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Medico-Ecological Approaches to Plant Research

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Abstract

The primary task of environmental protection is to ensure the safety of territories and promote their sustainable development. At the same time, analysing the current ecological situation in a specific area, including identifying spatio-temporal changes within its boundaries, is a complex undertaking that requires an integrated approach. This article proposes methods that use annual and perennial plants as natural targets for analysing the accumulation and migration of potentially toxic elements in the soil. In this sense, studying plant ecotypes already adapted to specific growing conditions is of significant practical importance. To gain a complete understanding of current circumstances, the study compared the indicators of geoecological indices related to the accumulation and migration of potentially toxic elements in the soil with the antioxidant potential of plants and their bioaccumulate capacity is important to emphasise, as the accumulation of potentially toxic elements in plants is most influenced by their genotype. Primary and secondary metabolic processes also play a role in the process of peroxidation in lipid-containing structures that are sensitive to pH, temperature, and other physicochemical parameters. Therefore, the acquisition of quantitative and qualitative data on the movement of potentially toxic elements in the soil-plant system is essential for a reliable assessment of the degree of environmental pollution.

Keywords: plants, potentially toxic elements, environmental pollution, geo-ecological index.

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1. Introduction

Conservation and rational use of biological and landscape diversity have become global priorities. However, scientific and technological progress and climate change have had an irreversible impact on ecosystems due to anthropogenic factors. In this context, significant changes have occurred in plant cover, primarily altering the endemic diversity of the region (Terschanski et al., 2024). The substantial descriptive material accumulated for most plants continues to serve as a valuable source for obtaining and using natural compounds that are of significant interest in medicine, veterinary medicine, biochemistry, and culinary studies, among others (Torosyan, 1983). In certain cases, this knowledge has been meticulously documented in historical scientific and applied works, which have served as a foundation for contemporary biological and clinical research (Khamkar et al., 2015).

Many nationalities have a unique collection of traditional plants that are endemic to their specific geographical locations. It is equally important to recognise that the use of such plants has consistently produced favourable results, thus validating their efficacy (Ekor, 2013). Here the tradition of the name of drug plants (DPs) has its origin. Numerous investigations on DPs, many of which were founded on subjective assessments of the plants' taste, smell and appearance, as well as on the spices, infusions, tinctures and other preparations derived from them. In some cases, this knowledge has been meticulously documented in historical scientific and applied works, which have served as a foundation for contemporary biological and clinical research (Petrovska, 2012). Integration of geo-ecological and biochemical studies is driven by the necessity to evaluate and predict the anthropogenic impact on the environment, to address one of the most significant fundamental ethno-geological challenges, namely the analysis of biologically active compounds found in plants. Armenia's landscape is notable for its pronounced relief features and vertical zonation, which can be defined by ten distinct climate and landscape regions. Consequently, this results in the formation of rich and unique plant diversity. The considerable biodiversity of the Armenian flora is attributable to a confluence of geographical, geological and topographical factors (Hayrapetyan, 2016).

Consequently, the study of plant ecotypes adapted to specific growth conditions is a pertinent research area. The aim of the presented work is to compare the antioxidant potential of plants with their bioaccumulation potential.

2. Materials and methods

The geographic coordinates for each sampling site are presented in Table 2. Soil sampling was conducted in accordance with the specified criteria, employing the envelope method as outlined by (43). All the elemental analysis of soil samples was conducted using a Termo Scientific™ Niton™ portable XRF analyzer and the geoaccumulation index and biomobility index were calculated using (Muller, 1981; Sukiasyan, 2018).

The concentrations of primary (diene conjugates) and secondary (malonic dialdehyde) oxidation products of lipid-containing structures of DPs extracts of diene conjugates and malonic dialdehyde were determined by the method of (Stalnaya, Garishvili, 1977). The antioxidant potential of corn (malonic dialdehyde, FRAP, polyphenol, flavonoids) was determined according to the protocol described in (Sukiasyan, 2016; Sukiasyan, 2019). The value of the redox potential was determined in DPs extracts by the potentiometric method using a potentiometer, measuring the electromotive force of the chain according to (Sumarukov, 1970).

