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Assessment of the condition and mutagenic potential of arable soils based on the soil-plant system

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Regional soil monitoring of natural ecosystems with multicomponent heavy metals contamination requires both geochemical studies and express-methods for soils' mutagenicity assessment using reliable test systems. We studied the mutagenic potential of arable soils in Armenian agricultural landscapes using *Tradescantia* clone 02 in the soil–plant system. Biotesting was carried out using two main bioassays: *Tradescantia* stamen hairs mutations test (Trad-SHM) and *Tradescantia* micronucleus test (Trad-MN). According to Trad-SHM bioassay, the level of recessive mutations (pink cells – PC) in the studied soil samples significantly increased the conditional background. The maximum PC and genetically undefined mutation (colorless cells – CC) manifestation was in a soil sample from the Hrazdan region, 600 m away from the industrial zone, where the values of the studied parameters exceeded the background level by 28 and 3.4 times, respectively ($p<0.001$). In terms of stunted stamen hairs the highest value was in the soil variant Martuni-2 and exceeded the control level by 3.7 times. According to Trad-MN, a significant increase in the frequency of both test criteria was also observed in soil samples from the Hrazdan region, located 400 m, 600 m and 800 m away from the industrial zone, as well as in samples from the Gavar region by 1.8–2 times ($p<0.05$). A correlation analysis of the dependence of the level of genetic effects on the chemical element (V, Cr, Mn, Fe, Co, Ni, Cu, Zn) content in the studied soil samples was carried out. To determine the level of soil pollution based on the concentration coefficient, the total pollution coefficient (Z_c) and the sanitary-hygienic series were calculated.

Keywords: arable soil contamination, agrolandscapes, bioassay, genotoxicity, clastogenicity, *Tradescantia* (clone 02).

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Оценка состояния и мутагенного потенциала пахотных почв на основе системы почва–растение

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Для регионального почвенного мониторинга природных экосистем при многокомпонентном загрязнении тяжёлыми металлами (ТМ) необходимо проведение не только геохимических исследований, но и применение экспресс-методов оценки мутагенности почв с использованием надёжных тест-систем. В системе генетического мониторинга в качестве чувствительного индикатора уровня мутагенности окружающей среды используется традесканция (клон 02). Проведено исследование мутагенного состояния пахотных почв агроландшафтов Армении с применением клона 02 традесканции в системе почва–растение. Биотестирование проводилось с использованием двух основных биотестов традесканции. По данным биотеста волосков тычиночных нитей традесканции (Трад-ВТН) показано достоверное повышение уровня соматических мутаций в изученных почвенных образцах по сравнению с условно фоновым вариантом. Максимальное проявление рецессивных мутаций (розовых клеток) и генетически неопределённых мутаций (бесцветных клеток) наблюдалось в почвенном образце из региона Раздан, удалённого от промышленной зоны на 600 м, где значения изученных параметров превысили уровень фона в 28 и 3,4 раза соответственно (p<0,001). По данным микроядерного теста (Трад-МЯ) также наблюдалось достоверное повышение частоты обоих тест-критериев в почвенных образцах региона Раздан, удалённых от промышленной зоны на 400, 600 и 800 м, а также – в образцах региона Гавар в 1,8–2 раза (p<0,05). Проведён корреляционный анализ зависимости уровня генетических эффектов от концентрации химических элементов (V, Cr, Mn, Fe, Co, Ni, Cu, Zn) в изученных почвенных образцах. Для определения степени загрязнённости почв были рассчитаны коэффициент концентрации и суммарный коэффициент загрязнения (Z_c), а также построены санитарно-гигиенические ряды для каждого почвенного образца.

Ключевые слова: загрязнение пахотных почв, агроландшафты, биотестирование, генотоксичность, кластогенность, традесканция (клон 02).

In conditions of increasing anthropogenic load on agrolandscapes, assessing the condition of agricultural lands, especially near technogenic objects, deserves more and more attention. As is known, regional environmental problems of agrocenoses located near industrial infrastructure facilities are largely associated with toxic substances accumulation, especially heavy metals (HMs) in the soil cover [1–3]. As agrolandscape soils are a source of pollution for agricultural products, regional monitoring of soil contamination is an essential part of the ecotoxicological testing system. In this regard, it is necessary to carry out genetic monitoring of the soil environment using express methods for assessing the potential toxicity degree of the studied area [4, 5].

