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A Combined Approach to the Assessment of the Level of Pollution and Toxicity by Macro- and Trace Elements of the Territories of Armenia with Different Anthropogenic Loads

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Abstract: Both natural and anthropogenic factors can cause long-term and irreversible damage to the environment, in particular to the soil. To solve several environmental problems, it is important to study the seasonal changes in the concentration of macro- and trace elements in soil samples and to identify the factors that regulate the concentration of chemical elements in soil and their toxicity. This study employs an innovative integrated methodology to detect alterations in the concentrations of certain chemical elements in soil samples from various regions of Armenia throughout the current agricultural seasons. Elemental analysis of soil samples is carried out using a portable XRF analyzer with direct X-ray exposure. The clastogenicity of the soil-plant system was evaluated using *Tradescantia (clone 02)*. A pollution index based on the established Clark concentration for chemical elements was calculated. Trace elements exhibited a higher pollution index than those in other groups. The genotoxic analysis of soils from the study sites revealed a significant increase in soil clastogenicity, beginning in autumn and peaking in the spring season. During the subsequent summer sampling season, all genotoxicity evaluation criteria for the materials under study notably decreased. The results analysis revealed positive and negative correlations among all chemical elements in the soil samples from all sites during the experimental cycle, with varying degrees of significance.

Keywords: soil; macro elements; trace elements; seasons; genotoxicity; the pollution index

1. Introduction

Armenia is one of the regions that is exceptionally rich in a variety of natural resources in a relatively small area. The country's climate varies due to factors such as its location, atmospheric circulation and mountainous terrain, as well as the height and position of the mountains. Under conditions of increasing anthropogenic impact on nature, potential changes in the environment are manifested primarily in the biogeochemical processes of land cover [1]. Changes in climatic conditions have a profound effect on soil formation processes [2]. This leads to changes in soil biochemistry in terms of composition and quantity [3], erosion and weathering [4,5], and irreversible processes for living organisms [6]. A noticeable redistribution of elements in soil composition due to their migration is associated with human industrial and agricultural activities [7,8].

Different surface soil horizons, formed by the intravital secretions of living organisms, plant root systems, and groundwater, provide for the physicochemical sorption of nitrogen, phosphorus, potassium, and calcium, as well as the biogenic accumulation of humic organic compounds from chemical elements [9]. In general, these elements are conventionally classified as macro- and trace elements (ME and TE). Their importance for plant growth is generally recognized in the scientific literature [10,11]. As participants in specific biochemical and physiological processes responsible for the synthesis of certain substances, both ME and TE have concentration limits when taken up by plants from the soil [12].

As a result, soils are irreversibly degraded, losing their beneficial properties and agricultural functionality, making it difficult to leave the environment unpolluted, which has adverse effects on the food chain [13–15]. That is why, before assessing the degree of soil contamination, it is important to distinguish between the causes of its occurrence: anthropogenic and abiotic [16,17].

The Clark-Vernadsky law emphasizes the ubiquitous presence of chemical elements in nature [18,19]. This situation can be clarified by introducing Clark's concentration (CC) of chemical elements in the continental crust, which serves as a standard method for comparing geochemical systems [20,21]. According to this concept, regional geochemical background landscapes are identified by analyzing the technogenic-geochemical transformation of the chemical composition of natural environments [22,23].

The proportion of ME present ranges from hundredths to whole percentages. They are evenly distributed in the various tissues of the body. These elements include chlorine (Cl), calcium (Ca), sulfur (S), phosphorus (P), magnesium (Mg), sodium (Na), and potassium (K). Most ME are an essential component of living organisms [24]. Their absence has serious consequences, such as disrupted metabolism, impaired cell division, and impaired transfer of genetic material [25–27].

TE groups are distributed unevenly and accumulate in living organisms in negligible quantities, typically in concentrations of thousandths and hundred-thousandths of a percent [28,29]. TE are essential for plant growth and development [30,31]. Among these are strontium (Sr), rubidium (Rb), iron (Fe), copper (Cu), selenium (Se), iodine (I), chromium (Cr), zinc (Zn), fluorine (F), manganese (Mn), cobalt (Co), molybdenum (Mo), cesium (Cs), silicon (Si), bromine (Br), vanadium (V), and boron (B).

For the differentiation of pollution sources, monitoring of the transformed soil cover with sensitive plant test objects is necessary [32–34]. Plants are preferred bioindicators over animals because they produce energy and provide food for many organisms at higher trophic levels [35,36]. Among the model plants for bio-testing, *Tradescantia (clone 02)* deserves special mention. Its use makes it possible to assess the induction of genetic disorders, point mutations, and clastogenic effects under the influence of sufficiently low concentrations of xenobiotics [37,38]. The main marker tests for *Tradescantia clone 02* in biotesting are the micronucleus test to detect disturbances in the process of microsporogenesis with the formation of micronuclei (MN) in tetrads of microspores (clastogenic effect - Trad-MN test). Using Trad-MN test allows the detection of the appearance of chromosomal aberrations (acentric fragments or lagging chromosomes), recorded in the form of MN at the stage of microspore tetrads when microsporogenesis is disturbed [39].

Based on the concept that comprehensive assessment and monitoring of natural potential are important national priorities of Armenia in the field of environmental safety, the following regions of Armenia were selected Hrazdan, Gavar, and Martuni. In general, the formation of the land cover on the territory of modern Armenia began in the lower Pliocene. Moreover, volcanic eruptions, which dramatically changed the orography of the country, had a great influence on the formation of land cover [39]. Geographically, Hrazdan is mainly located in a steppe area surrounded by open forests. A short distance from the city, in the basin of the Hrazdan River, there are several deposits. The climate of the region is distinctly continental, with moderately cool, rainy summers and rather cold, snowy winters with an average annual temperature of 4.8°C [40].

