Ecotoxicological assessment of the mutagenicity level of agricultural soils based on the soil-plant system

A. L. Atoyants, *E. A.* Aghajanyan, and *R. E.* Avalyan*

Yerevan State University, Research Institute of Biology, Laboratory of General and Molecular Genetics, Yerevan, Armenia

Abstract. Under modern conditions of agricultural production, soil pollution with heavy metals (HM) and pesticides is a pressing issue, including for Armenia. Biotesting of the potential mutagenicity level of soil samples from three agricultural land plots in the coastal areas of the Lake Sevan basin was conducted in the spring-autumn period, taking into account the content of HM and pesticides in them. To determine the genetic effects in soil samples, the micronucleus test (Trad-MN) of the model test object Tradescantia (clone 02) was used. According to the main test criteria of the Trad-MN test (frequencies of tetrads with MN and frequencies of MN in tetrads), the maximum level of the studied indicators was established in soil samples of the Lichk region in all time periods of observation, where their values were 2.5-3.5 times higher, respectively. A reliable positive correlation was shown between the frequency of both test criteria and the concentration of As ($p<0.01$; $p<0.001$) in the studied soil samples.

1 Introduction

In the context of increasing anthropogenic load on agroecosystems, the state of agricultural soils, especially in terms of the content of heavy metals (HM) and pesticides, deserves more and more attention. As is known, soil is the most sensitive indicator of the ecological and geochemical state of natural areas, since it is where the migration routes of many chemical elements, including toxicants, intersect. Soils of agricultural landscapes are a source of pollution of agricultural products for living organisms, therefore, regional monitoring of soil pollution is an essential part of the ecotoxicological testing system for the state of the environment.

Despite the fact that pesticides play an important role in ensuring food security by protecting crops from pests and diseases, along with their benefits, there are many environmental and sanitary risks associated with the use of these chemicals. [1–2].

One of the most pressing issues is the accumulation of pesticides and heavy metals (HM) in the soil, where they can persist for a long time, exerting toxic effects on living organisms. [3–4]. Particularly dangerous are heavy metals such as cadmium, lead and arsenic, which are

* Corresponding author: re_avalyan@mail.ru

not subject to microbiological or chemical decomposition, making them a constant source of threat to the environment [5–7].

Pollution problems are especially relevant for resource-limited countries such as Armenia, where the agricultural sector plays a key role in the economy. There are a number of environmental challenges in the republic related to the violation of environmental safety in agriculture, which is aggravated by the impact of chemical fertilizers, pesticides and emissions from industrial facilities [8–9]. Particularly dangerous is the pollution of lands used for agricultural production, as well as the pollution of water bodies as a result of the use of chemicals [10]. These problems can lead to a deterioration in the quality of life of the population, disruption of ecosystems and loss of biodiversity.

The widespread practice in Armenia of improper use of pesticides, their excessive use to control pests or diseases, as well as mixing and disposal of pesticides without taking precautions to protect soil, water and natural vegetation may affect the ecological state of natural agroecosystems. In addition, insufficient attention is paid to the study of the environmental situation in relation to the content of HM and pesticides in agricultural soils, as well as their impact on cultivated crops, in the republic.

To solve these problems, an integrated approach is needed, which includes not only strict control over the use of pesticides, but also the use of innovative methods for monitoring environmental pollution. In this case, to determine the ecotoxicological situation in a specific natural and economic region, it is especially important not only to develop and apply express methods for assessing the degree of potential toxicity of the studied territory, but also to use reliable and sensitive test objects for the purpose of biotesting the degree of anthropogenic pollution of natural territories.

In the genetic monitoring system *Tradescantia* (clone 02) is a reliable indicator in biotesting the mutagenic effects of the natural environment. The micronucleus assay (Trad-MN) is used as the main marker bioassay for *Tradescantia*, allowing detecting genetic disorders in the process of microsporogenesis, manifested in the formation of micronuclei in microspore tetrads, under the influence of toxic substances, including pesticides and heavy metals. This bioassay is part of the International Program for Plant Tests (IPPB) under the auspices of the United Nations (UNEP) on the environment.

