concentrations was *z*-transformed to reduce the influence of differences in the magnitude of variables, and the non-parametric cytogenetic data was *log*-transformed and reanalysed to verify if normality conditions were met. For all statistical tests, the significance was accepted at a probability level of $p < 0.05$.

Average values of indices for industrial sites were calculated using pooled raw analysis data from all sampling points from industrial areas. The same approach was employed for the landfills. A principal component analysis (PCA) was carried out on geochemical data, and principal components were extracted when they shared eigenvalues greater than 1 using Past3 software [35]. For each principal component, factor loadings > 0.7 were regarded as significant.

All cytogenetic experiments were performed in triplicate and continuous data variance between the groups and between the controls and treatment groups were tested using a one-way ANOVA test followed by Tukey and Dunnett's multiple comparisons tests in GraphPad Instat version 3.05 (GraphPad Software, San Diego California USA, www.graphpad.com). Statistical significance of the categorical binary genotoxicity data was evaluated by χ^2-test. Spearman rank order correlation analysis was performed for relationship testing of the cyto- and geoindices and metal content in the soil using Statistica version 7 (StatSoft. Inc., www.statsoft.com).

The electrophoretic data was codified as (1) for the presence of bands and (0) for their absence. Binary data were analysed using 1000 permutations and UPGMA dendrograms generated in TreeCon v1.3b [36]. Genetic distance matrices were compared performing a Mantel test in GenAlEx v6.5 [37,38].

3. Results and Discussion

3.1 Soil geochemistry and ecotoxicological ranking

We selected 15 PHEs to be determined in test-soils, as Cu, Ni, Zn, Cd, Cr and Pb are commonly monitored elements with recommendations to monitor for Ba, Co, Sn, As, Hg, Mo, and Mn to ensure there is no accumulation in the soil or deviations from normal composition [39]. Two types of soil contamination sources, represented by 4 areas of different industrial activities and 3 landfills of household waste (Table 1), received our attention due to their long-term environmental impacts [4] despite being inactive for more than a decade. We classified sampling areas according to the proposed grades of the risk and hazard assessment based on their geochemistry (Table 2).

Analysis of the local geochemical background composition of soil showed low levels of all the determined elements (Table A.3) and none of them exceeded SGV (Table A.1) with Ba being the most abundant among studied elements in all background soil samples. Soils from industrial areas and landfills had different dominating elements, along with different proportions of PHEs in a total bouquet of the 15 analysed elements. In PCA analysis, soils were grouped according to their normalised total PHE content and I1 and I2 were the most distinct from the rest of the soil samples while landfill soils grouped together with the background control (Fig. A.1).

The single-element concentration coefficient C_c showed that each PHE exceeded its background level in at least one soil sample, but Cu and Se were the only PHEs exceeding background values in all soil samples independent of the contamination type (Table 3). When element concentrations were compared to SGV, striking enrichment was determined in all soils from the industrial sites; namely, 4–11 PHEs exceeded or were equal to their SGVs. Even more elements were enriched when compared to the local geochemical composition indicating serious anthropogenic pollution [26]. Concentrations higher than established SGV indicate which excessive PHEs may pose widespread health-based risks and the elements exceeding SGV by more than 10-fold show extreme hazard for contamination (Table 2). Pb concentrations higher than the consensus-based probable effect concentration of 128 mg/kg are likely to cause harmful effects [40]; it was overrun in test-soil I1 (3266 mg/kg) (Table A.3). Zn is the most commonly enriched metal in city soil [39]; it was excessive in all soils from industrial areas and in only one from the landfills. It is important to note that we selected only one of the most polluted soil samples at each sampling location and data for less contaminated samples were excluded from this survey; therefore, it does not reflect the whole territory situation, which might overestimate its contamination level [4]. Cd, Cr, Hg in I1, Mo in I2, and Se in I3 soil exceeded SGV values by 3–10 fold. In other studies, Cd showed the highest ecological risk compared to the other metals, when soil enzyme activities were analysed in industrial regions [41]. According to Hakanson [26], Hg and Cd have the highest toxicity-response index (40 and 30, respectively). There are studies associating animal liver and blood Cd concentration with metastatic kidney tissue, indicating that the lesion might have been caused by Cd exposure [42]. Also, due to the ingestion and inhalation exposure [4] to carcinogenic elements (i.e., As, Cd, Co, Cr, Ni and Pb), people may develop cancer [43]. There are studies clearly showing harmful element concentrations in children's hair are depended on the environment where kids spent the most of their time [44].

Geoindices that we employed in this survey have different classification systems (Table 2). We attributed every soil sample to the risk/hazard categories based on the corresponding geoindex limits or according to the maximum value of a geoindex in single-element quantifications (Table 4). Maximum values of C_{SGV} and E^i_r between industrial and landfill areas differed by 4-fold (Table A.4), while multi-elemental RI and Z indices between two contamination types differed by 11-fold, revealing a considerably greater contamination at industrial sites and that landfills may pose a reduced risk to the environment or human health (Table 4, Fig. A.2). Our results agree with an urban soil contamination study where, among the five studied regions (utilities, commercial, industrial, tourism, and roadside), the industrial areas had the highest metal concentrations [41].

Table 3

Comparison of test-soil geochemistry through excess (C_{SGV}) and concentration (C_c) coefficients.

Soil sample		Potentially harmful elements [a]
Industrial areas		
I1	C_{SGV} [b]	$Pb^{41} > Cu^{26} > Sn^{12} > Cr^{9} > Zn^{8.8} > Cd^{8} > Hg^{5} > Ni^{2.4} > Mo^{1.9} > Se^{1.6} > As^{1.3}$
	C_c [c]	$Cu^{330} > Pb^{208} > Sn^{87} > Cd^{73} > Zn^{69} > Hg^{31} > Cr^{27} > Ni^{18} > Mo^{14} > Se^{11} > As^{10} > Co^{5} > Mn^{3} > Ba^{1.3}$
I2	C_{SGV}	$Mo^{7} > Hg^{3} > Zn^{2} > Pb^{1.3} > Cr^{1.2} > Ba^{1}$
	C_c	$Mo^{51} > Hg^{18} > Zn^{16} > Pb^{6.9} > Cu^{5.7} > Se^{4.6} > Cr^{3.5} > V^{3.1} > Ba^{1.8} > Co^{1.75} > Sn^{1.72} > Ni^{1.71} > As^{1.3}$