Gavar lies on the banks of the Gavaraget River at an altitude of 1982m. The city is dominated by the Gegham Mountains to the west and Lake Sevan to the east. Gavar has a humid continental climate with an annual rainfall of 450 mm. Gavar has pleasant, dry, and clear summers, but frosty, snowy, and sometimes cloudy winters. Temperatures typically range from -12°C to 24°C throughout the year.

Martuni is situated on the southwestern shore of the high mountain Lake Sevan, at an altitude of 1950 m above sea level. To the north of the town, there is a forest belt around Lake Sevan, and to the south, there is the Vardenis Range. Martuni is pleasant, dry, and clear in summer and frosty, snowy, and sometimes cloudy in winter. Temperatures throughout the year typically range from -12°C to 25°C.

In consideration of the above, the experimental study aimed to investigate the distribution of concentration of some chemical elements from two groups (ME and TE) in soil samples taken from regions of Armenia (Hrazdan, Gavar, and Martuni). These regions have almost similar soil structures, but they differ from each other in terms of climatic conditions. For this reason, we aimed to determine the concentration changes of ME and TE uptake during the four seasons (autumn 2021, winter 2021/22, spring 2022, and summer 2022). As a bioassay of our study, we first used a two-criteria genotoxicity test on soil samples to identify the correlation with the concentrations of ME and TE in soil samples.

2. Materials and Methods

2.1. Soil sampling

The experimental study determined the concentrations of ME such as Ca, S, and K; and TE consisting of Sr, Rb, and Cs in soil samples collected during the agricultural year: autumn 2021, winter 2021/22, spring 2022, and summer 2022. Soil samples were collected from several regions of Armenia, including Hrazdan (two locations), Gavar (two locations), and Martuni (two locations). Geographical coordinates for each sampling site are documented in Table 1. Sampling areas were identified on private agricultural land with moderate traffic, away from the industrial zone [6]. Only the Hrazdan soil was located in the south-western direction of the complex industrial comprising the Hrazdan Thermal Power Station and the Hrazdan Cement Plan. To account for the anthropogenic load and the regional wind rose, the sampling process was carried out meticulously [41]. Soil samples were collected from the control points under dry weather conditions by employing the envelope method at a depth of up to 20 cm. Non-metallic tools were used for point sampling. To prepare the soil sample, at least five incremental samples obtained from the same site were mixed. The samples were subsequently placed in dark glass containers and transported at +4°C for 24 hours for instrumental measurements in the laboratory [42]. They are mountain semi-desert grey soils formed at altitudes of 850-1350 m above sea level. These soils were formed on alluvial, alluvial-diluvial-pebble, or clastic-pebble carbonates, often gypsum loams. Most of the parent rocks are saline because the area is in a very arid zone. The absence of waterlogging and high porosity characterizes the loose sediments and lava deposits in this region. The density in the upper humus horizons of the non-moor semi-desert grey soils is high, reaching 1.42-1.34 g/cm^3. Mountain semi-desert grey soils are very poor in humus because poor plant residues mineralize rapidly at high temperatures.

Table 1. Geographical coordinates of the sampling sites**.**

Sampling sites		North	West
Hrazdan region	H1	40°33'04.6"	44°44'41.9"
	H2	40°33'16.4"	44°44'42.0"
Gavar region	G1	40°21'02.9"	45°11'49.7"
	G2	40°21'00.3"	45°11'46.5"
Martuni region	M1	40°24'10.4"	45°08'07.7"
	M2	40°24'07.5"	45°08'06.4"

2.2. Elemental analysis

After removing any debris from the root system, insects, and other solid components, the soil was ground using a mortar and pestle, and sifted through a 1 mm diameter sieve. An air-dried sample of the tested soil was weighed first using a calibrated analytical balance (OHAUS EP 6102). The sample was then spread thinly onto a clean, flat glass, situated beneath a fume hood where it was stirred continuously for roughly an hour using either a glass or polymer rod. After each 1-2 agitations, the sample was reweighed, and the weight difference was recorded. This process was repeated until a stable dry mass of the samples was established. If the last three weightings' results did not differ by more than the balance's error, we determined the average dry mass of the sample. Subsequently, we ground the obtained soil sample into a powdery mass using a ceramic mortar. The sample was transferred under specific pressure into a sealed container with a 32mm diameter and XRF sample cups made of polypropylene film. Direct X-ray exposure was used to analyze all soil samples for elemental analysis using a portable XRF analyzer by Termo Scientific™ Niton™ [43].

2.3. Chemical analyses

A 30 g soil sample (within a precision of 0.1 g) was added to a conical volumetric flask. Distilled water was then poured into the flask until it reached the 150 cm mark. The solution was stirred with a propeller stirrer for 3 minutes and left to settle for 5 minutes. Subsequently, a 25 cm^3 volume of the filtrate was poured into a porcelain cup that had been dried and weighed (with a tolerance of 0.001 g). The filtrate was finally evaporated in a water bath. After the evaporation process, the cup was set in a thermostat at 105°C for three hours, cooled in a desiccator, and weighed with a tolerance of 0.001 g. To determine the pH and electrical conductivity of the soil extract, a HACH LANGE HQ 14d water analyzer was utilized. The dense residue's mass fraction in the analyzed soil aqueous extract was calculated employing a formula.

$$X = ((m-m_1) \times 500)/25, \tag{1}$$

where m is the mass of the beaker containing the residue, g; m_1 is the mass of an empty beaker, g; 500 is the conversion factor in percent; 25 is the volume of the extracted sample, cm^3. These analyses, and the total relative error of these analyses, are provided by standard [44]. This standard establishes methods for determining the specific electrical conductivity, pH, and residual density of water extracts from saline soils to assess the total concentration of salts when conducting soil, agrochemical, and land reclamation surveys, monitoring the status of the salinity regime of soils. Details of soil chemical characteristics are shown in Table 2.

