Genotoxicity assessment of polluted soils from Vilnius city using *Tradescantia* clone #4430

Skaistė Bondzinskaitė[1], Violeta Kleizaitė[1], Donatas Žvingila[1], Raimondas Šiukšta[1,2], Tatjana Čėsnienė[1]

[1]*Institute of Biosciences, Life Sciences Center,* [2]*Vilnius University Botanical garden, Vilnius University*

Rapid urban growth and changes in their structure modify the nature of the territory use. Some manufacturing companies move out of the city center or suspend their activities, and their territories often are occupied by residential quarters. The soil surface is a major natural system that accumulates pollutants and allows researchers to disclose the present state and history of contamination of an area with toxic pollutants. Unfortunately, chemical analysis of the soil and air does not always reveal the potential impact of pollution on living organisms. Air, water and soil pollution sensitive *Tradescantia* clone 4430 test-system was used to assess soil genotoxicity of few urban areas, including the territory of former 'Skaiteks' and drills factories 'Grąžtai', and two closed dumps – Fabijoniškės and Polockas. In former factory territories now are establishing residential areas, Fabijoniškės dumps site is near a housing development. In this study the soil genotoxicity was evaluated by *Tradescantia* stamen hair mutation (SHM) test. Short-term genotoxic effects were assessed using soil H_2O and DMSO extracts, while long-term genotoxic effects – growing *Tradescantia* plants on investigated soils for 0.5 and 1.0 years. In all cases, elevated frequency of pink cells (PC), colorless cells (CC) and branched hair (BH) mutation were detected.

Tradescantia, Trad-SHM, soil genotoxicity, urban areas contamination

Introduction

Environmental pollution is the problem, invariably relevant to intensively growing urban areas. People are coming to live in areas, where an intensive industrial activity, that left substantial soil, air, water pollution, was performed in the past. The pollution monitoring is constantly performed in the cities (Taghipour et al., 2013, Adamcova et al., 2016, Pariente et al., 2017). However, chemical analysis of test material (for example, soil) commonly is not sufficient to describe the potential impact of pollution on living organisms, therefore, it necessary to study the impact of pollutants on the living systems and their genetic integrity, i.e. to perform genotoxicological research (Ferreira et al., 2003, Атоянц и др., 2012). The soil is a natural system that accumulates contaminants and allows researchers to disclose the history and the present state of contamination of an area with (geno)toxicants. Genotoxicological tests are widely carried out using the most popular plant test systems – *Allium cepa*, *Vicia faba* and *Tradescantia* (White, 2004).

In this work, the genotoxicity of a few urban areas formerly used as industrial or dumping territories were studied using sensitive *Tradescantia* clone 4430. Exactly, 'Grąžtai' and 'Skaiteks' factory areas were transformed from industrial to residential character. The other two studied areas are closed Fabijoniškės and Polockas dumps that are also located in the Vilnius city territory: Polockas dump – on the eastern part of the city, Fabijoniškės – in the northern part.

Materials and methods

Soil sample characteristics. Soil samples were collected in Vilnius industrial areas and waste dumps: the closed former drill factory 'Grąžtai' (abbreviation – GF); the precision instrument factory 'Skaiteks' (SF); the closed Polockas dump (PD), which now represent a part of territory of a regional park; the closed Fabijoniškės dump (FD) located near housing development (Fig. 1). The coordinates of these points are shown in Table 1.

Fig. 1. Territory of 'Skaiteks' factory and central recultivated area of closed Fabijoniškės dump; 2014 years
1 pav. *Gamyklos „Skaiteks" teritorija ir Fabijoniškių sąvartyno centrinė rekultivuota dalis; 2014 m.*

A marketable soil was applied as a control (C). Soil samples were collected from the topsoil using the envelope method for all tested areas (Kumpienė et al., 2011). The soil samples were mixed, sieved out, dried to a constant weight and used for further analysis.

Table 1. Soil sample coordinates
1 lentelė. *Dirvožemio ėminių koordinatės*

Sample point *Dirvožemio ėminiai*	Coordinates of territory margins *Teritorijų ribų koordinatės*
Industrial areas *Pramoninės teritorijos*	
GF (1-8 points)	54° 40' 18.14", 25° 15' 37.09" – 54° 40' 35.2", 25° 15' 46.01"
SF (1-9 points)	54° 40' 43.5", 25° 18' 7.94 – 54° 40' 45.69", 25° 18' 9.63"
Closed dumps *Uždaryti sąvartynai*	
PD (1-6 points)	54° 41' 16.84", 25° 20' 52.84" – 54° 41' 11.74", 25° 20' 51.73"
FD (1-6 points)	54° 44' 37.29", 25° 13' 52.47" – 54° 44' 42.66", 25° 14' 2.67"