I3	C_{SGV}	$Se^{6} > Zn^{2} > Hg^{1.6} > Pb^{1.4}$
	C_c	$Se^{41} > Zn^{15} > Cu^{12} > Hg^{10} > Pb^{7} > Sn^{4.5} > V^{2.12} > Ni^{2.09} > Cr^{1.5} > Mo^{1.4} > As^{1.2} > Co^{1.1}$
I4	C_{SGV}	$Cr^{5} > Ni^{2.1} > Mo^{1.9} > Zn^{1.1} > Cu^{1.01} > Pb^{0.9}$
	C_c	$Ni^{15} > Cr^{14.1} > Mo^{14} > Cu^{13} > Zn^{9} > Pb^{5} > Co^{3} > Se^{2.8} > V^{1.2}$
Landfills		
L1	C_{SGV}	Zn^{13}
	C_c	$Zn^{98} > Mo^{6} > Se^{4} > Cu^{2.7} > Pb^{2.3} > V^{1.5} > Cr^{1.4} > As^{1.3} > Ni^{1.2}$
L2	C_{SGV}	$Cd^{2.3}$
	C_c	$Cd^{22} > Se^{2.5} > Cu^{1.5} > V^{1.4} > Mo^{1.14} > Pb^{1.05} > Cr^{1.04} > Ni^{1.03}$
L3	C_{SGV}	$Sn^{1.6}$
	C_c	$Sn^{11} > Se^{2} > Co^{1.2} > Cu^{1.03}$

[a] Represents only enriched elements with the respective excess value in superscript.

[b] Only excess elements exceeding SGV limits given in descending order.

[c] Only excess elements exceeding the local background values given in descending order.

Soils were ranked into 4 hazard categories (Table 4) based on the potential ecological risk index E^i_r for individual PHEs. Only Cd, Cu, Hg, and Pb showed moderate to very high risk of PHE excess in soils (data not shown). I1 was the only soil bearing RI values greater than 600 which is considered as a very high risk for human health while the other sites were at a low-risk level determined by RI index (Table A.4). When soils were analysed as of industrial or landfill contamination origin, they were attributed to the different risk categories based on RI values (268 and 41, respectively).

Table 4

Test-soil contamination categories according to the risk (E^i_r, RI), hazard (C_{SGV}, Z) and geoaccumulation (I_{geo}) indices applicable in the incidences of single- ([a]) or multi-elemental ([b]) excess determined for each sampling site and averaged for industrial and landfill areas.

Site	E^i_r [a]	RI [b]	C_{SGV} [a]	Z [b]	I_{geo} [a]
Background	*low risk*	*low risk*	*permissible*	*permissible*	-
I1	very high risk	very high risk	extreme hazard	extreme hazard	extremely contaminated
I2	high risk	low risk	hazard	hazard	extremely contaminated
I3	moderate risk	low risk	hazard	hazard	extremely contaminated
I4	low risk	low risk	hazard	hazard	extremely contaminated
Average for industrial areas [c]	*high risk*	*moderate risk*	*extreme hazard*	*extreme hazard*	*extremely contaminated*
L1	low risk	low risk	extreme hazard	hazard	extremely contaminated
L2	moderate risk	low risk	average hazard	average hazard	extremely contaminated
L3	low risk	low risk	average hazard	permissible	extremely contaminated
Average for landfills	*moderate risk*	*low risk*	*extreme hazard*	*hazard*	*extremely contaminated*

[a] Represents only the maximum evaluation (extracted from the individual PHE analysis data) by a single-element index.

[c] Average index value for the industrial areas was determined from averaged PHE content in all samples (I1–I4), the same applies to the landfills.

In the cases where only one element was abundant, an enrichment coefficient C_{SGV} was used for soil hazard evaluation, and the total soil pollution Z index was determined when the environmental sample possessed many different elements reaching a dangerous contamination level (Table 1). The Z index was used to identify areas where the danger for the total sickness rate was elevated [44]. In our study, we determined the intense pollution at most sites using both C_{SGV} and Z indices (Table 4). This situation arouses great attention and warning because the analysed sites with elevated amounts of toxic elements are near residential sites in the city. Soil I1, having the highest levels of danger determined by all 5 indices, was from the city centre. This hot spot of soil pollution was a former factory of electrical gauges that used a wide spectrum of HM in their production processes and was also impacted by the high technogenic load from motor vehicles. The extent data, to which I_{geo} calculations for Ba, Cr, Cu, Mn, Ni, Pb, V and Zn exceeded preindustrial level (data not shown), designates an extreme contamination pattern in every test-soil indicating the intensive anthropogenic load of PHEs and disclosing no differentiation in the intensity of test-soil contamination. The maximum values of C_{SGV} and E^i_r together with the Z index ranked soil samples into more variable hazard and risk categories and were more efficient in soil contamination discrimination compared to I_{geo}, values, which have ranked all soils as extremely contaminated, or RI discerning only 2 risk categories for the soils (Table 4). RI was calculated only for those 11 PHEs

that have defined T^i_r values (Table A.1), while the *Z* index was calculated for all 15 PHEs. Despite that difference, a statistically significant correlation was found between RI and *Z* ($r = 0.80$), as well as RI and C_{SGV} ($r = 0.80$) (Table A.5). Application of several geoindices provided a different classification of the test-soils as either contaminated, less or noncontaminated indicating their variable risk/hazard levels dependent on the selected geoindex.

3.2 Test-soil cyto/genotoxicity evaluation

Environmental samples are complex mixtures comprised of various substances and a wider spectrum of approaches is needed for a primary screening of possible biological effects in living systems [45]. We applied a test battery of several *Allium* bioassays to assess the macroscopic, microscopic and genetic changes induced by HM-contaminated urban soil from anthropogenic pollution hot spots.

Phytotoxicity is one of the many adverse effects induced by chemicals in plants and may be measured through the growth restriction – a parameter expressing the total of all damage effects [46]. We checked test-soils for cytotoxicity in *Allium* using an RGI test often used as a primary indicator in screening surveys for its suitability to monitor potentially cytotoxic agents that affect root growth well before DNA damage occurs. Onion root length changed significantly ($p < 0.001$) in every test-soil, except L2 (Fig. 1A). Root growth was stimulated in I1 and I4, while the rest soils inhibited root growth, showing possible toxic effects on plant growth and development [47]. Root inhibition was about 3-fold stronger in landfill soils (Fig. 1A), and disagreed with Vujošević *et al.* [48], who pointed that the presence of organic pollution, often present in landfills, may stimulate root growth. RGI results were conflicting with the risk prediction as the greater risk was calculated for industrial soils (Fig. 1A, Table 4) and with a study by Liu *et al.* [49] where Pb, an element that is in excess in all of our test-soils from industrial sites, significantly inhibited onion root growth. RGI showed no dependence on any element concentration nor geoindex except a correlation with E^i_r for Cd, Cu and Ni ($r = 0.749$, 0.767, and 0.767, respectively, $p < 0.05$), designating that the growth response was determined by complex interrelationships of many substances in the soil (Table A.5).