The aim of this work was to assess the level of mutagenicity of soil samples from agricultural lands in the coastal areas of the Lake Sevan basin using the micronucleus test (Trad-MN assay) of *Tradescantia* (clone 02) in the soil-plant system.

2 Materials and methods

The study material was soil samples from agricultural lands for growing vegetable crops in the coastal areas of the Lake Sevan basin. Soil samples were collected from three regions (Gavar – 40°35′40″N 45°11′29″E, Lichk – 40°15′62″N 45°23′06″E, Martuni – 40°13′10″N 45°30′52″E), two plots for each - the first plot of soil treated with pesticides; the second plot of untreated natural soil, located at a distance of 500 m from the first. Sampling was carried out in three seasons: a) spring (May), when fertilizers have not yet been applied to the field and sowing has not been done; b) summer (July), after fertilization; and c) autumn (October), after harvesting. All three sites were located in mountain-steppe landscapes, where mountain-chestnut soil types predominate, at an altitude of 1780–1982 meters, surrounded by untouched natural areas. Soil sampling was carried out in accordance with the methodology of agrochemical study of agricultural soils.

Soil samples were collected in dry weather conditions from a depth of 5–20 cm using the envelope method at a distance of 100–200 m from each other. In each regional soil group, at least five samples were collected from one test plot to form a combined soil sample for the purpose of a more objective assessment of the degree of accumulation of chemical components. The mass of each point sample was 200 g, the combined one – 1 kg. The soil of the ESU greenhouse (mountain chestnut soil type), located at a distance of 30-50 km from the main study areas, was used as a conditional background sample. The concentration of heavy metals and qualitative analysis of pesticides in the soil were carried out at the RA Monitoring Center using atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS). Pesticide analysis in the soil was carried out by gas chromatography on *PerkinElmer Clarus 680 GC* devices.

Plants of the model test object *Tradescantia* (clone 02) were used as the object of the research. The vegetation experiment in the soil-plant system was conducted in the greenhouse of YSU. Tradescantia plants were grown in pots with the studied soil samples under the same vegetation conditions: T = 20–25 °C, day/night light regime - 18 h/6 h. For each variant of the regional soil sample, three vegetation vessels with 300–400 g of soil in each were used, in which 5–7 *Tradescantia* plants at a certain stage of organogenesis were planted. The plants were watered with tap water. After 3–4 weeks, after the formation of inflorescences consisting of 16–20 flower buds, the small buds were fixed to conduct the Trad-MN assay according to the generally accepted method.

The use of the micronucleus test (Trad-MN) allows recording the occurrence of such genome disorders as chromosomal aberrations (acentric fragments or lagging chromosomes), which are recorded as micronuclei (MN) at the stage of microspore tetrads, when the process of microsporogenesis is disrupted. When testing using this method, two test criteria are taken into account: the frequency of tetrads with micronuclei and the frequency of micronuclei in tetrads. Fixation of buds was carried out using ethanol-glacial acetic acid (3:1). Temporary acetocarmine preparations were prepared. Microspore tetrads were analyzed at a magnification of 10x40 using a *Motic Images Swift M10L* microscope. The number of tetrads with micronuclei was determined and the number of micronuclei per 100 tetrads was recalculated using the standard method. For each soil sample, 3000 tetrads were examined. A correlation analysis was also conducted between the frequency of micronuclei in microspore tetrads and the concentration of chemical elements in the water samples under study. All the results obtained were statistically processed using the *Stastgraphics Centurion 16.2* computer program.

