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Relation between soil contamination with metals in military training grounds and genotoxicity revealed by *Tradescantia* assays

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The genotoxicity of H_2O and DMSO extracts from four military grounds left after the Soviet Army was examined by Trad-MCN and Trad-SHM assays. In each military ground, two contrasting points were chosen and the content of 29 elements in soil samples used for genotoxicity investigation was determined. The highest contamination with metals in soil samples from Zokniai was in full agreement with the highest genotoxicity both for H_2O or DMSO extracts. The Trad-MCN assay was better to reveal it. Multiple regression analysis showed a statistically significant correlation (R^2) between MCN frequencies and soil contamination with Co, Zn, Mo, Cr, Sc, V, and only the Trad-SHM assay Yb revealed.

Key words: soil genotoxicity, military grounds, *Tradescantia*, MCN and SHM assays, correlation metal / MCN

INTRODUCTION

Chemical composition assays of environmental habitat give important information on the extent of its contamination. However, they do not show the real effect on biological systems, because the 'cocktail' of many factors acts simultaneously and reveals a summary biological effect. For this reason, biological assays of genotoxicity evaluation have been elaborated.

For genotoxicity evaluation of contaminated soils, various biological assays are also used. According to White and Claxton [1], the most popular is the *Salmonella* mutagenicity assay (about 38% of all publications reviewed), followed by *Tradescantia* micronucleus (Trad-MCN) and stamen hair mutation (Trad-SHM) assays. *Tradescantia* is preferable not only as a complex multicellular organism, but also as a direct consumer of the mineral composition of soil. For terrestrial plants, soil is the ground of their existence, and there exist plant species very sensitive or, on the contrary, tolerant to soil contamination [2–4]. *Tradescantia* Trad-SHM and especially Trad-MCN assays were originally developed by Ma et al. [5] for gaseous and airborne mutagens and afterwards for mutagens in contaminated soils [6–11].

In the present work, military grounds and other territories formerly occupied for various purposes by the Soviet Army were investigated within the NEAP [NATO / EST (Environmental and Earth Science and Technology) / ASI (Advanced Study Institute) Program] on the initiative of Director of the Institute Prof. Te-Hsiu Ma. The extent of contamination of military grounds in Lithuania was reported elsewhere [12–17], but the genotoxicity of these territories has never been investigated before.

In the present work, four territories of different history and fate were chosen and examined employing the Trad-MCN and Trad-SHM assays. The complex action of water and DMSO extracts from soils was compared. As the different content of various metals was characteristic of the chosen soils, to study the correlation between the genotoxic effect and a concrete metal was the main aim of the present work. Comparison of the genotoxic effect of water and DMSO extracts allows us to discriminate between the effects evoked by water-soluble or insoluble agents.

MATERIALS AND METHODS

All experiments were conducted using cuttings of the *Tradescantia* clone 4430, a sterile hybrid (2n = 12) between *Tradescantia hirtusiflora* × *T. subcaulis.* This clone is especially sensitive to mutagens in its environment. Initial material of clone 4430 was obtained from Prof. T.-H. Ma's collection of Western Illinois University (Macombo, USA). To escape de-

Content of elements

Point	Low	High
C1	Sr–Mg–Zn	Al–Co–Fe–Ti–P
P1	Y–Ag–B–Ba–Cr–Fe–Ni–Sr–**Ti**–Yb–Zn–**Zr**	**P**–Mg
P2	Y–Ga–B–Pb Cr–Fe–Ni–Sr–**Ti**–Yb–Zn–Zr	P–Mg
Pc*	Y B Cr–Fe–Ni–Sr–**Ti**–Yb–Zn–Zr	P–Mg
R1	Mo	Ca–**Cu**–**Zn**
R2	Mg	**Pb**–Zr–Ag
Rc*		
KR1	Li	Ag–B–Ba–Co–Mg–P
KR2	Li–Mn–Sr	Ag–B–La–**Ti**–Mg–P–**Zr**
KRc*	Li	Ag–B Mg–P
Z1	P	Zn–**V**–Ti–Sr–**Ni**–Mo–**Al**–**Ba**–**Ca**–**Co**–**Cr**–**Fe**–**Ga**–La
Z2	P	Zn–**Y**–V–Ti–**Sr**–Ni–**Ag**–Al–**Ba**–Ca–Co–Cr–**Fe**–**Ga**–**La**–Mn
Zc*	P	Zn–V–Ti–Sr–Ni Al–**Ba**–Ca–Co–Cr–**Fe**–**Ga**–La