As a result of technogenic factors impact on the Armenian natural landscapes various processes of soil degradation are intensifying. This leads to the emergence of anthropogenically transformed ecosystems. Environmental situation assessment on the territory of natural and anthropogenically transformed landscapes, especially near industrial infrastructure, carried out not active enough in the Republic of Armenia. Along with geochemical studies, it is also necessary to obtain information on the level of soil mutagenic activity. As a rule, bioassay in the soil–plant system carry out using highly informative plant test-objects to determine the degree of the potential toxicity of the studied area [6, 7].

Tradescantia (clone 02) is a sensitive indicator of the air, water, and soil pollutants mutagenicity in the genetic monitoring system *in situ*. The frequency of somatic mutations in the stamen hairs (Trad-SHM test) as well as the frequency of the micronuclei appearance in pollen mother cells (Trad-MN test) assessed with the application of this clone. High sensitivity to xenobiotics, the undemanding nature of plants when grown in greenhouse conditions, and the ability of simultaneous study of mutations in both somatic and sporogenic cells on the same plants is the advantage of this clone as a test-object [8–10].

The purpose of our study was to bioassay the level of genotoxicity and clastogenicity of soil samples from arable lands of some Armenian regions in the soil–plant system using the stamen hairs (Trad-SHM) and the micronucleus (Trad-MN) bioassays of *Tradescantia* clone 02 as model test-object.

Objects and methods of research

The research material was soil samples from arable lands from three Armenian regions – Hrazdan, Gavar and Martuni. Private agricultural lands with moderate traffic were sampling sites. Landscapes of moderately humid steppe and mountain chernozem soil type dominated in the Hrazdan region. Dry steppes and mountain-chestnut soils prevail in the Gavar and Martuni regions. Soil sampling sites (5) in the Hrazdan region were located at a distance of 200, 400, 600, 800 and 1000 m from the industrial zone, including the Hrazdan Thermal Power Plant (HTPP) and the Hrazdan Cement Plant (HCP). Soil samples in the Gavar (3) and Martuni (2) regions were taken from the arable lands of the Lake Sevan basin and were located at a distance of 30 km from each other. Soil sampling was carried out taking into account the regional wind rose. The soil samples were labeled according to their territorial location: samples from the Hrazdan region – H-1, H-2, H-3, H-4, H-5; as well as from the Gavar region – G-1, G-2, G-3, and from the Martuni region – M-1, M-2.

Soil sampling was carried out according to the methodology for agrochemical soil analysis of agricultural lands [11]. Soil samples were collected in dry weather from a depth of up to 20 cm using the envelope method at a distance of 200 m from each other. In each regional soil group, at least five samples were taken from one test site to form a combined soil sample in order to more objective assessment of the chemical accumulation degree. The soil samples chemical analysis for the HMs (V, Cr, Mn, Fe, Co, Ni, Cu, Zn) content was carried out using a portable X-ray fluorescence analyzer Niton XRF (Thermo Scientific™ Niton™ Analyzers, USA).

Tradescantia (clone 02) plants were the object of research. When conducting a vegetation experiment, plants grew in flowerpots with the studied soils in a greenhouse, where was the same vegetation regime: 20–25 °C, 18/6 h day/night cycle. For each soil sample (depending on the region), three vegetation vessels with 300–400 g of soil in each were used, into which 5–7 *Tradescantia* plants at a certain stage of organogenesis were planted. The plants watered with tap water. A soil sample (mountain-chestnut soil type) from the Yerevan State University greenhouse, located 40–50 km from the main study regions, was a conditional background sample.