Bioassay for determining the extent of clastogenicity in the contaminated soil is included in the International Programme for Monitoring and Testing of Environmental Contaminants (Mišík et al., 2019).

All experiments had up to 5 technical replicates. The data were statistically processed. The Student's t-criterion was used to process the results. The observed differences are statistically significant. The calculated values of the criterion were greater than the critical value at a significance level of $p < 0.05$.

3. Results and discussion

Nowadays, the forecasts are not encouraging and entail the destruction and transformation of many species of plants. In the context of anticipated changes in temperature and precipitation, a scenario of progressive upward displacement of landscape zones can be postulated (Figure 1).

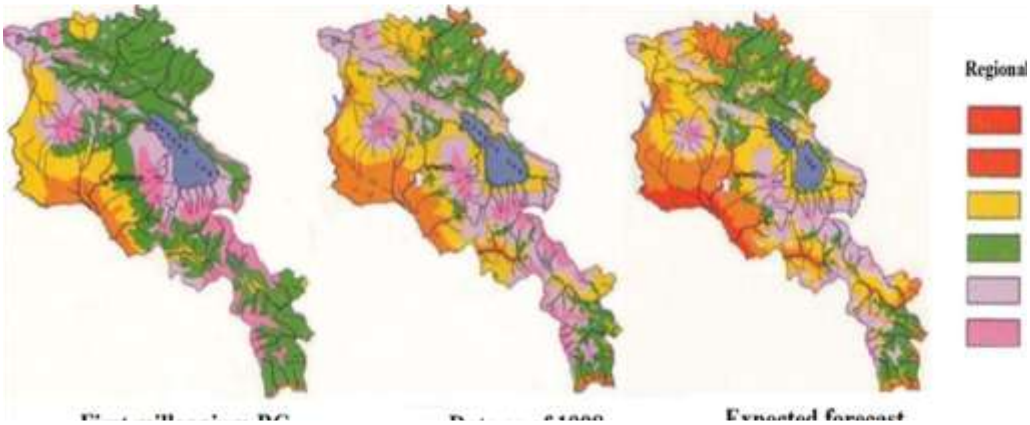

Fig. 1. Prognosis of dynamics of the spatial distribution of flora zones in Armenia
Source: Hayrapetyan, 2016

Recent years have seen rapid advancements in the field of biogeochemistry, with the development of novel methodologies including the use of plants for the monitoring of changes in the concentration of potentially toxic elements (PTEs) in soil. In this context, biochemical analysis of plant organism metabolism, particularly changes in antioxidant status, has been instrumental (Thalassinos et al., 2023; Sukiasyan et al., 2024).

Nevertheless, the response of secondary metabolite formation in plants to metal stress remains to be fully elucidated. It is imperative to acknowledge that a certain concentration pool of PTEs with variable valence is necessary also for plants as an example of a living organism (Figure 2). It is well established that such elements are actively involved in many metabolic processes that are integral to the growth and development of plants (Reshi et al., 2023).