Fig. 1. Distribution of elements in soil samples tested:
C1 – control from Kairėnai; P1–P2 – Pabradė; R1–R2 – Rukla; KR1–KR2 – Kazlų Rūda; Z1–Z2 – Zokniai; Pc*, Rc*, KRc*, Zc* – common situation for both points in the same territory. There is a bolded element whose content was exceptionally low or high

pendence of results on the growing conditions [18], all material was preliminarily grown and propagated in the greenhouse of Department of Botany and Genetics of Vilnius University, applying a 16-h day/8-h night cycle at 20 ± 2 °C. All cuttings were prepared and treated with soil extracts in the same conditions. Two soil samples were taken from different points according to a standard protocol [12–16] from the 0–20 cm layer in four military ground territories.

Their brief characteristics:

1. Rukla (Gaižiūnai) military ground, established in 1930 as a summer armed camp of the Lithuanian army; from 1939 a base of the Soviet Army; at present it is divided into three firing grounds (Central, Gaižiūnai, Kairiai). Sampling:

R1 – in the former soviet tank training ground (N55º1'44''; E24º21'33'');

R2 – acting firing ground (N55º1'16''; E24º22'0'');

2. Kazlų Rūda, formerly a special base of the Soviet Army. Samplings points:

KR1 – mine fighter field (N54º47'5''; E23º26'50'');

KR2 – forest near the airport where large quantities of fuel got into soil (N54º49'3''; E23º29'50'');

3. Pabradė, central military ground, established during the Russian–Japanese War (1904–1905), operates also at present. Sampling:

P1 – firing ground (N55º6'30''; E25º48'15'');

P2 – mine fire field (N55º2'0.1''; E25º48'15'').

4. Zokniai aviation base is restricted but acting at present. One of the biggest aviation bases of the Soviet Army, about half of the territory is very strongly contaminated with oil products:

Z1 – formerly fuel reservoirs (N55º52'46.6''; E21º24'10'');

Z2 – formerly airplane repair shop, very close to living houses (N55º52'40''; E21º24'165'').

Table. **Summary results of genotoxicity analysis of soils in the study territories, comparison of Trad-MCN and Trad-SHM assays and of different solvents (H_2O and DMSO)**

Point	Trad-MCN				Trad-SHM			
	n*		MCN%		SHM%		n**	
	H_2O	DMSO	H_2O	DMSO	H_2O	DMSO	H_2O	DMSO
P1	2022	1395	3.34 ± 0.92	4.65 ± 1.42	2.15 ± 0.29[2]	2.72 ± 0.66	26446	5572
P2	1763	1877	5.59 ± 0.74	6.01 ± 1.03	3.55 ± 0.47[1]	4.54 ± 0.49[1,2]	15326	23286
R1	1789	1581	3.58 ± 0.50	4.79 ± 0.74	3.99 ± 0.41[1]	4.02 ± 0.63	27976	18812
R2	3430	2387	2.78 ± 0.28	3.41 ± 0.58	3.61 ± 0.57[1]	3.64 ± 0.36	10837	21304
KR1	1658	1819	3.56 ± 0.94	4.68 ± 1.05	2.99 ± 0.35	3.75 ± 0.44	23632	21864
KR2	2440	2455	2.75 ± 0.27	3.33 ± 0.46	3.75 ± 0.63[1]	3.81 ± 0.42	11639	23536
Z1	1566	3398	5.31 ± 0.86	5.86 ± 0.87	4.03 ± 0.41[1]	4.18 ± 0.47	25017	26528
Z2	2126	1978	8.23 ± 1.06	9.63 ± 1.65	4.52 ± 0.49[1,2]	5.11 ± 0.39[1,2]	32031	36062
$C1_{soil}$	3024	3779	0.44 ± 0.18	0.79 ± 0.22	2.33 ± 0.27	3.28 ± 0.39	31492	29114
$C_{solv.}$	2376	2380	1.02 ± 0.36	2.27 ± 0.50	3.33 ± 0.39	3.35 ± 0.39	22146	19344

For Trad-MCN: all test samples were in the range $p < 0.05$, according to Student's t coefficient for both comparisons – soil or solvent controls.