3 Results and discussion

The analysis of the results of determining the gross content of the studied chemical elements in the studied soil samples of the Gavar, Lichk, Martuni districts showed that their content was heterogeneous. Although the soil samples from the treated agricultural plots were distinguished by an increased content of TM compared to the untreated plots, for some elements in both cases there was an excess of generally accepted norms (MPC) for soil (Table 1). The table presents data only for the summer period, when the soils were exposed to the most intense impact of toxic substances. In all the studied soil samples, there was an excess of the norm of the elements - As, Co, Ni, as well as - Cr and Cu in all samples, except for the Martuni sample. The main sources of the studied elements in agrocenoses were organic fertilizers.

Table 1. Content of chemical components in soil samples (mg/kg).

Soil samples	**Content of chemical components (mg/kg)**								
	As	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
Gavar	**7,2**	**155,9**	**133,5**	**73,6**	33265,3	770,3	**86,0**	17,0	89,8
Gavar/c	**4,2**	**103,2**	**109,9**	**65,3**	30907,4	704,5	**68,0**	12,0	86,4
Lichk	**10,5**	**80,9**	**140,6**	**83,6**	40194,2	834,5	**86,2**	31,5	90,5
Lichk/c	**9,0**	**74,3**	**130,0**	**78,4**	36835,8	804,8	**75,5**	27,0	87,0

Continuation of Table 1.

Soil samples	Content of chemical components (mg/kg)								
	As	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
Martuni	**4,8**	**56,1**	82,4	44,2	32189,5	736,0	**60,6**	16,0	86,0
Martuni/c	**3,2**	**36,2**	60,7	31,4	22514,2	420,0	**53,4**	18,0	84,9
MAC/soil	2	20	100	55	40000	1500	50	32	100

Note: Concentrations exceeding the Maximum Allowable Concentrations (MAC) for soil are highlight in bold.

The soils of the treated plots of agricultural land differed in the content of the pesticides used (mainly Pendimethalin, Fipronil, Tefluthrin, Folpet), which have a dangerous and toxic effect on biota. Folpet is a systemic pesticide, organochlorine fungicide. It is intended for processing grapes, vegetables and fruits from pathogenic fungi. This fungicide is considered to be low-toxic to plants. Tefluthrin is an insecticide, a contact pesticide, which is used in agriculture to control harmful insects. Tefluthrin-based preparations belong to hazard classes 2 and 3 for humans and require careful handling. Fipronil is an insecticide, a contact-intestinal pesticide. It is used in the processing of seed material (seed dressing) and spraying of plants. When used moderately according to the regulations, it does not exhibit phytotoxicity. However, large-scale use of this compound is highly toxic. Pendimethalin is a soil herbicide. It has herbicidal activity only in relation to germinating seeds, therefore it is intended only for soil application. Almost completely decomposes in the soil by the end of the growing season, as a result of which it does not have a phytotoxic effect on subsequent crops. Unfortunately, this study only determined the presence of the mentioned pesticides without data on their concentrations in soil samples.

When conducting biotesting of the mutagenicity level of the studied soil samples to detect disturbances in the microsporogenesis process with the formation of micronuclei (MN) in microspore tetrads (clastogenic effect) based on the analysis of the micronucleus test of *Tradescantia*, a significant increase in the frequency of MN was shown compared to the control (conditional background) in the soil samples of all the studied areas. At the same time, the treated soils of agricultural areas showed a higher level of clastogenicity than untreated (control) areas (Table 2).

Table 2. Induction of clastogenic effects in soil samples of agricultural areas by Tradescantia (clone 02).