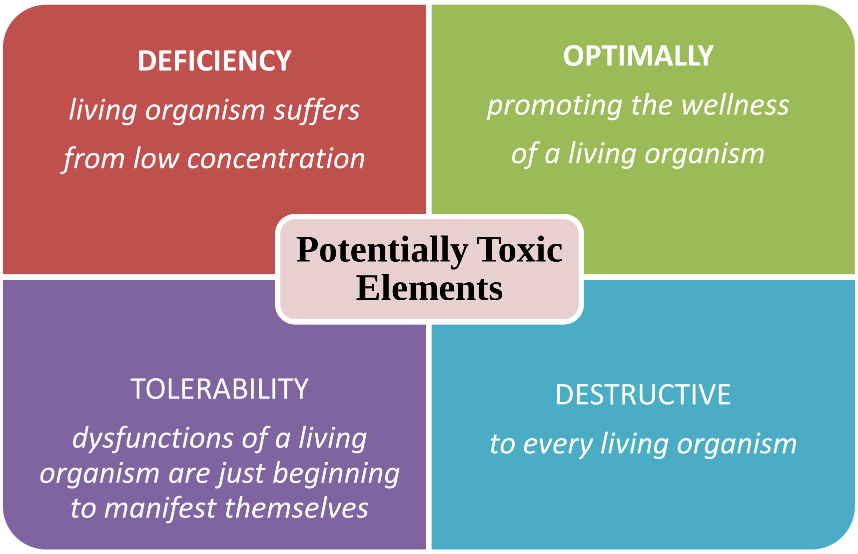

Fig. 2. The borders of the concentration pool of potentially toxic elements with variable valency necessary for a living organism

Uncontrolled concentration changes of PTEs are one of the resulting factors responsible for biodiversity decline. However, to comprehensively comprehend the concentration fluctuations of PTEs within the environment, it is imperative to consider geochemical alterations to identify areas of potential risk and to differentiate between abiotic and anthropogenic contamination.

The external anthropogenic threat of environmental pollution is a significant concern for endemic plants, which are not evolutionarily equipped to cope with it. The concentration changes of PTEs in the trophic chain have become a critical issue, as these contaminants can accumulate and migrate in ecosystems via contaminated water, soil, and air (Vodyanitskii, 2008). Consequently, the study of PTEs in the environment, their levels in water, their accumulation in soil, and their subsequent bioaccumulation in plants is a vital aspect of environmental risk assessment (Sukiasyan, Kirakosyan, 2024; Shtangeeva, 2022; Kaur et al., 2023).

Plants as a natural source of antioxidants. A significant proportion of DPs have been recognized as valuable sources of natural antioxidants for quite some time. The bioactive compounds present in these matrices have been shown to play a pivotal role in regulating speed of oxidative stress. Primary metabolism includes proteins, carbohydrates, fats, vitamins, organic acids, and enzymes, while secondary metabolism consists of alkaloids, phenolic compounds, terpenes, and terpenoids. For example, phenolic compounds, which include tannins, flavonoids and phenolic acids, are a constant source of antioxidant potential in these plants, scavenging free radicals and thereby inhibiting lipid peroxidation (LPO) (Halliwell, Gutteridge, 2015). In addition, tannins have been shown to be superior to phenolic acids in tests of LPO activity (Dorman, Deans, 2000). Conversely, the antioxidant activity (AOA) of plant flavonoids depends on their concentration and type (Manach et al., 2004). The synergistic effect of carnosic acid and other diterpenes has been shown to enhance AOA in lipid-containing structures (Wang et al., 2023). The synergistic effect of mixtures of many plants has been shown to enhance antioxidant efficacy, providing insight into their therapeutic potential (Bahadori et al., 2015; Krzyżek et al., 2023). A distinction is made between primary and secondary metabolism, which are sensitive to pH value, temperature, and other physical and chemical parameters (Table 1).

Table 1. Antioxidant potential of plant extracts depending on their thermal treatment

Plant species	Malondialdehyde. nmol		Diene conjugates. μmol		Eh		rH	
	A	B	A	B	A	B	A	B
Quercus robur L.	4.1±0.5	8.3±0.7	1.52±0.02	1.19±0.03	+347±17	+354±19	20.41	19.34
Urtica dioica L.	8.3±0.8	9.3±0.7	1.78±0.04	1.55±0.03	+70±13	+93±11	17.43	17.76
Salvia officinalis L.	9.9±0.4	12.4±1.0	1.91±0.03	1.90±0.03	+115±14	+183±22	15.33	16.46
Hypericum perforatum L.	11.1±0.5	18.8±0.8	2.53±0.02	2.27±0.03	+264±16	+237±19	18.37	18.04
Chelidonium majus L.	13.1±0.9	13.69±0.9	2.86±0.03	2.27±0.03	+217±20	+200±21	18.37	17.61
Artemisia absinthium L.	21.1±0.9	15.9±1.04	2.86±0.03	2.15±0.02	+233+28	+213±14	18.23	17.36