A decrease of MI below 50 % of the control level is the cytotoxic limit that induces sublethal effects in the test-organism and a decrease below 22 % is lethal [50], but none of the test-soils had such strong effects on onion root cells in this study. Nonetheless, the MI values in exposed onions changed significantly (Fig. 1B). Onions grown in industrially polluted soils showed no mitotic recovery in tap water (Fig. 1C), whereas two landfill soils had a detrimental effect on onion MI that disappeared after recovery in tap water showing easier recovery after exposure, yet, the change was not statistically significant in L2 ($p > 0.05$). Possibly, the inhibition of mitotic activities could happen due to physical factors like a lack of aeration and a physical hindrance to growth [50]. A significant MI increase from the control indicates the impact of substances with mitogenic capacity, causing an increased rate of cell division, which can lead to uncontrolled cell proliferation and phytocarcinoma induction [51]. The majority of the soils had affected MIs in a stimulating way, showing a potential to stimulate cell division, but that disagrees with our findings of the soil's potential to inhibit root growth. The stimulation of mitosis may be due to the waste that is rich in phosphorus and nitrogen, which are abundant in domestic sewage [52].

RGI results correlated only with the recovery MI_{48} ($r = –0.70$, $p < 0.05$) and it might be that elongation during exposure was not occurring appropriately because its responsible enzymes were affected by substances present in the soils [53]. Only exposure MI_{24} was positively dependent on the Cu, Mo and Ni amount in the soil (Table A.5). MI had no significant relationship with any of multi-elemental geoindices, thus no association between geochemistry and cell proliferation was found.

Application of a modified *Allium* test when just anaphase-telophase cells were analysed revealed that only L3 soil failed to induce CA in onion cells (Fig. 1B), agreeing with its lowest hazard and risk evaluation according to the multi-elemental geoindices (Fig. 1A, Table 4). Lower mitotic activity is often correlated with higher CA frequency [54], as this relationship shows an adverse effect of exposure [1,9] but we did not find such relationship in our research. Nevertheless, the CA frequency was dependent on RI, *Z* and C_{SGV} indices, indicating that the CA rate was consistent with the intensity of the soil contamination level. Correlation analysis revealed that CA induction depended on the concentrations of 9 PHEs (Table A.5). Our results agree with other studies where genetic damage was positively correlated with high levels of HM, resulting in genetic instability in plants [55].

The most hazardous soil I1 induced the highest rate of cell death (Fig. 1C), indicating acute soil phytotoxicity when cells with multiple alterations did not survive. Soils from industrial areas induced almost 4-fold higher CDR in onion

cells (Fig. 1C); DNA damage resulting in apoptotic cell death was evident from other studies using genotoxicity assays [56]. CDR and CA were strongly interdependent (Table A.5) and both changed in a dose-dependent manner relative to the *Z* index. It is noted that only cytogenetic preparations where MI ≥ 1 % are suitable for CA and MN frequency evaluation and only such slides were included in the analysis. Despite that, cell death induction diminished the ability to evaluate MN frequency correctly and the most hazardous I1 did not induce the MN at a rate that may be expected from its risk evaluation. Nevertheless, it correlated with CDR ($r = 0.733$, $p < 0.05$). A similar trend was found in Čėsnienė *et al.* [20], where the most contaminated test-soil induced the MN rate in *Tradescantia* at the similar potency compared to the less contaminated (about 10 times lower *Z* value) test-soils. MN induction in exposed onions was not dose-dependent to the PHE content in the test-soil, and this coincides with other studies where MN frequency was low and did not correlate with Zn, Pb, Cu, Ni, Cd or As [50]. A positive MN relationship was determined with E^i_r for As, and a negative one with E^i_r for Cd and V. Dose-dependent relationships were determined between MN rate and two indices, *Z* and C_{SGV}, in individual soil analyses. Onions exposed to landfill soils had a significantly higher average MN rate compared to those exposed to the soils from industrial sites contaminated at a higher degree. Plants differ significantly in their tolerance to HM according to the mechanisms of tolerance [57] and there are some plants that are capable of surviving in the presence of HM [58]. At the time the *Allium* test was suggested as a standard in environmental monitoring in 1985, several metals (Al, Be, Cd, Cu, Hg, Li, Mn, Ni and Se) were shown to induce chromosomal abnormalities or root growth retardation in onions [46]. Subsequent research determined various cytogenetic effects in onions exposed to aqueous solutions or effluents contaminated with an even wider spectrum of metals: As, Cr, Fe, Pb and Zn [59]. Some studies conclude that the classical *Allium* test can give more comprehensive data when done in combination with other assays [50] and therefore, we also screened test-soil for its possible deleterious effects using two systems of polymorphic molecular DNA markers, RAPD and ISSR, after direct 96 h onion root exposure to the soil.

Nine RAPD primers amplified 166 bands in total, of which 149 were polymorphic, while five ISSR primers generated 112 bands in total, and 104 were polymorphic. Polymorphic bands were employed in the generation of genetic distance matrices further used for dendrogram construction and molecular assay correlation analysis. RAPD and ISSR dendrograms revealed the same tendency – the pairs of the most heavily polluted I1 and L1, less polluted I2 and I3, and least polluted I4 and L2 soils (Table 4) were grouped on the same dendrogram branches (Fig. 2). This indicates similar genetic changes induced in onion roots by HM-contaminated soils of similar contamination hazard level.