A test system of *Tradescantia* stamen hairs (Trad-SHM bioassay) was used to determine the genotoxicity level of the studied soil samples. Changes in the stamen hair cells color from blue to pink (recessive point mutations – pink cells (PC)) were taken into account as marker test criteria, as well as the appearance of genetically undefined mutations (colorless cells – CC). Stunted stamen hairs (SSH) and branched stamen hairs (BH) were also recorded as morphological changes during bioassay. In each variant 10–14 thousand of stamen hairs were analyzed. The frequency of mutation events was calculated on average per 1000 hairs according to the generally accepted method [12]. When conducting the micronucleus test (Trad-MN) two main test criteria were taken into account: the frequency of tetrads with MN (Tetr/MN) and the frequency of MN in tetrads (MN/tetr). For each the soil sample 3000 tetrads were examined. Calculations of clastogenic effects were carried out on 100 tetrads according to the generally accepted method [13]. Both bioassays are part of the International Plant Test Program (IPPB) under the auspices of the United Nations Environment Program (UNEP) [14].

For the integral geochemical characteristics of the regions qualitative geochemical series were constructed based on the average of HMs concentration in soil samples by normalizing the components according to the MPC level (as a sanitary and hygienic assessment) (GN 2.1.7.2041-06). The coefficient of concentration (K_h) was calculated according to the generally accepted formula:

$$K_h = C / C_{mpc}, \qquad (1)$$

where C is the gross content of the element in the soil, C_{mpc} is the maximum permissible content of the element.

The total pollution index (Z_c) was also calculated for each soil option using the formula:

$$Z_c = \sum K_n - (n - 1), \qquad (2)$$

where K_n is the concentration coefficient for each soil option, n – number of chemical elements.

The level of soil contamination and the degree of danger were determined according to the generally accepted scale [15].

All results were statistically analyzed using the Student's t-test and the Pearson correlation test using the Statgraphics Centurion 16.2 computer program (StatPoint Technologies, Inc. USA; Warrenton, VA).

Results and discussion

Qualitative soil analysis by the contamination degree is crucial for solving the problem of the ecological status of the region. The soil cover in our studies is represented mainly by mountain chernozem and mountain chestnut soils. These soils are characterized by the loss of the top fertile layer due to technogenic pollution [16]. Analysis of the gross content of the studied chemical elements in soil samples showed that their content was heterogeneous and for some elements exceeded generally accepted standards (MPC) for soil (Table 1).

Based on the data in Table 1, it should be noted that the high Ni and Cu content in all studied soil samples exceeding the maximum permissible levels. It is especially necessary to highlight the group of soil samples from the Gavar region, where a high Cr, Co and Ni content were observed (exceeded the MPC). In addition, in Martuni soil samples an increased content of Cr, exceeding the permissible levels was found.

Sanitary-hygienic series based on the coefficients of technogenic concentration of HMs were compiled for each soil sample in order to obtain qualitative and quantitative characteristics of the studied soils (Table 2).

At the same time, the dominant pollutants in each soil sample were identified, and the share of each chemical element in the total pollution index was determined depending on the soil-

Table 1

Concentration of some heavy metals in soil samples from different sites

Soil sample / Soil type	Gross concentration, mg/kg							
	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
H-1 / MMC	135.9	**117.0**	825.4	32705.3	**84.0**	**71.4**	**92.8**	**115.6**
H-2 / MMC	119.8	**117.6**	827.5	28123.3	0.0	**72.4**	**72.6**	89.0
H-3 / MMC	128.9	97.8	943.5	31593.3	0.0	**57.7**	**87.3**	98.5
H-4 / MMC	140.1	**109.2**	843.1	29543.8	0.0	**70.1**	**79.9**	**101.9**
H-5 / MMC	109.8	78.4	778.3	24600.7	0.0	**56.9**	**75.2**	83.7
G-1 / MCS	129.3	**110.9**	834.5	**40194.2**	**103.6**	**85.9**	**84.2**	88.6
G-2 / MCS	142.7	**127.3**	838.1	39025.8	**165.4**	**86.2**	**79.2**	89.8
G-3 / MCS	131.8	**115.7**	804.8	36835.8	**170.4**	**75.5**	**84.6**	86.4
M-1 / MCS	114.2	**130.0**	704.5	30907.4	**75.9**	**60.6**	**73.8**	84.9
M-2 / MCS	118.9	**140.6**	770.3	32189.5	**81.7**	**53.4**	**69.8**	86.0
Background / MCS	115,3	**109,5**	803,8	30591,5	**60,1**	**63,4**	**67,0**	76,6
MPC in soils	150	100	1500	40000	20	50	55	100

Note: MMC – mountain-meadow chernozem; MCS – mountain-chestnut soil; values exceeding MPC are highlighted in bold.