Notes: A – fresh plant extract. B – hot treatment plant extract

The results of the redox potential (Eh) determination in fresh DPs extracts were positive for all samples (Table 1). The heat treatment of DPs extracts generally did not result in alterations to the Eh levels within the comparison series. The value of the redox potential is contingent on the acid-base environment, and the value of rH was calculated based on this premise, thus allowing for the elimination of the effect of pH. The values of rH for all DPs extracts fall within the range corresponding to the reduction potential of hydrogen (from 0 to 41). This finding is indicative of their antioxidant nature, given that hydrogen ions are the principal reducing agents in free radical oxidation processes.

Plants as bioindicators of contaminated soil. The degree of ecological safety of the environment is typically evaluated by comparing the existing changes with the level of permissible content of pollutants (Sukiasyan, 2018). The impact of PTEs as pollutants is twofold: firstly, they directly affect biodiversity, and secondly, they reduce the tolerance limits of plants, reducing resistance to natural factors (Milyutina et al., 2019; Ali et al., 2013; Bielen et al., 2013; Wuana, Okieimen, 2011). It has been established that an increase in abiotically derived background levels of PTEs is to be expected in response to intensified human activities, which will be compounded by alterations in the water balance of the environment (Bray et al., 2000). In case the river water quality remains tense, but the effect is weakening, since the coastal soil itself acts as a "natural filter" for plants growing in these areas (Sukiasyan, Pirumyan, 2018).

Genotoxic potential of plant use. The accumulation of PTEs in plants is also largely determined by their genotype, but they an excess affects the absorption of water from the soil, reducing the water content in the root system. The initiation of plant response mechanisms in response to the intake of PTEs occurs at the level of the root system (Rucinska-Sobkowiak, 2016; Pirumyan et al., 2011).

The sampling of endemic Artemisia plants was arranged according to the following defined criteria: slightly disturbed natural flora, with fairly uniform saturation, known geochemical and geological study of the region, a certain distance from large industrial centers. The following locations were sampled: Sevan town (site1); Fantan village near Hrazdan town (site 2); Yerevan botanical garden (site 3) and, as a control, soil from the greenhouse of Yerevan State University (site 4).

Thereafter, the concentration values for each of the elements in the plant samples and the corresponding growing soil were determined. Using the values obtained, the biological mobility index coefficient was calculated as follows. The results obtained are summarized in Table 2, which indicates that an index value of less than 1 suggests an increased content of PTE in the soil sample, and vice versa. In practical situations, the assessment of contamination is often difficult to carry out, which hinders the assessment of the toxicity and mutagenicity of potential environmental hazards. In this context, transgenic organisms have emerged as a particularly promising avenue of research (Atoyants et al., 2009). To determine the extent of clastogenicity in the contaminated soil samples in the soil-plant system, Tradescantia (clone 02) was studied as a model plant (Figure 3).

Fig. 3. General view of flowers of the model plant *Tradescantia (clone 02)*

The results of this study showed a significant increase in the frequency of pink cells (PC) in the soil samples compared to the control (Table 2). The elevated incidence of PC frequency observed in the soil samples from points 2 and 4 can be attributed to the elevated concentrations of certain PTEs (Cu, Zn, Pb) in it. It has been demonstrated that under the influence of solutions of Pb^{2+} and Zn^{2+} salts, along with an increase in PC, the frequency of colorless cells (CC) increases linearly.

Furthermore, it was established that ions of the same metals with different valences exhibit a pronounced divergence in mutation activity. Specifically, Cr^{6+} was found to enhance the frequency of recessive mutations by a factor of 1.5 in comparison with the trivalent chromium ion (Table 2).

For certain PTEs ions (Cr^{6+}, Ni^{2+}, As^{3+} and Cd^{3+}), patterns analogous to those previously identified in our research were observed (Huang et al., 2012; Kumar et al., 2015). In addition to the mutational disorders previously described, the treated Tradescantia clone 02 plants exhibited various types of morphological change. Of these, the most prevalent were dwarf and branched hairs (Sukiasyan et al., 2009).