RAPD and ISSR markers cover different regions of the genome (variable regions of DNA and tandem-repeat regions, respectively), therefore we initially selected both marker systems for the analysis. Application of different markers enables a wider screening of sites where mutations can occur especially when a priori information about the nature of genetic damage is missing. And despite the different nature of DNA markers, RAPD and ISSR marker systems differentiated onion DNA profiles when grown in uncontaminated background soil from those grown in contaminated soil. A Mantel test, correlating genetic distance matrices generated from RAPD and ISSR data, revealed concordance between results of both marker systems ($r = 0.473$, $p = 0.032$). The result indicates contaminated soil had a different access to DNA material when less contaminated or uncontaminated soil induced different exposure effects probably with fewer mutation events. With the increasing frequency of incidents of genetic impairments, seen as appeared/disappeared RAPD or ISSR bands, consistently increases the possibility of occurrence of more severe mutations, chromosomal lesions or large rearrangements. Though, any changes in RAPD or ISSR profiles can be harmful to the organism as those changes may become heritable [16,21]. Different combinations of HM were responsible for general toxicity in plants, and besides the induction of oxidative stress, it also induces alterations in the DNA damage response [17,60]. Even though molecular marker results revealed a change in the amplification profiles of exposed and unexposed onions, our results do not have the same tendency of a dose-dependent band loss or gain resulting from *Allium* exposure to the tested compounds as in other studies [12,18,61]. Nevertheless, there is a deficiency of soil genotoxicity studies involving *Allium* molecular markers, and our study confirms that RAPD and ISSR marker systems can be successfully applied to evaluate how environmental pollutants modify the structure of DNA in onions. Since both dendrograms clearly show genetic divergence among onions grown in soil of different pollution level, we suggest the use of ISSR analysis over RAPDs as a more robust assay.

Chemical analysis and risk indices can over- or underestimate the detrimental effects in different organisms as their tolerance and sensitivity may differ by a great magnitude. The ease of sample preparation and the cost, accuracy and reliability of complex of analytical methods are the most important characteristics and are desirable in all research studies. Studies of environmental monitoring that design and employ biomarkers and methodologies for the application of fast and simplified assays are increasingly valuable as a measure of potential pollution [44,52]. *Allium* showed

genotoxicity for each test-soil and the highest rate of every cytogenetic index was induced by the different soil. Moreover, in every test-soil, the elemental composition was different and the risk to induce adverse effects in living organisms along with the elemental interactions also depend on each element availability, stability, mobility, etc. [1,45,62]. Thus, without testing in model organisms or test-systems, it is difficult to predict the final outcomes of organism exposure to such complex mixtures of pollutants as individual detailed chemical analysis is limited in its ability to do and thus, knowledge of the behaviour of PHEs is far from complete [1,39,45]. All cytogenetic indices were checked for correlation with each PHE concentration, and some chemical elements were related to *Allium* indices (Table A.5). This finding reflects a more complex plant response to total soil contamination compared to the effects induced by a single excessive element. Results from *Allium* bioassay application confirm that SGV can only predict possible risks but cannot forecast the real detrimental effects on organisms. Even multi-elemental geoindices are probably insufficient to predict potential genotoxicity and cannot be extrapolated straight into potential detrimental effects in organisms. Despite a relationship between RI and *Z* ($r = 0.8$, $p < 0.05$), both geoindices correlated with different cyto/genotoxicity parameters in *Allium*, and the *Z* index showed more numerous relationships. We determined a positive relationship between RI and CA frequency, while *Z* index was related to MN, CA and CDR. The findings suggest that the *Z* index should be one of the first choices in soil ecotoxicity testing employing *Allium* assays. To minimize the health risks to the public, impact on the natural environment and resources, industrial sites and landfills should undergo safety evaluation for which an application of a test battery that includes several bioassays is strongly advised. We recommend the simultaneous use of all the methods presented to obtain an objective assessment of soil genotoxicity.

4. Conclusions

We employed several geoindices along with a battery of *Allium* cytogenetic and molecular bioassays to assess the possible deleterious effects of environmental inorganic pollution. In this research, seven hot spots of anthropogenic soil pollution were evaluated geochemically. The soil from the surroundings of industrial facilities was more severely polluted with heavy metals compared to landfill soil. However, in this study, the outcome of soil exposure in an *Allium* test-system was the same for all test-soils – the detrimental cyto/genotoxic effects were induced by every test-soil, despite their different contamination level and hazardousness. Thus, test-soils, although a fraction of them had permissible contamination levels, could not be classified as non- or genotoxic in that their genotoxic effects were revealed by at least one plant bioassay. Our results revealed that sediment solo chemical analysis was not sufficient to predict genotoxic potential, and all *Allium* bioassays complemented each other by depicting different aspects of exposure to toxic substances. In conclusion, the evaluation of cyto/genotoxicity using a battery of different plant bioassays is important in the risk assessment and ecotoxicological evaluation of the hot spots of soil pollution.

Declaration of interest

None.

Acknowledgements

This work was supported by the Research Council of Lithuania [grant number MIP-042/2015].

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at […]

References

[1] P.A. White, L.D. Claxton, Mutagens in contaminated soil: a review, Mutat. Res. 567 (2004) 227–345. doi:10.1016/j.mrrev.2004.09.003.

[2] R. Anjum, A. Malik, Mutagenicity assessment of contaminated soil in the vicinity of industrial area, Environ. Monit. Assess. 184 (2012) 3013–3026. doi:10.1007/s10661-011-2167-7.

[3] V. Kadūnas, V. Katinas, A. Radzevičius, R. Taraškevičius, R. Zinkutė, Geochemical atlas of Lithuania – A basis for detailed geochemical investigations of long-term ecological research sites, Acta Zool. Litu. 15 (2005) 131–135. doi:10.1080/13921657.2005.10512389.

[4] P.W. Hadley, S.D. Mueller, Evaluating "hot spots" of soil contamination (Redux), Soil Sediment Contam. An Int. J. 21 (2012) 335–350. doi:10.1080/15320383.2012.664431.

[5] A.V. Gorbunov, S.M. Lyapunov, O.I. Okina, M. V. Frontas'eva, S.S. Pavlov, I.N. Il'chenko, Nuclear-physical analysis methods in medical geology: Assessment of the impact of environmental factors on human health, Phys. Part. Nucl. 46 (2015) 424–451. doi:10.1134/S1063779615030065.

[6] M. Staniszewska, H. Boniecka, Managing dredged material in the coastal zone of the Baltic Sea, Environ. Monit. Assess. 189 (2017) 46. doi:10.1007/s10661-016-5734-0.

[7] Z. Yuan, J. Yao, F. Wang, Z. Guo, Z. Dong, F. Chen, Y. Hu, G. Sunahara, Potentially toxic trace element contamination, sources, and pollution assessment in farmlands, Bijie City, southwestern China, Environ. Monit. Assess. 189 (2017) 25. doi:10.1007/s10661-016-5755-8.