Table 2. Some of the chemical characteristics of all sampled soils*.

Soil characteristic	Season of soil sampling	Hrazdan site		Gavar site		Martuni site	
		H1	H2	G1	G2	M1	M2
Conductivity, µm/cm	Autumn, 2021	174	194	251	163	185	217
	Winter, 2021/22	236	322	741	167	433	533
	Spring, 2022	408	634	726	299	434	407
	Summer, 2022	342	861	300	204	519	468
Salinity, %	Autumn, 2021	0.08	0.13	0.70	0.10	0.01	0.01
	Winter, 2021/22	0.15	0.52	0.57	0.52	0.47	0.08
	Spring, 2022	0.07	0.01	0.06	0.01	0.31	0.19
	Summer, 2022	0.15	0.28	0.25	0.16	0.16	0.18
pH	Autumn, 2021	7.10	6.93	7.80	7.78	7.30	6.91
	Winter, 2021/22	7.66	7.19	6.88	8.28	7.45	7.74
	Spring, 2022	7.46	7.85	7.94	7.60	7.56	7.68
	Summer, 2022	7.03	7.87	7.73	7.87	7.76	7.01

Note: * The total relative error is 7.5% - when determining the electrical conductivity up to 0.3 mS/cm, 5% - over 0.3 mS/cm; 20% - with a mass fraction of a dense residue from 0.1 to 0.3%; 7.5% - from 0.3% to 1%; 5% - over 1%; when measuring pH, the total error of the method is 0.1 pH units.

2.4. Bioassays

Tradescantia (clone 02) was used as a model plant to determine the extent of clastogenicity in the contaminated soil samples. This bioassay is included in the International Programme for Monitoring and Testing of Environmental Contaminants [45]. During the experiment, 5-10 plants per option underwent cytogenetic monitoring. The plants were planted directly in the pot with soil from the study sites. A few weeks after the formation of inflorescences containing 16-20 flower buds (the 7th or 8th pair of buds from the top of the inflorescence pyramid), tetrads in early prophase I (pachytene and diplotene) show sensitivity to mutagens. The buds were then fixed in acetoacetic acid fixative consisting of three parts 96% alcohol and one-part glacial acetic acid. Temporary acetocarmine preparations were then made according to standard methods [46]. Early tetrads have complete membranes around the four nuclei. As a result, the four cells of the tetrads do not separate during preparation and the resulting micronuclei, which are indicators of contamination, are close together. One to five micronuclei can form in each tetrad. The number of tetrads with micronuclei was determined and the number of micronuclei per 100 tetrads was recalculated according to the standard method [47]. Cells were analyzed at 10x20 or 10x40 magnification using a Motic Images Swift M10L series microscope.

2.5. Calculation of geochemical coefficients

The total pollution index (Z_c) by characterizing the accumulation of chemical elements in soil samples, was calculated using the formulas

$$\boldsymbol{Z_c = \sum_{i=1}^{n} K_c - (n-1)} \tag{2}$$

where n is the number of chemical elements that are measured in a sample of soil, K_c is the ratio of the concentration of a chemical element in the investigated soil samples to the value (C_s, mg/kg) of its background (i.e. CC) concentration (B_f, mg/kg) [48,49].

2.6. Statistical analysis

All results were averaged and statistically processed using the computer program Stastgraphics Centurion 16. 2. - (STATGRAPHICS Centurion XVI Version 16.2.04). The calculation of mean concentrations of elements and analysis of variances to estimate statistically significant differences between groups of the samples was performed.

3. Results and discussion

3.1. Distribution of elements in soils

The qualitative and quantitative composition of soil depends on the environment in which it was formed [50,51]. Special attention is given to soil formation processes that are directly related to climatic conditions [52]. The process of soil chemistry formation can be influenced by abiotic and anthropogenic factors, depending on the specific natural conditions of the location. However, the direct influence of various pollutants on the qualitative composition of the soil is a determining factor [53]. Therefore, it is important to study the changes in the concentration of ME and TE present in the soil, as well as to determine the factors that control the concentration of these elements, and their toxicity [54–56]. These spatial-climatic investigations can help solve many environmental problems.

3.1.1. Macro-elements

The concentration of K, Ca and S in soil samples from different locations is shown in Figure 1. In the ME group, the concentration of K, Ca and S was determined in soil samples from private agricultural land during the whole sowing period from autumn 2021 to summer 2022. The concentration of K showed statistically significant differences, which slightly increased during the summer. At the same time, the highest accumulation of K was found in G1 soil samples in autumn, with an average of 17368 mg/kg. In winter and spring, the highest accumulation was found in the G2 soil samples.