Geo-ecological aspects of plant use. PTEs migration in the soil-plant system also depends on the vegetative cycle of plant growth and development. In soil, PTEs are initially adsorbed by fast reactions (lasting minutes to hours), followed by slow adsorption reactions (lasting days to months). Thereafter, they are redistributed into different chemical forms, exhibiting excellent biodistribution, mobility, and toxicity. A comparative analysis revealed that annual plants accumulate Cu, Zn and Mo much more intensively than perennial plants. Conversely, a contrasting trend was observed for Pb, with perennial plants exhibiting a fivefold accumulation in comparison to annual plants.

This phenomenon can be attributed to the notion of an "ecological memory" in perennial plants, which refers to the capacity to accumulate pollutants in the growing area. In contrast to annual plants, the accumulation of PTEs in perennial plants is accompanied by their active participation in metabolic processes associated with growth and development throughout the entire growing period (Juc et al., 2006).

Table 2. Induction of clastogenic effects in sporogenic cells of *Tradescantia (clone 02)* in soil samples according to bioassay tests

Soil sampling		Biological mobility index				Genotoxicity analysis			
Site	Coordinates	Cu	Zn	Pb	Cr	Total quantity	Pink cells in stamen hairs (1000±m)	Colorless cells in stamen hairs (1000±m)	Stunted hairs (1000±m)
1	40°37'20.8" N, 44°57'33.5" E	1.26±0.03	0.14±0.01	2.50±0.13	1.97±0.08	15804	0.76±0.22*	2.53±0.4	2.66±0.41
2	40°24'02.9" N. 44°41'34.4" E	0.43±0.02	0.14±0.01	5.71±0.23	1.19±0.05	17204	1.51±0.3***	4.82±0.53	3.78±0.47
3	40°12'41.9" N. 44°33'31.6" E	0.73±0.03	0.12±0.01	0.04±0.002	0.09±0.002	14526	0.96±0.26**	5.44±0.61**	1.86±0.36
4	40°18'29.04" N, 44°52'65.07" E	Concentration of potential toxic elements in soil sample				15322	0.2±0.11	3.59±0.48	1,44±0,31
		2.9	65.2	0.2	4.2				

Notes: biological mobility index is the ratio of an element's concentration in a plant to its content in the soil; statistically significant difference compared to the control is indicated at * – p <0.05, ** – p <0.01, *** – p <0.001.

Conversely, annual plants can serve as bioindicators of soil pollution only within a specific growing season (Figure 4). The reaction of the plant itself to changes in the concentration of PTEs in the environment includes complex signal transduction, according to which PTEs bind to receptors of the plant plasma membrane, generating a stress signal (Yang et al., 2021). As is evidenced by research findings, there is a direct relationship between the study of endemic plants and the ecological condition of their growing region (Sukiasyan et al., 2024; Sukiasyan, 2019).

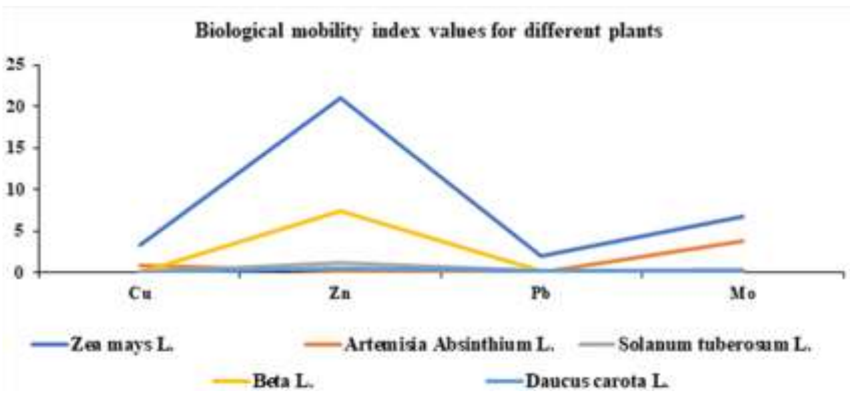

Fig. 4. Biological mobility index for annual and perennial different plant species