For Trad-SHM: 1 – $p < 0.05$ – in comparison with soil control; 2 – $p < 0.05$ – in comparison with solvent control, n – the number of tetrads *or cells** tested.

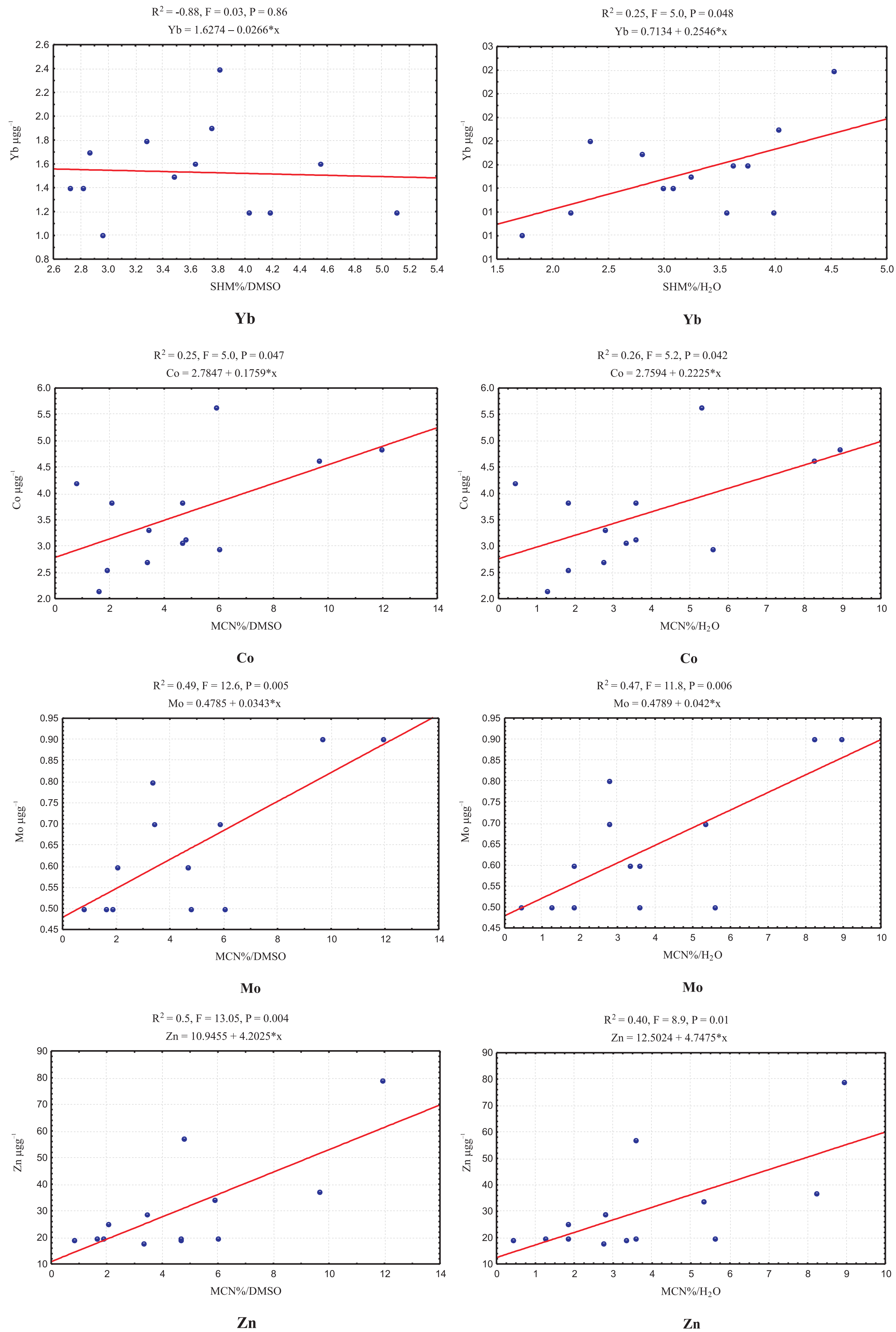

Fig. 2. Empirical relationships between mutation frequency in *Tradescantia* assays (mainly MCN) and soil contamination with various metals. The results are presented as data of multiple regression analysis