Soil samples	Micronucleus frequency in sporogenous cells of Tradescantia					
	Tetrads with MN (%±m)			**MN in tetrads (%±m)**		
	spring	summer	autumn	spring	summer	autumn
Gavar	3,8±0,35***	5,8±0,43***	5,5±0,42***	4,8±0,39***	7,1±0,47***	6,0±0,43***
Gavar/c	3,2±0,32*	4,7±0,39***	4,5±0,38***	3,8±0,35**	5,7±0,42***	5,1±0,40***
Lichk	5,6±0,42***	8,0±0,46***	5,8±0,43***	7,1±0,41***	8,7±0,52***	7,1±0,47***
Lichk/c	4,9±0,39	5,1±0,40***	4,7±0,39***	4,1±0,36***	6,0±0,43***	5,7±0,42***
Martuni	2,9±0,31	4,9±0,39***	4,5±0,38***	3,4±0,33*	5,5±0,42***	5,2±0,41***
Martuni/c	2,7±0,30	3,5±0,34**	3,5±0,34**	3,1±0,32	3,96±0,36**	3,9±0,35**
Fon	2,2±0,27			2,5±0,29		

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

In the spring, before planting vegetable crops (mainly melons), the lowest mutation rate was observed in the soil samples studied. It should be noted that the highest mutation rate was observed in the summer, when the crops were actively flowering and pesticides were applied to control pests. Despite the fact that in the autumn the soil was less polluted in terms of toxic substances, at the same time, the clastogenicity level retained high toxicity rates according to both criteria, exceeding the background level by 1.6-3.6 times. The most pronounced clastogenic effects were observed in the soil samples from the Lichk district, exceeding the background indicators by 2.1-3.6 times in the treated areas and by 2 times in the corresponding control areas. The lowest value of the soil clastogenicity level was observed in the samples taken from the Martuni district. Moreover, this trend persisted throughout the study period. Thus, based on the obtained results for both test criteria, it was shown that the soils from the treated areas had a higher level of clastogenicity than similar soils from untreated areas, and this trend was observed in all seasonal periods of monitoring.

The conducted correlation analysis between the concentration of chemical elements and the test criteria of the Trad-MN test showed the presence of a reliable positive correlation between both test criteria and the concentration of arsenic (As) in soil samples - MN in tetrads ($r=0.98$, $p<0.001$) and tetrads with MN ($r=0.84$, $p<0.05$). As is known, arsenic, along with cadmium and lead, are ecotoxicants that have a cumulative and carcinogenic effect on living organisms.

4 Conclusion

Agroecosystems are usually exposed to many types of pollutants, including biotic and abiotic byproducts of agricultural practices. Among the agricultural sources of heavy metals, pesticides are the most common. Excessive use of pesticides over a long period of time leads to the accumulation of heavy metals in agricultural soils, reduces soil fertility and, as a result, reduces plant growth and productivity. As a result of uncontrolled use, pesticide residues accumulate significantly in the soil and increase pollution, which directly or indirectly harms fauna and flora.

A comparative analysis of soil samples from agricultural lands in different regions of Armenia and a conventional background sample based on the results of the Trad-MN test of clone 02 of *Tradescantia* in the soil-plant system showed the presence of clastogenic effects in the studied soil regional groups with varying degrees of manifestation depending on the sample. This paper presents the results of the total mutagenic activity of the studied soil samples. The highest level of genetic disturbances during microsporogenesis was observed in the Lichk sample from the territory of the soil plot treated with pesticides. The absence of a correlation between clastogenic effects and the content of chemical components in the soil samples may indicate a combined multicomponent effect of ecotoxicants (additive toxic effect) in the soil-plant system and their influence on increasing the level of clastogenicity in sporogenous cells of Tradescantia. Therefore, further studies should take into account not only the content of heavy metals and pesticides, but also other environmental factors that may affect the mutagenic activity of agricultural soils.

Studies on the ecological and genetic assessment of the state of agricultural soils taking into account the content of toxic substances in them, in particular pesticides, using clone 02 of *Tradescantia* were conducted in Armenia for the first time. In the future, it is planned to continue studies aimed at identifying the long-term effects of heavy metals, heavy metals and pesticides on agroecosystems, especially taking into account seasonal changes. Based on the conducted research, the feasibility of using *Tradescantia* (clone 02) as a model test object for biotesting the degree of anthropogenic disturbance of the soil cover of agricultural agroecosystems was demonstrated.

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