Treatment of plants with soil extracts. Tradescantia clone #4430 (Fig. 2) was kindly provided by prof. T. H. Ma (Western Illinois University, USA). The initial plants were propagated in a marketable soil mixture in the greenhouse of the Life Sciences Center of Vilnius University. At least 15 plant cuttings with young inflorescences were prepared for each analysis. To study different spectra of dissolved substances, two solvents were used: bi-distilled water or 5% DMSO, added at a ratio of 1:2 (soil:solvent). The treatment of the cuttings was carried out according to the method described by Ma et al. (1994). The plants were incubated in the soil extracts for 6 h. After treatment, plants were recovered for 24 h. The analysis of somatic mutations in *Tradescantia* stamen hair was performed on the microscope slide in a drop of water and glycerol (1:1) solution (Fig. 2).

For evaluation of soil genotoxicity, the induction of pink cells (PC), colorless cells (CC) and branched hairs (BH) in stamens were assessed. As a control for the water and DMSO extract experiments, cuttings were treated with the respective solvents. The flowers were analyzed during 14 days after treatment. Frequencies of the somatic mutations and branched hair was estimated by counting the number of events per 1000 cells. For each soil sample, at least 5000-6000 stamen hairs were analyzed.

Fig. 2. *Tradescantia* clone 4430 plant: flower and stamen
2 pav. *Tradescantia klono 4430 augalo žiedas ir kuokelis*

Long-term plants growing in contaminated soils. *Tradescantia* plants were replanted in flower pots with the experimental soils (Fig. 3). Plant growth was carried out under standardized greenhouse conditions. At least 15 young inflorescences were used for each analysis.

The concentrations of metals and other elements were ascertained at the Nature Research Center (Vilnius) as described previously (Kumpienė et al., 2011).

Fig. 3. *Tradescantia* plants in flower pots for long-term exposure
3 pav. *Dirvožemio ilgalaikio poveikio Tradescantia augalams tyrimas*

Results and discussion

To assess the genotoxicity of soil samples from each study territory, at least six samples of soil were analyzed in each case. Chemical analysis of soil samples revealed the variation in pollution level inside each territory. This indicates the importance of covering the entire territory, choosing proper design of the sample collection sites and collecting the optimal number of soil samples. As expected, the results of closed dump soil chemical analysis are very different from that of industrial territories. The metal concentrations in the soil samples from closed dumps were 1.5-3 times higher than the control, but did not exceed the maximal allowable concentrations (MAC). Nevertheless, other investigations report that in some landfills the level of metals may exceed the MAC values (Adamcova et al., 2016).

Despite significant variations in the extent of soil contamination among separate points within the urban territories of Vilnius, all soils from test areas, in general, were heavily contaminated with metals and other elements that were tested for.

In four samples from the territory of former drills factory, the concentration of molybdenum (Mo) was 3.6 times higher than the MAC. In SF area, the soil samples have been continuously collected during several years: in a deserted area, when there was no activity (the genotoxicity research is presented in this paper), and later, when the demolition of old buildings and the soil removal works were started (the data is not presented). SF soil chemical analysis showed that concentrations of several metals, including Cr, Cu, Ni, Pb, Sn, Se and Zn, exceed MAC.

All samples of soil were genotoxic for the *Tradescantia* clone #4430 compared to the respective controls (Fig. 4).

Results for PC, CC mutations and BH alterations are presented in this paper. The scoring of CC and BH has fully justified oneself in our previous investigation on the soil samples of military polygons that have been extensively used and polluted during the Soviet period. Exactly, the CC test showed the genotoxicity of all tested soils, regardless of differences in chemical contamination of the soil samples as revealed by chemical analysis. The frequency of these mutations induced by test soils after short-term and after long-term exposure was significantly higher than in the control plants.