[8] R. Zinkutė, R. Taraškevičius, M. Jankauskaitė, Ž. Stankevičius, Methodological alternatives for calculation of enrichment factors used for assessment of topsoil contamination, J. Soils Sediments. 17 (2017) 440–452. doi:10.1007/s11368-016-1549-4.

[9] J. Rank, L.C. Lopez, M.H. Nielsen, J. Moretton, Genotoxicity of maleic hydrazide, acridine and DEHP in *Allium cepa* root cells performed by two different laboratories, Hereditas. 136 (2002) 13–18. doi:10.1034/j.1601-5223.2002.1360103.x.

[10] G. Fiskesjö, *Allium* test on river water from Braån and Saxån before and after closure of a chemical factory, Ambio. 14 (2011) 99–103. http://www.jstor.org/stable/4313118.

[11] C.P. de Souza, T. de A. Guedes, C.S. Fontanetti, Evaluation of herbicides action on plant bioindicators by genetic biomarkers: a review, Environ. Monit. Assess. 188 (2016) 694. doi:10.1007/s10661-016-5702-8.

[12] S. Correia, M. Matos, V. Ferreira, N. Martins, S. Gonçalves, A. Romano, O. Pinto-Carnide, Molecular instability induced by aluminum stress in *Plantago* species, Mutat. Res. Toxicol. Environ. Mutagen. 770 (2014) 105–111. doi:10.1016/J.MRGENTOX.2014.06.002.

[13] A. Stapulionytė, S. Bondzinskaitė, M. Stravinskaitė, R. Šiukšta, R. Taraškevičius, T. Čėsnienė, Soil genotoxicity biomonitoring in recultivated factory area using the cytogenetic and molecular assays in two plant test-systems, in: Proccedings Int. Sci. Conf. "Rural Dev. 2017," Aleksandras Stulginskis University, 2018. doi:10.15544/RD.2017.025.

[14] S. Pal, R. Kundu, Heavy metal induced genotoxicity detection by RAPD in Alligator Weed, Int. J. Eng. Technol. Sci. Res. IJETSR Www.Ijetsr.Com ISSN. 2 (2015) 2394–3386. http://www.ijetsr.com/images/short_pdf/1441338203_suparna_ijetsr.pdf (accessed November 14, 2017).

[15] R. Šiukšta, S. Bondzinskaitė, V. Kleizaitė, D. Žvingila, R. Taraškevičius, L. Mockeliūnas, A. Stapulionytė, K. Mak, T. Čėsnienė, Response of *Tradescantia* plants to oxidative stress induced by heavy metal pollution of soils from industrial areas, Environ. Sci. Pollut. Res. (2018). doi:10.1007/s11356-018-3224-3.

[16] I. Dogan, I.I. Ozyigit, G. Tombuloglu, M.S. Sakcali, H. Tombuloglu, Assessment of Cd-induced genotoxic damage in *Urtica pilulifera* L. using RAPD-PCR analysis, Biotechnol. Biotechnol. Equip. 30 (2016) 284–291. doi:10.1080/13102818.2015.1115371.

[17] F. Al-Qurainy, Application of inter simple sequence repeat (ISSR marker) to detect genotoxic effect of heavy metals on *Eruca sativa* (L.), African J. Biotechnol. 9 (2010) 467–474. http://www.academicjournals.org/AJB (accessed November 16, 2017).

[18] P.M. Bernardes, L.F. Andrade-Vieira, F.B. Aragão, A. Ferreira, M.F. da Silva Ferreira, Toxicity of difenoconazole and tebuconazole in *Allium cepa*, Water Air Soil Pollut. 226 (2015) 207. doi:10.1007/s11270-015-2462-y.

[19] A. Ziembińska-Buczyńska, M. Szulc, A. Zgórska, PCR-RAPD based estimation of hospital wastewater genotoxicity on *Allium cepa*, BioTechnologia. 3 (2016) 195–201. doi:10.5114/bta.2016.62357.

[20] T. Čėsnienė, V. Kleizaitė, S. Bondzinskaitė, R. Taraškevičius, D. Žvingila, R. Šiukšta, V. Rančelis, Metal bioaccumulation and mutagenesis in a *Tradescantia* clone following long-term exposure to soils from urban industrial areas and closed landfills, Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 823 (2017) 65–72. doi:10.1016/j.mrgentox.2017.09.004.

[21] S. Sukumaran, A. Grant, Effects of genotoxicity and its consequences at the population level in sexual and asexual *Artemia* assessed by analysis of inter-simple sequence repeats (ISSR), Mutat. Res. Toxicol. Environ. Mutagen. 757 (2013) 8–14. doi:10.1016/J.MRGENTOX.2013.03.015.

[22] R. Taraškevičius, R. Zinkutė, Ag, Ba, Co, Cr, Cu, Mo, Ni, Pb, Sn, V, Zn in topsoil of transport and metal processing works of Vilnius, in: Ż. Bargańska, A. Beyer, K. Klimaszewska, J. Namieśnik, M. Tobiszewski, I. Rutkiewicz (Eds.), Proc. 15th Int. Conf. Heavy Met. Environ., Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, Gdańsk, 2010: pp. 1030–1033. https://inis.iaea.org/collection/NCLCollectionStore/_Public/41/131/41131147.pdf (accessed November 4, 2018).

[23] SĮ Vilniaus planas, Vilniaus aplinka, (2016). http://aplinka.vilnius.lt/lt/index.php/aplinkos-kokybe/uzterstos-teritorijos/uzterstos-teritorijos-monitoringo-ataskaitos/ (accessed July 19, 2016).

[24] Taraškevičius R., The problems of biogeochemical analysis of municipal wastes landfill, in: J. Conf. Abstr., Cambridge Publications, Heidelberg, 1996: p. 614. https://goldschmidt.info/1996/614.html (accessed November 4, 2018).

[25] R. Taraškevičius, J. Radžiūnienė, R. Zinkutė, A. Petrauskienė, M. Jankauskaitė, Conditions to obtain results analysing a small amount of plant material by EDXRF, Chemija. 27 (2016) 114–122. http://www.lmaleidykla.lt/publ/0235-7216/2016/2/114–122.pdf (accessed October 7, 2016).

[26] L. Hakanson, An ecological risk index for aquatic pollution control. A sedimentological approach, Water Res. 14 (1980) 975–1001. doi:10.1016/0043-1354(80)90143-8.