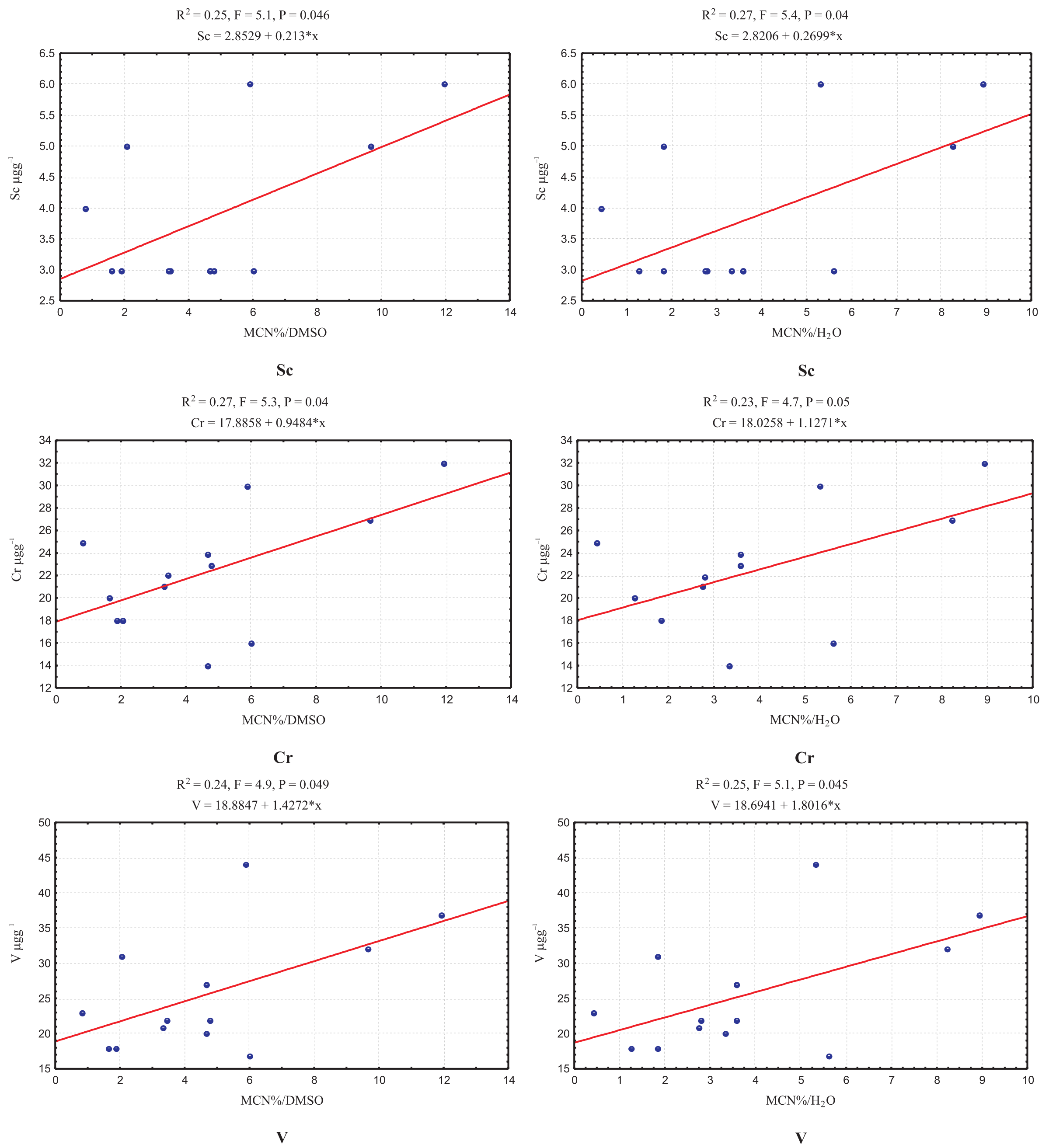

Fig. 2 (continued)

Soil extract preparation. After drying to a constant weight, the soil samples were sieved and extracted with distilled water or with 5% dimethyl sulfoxide (Sigma) added in proportion 1:3. The samples were intensively shaken for 1 h and kept for extraction for 24 h at room temperature, then filtrated and kept in a cool room.