The following analysis, the changes in the concentration of TBA-active oxidation products in maize, was grown in the Tekhut and Shnogh regions. The results demonstrate that the plant samples harvested from Tekhut exhibited a 50 % increase in the parameter values compared to the control sample (Figure 5). When studying the samples from the Shnogh settlement, the changes in the malondialdehyde (MDA) value remained within the mean square. Samples of sweet maize from the Armenian population showed variation based on the area of growth, including the concentration of iron in the soil cover (Sukiasyan, 2016). In addition, concentration changes in the reducted of low-molecular iron by antioxidants (ferric reducing/antioxidant power, FRAP (Benzie, Strain, 1996)) revealed that the level in the maize sample harvested from Tekhut was 2.4 times higher than in others. Polyphenols have been shown to possess strong antioxidant properties, which contribute to the regulation of free radicals within biological systems. Concurrently, flavonoids meticulously regulate the development of individual organs and the entire plant (Brunetti et al., 2023). As was also the case in previous cases, in this instance too, the concentration changes of polyphenols and flavonoids were the most significant in the comparison series (Figure 5) in the samples.

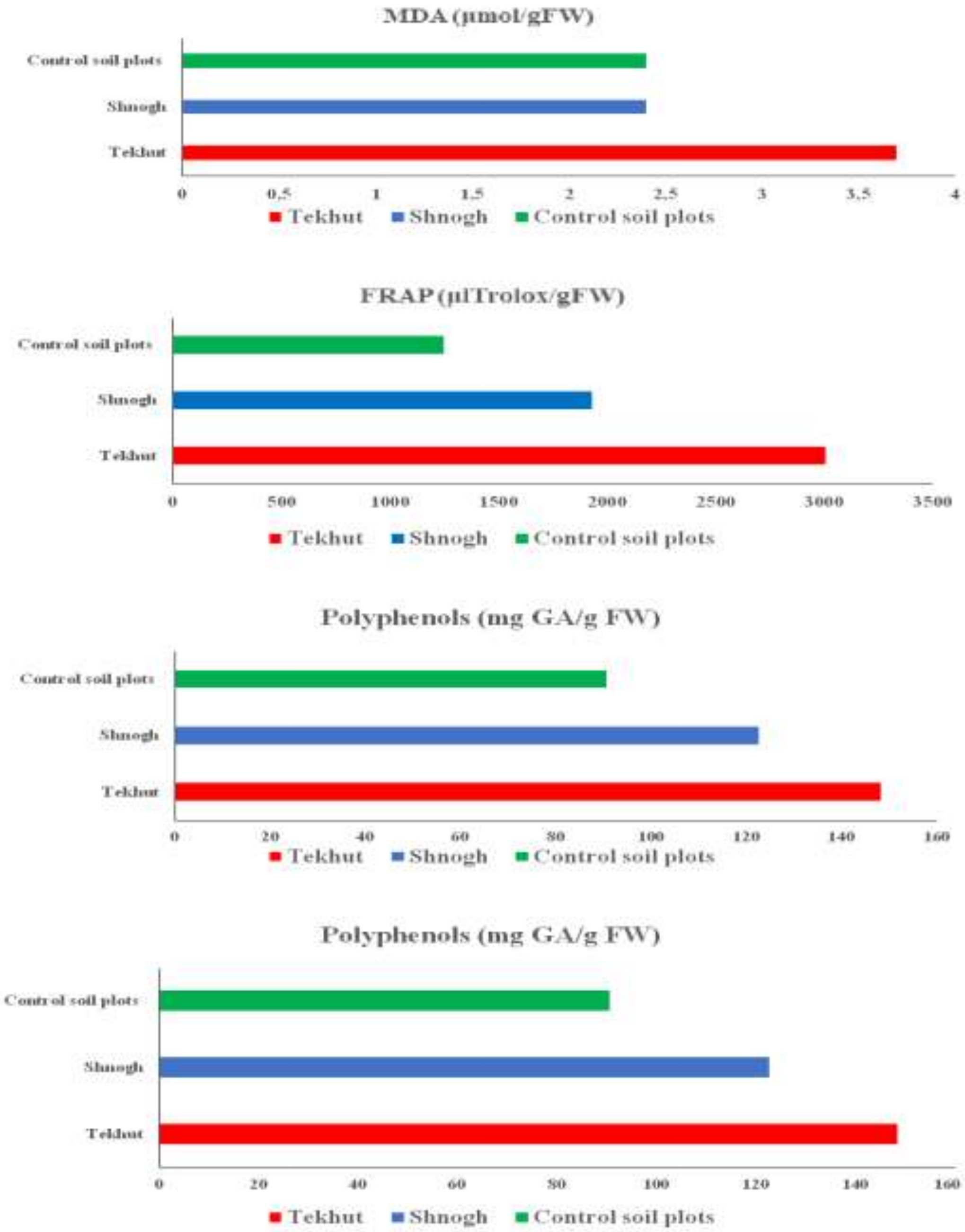

Fig. 5. Comparative analysis antioxidant indicators of samples of *Zea mays L.* plants from different soil-climatic regions of Armenia