Table 2

Sanitary-hygienic series and value of the total pollution index (Z_c) for soil samples from different sites

Soil sample	Sanitary-hygienic series*	Z_c
H-1	$Mn_{(0.5)} – Fe_{(0.8)} – V_{(0.9)} – Zn, Cr_{(1.2)} – Ni_{(1.4)} – Cu_{(1.7)} – Co_{(4.2)}$	11.9
H-2	$Mn_{(0.5)} – Fe_{(0.7)} – V_{(0.8)} – Zn_{(0.9)} – Cr_{(1.2)} – Cu_{(1.3)} – Ni_{(1.4)}$	6.8
H-3	$Mn_{(0.6)} – V, Fe_{(0.8)} – Zn, Cr_{(0.9)} – Ni_{(1.2)} – Cu_{(1.6)}$	6.8
H-4	$Mn_{(0.6)} – Fe_{(0.7)} – V_{(0.9)} – Zn_{(1.0)} – Cr_{(1.1)} – Cu, Ni_{(1.4)}$	7.1
H-5	$Mn_{(0.5)} – Fe_{(0.6)} – V_{(0.7)} – Zn, Cr_{(0.8)} – Ni_{(1.1)} – Cu_{(1.3)}$	5.8
G-1	$Mn_{(0.6)} – V, Zn_{(0.9)} – Fe_{(1.0)} – Cr_{(1.1)} – Cu_{(1.5)} – Ni_{(1.7)} – Co_{(5.2)}$	12.9
G-2	$Mn_{(0.6)} – V, Fe_{,} Zn_{(0.9)} – Cr_{(1.3)} – Cu_{(1.4)} – Ni_{(1.7)} – Co_{(8.3)}$	16.0
G-3	$Mn_{(0.5)} – V, Fe_{,} Zn_{(0.9)} – Cr_{(1.2)} – Cu, Ni_{(1.5)} – Co_{(8.5)}$	15.9
M-1	$Mn_{(0.5)} – V, Fe_{,} Zn_{(0.8)} – Ni_{(1.2)} – Cr, Cu_{(1.3)} – Co_{(3.8)}$	10.5
M-2	$Mn_{(0.5)} – V, Fe_{(0.8)} – Zn_{(0.9)} – Ni_{(1.1)} – Cu_{(1.2)} – Cr_{(1.4)} – Co_{(4.1)}$	10.8

*Note: * – K_h value in relation to MPC is in parentheses.*

sampling region. Priority pollutants Cu and Ni (K_n>1) were found in the soil samples from the Hrazdan region, excepting the H-1 sample, where the dominant pollutants were Co and Cu (K_n>1). In the soil samples from the Gavar region the priority pollutants were Co and Ni, and in the soil samples from the Martuni region – Co, Cu and Cr (K_n>1). The occurrence of high levels of priority pollutants in these regions may be due to the close proximity of highways. In addition, the processes of HMs migration over long distances may be due to the predominance of the southwest wind rose in these regions. As is known, soil pollution from industrial enterprises is often traced at a significant distance from the source of metal-containing emissions (in this case, from the HTPS and the HCP, located in the Hrazdan industrial zone).

The results of genetic monitoring of soil samples from different regions using *Tradescantia* clone 02 to for accounting somatic mutations based on the Trad-SHM bioassay data showed a significant increase in the frequency of both PC and CC in all studied samples compared to the conditional background sample (Table 3).

The level of frequency of PC appearance in the stamen hairs, depending on the soil sample, exceeded the background level by 1.6–28 times, with a maximum value in the H-3 sample (p<0.001). A high frequency of point mutations was also observed in G-3 and H-4 samples, where the level of mutations was 3–3.5 times higher than the background level (p<0.001) respectively.