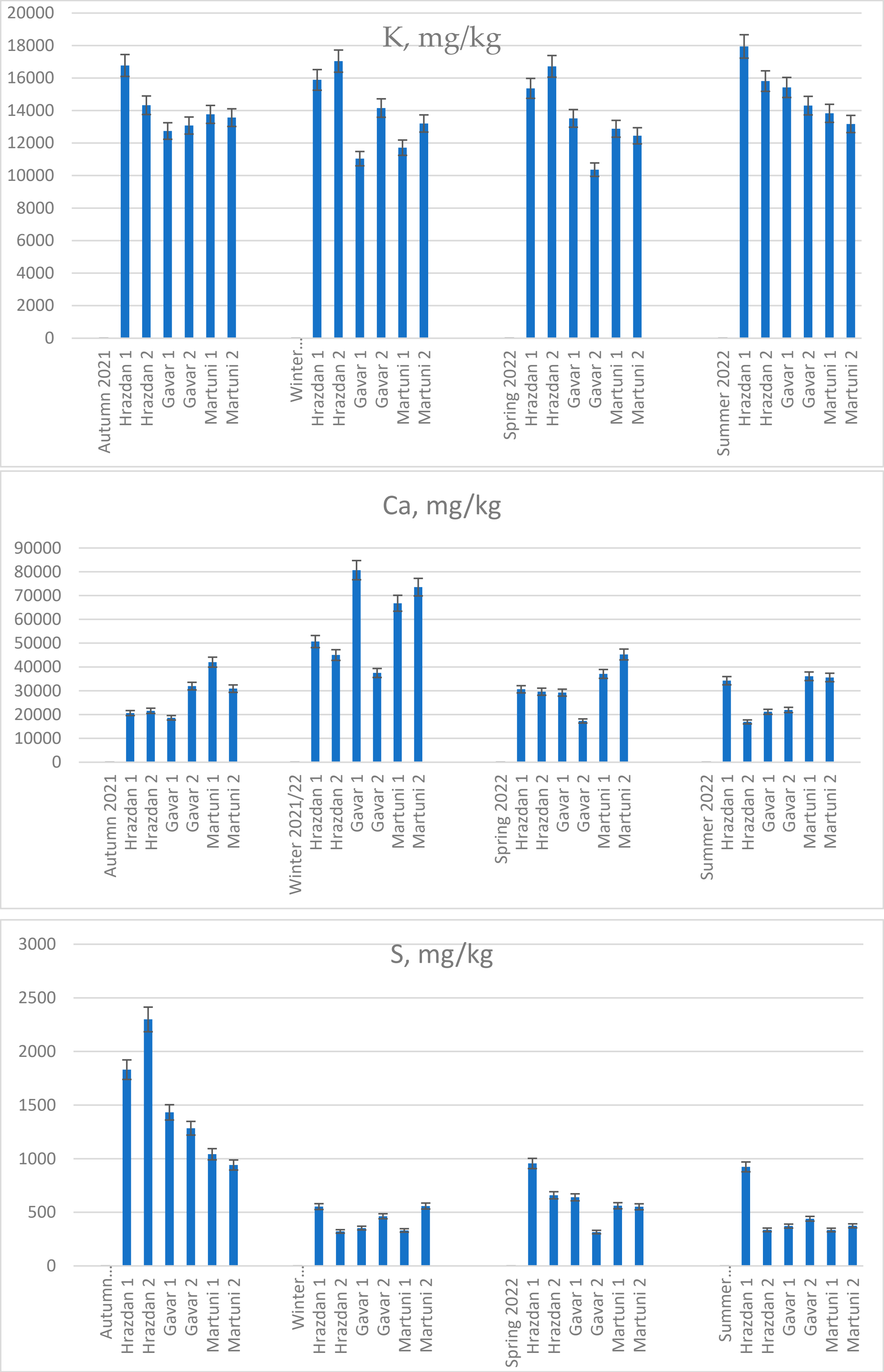


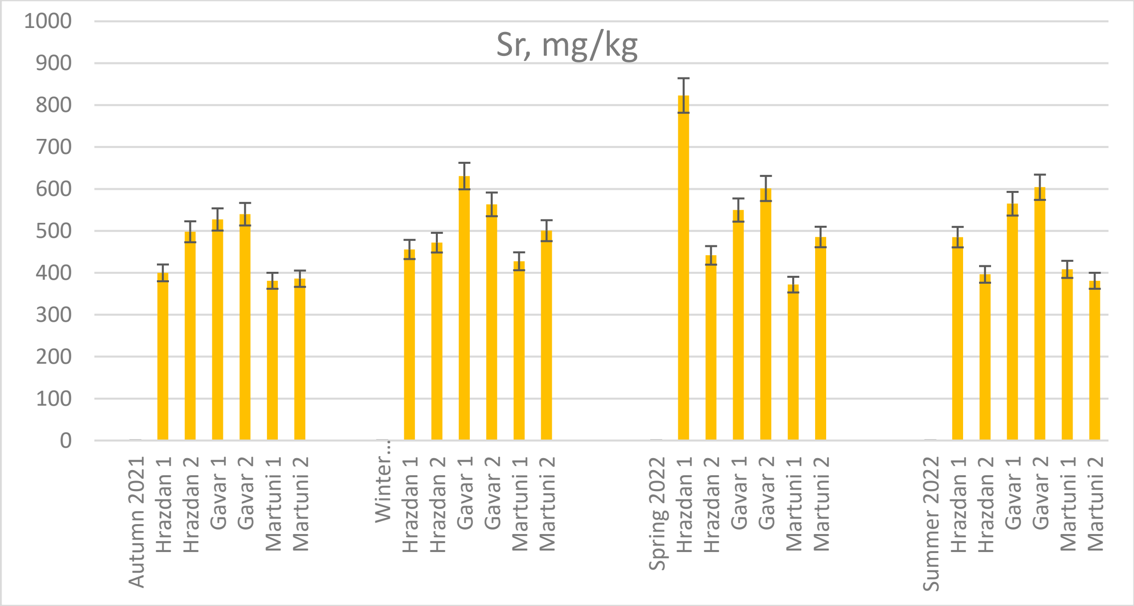
Figure 1. The concentration of K, Ca, and S in soil samples from different sites.

High concentrations of Ca were found at all soil sampling sites in winter. In soil samples from H1, the average concentration in autumn, spring and summer was 28514 mg/kg, which was 44% lower than in samples from the same region in winter. Soils from G1 and G2 differed in relatively low Ca concentrations in autumn, spring and summer. According to the obtained results, high Ca concentrations were found in all investigated sites of soil sampling in winter in the sites of Hrazdan H1 > H2. In the sites of Gavar, the situation was not as simple as it seemed. The highest Ca concentration in G1 was 80685 mg/kg and the lowest in G2 was 37500 mg/kg. A similar picture is observed both in autumn and in spring, but with a fourfold lower concentration.