[27] Z.Q. Xu, S.J. Ni, X.G. Tuo, C.J. Zhang, Calculation of heavy metals' toxicity coefficient in the evaluation of potential ecological risk index, Environ. Sci. Technol. 31 (2008) 112–115. http://en.cnki.com.cn/Article_en/CJFDTOTAL-FJKS200802029.htm (accessed January 30, 2018).

[28] R. Zinkutė, V. Baltrūnas, R. Taraškevičius, B. Karmaza, R. Stakėnienė, V. Šeirienė, D. Kisielienė, Quaternary interglacial sediments as possible natural sources of arsenic and molybdenum anomalies in stream sediments in Lithuania, J. Environ. Eng. Landsc. Manag. 23 (2015) 60–70. doi:10.3846/16486897.2014.926909.

[29] V. Gregorauskienė, R. Taraškevičius, V. Kadūnas, A. Radzevičius, R. Zinkutė, Geochemical characteristics of Lithuanian urban areas, in: Mapp. Chem. Environ. Urban Areas, John Wiley & Sons, Ltd, Chichester, UK, 2011: pp. 393–409. doi:10.1002/9780470670071.ch23.

[30] G. Müller, Die Schwermetallbelastung der Sedimenten des Neckars und Seiner Nebenflusse, Chem. Zeitung. 105 (1981) 157–164.

[31] J. Rank, M.H. Nielsen, A modified *Allium* test as a tool in the screening of the genotoxicity of complex mixtures, Hereditas. 118 (1993) 49–53. doi:10.1111/j.1601-5223.1993.t01-3-00049.x.

[32] M.H. Nielsen, J. Rank, Screening of toxicity and genotoxicity in wastewater by the use of the *Allium* test, Hereditas. 121 (1994) 249–254. doi:10.1111/j.1601-5223.1994.00249.x.

[33] J. Rank, M.H. Nielsen, Genotoxicity testing of wastewater sludge using the *Allium cepa* anaphase-telophase chromosome aberration assay, Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 418 (1998) 113–119. doi:10.1016/S1383-5718(98)00118-1.

[34] J. Rank, The method of *Allium* anaphase-telophase chromosome aberration assay, Ekologija. 1 (2003) 38–42.

http://www.lmaleidykla.lt/publ/0235-7224/2003/1/E-38.pdf.

[35] P.D. Ryan, Ø. Hammer, D.A. Harper, D.D. Paul Ryan, PAST: paleontological statistics software package for education and data analysis, Palaeontol. Electron. 178kb. T. Harper. Geol. Museum. 4 (2001) 5–7. http://palaeo-electronica.org (accessed June 20, 2018).

[36] Y. Van de Peer, R. De Wachter, TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment., Comput. Appl. Biosci. 10 (1994) 569–570. http://www.ncbi.nlm.nih.gov/pubmed/7828077 (accessed November 13, 2017).

[37] R. Peakall, P.E. Smouse, Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research, Mol. Ecol. Notes. 6 (2006) 288–295. doi:10.1111/j.1471-8286.2005.01155.x.

[38] R. Peakall, P.E. Smouse, GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update., Bioinformatics. 28 (2012) 2537–2539. doi:10.1093/bioinformatics/bts460.

[39] A. Kabata-Pendias, Trace Elements in Soils and Plants, CRC Press, 2011. doi:10.1201/b10158-25.

[40] H. Haris, L.J. Looi, A.Z. Aris, N.F. Mokhtar, N.A.A. Ayob, F.M. Yusoff, A.B. Salleh, S.M. Praveena, Geo-accumulation index and contamination factors of heavy metals (Zn and Pb) in urban river sediment, Environ. Geochem. Health. (2017) 1–13. doi:10.1007/s10653-017-9971-0.

[41] Y. Li, H. Li, F. Liu, Pollution in the urban soils of Lianyungang, China, evaluated using a pollution index, mobility of heavy metals, and enzymatic activities, Environ. Monit. Assess. 189 (2017) 34. doi:10.1007/s10661-016-5740-2.

[42] W.G. Brumbaugh, M.A. Mora, T.W. May, D.N. Phalen, Metal exposure and effects in voles and small birds near a mining haul road in Cape Krusenstern National Monument, Alaska, Environ. Monit. Assess. 170 (2010) 73–86. doi:10.1007/s10661-009-1216-y.

[43] E. Zajusz-Zubek, T. Radko, A. Mainka, Fractionation of trace elements and human health risk of submicron particulate matter (PM1) collected in the surroundings of coking plants, Environ. Monit. Assess. 189 (2017) 389. doi:10.1007/s10661-017-6117-x.

[44] R. Taraškevičius, R. Zinkutė, L. Gedminienė, Ž. Stankevičius, Hair geochemical composition of children from Vilnius kindergartens as an indicator of environmental conditions, Environ. Geochem. Health. (2017) 1–24. doi:10.1007/s10653-017-9977-7.

[45] G.I. Paton, K. Killham, H.J. Weitz, K.T. Semple, Biological tools for the assessment of contaminated land: applied soil ecotoxicology, Soil Use Manag. 21 (2005) 487–499. doi:10.1079/SUM2005350.

[46] G. Fiskesjö, The Allium test as a standard in environmental monitoring, Hereditas. 102 (1985) 99–112. doi:10.1111/j.1601-5223.1985.tb00471.x.

[47] R. Kumar, R.K. Mishra, V. Mishra, A. Qidwai, A. Pandey, S.K. Shukla, M. Pandey, A. Pathak, A. Dikshit, Detoxification and tolerance of heavy metals in plants, in: Plant Met. Interact., Elsevier B.V., 2016: pp. 335–359. doi:10.1016/B978-0-12-803158-2.00013-8.

[48] M. Vujošević, S. Anđelković, G. Savić, J. Blagojević, Genotoxicity screening of the river Rasina in Serbia using the *Allium* anaphase–telophase test, Environ. Monit. Assess. 147 (2008) 75–81. doi:10.1007/s10661-007-0099-z.

[49] D. Liu, J. Zou, Q. Meng, J. Zou, W. Jiang, Uptake and accumulation and oxidative stress in garlic (*Allium sativum* L.) under lead phytotoxicity, Ecotoxicology. 18 (2009) 134–143. doi:10.1007/s10646-008-0266-1.

[50] R. Chakraborty, A.K. Mukherjee, A. Mukherjee, Evaluation of genotoxicity of coal fly ash in *Allium cepa* root cells by combining comet assay with the *Allium* test, Environ. Monit. Assess. 153 (2009) 351–357. doi:10.1007/s10661-008-0361-z.