The high frequency of CC appearance was especially noted in soil samples from the Hrazdan industrial region, with the maximum value in the H-2 and H-3 variants, where the background value was exceeded by 3.0–3.4 times, respectively. In soil samples from the Gavar region (G-1 and G-2), as well as in the M-1 sample from the Martuni region, a minimum value of the CC frequency was observed, which was below the background level.

In addition to somatic mutations (pink and colorless cells), the appearance of SSH was also recorded during biotesting. As is known, this type of morphological abnormalities appearance is an additional test for cell survival in the SH of *Tradescantia*. The level of this indicator exceeded the background level by 2.0–3.7 times with the highest value in the M-2 sample ($p<0.001$), which may indicate the presence of toxic substances with increased genotoxic activity leading to decreased survival of *Tradescantia* stamen hair cells. The minimum level of SSH frequency was observed in the H-5 and G-2 samples, where it was at the background level.

The correlation analysis between the concentration of chemical elements and genetic markers of the Trad-SHM test revealed a significant positive correlation between the level of PC frequency and the Mn concentration (r=0.62, $p<0.05$). In addition, the increased level of point mutations observed in H-3 sample corresponded to the highest Mn concentration in this sample compared to other soil samples. A significant positive correlation was shown between the frequency of point mutations (pink cells) and the level of colorless mutations (r=0.63, $p<0.05$) (Table 4).

The study of clastogenic effects in sporogenic *Tradescantia* cells based on the Trad-MN bioassay showed an increase in the frequency of both tetrads with MN and MN in tetrads forming in almost all soil samples. The exception is the nearest and the most remote industrial zone samples (H-1 and H-5) of the Hrazdan region, where the MN frequency was at background levels (Fig.).

Table 3

Induction of genotoxic effects in *Tradescantia* clone 02 somatic cells in soil samples from different sites

Soil sample	Somatic mutations		Morphological changes
	PC/1000 (pink cells)	CC/1000 (colorless cells)	SSH/1000 (stunted hairs)
H-1	1.29±0.35***	18.3±1.3**	3.89±0.6*
H-2	1.00±0.26***	24.9±1.3***	3.9±0.5*
H-3	12.8±1.1***	27.9±2.5***	4.0±0.6**
H-4	1.51±0.34	19.7±1.2**	4.1±0.6**
H-5	0.68±0.26	9.5±1.0**	1.9±0.4
G-1	0.91±0.27***	4.1±0.6	2.7±0.5*
G-2	1.08±0.33**	7.1±0.8	1.9±0.4
G 3	1.60±0.37	14.4±1.1**	4.0±0.6*
M-1	1.05±0.31	7.6±0.8	2.5±0.5*
M-2	0.77±0.23***	9.4±0.8*	7.1±0.7***
Background	0.46±0.23	8.2±1.0	1.9±0.5

*Note: the differences are significant at: * – p<0.05, ** – p<0.01, *** – p<0.001.*

Table 4

The correlation coefficient (*r*) between the Trad-SHM and Trad-MN genetic parameters and heavy metals concentration in the soil samples

Chemical elements	Trad-SHM bioassay			Trad-MN	
	PC/1000	CC/1000	SH/1000	MN with tetrads	Tetrads with MN
V	0,1	0,1	-0,11	0,19	0,16
Cr	-0,31	-0,33	-0,02	-0,33	-0,35
Mn	0,62*	0,49	-0,08	0,27	0,26
Fe	-0,08	-0,45	-0,23	0,12	0,07
Co	-0,36	-0,6	-0,51	-0,28	-0,32
Ni	-0,34	-0,3	-0,51	0,17	0,12
Cu	0,34	0,19	-0,25	-0,18	-0,18
Zn	-0,06	-0,04	-0,26	-0,42	-0,43