The situation was completely different for the accumulation of S in the soil samples. This element had a high concentration in all soil samples only in autumn: at the Hrazdan site (H1<H2), then decreasing in order at the Gavar (G1>G2) and Martuni (M1>M2) sites. In the other sampling seasons, 4-5 times decrease in the concentration of the element in soil samples is observed in winter, spring and summer. The comparative series was maintained in the sampling sites for S concentration.

3.1.2. Trace elements (TE)

The concentration of Sr, Rb, and Cs in soil samples from different sites is shown in Figure 2. In most cases, except soil taken from H1 in spring 2022, the soil samples from Gavar sites contained the highest Sr. The seasonal changes in the Sr accumulation were the following: winter` 21/22≥ summer` 22> autumn` 21. However, relatively low levels of this element data were obtained in the soil samples from the Martuni sites, with the highest levels of accumulation occurring in the spring at M2.



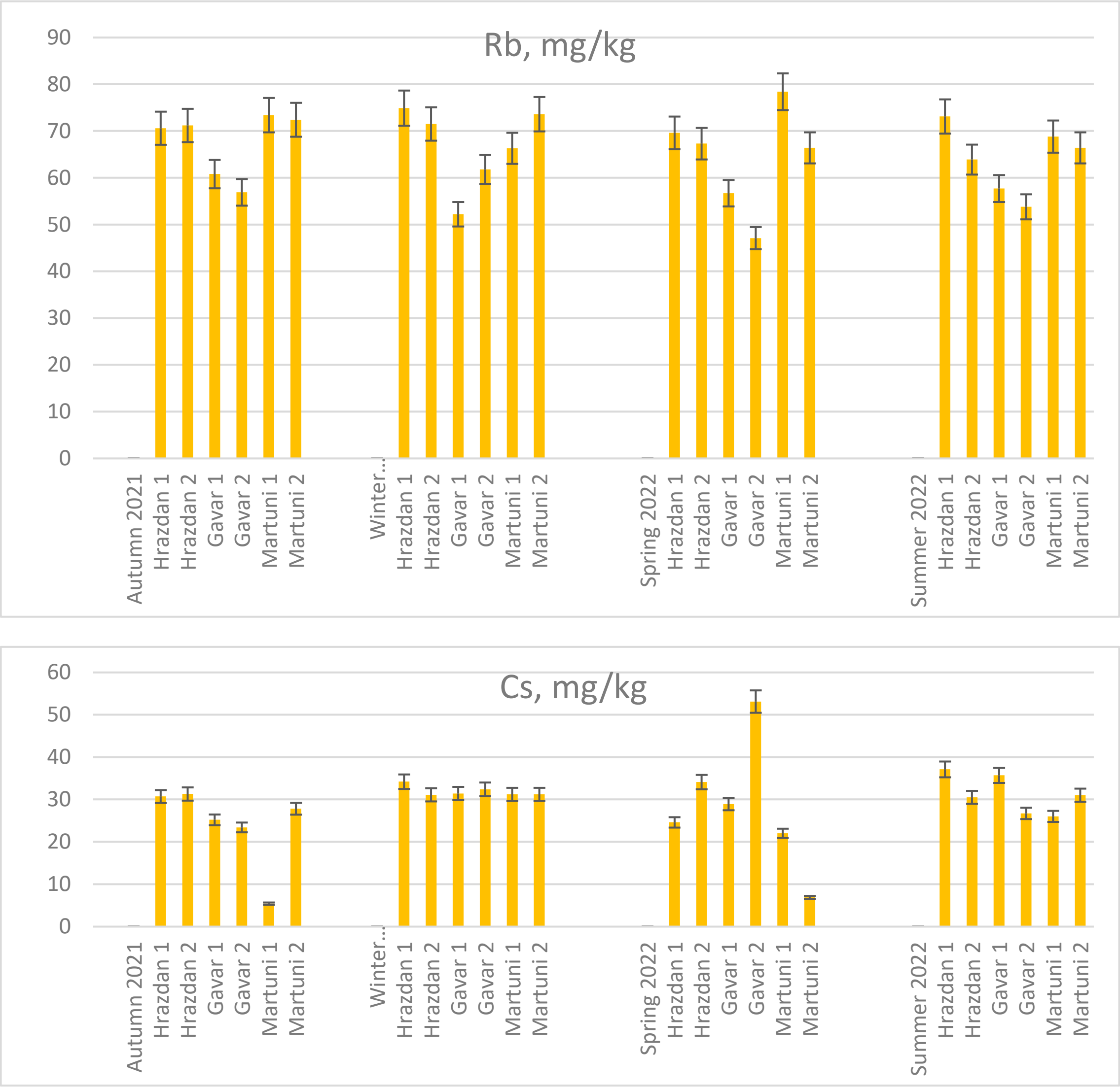


Figure 2. The concentration of Sc, Rb, and Cs in soil samples from different sites.

Compared the Rb concentration in soil sampling sites was found in winter G1<G2. In spring` 22 the Rb concentration is less than 50 mg/kg in the soil collected from site G2.

The Cs concentration in the general context of the studies was maintained at an average level of 29 mg/kg in the soil samples from all sites. Furthermore, on the one hand, the lowest Cs concentrations were found in the Martuni soil samples in autumn (M1- 5.4 mg/kg) and spring (M2 - 6.9 mg/kg). On the other hand, the highest Cs concentrations were found in soil samples from the Gavar sites (G2 - 53.1 mg/kg).