[51] E. Düsman, M. Luzza, L. Savegnago, D. Lauxen, V.E.P. Vicentini, I.B. Tonial, T.P. Sauer, *Allium cepa* L. as a bioindicator to measure cytotoxicity of surface water of the Quatorze River, located in Francisco Beltrão, Paraná, Brazil, Environ. Monit. Assess. 186 (2014) 1793–1800. doi:10.1007/s10661-013-3493-8.

[52] M.F. Amaral, R.S. Gates, D.G. Overhults, I.F. Tinôco, E.G. Wilkerson, H. Li, R.T. Burns, H. Xin, J.E. Earnest, Comparison between two systems for ammonia emission monitoring in broiler houses, in: Int. Symp. Air Qual. Waste Manag. Agric. 16-19 Sept. 2007, Broomfield, Color., American Society of Agricultural and Biological Engineers, St. Joseph, MI, 2007: p. 20. doi:10.13031/2013.23825.

[53] G.L. Silveira, M.G.F. Lima, G.B. dos Reis, M.J. Palmieri, L.F. Andrade-Vieria, Toxic effects of environmental pollutants: Comparative investigation using *Allium cepa* L. and *Lactuca sativa* L., Chemosphere. 178 (2017) 359–367. doi:10.1016/j.chemosphere.2017.03.048.

[54] J.R. Lazutka, A. Stapulionyte, D.K. Bjerketvedt, A. Odland, Seasonal variation in the frequency of abnormal anaphases and mitotic index values in wild populations of herb-Paris (*Paris quadrifolia* L., *Trilliaceae*): Implications for genetic monitoring, Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 534 (2003) 113–122. doi:10.1016/S1383-5718(02)00247-4.

[55] T. Li, M. Zhang, Z. Lu, U. Herman, D. Mumbengegwi, J. Crittenden, Effects of heavy metals from soil and dust source on DNA damage of the *Leymus chinensis* leaves in coal-mining area in northwest China., PLoS One. 11 (2016) e0166522. doi:10.1371/journal.pone.0166522.

[56] M. Ghosh, S. Bhadra, A. Adegoke, M. Bandyopadhyay, A. Mukherjee, MWCNT uptake in *Allium cepa* root cells induces cytotoxic and genotoxic responses and results in DNA hyper-methylation, Mutat. Res. - Mol. Mech. Mutagen. 774 (2015) 49–58. doi:10.1016/J.MRFMMM.2015.03.004.

[57] V. Rancelis, T. Cesniene, D. Zvingila, D. Barysas, L. Balciuniene, S. Dapkuniene, Polymorphism of response to cobalt excess in individual Vicia faba plants, Environ. Exp. Bot. 55 (2006) 221–234. doi:10.1016/j.envexpbot.2004.11.002.

[58] S.K. Pramanik, S. Joarder, S. Das, S. Roy, A. Bhattacharyya, Photodegradation of oryzalin in aqueous isopropanol and acetonitrile, J. Environ. Sci. Heal. Part B. 51 (2016) 287–297. doi:10.1080/03601234.2015.1128741.

[59] D.M. Leme, M.A. Marin-Morales, *Allium cepa* test in environmental monitoring: A review on its application, Mutat. Res. Mutat. Res. 682 (2009) 71–81. doi:10.1016/j.mrrev.2009.06.002.

[60] A. Simonyan, G. Hovhannisyan, A. Sargsyan, M. Arakelyan, S. Minasyan, R. Aroutiounian, DNA damage and micronuclei in parthenogenetic and bisexual *Darevskia* rock lizards from the areas with different levels of soil pollution, Ecotoxicol. Environ. Saf. 154 (2018) 13–18. doi:10.1016/j.ecoenv.2018.02.025.

[61] C. Conte, I. Mutti, Puglisi P, Ferrarini A, Regina G, Maestri E, Marmiroli N, DNA fingerprinting analysis by a PCR based method for monitoring the genotoxic effects of heavy metals pollution, Chemosphere. 37 (1998) 2739–2749. doi:10.1016/S0045-6535(98)00317-8.

[62] F.E. Prado, M. Hilal, S. Chocobar-Ponce, E. Pagano, M. Rosa, C. Prado, Chapter 6 – Chromium and the plant: A dangerous affair?, in: Plant Met. Interact., 2016: pp. 149–177. doi:10.1016/B978-0-12-803158-2.00006-0.