Treatment of cuttings was made according to a standard protocol [19]. As the control we used soil extracts prepared by the same procedure from the soil of a greenhouse where *Tradescantia* plants were grown, and as a negative control the extracts from uncontaminated territory of Botanical Garden of Vilnius University (Kairėnai) were used. Solvents H_2O and DMSO were also used for control.

At the minimum 20 plants were used for Trad-MCN or Trad-SHM assays. Before treatment, cuttings were kept for 24 h in filtered tap water, then treated for a period of 6 h, and during this period the plants were illuminated with artificial light and aerated. The recovery period followed after treatment in tap water for 24 h. This time was necessary for the formation of micronuclei in tetrads and for pink mutations in stamen hairs.

The target cells in the Trad-MCN assay were pollen mother cells at their early prophase 1 (pachytene and diplotene), and for Trad-SHM assay the targets were mitotic cells in the early growth of the stamen hair (4 cells' stage).

After exposure, the inflorescences were harvested and fixed in a 3:1 ethanol – acetic acid mixture, and the samples were conserved in 70% ethanol.

Scoring of SHM and MCN was carried out according to the procedures described by Ma et al. [19]. For MCN staining the aceto-orsein was used. On each slide, 300 tetrads were scored at a 400-fold magnification under the light microscope.

Metal concentration in soil samples was determined by atomic absorption spectrometry (AAS, model DFS-13-A, Russia) by a standard protocol for soil samples [13] at the Institute of Geology and Geography (Vilnius) under conditions certified in agreement with ISO 5725.

RESULTS AND DISCUSSION

Soil sampling in contaminated territories is usually randomised [1, 12–15, 17]. Significant variations of separate metal content are observed in various points of the same territories [12–15, 17]. In the present work, the points in each territory were preliminarily chosen as contrasting. The choice was based on the history of a chosen point and on the kind advice of army ecologists. However, results of a concrete investigation did not fully coincide with those expected, even for sample C1 from the Botanical Garden of Vilnius University, which supposedly could serve as a negative control from uncontaminated soil. However, the sample from that point contained significant quantities of Al (4.1 μg g^{-1}), Co (4.19 μg g^{-1}), Fe (1.36 μg g^{-1}), P (779 μg g^{-1}) and Mn (448 μg g^{-1}). Nearly the same result was obtained also in soil samples from that territory in our previous work [20]. However, in fact these metal concentrations in soil did not exceed the permissible levels [13, 17]. A higher content of Al, Fe, and Co was found only in both samples from Zokniai (Z1 and Z2, respectively 5.6–4.6, 1.95–1.64 and 5.64–4.03 μg g^{-1}) and of Mn in samples from points Z2 and P1 (Pabradė) (Fig. 1). Only in respect of Mn points Z1–Z2 and P1–P2 were really contrasting. A high content of phosphorus was found in both points of Kazlų Rūda and especially of Pabradė (Fig. 1).

Despite the difficulties of a preliminary prognosis of contamination extent in the study points, direct analysis of soil samples allowed us to make several conclusions.

• First, most contaminated were soils from Zokniai and, *vice versa*, decreased concentrations of many elements were found in soils from Pabradė. In this respect, Rukla and Pabradė are excellently chosen for acting firing grounds.

• Second, specific elements are characteristic of the study territories: an especially low Ti content in samples from Pabradė and a significantly increased content of several elements (Zn, V, Ti, Sr, Ni, Al, Ca, Co, Cr, La and especially Ba, Fe, Ga) in the Zokniai samples.

• Third, separate points may be also characterized by specific contamination. So, for P1 such element was P, for R1 it was a high concentration of Cu and Zn and for R2 of Pb. Point 2 in Kazlų Rūda (KR2) was characterized by increased concentrations of Ti and Zn. Despite the very strong common contamination with the study elements, both points in Zokniai (Z1 and Z2) also had some peculiarities (Fig. 1).