3.2. Geo-ecological coefficient of ME and TE assessment of soil pollution

In order to assess the degree of soil contamination, an unconditional constant value was introduced as no maximum allowable concentration values were established for all chemical elements [19,57]. CC of chemical elements has often been used to assess pollution levels in available environmental studies to identify the type of chemical element accumulation: anthropogenic or abiotic [58].This indicator characterizes the relative concentration of chemical elements in nature. The pollution index (Z_c) was calculated considering the CC to interpret the results obtained on the chemical element concentration in soil samples [21,59]. This methodology also aimed to differentiate between anthropogenic and absolute impacts.

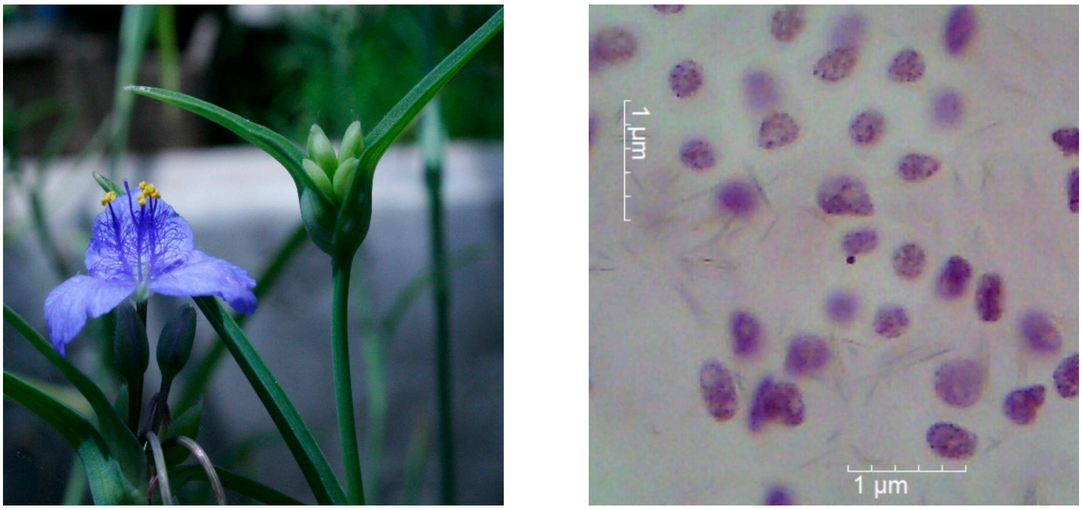
Table 3 shows the comparative pollution index for each group of ME and TE elements in soil samples collected from all sample sites during the study period. Regardless of the season, the meaning of the pollution index for TE is higher than the value of the other groups of elements. This could be attributed to the fact that TE typically comprise heavy metals that had demonstrated harmful effects on the environment in various literature [60,61]. In the autumn, the superiority of the ME in the total pollution index was observed in all soil samples.

Table 3. The value of the total pollution index (Z_c) for soil sampling from collected sites.

Sampling sites		**Macro Element (ME)**				**Trace Element (TE)**			
		Autumn, 2021	**Winter, 2021/22**	**Spring, 2022**	**Summer, 2022**	**Autumn, 2021**	**Winter, 2021/22**	**Spring, 2022**	**Summer, 2022**
Hrazdan region	H1	3.26	1.53	1.68	1.84	7.94	9.08	7.53	9.94
	H2	4.20	0.89	1.07	−0.08	8.40	8.27	8.96	7.84
Gavar region	G1	2.19	1.92	0.89	0.13	6.77	8.69	7.81	9.70
	G2	2.34	0.82	−0.33	0.25	6.29	8.83	14.43	7.35
Martuni region	M1	2.19	1.43	0.96	0.49	1.07	8.13	5.56	6.69
	M2	1.59	2.20	1.20	0.53	7.13	8.40	1.74	7.94

3.3. Biotesting of soil samples using the Trad-MN testing system

The Trad-MN test of the model plant *Tradescantia (clone 02)* was used in the plant-soil system to biotest the degree of clastogenicity of the investigated soil samples (Figure 4). The percentage of tetrads with MN and MN in microspore tetrads were used as marker test criteria. The use of the test makes it possible to detect the appearance of chromosomal aberrations (acentric fragments or lagging chromosomes), which was recorded in the form of MN at the stage of microspore tetrads when the process of microsporogenesis is disrupted.



(**a**) (**b**)

Figure 4. General view of *Tradescantia (clone 02) plant*: (**a**) General view of flowers of model plant; (**b**) micronuclei in tetrads of microspores in sporogenous tissue.

This test takes into account two criteria: the percentage of tetrads with micronuclei and the percentage of MN in tetrads (Table 4). A considerable rise in the occurrence of both testing criteria was noted in all the studied variants during the examination of clastogenic outcomes in sporogenic cells of a model plant, based on Trad-MN results, compared to the experimental conditioned average background.

Table 4. Induction of clastogenic effects in sporogenic cells of *Tradescantia (clone 02)* in soil samples according to the Trad-MH biotest.