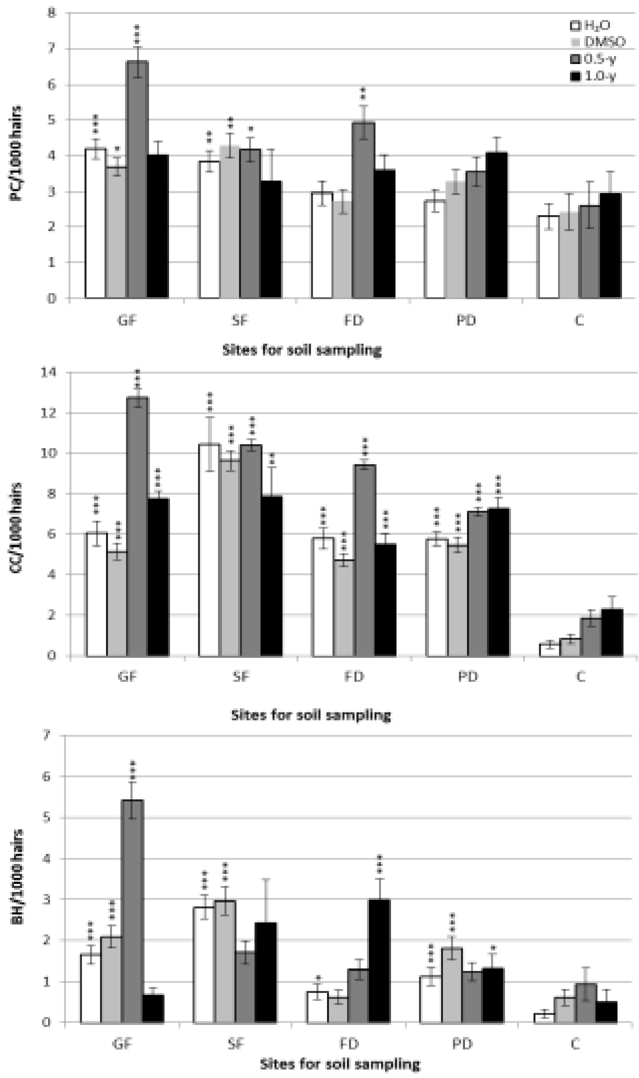

Fig. 4. Comparison of soil genotoxicity after *Tradescantia* plant long-term and short-term exposure to different test soils: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ in the comparison with the respective control

4 pav. *Ilgalaikio ir trumpalaikio tiriamų dirvožemių genotoksinio poveikio tradeskantės augalams palyginimas: *P <0,05; **P <0,01; ***P <0,001 palyginus su atitinkama kontrole*

However, the sudden increase of separate variations in *Tradescantia* grown in soils for a long-term, similar to the 'burst' observed in a previous, was observed after 0.5 years only in soils from the GF industrial area and FD, and only for GF with all *Trad*-SHM tests. For soil samples from GF, the higher mutation rate was possibly influenced by Mo, concentration of which exceeds the MAC value. Researchers from Yerevan University investigated the genotoxicity of urban soils contaminated with different technogenic pollutants using *Tradescantia* clone 02 as well as the accumulation of chemical elements in the above-ground parts of plants. It was concluded that some metals, including Zn and Cu, were not accumulated in *Tradescantia* vegetative organs, whereas the amount of Mo in plants grown in soils with elevated Mo concentration appeared to be higher as well as the MN and SHM rates, in comparison with control plants (Атоянц и др., 2012). These observations agree with our results obtained from *Tradescantia* plants grown in GF soil.

Considerable increase of PC and CC mutation frequency was observed in FD. This result agreed with the results of the previous study because the 'burst' was common for military territories, but was observed only in one point of Vilnius (Čėsnienė et al., 2014). For BH, the 'burst' was also observed in plants grown for 0.5 years in control soil (t = 18.7, in comparison to H_2O extract). It is noteworthy that the genotoxicity of the H_2O and DMSO extracts did not differ statistically, which shows that water-insoluble soil components had no significant influence on the genotoxicity of the test soils.

Two common patterns were found after comparing the long-term, 0.5- and 1.0-year, plant growth in contaminated soils (Fig. 4). At first, a dependence on the variation types was noted. The clearest differences were found between the 0.5- and 1.0-year exposures partially for PC and CC induction. Second, statistically significant differences for PC and other variation types correlated with the exposure duration in soil from GF, but with different relations. The levels of PC and CC decreased after a more prolonged 1-year exposure in all cases except PD. However, the measures of all *Trad*-tests for PD did not show correlation with treatment time. The cases, when after 1.0 years of exposures in polluted soil, the PC and CC mutation frequency returns to the level of short-term impact, cannot be strictly considered as an adaptation would not be fully correct, because it is important to assess the plant fitness and the overall "health". In this study, after 1.0 years of growth in highly polluted SF soils, plants looked rather poor and the flowering was suppressed. Although in GF case, as was mentioned previously, in all three tests the same trend was observed, independently whether it was a short-term or long-term exposure. Unexpected was FD soil long-term (1.0 year) exposure effect, expressed by significantly increased stamens hair BH frequency, despite of the soil chemical analysis that did not show significant pollution.