*Note: * – the differences are significant at p<0.05.*

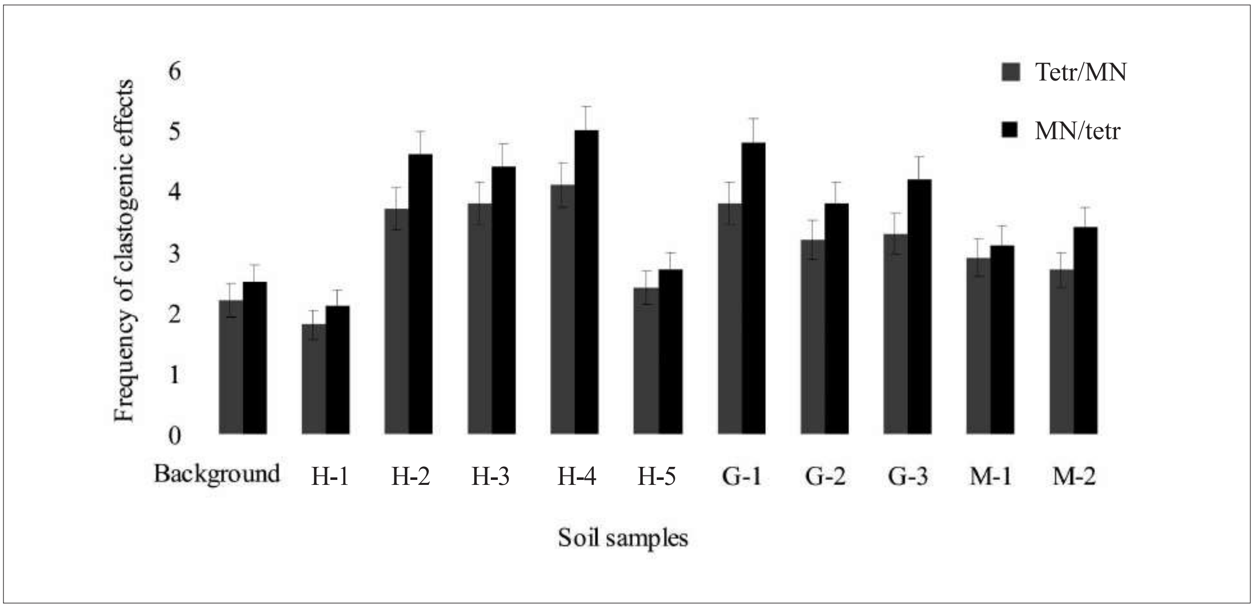

Fig. Frequency of clastogenic effects in *Tradescantia* (clone 02) sporogenous cells. Tetr – the stamen hairs bioassay, MN – micronucleus bioassay

The pronounced clastogenic activity according to both marker criteria was the characteristic for the H-2, H-3, and H-4 samples ($p<0.01$), where the MN frequency exceeded the background level by 1.8–2.0 times, as well as for G-1 and G-3 samples ($p<0.01$), where these indicators were almost 2 times higher than the background (Fig.).

Conclusion

A comparative analysis of soil samples from agricultural landscapes of different Armenian regions and a conditional background sample based on the results of both bioassays of *Tradescantia* clone 02 in the soil-plant system showed the presence of genotoxic and clastogenic effects in the studied groups with varying degrees of manifestation depending on the soil sample. An increase in all marker test indicators is observed in the H-3 sample (the sampling point located 600 m from the industrial zone) according to both Trad-SHM and Trad-MN bioassays. The increased Mn content and the presence of a significant positive correlation between recessive mutations (PC) and this component is also characteristic for H-3 soil sample. Based on the results of the micronucleus test, we fixed a minimal clastogenic effect (at the background level) in samples from the nearby and most distant areas from the Hrazdan industrial zone (H-1 and H-5) compared to other soil samples.

Soil samples from the agricultural landscapes adjacent to the industrial zone were characterized by a total pollution index (Z_c in the range of 0–16). The sampling sites located under the atmospheric emissions plume in direction of the wind rose were particularly polluted.

The lack of correlation between mutational events and the HMs concentration in soil samples, apparently, may indicate the overall polymetallic effect of the components in the soil–plant system (additive toxic effect) and their effect on increasing the level of genotoxicity and clastogenicity in *Tradescantia* cells.

These studies on the ecological-genetic assessment of arable soils using *Tradescantia* (clone 02) are being conducted in Armenia for the first time. Based on the biotesting, the possibility of using the Trad-SHM and Trad-MN bioassays for further studies in genetic monitoring of the mutagenic potential of soils in technogenically disturbed agricultural landscapes has been demonstrated.

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