The soil has a selective capacity to accumulate certain chemical elements, thereby inducing a change in their accumulation rate in the vegetation growing on it (Brunetti et al., 2023; Tangahu et al., 2011). The capacity of a plant to manifest bioindicative behavior, predicated on the content of PTE in the soil, will be predominantly contingent on the condition of the underlying soil itself (Palansooriya et al., 2000). Information about quantitative alterations in some PTEs within the soil-plant system is imperative for identifying the level of environmental pollution. Numerical values of several PTEs were determined in soil samples and maize grains (Figure 6). Subsequently, the soils in which the plant is cultivated were classified by the geoaccumulation coefficient (I_{geo}).

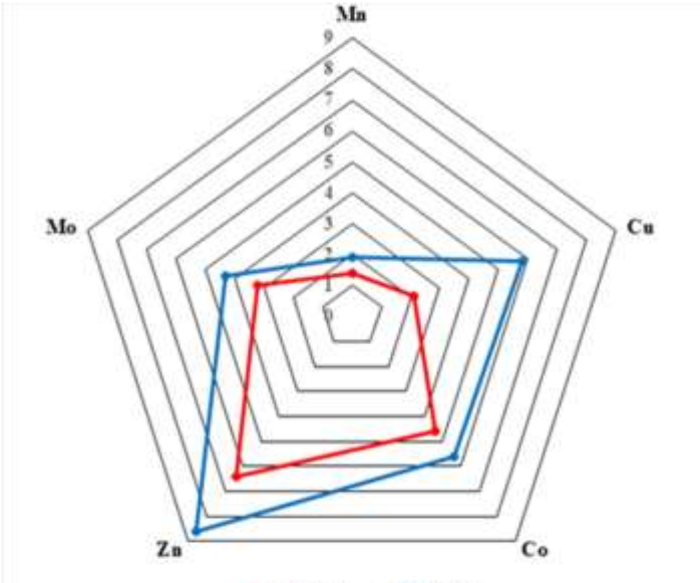

Fig. 6. The value of the geoaccumulation coefficient of some potential toxic elements

The analysis of soil samples from the Tekhut settlement revealed that they were slightly polluted with the concentration of Mo, Mn and Cu, but heavily polluted with Co and Zn. Furthermore, analysis of soil samples from Shnogh indicated their light pollution with manganese, and they were heavily polluted with molybdenum. Concurrently, these soil samples exhibited pronounced levels of copper, cobalt and zinc contamination.

4. Conclusion

The integration of endemic flora as a target indicator of environmental quality has the potential to facilitate the emergence of new types of ecological indicators. This interdisciplinary approach enabled the identification of correlations with respect to the cumulative activity of PTE based on the value of the geoaccumulation index and certain biochemical parameters of antioxidant potential. These parameters primarily depended on soil-climatic conditions and the degree of soil contamination on which the plants grew.

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