As for the relation between the genotoxic effect and element content, attention must be paid to three methodical aspects important for interpretation of the experimental data:

• the concentration of metals was determined in a concrete soil sample used for extraction and following treatment of *Tradescantia* cuttings;

• comparison of the mutagenic activity of H_2O and DMSO extracts, and if the mutagenic effect of DMSO extract would be higher it could indicate the presence of active organic compounds;

• all plants for experiments were grown in the same conditions of a decreased content of the many of the study elements in the greenhouse. It allowed us to avoid preliminary acclimation of plants to metal stress. Acclimation to metal stress is a well known phenomenon [3, 4] and must be taken into account in genotoxicity studies of soil samples. The greenhouse soil sample, marked as C2, had the lowest content of many elements: Ag, Al, Li, Mn, Mo, La, Ba, Nb, Pb, Sc, Sn, V, P, Yb and a relatively low content of Co, Fe, Ga, Mg, Ni, Ti, Zn. Especially low was the content of Al (1.9 μg g^{-1}), Li (5 μg g^{-1}) and P (390 μg g^{-1}).

The summary results of genotoxicity investigation are presented in Table. As could be expected, the highest genotoxic effect was shown by soil extracts from the most contaminated point Z2. This conclusion followed from both Trad-MCN and Trad-SHM assays used in the present work. However, the MCN test was more effective. Especially it is clear from a comparison of points Z1 and Z2. Unexpected results showed soil extract from point P2 characterized by a low content of several elements (Fig. 1).

In all cases, independently of the assay used in an experiment, DMSO extracts showed a slightly higher genotoxic effect than H_2O extracts. However, this effect could be attributed to the solvent because the same differences were shown also by the control solvents.

An impetus to the relation studies between separate metal content in soils and genotoxicity results was given by the review of White and Claxton [1] grounded on the works of Knasmüller et al. [21] and Majer et al. [22]. In the present work, correlation studies were made for all 29 elements whose concentrations were examined in the soil samples, and separately for both assays, Trad-MCN and Trad-SHM, and for both solvents, H_2O and DMSO. In general, it gave 116 separate figures. In the review of White and Claxton [1], the relationship between MCN frequency and metal content was shown only for eight metals: Cr, Pb, As, Cd, Sb, Cu, Ni, Zn, and correlation coefficients were in the range 0.16–0.42. We grouped all relationships into three groups:

the first group where R^2 was low and differences between Trad-MCN and Trad-SHM, H_2O and DMSO were absent (Sn, Pb, Cu, Ca, B, Nb, Li, Sr, Ti, Zr, P);

• the second group comprised the metals whose R^2 was in the range 2.7–4.6, but R^2 was statistically insignificant:

– for La, Ag, Ni, Ga, Fe, Al such result was obtained with both solvents, H_2O and DMSO, in the MCN assay and only with H_2O extract in SHM assay;

– for Mn, such result was observed only in the MCN assay;

• the third group containing metals whose R^2 was statistically significant (Fig. 2):

– such result was observed only for Yb and only if SHM assay and H_2O solvent were used;

– even for six metals (Co, Zn, Mo, Cr, Sc, V) R^2 was statistically significant ($p < 0.05$ for Co, Zn, and even $p < 0.01$–0.005 for Mo), but such results were observed only for MCN assay. The results did not depend on the solvent used (H_2O or DMSO). Only in one case, with Zn, DMSO extract gave a higher MCN frequency than did H_2O extract (Fig. 2).

As mentioned, in [1] results of Knasmüller et al. [21] and Majer et al. [22] are discussed. Statistically significant R^2 values were fixed for eight metals (Pb, Cr, Cd, As, Ni, Sb, Cu, and Zn).

Only Cr and Zn coincided with our results (Fig. 2). Of course, such discrepancy may be explained by the longer list of metals studied in our work: V, Sc, Mo and Yb were not analysed in the above works [21, 22]. However, many other factors may influence the results [10] as Ni, Cu, Pb were also tested in our work and clear input of these metals on genotoxicity of tested soils was not observed, but also formally, only in regression studies.