Sampling sites		**Frequency of clastogenic effects in the sporogenic cells of *Tradescantia (clone02)***							
		Tetrads with MN, %				**MN in tetrads, %**			
		Autumn, 2021	**Winter, 2021/22**	**Spring, 2022**	**Summer, 2022**	**Autumn, 2021**	**Winter, 2021/22**	**Spring, 2022**	**Summer, 2022**
Hrazdan region	H1	1.8 ± 0.3	2.1 ± 0.4	3.9 ± 0.4	3.0±0.3	2.1± 0.3	2.3 ± 04	4.4 ± 0.4	3.4 ± 0.3
	H2	2.4 ± 0.3	5.4 ± 0.4	5.1 ± 0.4	3.8 ± 0.4	2.7 ± 0.3	6.4 ± 0.5	5.9 ± 0.4	4.7 ± 0.4
Gavar region	G1	3.8 ± 0.4	no data	5.8 ± 0.4	3.5 ± 0.3	4.8 ± 0.4	no data	7.1 ± 0.5	3.9 ± 0.4
	G2	3.3 ± 0.3	6.7 ± 0.6	4.7 ± 0.4	5.5 ± 0.4	4.0 ± 0.4	10.0 ± 0.7	5.7 ± 0.4	6.0 ± 0.4
Martuni region	M1	2.9 ± 0.3	4.3 ± 0.4	3.5 ± 0.3	4.5 ± 0.4	3.1 ± 0.3	5.2 ± 0.4	3.9 ± 0.4	5.2 ± 0.4
	M2	2.7 ± 0.3	5.3 ± 0.4	4.9 ± 0.4	4.5 ± 0.4	3.4 ± 0.3	6.5 ± 0.5	5.5 ± 0.4	5.1 ± 0.4
Conditional background		2.4 ± 0.3				2.8 ± 0.3			

In soil samples collected in autumn, the lowest frequency of occurrence of tetrads in MN in the test -system compared to the conditional background was in the Hrazdan sites with H1<H2 (p<0.01), and the highest with an almost twofold increase was in the Gavar sites with the value G1 >G2 (p<0.01). Significant changes were within the average control range (p<0.05) in the Martuni sites.

In all the soil samples collected in the winter, the Trad-MN test showed an increase in the frequency of tetrads in MN, which is a sign of contamination of the samples. In the Hrazdan sites, the average increase in the frequency of tetrads in MN was up to 58%, in the Gavar and Martuni sites it was 2.8 times (but only in G2) and 3.1 times, respectively.

The highest percentage increase in the frequency of tetrads was observed in the spring samples from MN. Compared with the conditional background for soil samples from the Hrazdan sites was increased 2.1 times (with H1<H2, p<0.001), from the Gavar sites it was 2.7 times (with G1>G2, p<0.001) and from Martuni it was 2.0 times (with M1<M2, p<0.001).

When analyzing the results for the summer, a decrease in the frequency of occurrence of tetrads in the MN of the model plant was observed. The change in the frequency of their occurrence was 2.1 times greater in soil samples from the Hrazdan and Gavar sites compared to the conditional control (with H1<H2 and G1<G2). For soil samples from the Martuni sites, the test system showed an increase in the frequency of occurrence of tetrads in MN to 87.5% (with M1=M2, p<0.001). The comparisons for soil samples, according to the test criterion for the occurrence of tetrads in the MN of our model plant, are as follows

Autumn, 2021	Hrazdan region H1<H2	<	Martuni region M1>M2	<	Gavar region G1>G2
Winter, 2021/22	Hrazdan region H1<H2	<	Gavar region only by G2	<	Martuni region M1<M2
Spring, 2022	Martuni region M1<M2	<	Hrazdan region H1<H2	<	Gavar region G1>G2
Summer, 2022	Hrazdan region H1<H2	<	Martuni region M1=M2	<	Gavar region G1<G2

Another test, the frequency of occurrence of MN in tetrads of the model plant, was characterized by taking into account the sign of mutagenicity of the material tested [62]. Furthermore, in the seasonal soil sampling, the comparative ranges of biotests in the case of the tetrads in the MN were almost similar across sites (Table 4). The frequency of occurrence of MN in the tetrads of the model plant test system in the autumn soil samples in the Hrazdan sites is within the statistical mean of the conditional background (with H1<H2, p<0.001). Soil samples from the Gavar and Martuni sites showed a 57% and 17% increase in the frequency of MN in the tetrads of the test plant respectively.

A significant increase in the frequency of MN in tetrads occurring in *Tradescantia tetrads (clone 02)* compared to the spring was observed in all soil samples collected in winter. For this characteristic, the soil samples from the Hrazdan sites were 57% more mutagenic than the conditional background. Analysis of the results for soil samples from Martuni showed an average increase in the frequency of occurrence of MN in tetrads of 2 times and for samples from Gavar - 3.6 times (but only for G2).

The frequency of MN in tetrads was significantly highest in spring soil samples, ranging from 1.7 times (Martuni) to 2.3 times (Gavar) on average. At the same time, the highest percentage of occurrence of MN in tetrads was detected in soil samples from site G1 (7.5±0.5, $p<0.001$) and the lowest from site M1 (4.0±0.4, $p<0.01$).

The genotoxicity of soils collected during the summer was also assessed by mutagenicity. A decrease in the frequency of occurrence of MN in tetrads of sporogenic Tradescantia cells was observed. Moreover, in soils collected from the Hrazdan sites, their frequency was on average 1.4 times higher compared to the conditional background (with H1<H2, $p<0.01$). Analysis of the results of soil samples from the Gavar sites showed a 77% increase in clastogenicity. The highest percentage of MN occurrence in the test tetrads (84%) was found using soil samples from Martuni. It was noteworthy that M1≤M2 ($p<0.001$).

3.4. Correlation analysis

Table 5 shows the correlations between the ME and TE groups in soil samples across all sites during the experimental cycle. Both positive and negative correlations with varying degrees of significance were found. It should be noted that the results of the correlation analysis may be influenced by the similarity of the soil type. In the autumn, a strong negative relationship was observed between Rb and Sr, Cs and Ca. A direct strong correlation was observed in the concentration in the soil between such ME and TE, which are Rb and K, Cs and S. In the winter, a strong negative correlation occurred between K and Ca, Rb and Sr. A strong positive correlation was observed between ME and TE, such as Rb and K, Cs and S. In the soil samples collected in spring, there was only a positive correlation between Rb and Ca, S and K, and correspondingly a negative correlation between Cs and Ca (as in autumn), Cs and Rb. In the soil samples collected in summer, there was a strong positive correlation between Rb and Ca (as in spring), S and K (as in spring), Cs and S. In all soil samples a negative correlation between was between Rb and Sr.