The other observation was the apparent dependence of genotoxicity on the test area. Differences between the former industrial areas, GF and SF, and the closed landfills, FD and PD, were noted.

Such type of investigations is of relevant importance since the correlations of genotoxicological data from plant test-systems with the air, water and soil chemical analysis (Watanabe et al., 2001, Misik et al., 2011) are determined, furthermore, the link to dangerous/deadly human conditions like cardiovascular diseases and cancer (Mariani et al., 2009) may also be identified.

Conclusions

1. Soil chemical analysis revealed that pollution persists for a long time even in unexploited territories. The extent of pollution depends on the character of former activities:

• in the soil samples from drill factory 'Grąžtai' area the Mo concentration exceeded MAC 3.6 times; in former 'Skaiteks' factory soil samples, concentrations of eight metals exceeded MAC.

• surveyed dumps (FD, PD) soil metal concentrations do not exceeded MAC, but were 1.5-3 times higher than in controls.

2. Soil genotoxicity was detected by using all Trad-tests – after the short-term (H_2O/DMSO extracts of soil) and long-term (0.5/1.0 years) exposure of *Tradescantia* plants:

• in all cases, the frequency of observed colorless cells was significantly higher than in the controls;

• the increase in frequency of pink and colorless cells after 0.5-year exposure was detected only for GF soil;

• the decrease in frequency of pink cells to the level of short-term exposure cannot be unambiguously considered as an adaptation

3. All *Trad*-tests indicate that the level of genotoxicity in unexploited industrial areas differs from that of closed dumps.

4. Our study revealed that the analysis of chemical composition in is not sufficient to evaluate the potential environmental hazards and the assessment of genotoxicity and potential hazard to human health is of relevant importance.

Acknowledgments

We sincerely thank dr. R. Taraškevičius from the Institute of Geology and Geography, Nature Research Centre for the soil chemical analysis.

This work was supported by a grant from the Lithuanian Research Council MIP-042/2015.

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Vilniaus miesto užteršto dirvožemio genotoksiškumo įvertinimas, naudojant *Tradescantia* kloną # 4430

Santrauka

Augant miestams ir keičiantis jų struktūrai, kinta teritorijų panaudojimo pobūdis. Įvairios paskirties gamybos įmones perkėlus už miesto arba nutraukus veiklą, jų vietoje dažnai įkuriami gyvenamieji kvartalai. Tokių vietų dirvožemio ir oro taršos cheminės analizės rezultatai ne visada atskleidžia galimą poveikį gyviems organizmams. Kadangi dirvožemis natūraliai akumuliuoja įvairius teršalus, tai jo galimą genotoksiškumą tyrėme panaudodami oro, vandens ir dirvožemio taršai jautrų *Tradescantia* kloną 4430. Ištyrėme Vilniaus miesto buvusių gamyklų „Skaiteks" ir "Grąžtai" teritorijų bei jau uždarytų Fabijoniškių ir Polocko sąvartynų dirvožemius. Gamyklų teritorijose statomi gyvenamieji namai, o Fabijoniškių uždarytas sąvartynas yra šalia gyvenamojo mikrorajono. Dirvožemio genotoksiškumą įvertinome tradeskantės kuokelių plaukelių testu (*Trad*-SHM). Buvo tiriamas trumpalaikis ir ilgalaikis dirvožemio poveikis tradeskantės augalams. Trumpalaikį poveikį įvertinome naudodami dirvožemio H_2O ir DMSO ekstraktus, ilgalaikį – pusę metų ir vienerius metus augindami augalus tiriamuose dirvožemiuose. Visais atvejais buvo nustatytas padidėjęs plaukelių kuokelių rausvų ląstelių (RL), bespalvių ląstelių (BL) dažnis ir plaukelių išsišakojimų (ŠP) skaičius.

Tradescantia, Trad-SHM, dirvožemio genotoksiškumas, miestų dirvožemio tarša

Gauta 2017 m. kovo mėn., atiduota spaudai 2017 m. balandžio mėn.

Skaistė BONZDINSKAITĖ. Vilniaus universiteto Gyvybės mokslų centro Biomokslų instituto magistrantė. Adresas: Saulėtekio al. 7, LT-10223 Vilnius. Tel. (8 5) 23 98 261, el. paštas: skaiste.bond@gmail.com

Skaistė BONZDINSKAITĖ. Postgraduate student of Vilnius University, Life Sciences Center, Institute of Biosciences. Address: Saulėtekis avenue 7, LT-10223 Vilnius. Tel. (8 5) 23 98 261, email: skaiste.bond@gmail.com