Our work confirms a higher effectiveness of Trad-MCN assay as compared with Trad-SHM assay observed in previous works [1, 7, 19]. On the other hand, Trad-MCN and Trad-SHM assays show the different kinds of genome injuries, and a conclusion may be made that soil contamination with metals exerts a summary effect on *Tradescantia* as a clastogenic factor rather than an inducer of point mutations. However, it may depend also on the metal tested like in the case of Yb (Fig. 2).

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References

1. White PA, Claxton LD. Mutat Res 2004; 567: 227–345.
2. Baker AJM, Brooks RR. Biorecovery 1989; 1: 81–126.
3. Ernst WHO, Nelissen HJM, TenBookum WM. Environ Exp Bot 2000; 43: 55–71.
4. Pollard AJ, Powell KD, Harper FA, Smith JAC. Crit Rev Plant Sci 2002; 21: 539–66.
5. Ma T-H, Sparrow AH, Schairer LA, Nauman AF. Mutat Res 1978; 58: 251–8.
6. Baud-Grasset S, Baud-Grasset F, Bifulco JM, Meier JR, Ma T-H. Mutat Res 1993; 303: 77–82.
7. Ma T-H, Kong MS. Environ Mol Mutagen 1997; 29: 7–12.
8. Kong MS, Ma T-H. Mutat Res 1999; 426: 221–8.
9. Gichner T, Veleminsky J. Mutat Res 1999; 426: 163–6.
10. Cotelle S, Masfaraud J-F, Ferard J-F. Mutat Res 1999; 426: 167–71.
11. Cabrera GL, Rodriguez DMG. Mutat Res 1999; 426: 211–4.
12. Baltrėnas P, Ignatavičius G, Vaišis V. Aplinkos inžinerija 2001; 9(1): 3–8.
13. Taraškevičius R. Aplinkos inžinerija 2000; 8(3): 137–45.
14. Ignatavičius G, Baltrėnas P. Proceed Nato Advanced Research Workshop. Prague, Czech Republic, 14–19 June 2000. 2003: 347–51.
15. Baltrėnas P, Ignatavičius G. RTO Proceed 2000; NATO Publishing: Cedex, France 39(12): 1–7.
16. Baubinas R, Taminskas J. Karinė gamtonauda Lietuvoje sovietmečiu: ekologinės pasekmės. Vilnius. 1997–1998.
17. Vasarevičius S, Greičiūtė K. Environmental Engineering. 6th Int Conf, May 26–27, 2005. Vilnius. 300–5.
18. Klumpp A, Ansel W, Fomin A, Schnirring S, Pickl C. Hereditas 2004; 141: 142–8.
19. Ma T-H, Cabrera GL, Chen R, Gill BS, Sandhu SS, Vanderberg AL, Salamone MF. Mutat Res 1994; 310: 221–30.
20. Rancelis V, Cesniene T, Zvingila D, Barysas D, Balciuniene L, Dapkuniene S. Environ Exp Bot 2006; 55: 221–34.
21. Knasmüller S, Gottmann E, Steinkellner H, Fomin A, Pickl C, Paschke A, God R, Kundi M. Mutat Res 1998; 420: 37–48.
22. Majer BJ, Tscherko D, Paschke A, Wennrich R, Kundi M, Kandeler E, Knasmüller S. Mutat Res 2002; 515: 111–24.

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RYŠYS TARP KARINIŲ POLIGONŲ DIRVOS UŽTERŠTUMO METALAIS IR *TRADESCANTIA* BANDINIAIS NUSTATOMO GENOTOKSIŠKUMO

Santrauka

Tradeskantės (*Tradescantia*) Trad-MKB ir Trad-KPM bandiniais ištirtas dirvožemio genotoksiškumas keturiuose nuo Sovietų armijos likusiuose kariniuose poligonuose (po du punktus kiekviename). Didžiausias užteršimas sunkiaisiais metalais Zoknių poligone visiškai sutapo su didžiausiu genotoksiškumu, nustatytu Trad-MKB bandiniu. Šiuo bandiniu pavyko nustatyti ir statistiškai patikimą koreliaciją (R^2) tarp MKB dažnio ir konkrečių metalų kiekio dirvožemyje, būtent Yb, Co, Zn, Mo, Cr, Sc, V. Tik su Yb toks ryšys aptiktas ne Trad-MKB, bet Trad-MKP bandiniu.