Table 5. Coefficient for correlation between chemical elements in soil samples taken at different sites and seasons.

Autumn, 2021	**Ca**	**K**	**S**	**Sr**	**Rb**	**Cs**	**Winter, 2021/22**	**Ca**	**K**	**S**	**Sr**	**Rb**	**Cs**
Ca	1						Ca	1					
K	−0,30	1					K	−0,77	1				
S	−0,69	0,49	1				S	−0,08	0,20	1			
Sr	−0,44	−0,49	0,34	1			Sr	0,26	−0,42	−0,08	1		
Rb	0,23	0,49	0,07	−0,85	1		Rb	−0,334	0,67	0,50	−0,82	1	
Cs	−0,84	0,33	0,59	0,31	−0,12	1	Cs	−0,43	0,40	0,61	−0,14	0,29	1
Spring, 2022	**Ca**	**K**	**S**	**Sr**	**Rb**	**Cs**	**Summer, 2022**	**Ca**	**K**	**S**	**Sr**	**Rb**	**Cs**
Ca	1						Ca	1					
K	0,18	1					K	−0,17	1				
S	0,27	0,78	1				S	0,34	0,79	1			
Sr	−0,36	0,10	0,53	1			Sr	−0,43	0,20	0,19	1		
Rb	0,71	0,51	0,53	−0,28	1		Rb	0,72	0,35	0,51	−0,69	1	
Cs	−0,98	−0,23	−0,43	0,18	−0,68	1	Cs	−0,01	0,73	0,61	0,14	0,28	1

In addition, the comparative correlation of the soil samples according to the location of their collection is shown in Table 6. Subsequent elemental analyses throughout the experimental cycle showed a significant positive correlation, indicating a homogeneous structure of the soils selected during the research.

Table 6. Coefficient for correlation between different soil sampling sites and seasons.

Autumn, 2021	H1	H2	G1	G2	M1	M2	Winter, 2021/22	H1	H2	G1	G2	M1	M2
H1	1						**H1**	1					
H2	0,994	1					**H2**	0,998	1				
G1	0,994	0,997	1				**G1**	0,985	0,971	1			
G2	0,946	0,975	0,970	1			**G2**	0,997	1	0,971	1		
M1	0,917	0,955	0,948	0,997	1		**M1**	0,991	0,980	0,999	0,979	1	
M2	0,954	0,980	0,976	0,999	0,994	1	**M2**	0,991	0,980	0,999	0,980	1	1
Spring, 2022	*H1*	*H2*	*G1*	*G2*	*M1*	*M2*	**Summer, 2022**	*H1*	*H2*	*G1*	*G2*	*M1*	*M2*
H1	1						**H1**	1					
H2	0,998	1					**H2**	0,952	1				
G1	0,999	0,996	1				**G1**	0,983	0,990	1			
G2	0,993	0,998	0,991	1			**G2**	0,991	0,980	0,998	1		
M1	0,990	0,980	0,994	0,971	1		**M1**	0,992	0,907	0,956	0,971	1	
M2	0,978	0,964	0,984	0,952	0,997	1	**M2**	0,990	0,902	0,952	0,968	1	1

4. Conclusions

A combined assessment system is needed to facilitate efficient soil remediation, taking into account the current soil conditions and the factors influencing the biogeochemical processes there. Therefore, the study of several elements in soil samples requires special attention to their main parameters, distribution patterns, and assessment of correlations between groups, as they have not been extensively studied.

The analysis showed significant differences depending on the sampling sites and the season. In the ME group, the concentration of K showed statistically significant differences, slightly increasing during the summer season, with the highest accumulation in the Gavar sites (for G1 soil samples in autumn and for G2 soil samples in winter and spring). High concentrations of Ca were found at all soil sampling sites in winter. However, soil samples from G1 and G2 differed in having relatively low Ca concentrations in autumn, spring, and summer. As for the accumulation of S in the soil samples, a high concentration was only observed in autumn. During the winter, spring, and summer sampling periods, a 4-5 times lower concentration of this element was observed in the soil samples. In most of the cases considered, seasonal changes in Sr, Rb and Cs accumulation concentrations in soil samples were as follows: winter'21/22≥ summer'22> autumn'21. However, relatively low levels of TE were found in the soil samples from the Martuni sites. In comparison, the Rb concentration in the soil sampling sites was found in winter G1<G2. The Cs concentration in the general context of the studies was maintained at an average level of 29 mg/kg in the soil samples from all sites.

Irrespective of the season, TE group elements showed a higher pollution index than other element groups. But in autumn, ME showed a higher total pollution index in all soil samples. Similarly, in the spring soil sampling, ME was a higher value Zc at the following sites: H1, G1, M1 and M2.

Summarizing the results of genotoxic analysis of soils from the study sites, it can be stated that there was a considerable increase in soil clastogenicity, according to both test criteria starting from autumn. During the spring of soil sampling, there was a peak in the percentage of occurrence of both tetrads criteria in MN and MN in tetrads of *Tradescantia (clone 02).* In the subsequent summer sampling, a significant decrease was seen in all genotoxicity assessment criteria for the materials under study.

Thus, the observed characteristic trends in the seasonal distribution of the studied series of elements from the group of both ME and TE in soil samples from regions of Armenia with different climatic indicators indicate the presence of common factors influencing the chemical composition of soils, to understand which it is necessary to know the features of the spatial-temporary distribution of elements in the studied areas. The study conducted a comparative analysis of the concentration of some chemical elements of the ME and TE groups in soil samples from three regions of Armenia, which differ from each other in their agro-geographical indicators - Hrazdan, Gavar and Martuni was carried out.

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