Excel graphs in word file

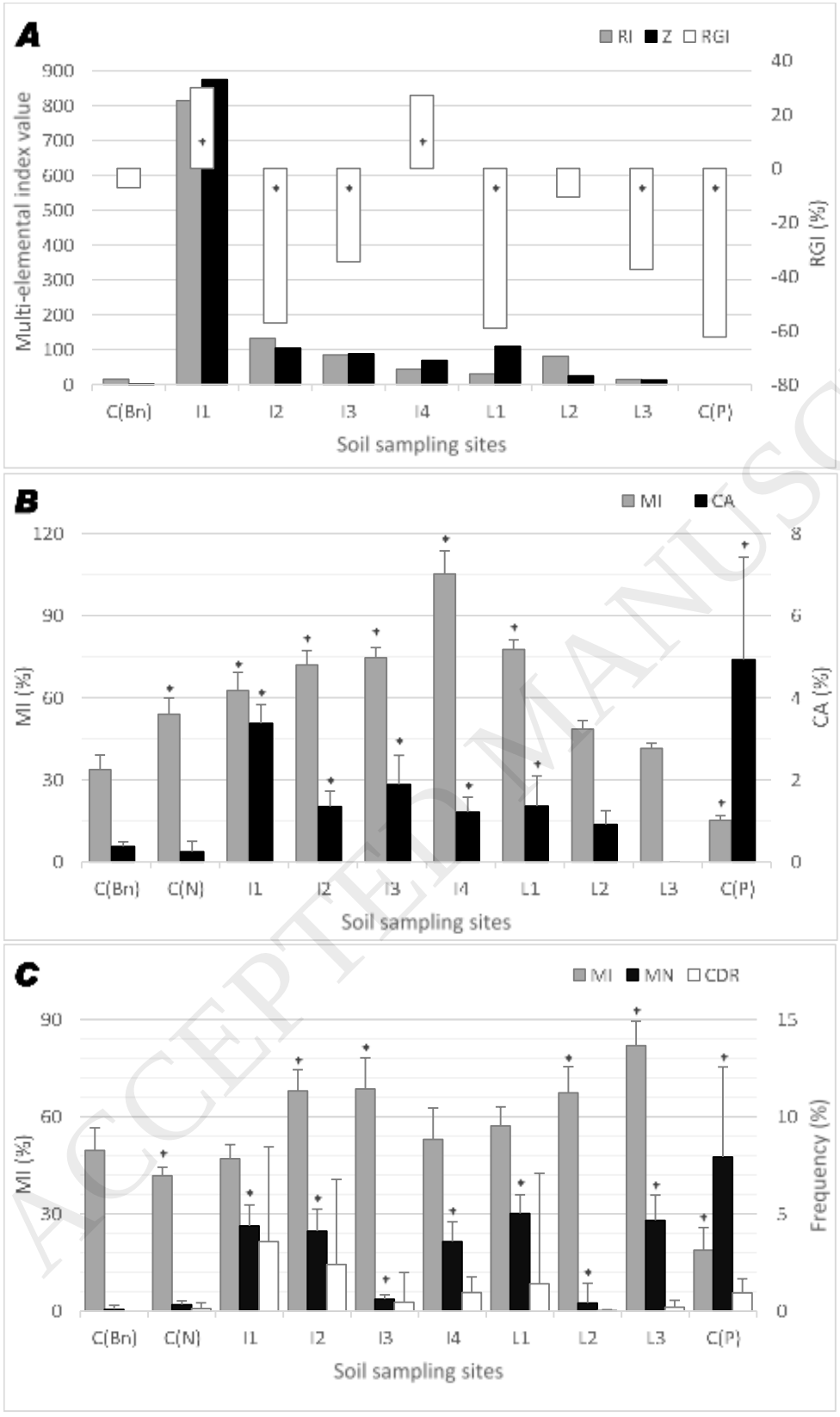

Fig. 1. Cyto/genotoxicity analysis of soil from industrial (I1-I4), and landfill (L1-L3) sites using *Allium* bioassays. A. Onion root growth inhibition index (RGI) after 96 h of growth in test-soil in relation to soil's multi-elemental risk (RI)

and contamination (Z) indices. B. Test-soil cytogenotoxicity evaluation by determining the mitotic index (MI_{24}) and frequency of chromosome aberrations (CA) in onions exposed to the test-soil for 24 h. C. Test-soil cytogenotoxicity evaluation by determining MI_{48}, frequency of micronuclei (MN) and frequency of cell death rate (CDR) in onions exposed to the test-soil for 24h and recovered in water for another 24 h; C(Bn) – background soil control; C(N) – negative tap water control; C(P) – positive control (10 mg/l methyl methanesulfonate). Test-soil sampling site acronyms and full characteristics are given in Table 1. Error bars indicate the standard error of the mean (SEM). SEM calculations for MI, MN and CDR are based on at least 18000 cells, for CA are based on at least 1500 cells scored from 5 onions in three experimental replicates per soil sampling site; * $p < 0.05$ indicates a statistically significant difference when compared to the background uncontaminated soil control.

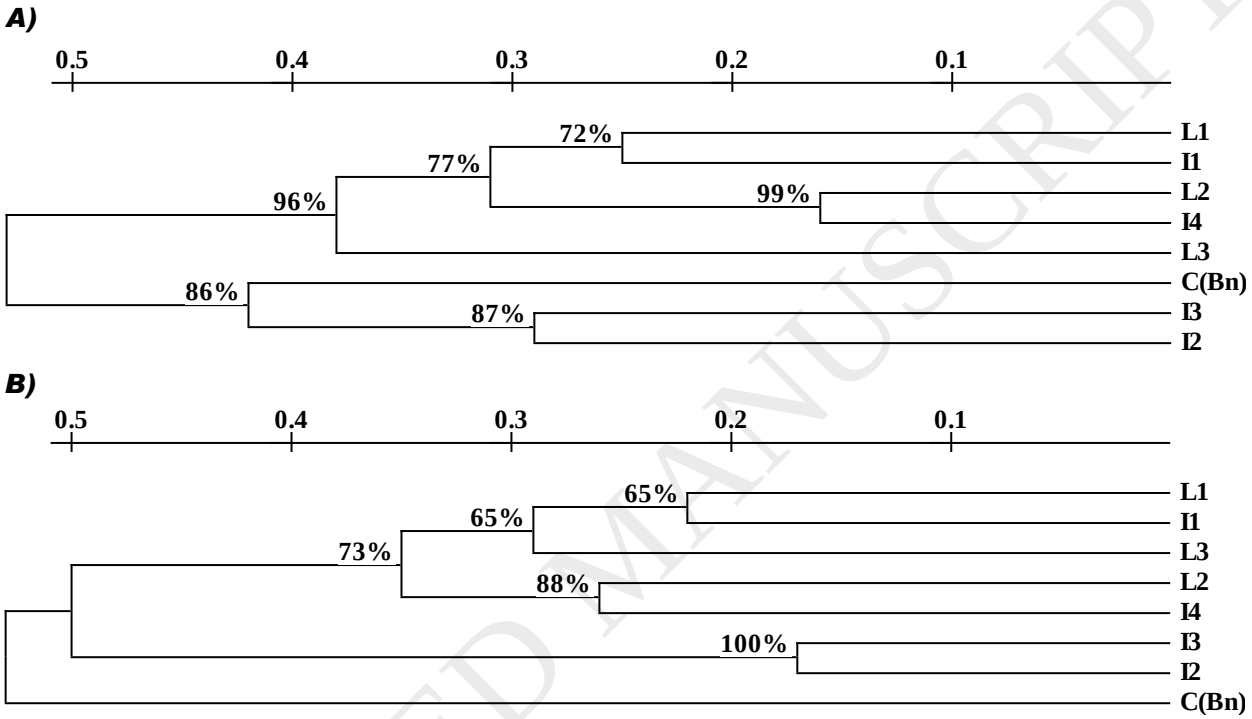

Fig. 2. UPGMA dendrograms among treated (grown in contaminated soil from industrial I1-I4, and landfill L1-L3 sites) and untreated (grown in background C(Bn) soil) *Allium* using RAPD (A) and ISSR (B) biomarkers. Nei's genetic distance presented on the axis, bootstrap values (%) from 1000 permutations given at the branch nodes. Test-soil acronyms and full characteristics are given in Table 1.