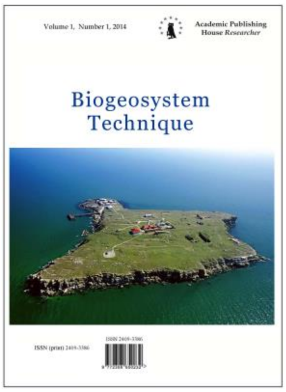
Published in the USA
Biogeosystem Technique
Issued since 2014.
E-ISSN: 2413-7316
2021. 8(2): 93-101

DOI: 10.13187/bgt.2021.2.93
https://bgt.cherkasgu.press



Geoecological Aspects of Migration of Heavy Metals in Environment and Antioxidant Status of Plants

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Paper Review Summary: Received: 2021, November 01
Received in revised form: 2021, December 3
Acceptance: 2021, December 22

Abstract

The geoecological assessment of the multi-component impact of technogenic pollution of natural soil zones is necessary for the comprehensive evaluation of environmental investigation of the impact of climate change on biota. The aim of a study was to use a combined assessment method of pollution in a chain soil-plant based on the values of a number of biogeochemical coefficients in the chain of soil-plant (1), and bioaccumulation of plants to assess the level of pollution for some HMs in the different regions of Armenia (2). The object of the study was the Armenian species of *Zea mays L.* and samples of soil near the river's coast. To quantitatively determine the indicators of the antioxidant system, the fifth leaf of *Zea mays L.* was used to determine the concentration of malondialdehyde in the presence of 2-thiobarbituric acid; the concentration of Ferric reducing the ability of plasma; concentration of polyphenols and flavonoids. The potential biochemical mobility of HMs from soil to plant (K_m), index of pollution (I_{pol}), and the value of the span of pollution (SP) were calculated in samples of soils. The excess concentration changes the K_m coefficient for Mo, Zn, and Cr for the studied samples of maize from different soil and climatic conditions are shown. It was found that, depending on the soil conditions, in almost all studied territories, Mo and Zn are strong concentrators, and the remaining HMs are deconcentrators in the range from strong (0.04-0.0025) to weak (0.4-0.25). We are inclined to assert that the antioxidant system is that sensitive component of the plant organism, which primarily reacts to changes in the environment caused by uncontrolled changes in micro and macro elements in biota.

Keywords: heavy metals, pollution, the chain plant-soil, biogeochemical coefficients, antioxidant system, geoecological assessment.

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1. Introduction

The investigation of spatio-temporal changes in the environment have recently become the most urgent relevant in nature management and geoecology. Changes in climatic conditions lead to the dramatic development of situations, provoking qualitative and quantitative changes in the biota. Most of the pollutants enter the environment as waste from a specific source of pollution. Heavy metals (HMs) are the main part of these pollutants or are present in trace concentrations (Seregin, Ivanov, 2001; Kasimov et al., 2016). HMs themselves are natural components of the earth's crust, determining the natural background of their content in the environment. The deposition of HMs ions in the soil in a certain way "controls" its physicochemical properties, changing such basic characteristics of the soil cover as mobilization, sorption, and adsorption (Wuana, Okieimen, 2011).

In the geochemical assessment of the state of the environment to take into account the associativity in the distribution of chemical elements is very important. This makes it possible to group HMs according to the degree of danger of environmental impact in order to comprehensively solve the problem (Hossain et al., 2012). In 1973, in the UN Global Monitoring Program, only Pb, Cd, and Hg were listed as hazardous HMs (Dobrovolsky, 1983). Later, according to the UN Environment Program, the list was expanded and consisted of both HMs (Cu, Sn, V, Cr, Mo, Co, Ni) and metalloids (Sb, As, and Se) (State of the environment, 1980). As highly hazardous HMs include As, Cd, Hg, Se, Pb, Zn, moderately hazardous ones – Ni, Mo, Cu, Sb, and low hazardous ones – Ba, V, W, Mn, Sr (GOST RF, 2008). In general, anthropogenic pollution itself is inherently multielement. That is why the content of HMs in soils, according to the study of statistical parameters of their distribution, can serve as a regional characteristic of soil contamination.

One of the important properties of the soil is its buffering capacity, which determines its resistance to anthropogenic influences. Irreversible both concentration and chemical modifications lead to the formation of complexes, the toxicity of which is detrimental to the habitat of living organisms (Pierzynski et al., 2000). Soil contamination with an increase in HMs content contributes to the stage-by-stage degradation of the environment, and the process of its restoration depends on the dynamics of HMs migration, their sorption, and accumulation in plants (Kabata-Pendias, Pendias, 2001). In this case, the predisposition of one plant species to a particular chemical element is very individual, and to a greater extent depends on the intensity of metabolic processes where it is involved (Sukiasyan, 2018). In fact, it is possible to extract positive effects from the current situation. Because of HMs accumulating and concentrating abilities of various chemical elements plants can be used in geoecological studies of the pollution of a certain territory on the basis of biomonitoring, but this will require a special understanding of the processes of soil solubilization and the mechanisms of absorption by plants (Sukiasyan, Kirakosyan, 2020).

In this study, we will use a combined assessment method based on the values of a number of biogeochemical coefficients in the chain of soil-plant and bioaccumulation of plants to assess the level of pollution for some HMs in the coastal areas of a number of rivers in Armenia in different soil-climatic regions.

2. Materials and methods

The object of the study was the Armenian species the of *Zea mays L.*, which was grown in Lori Marz along the Debet River (Odzun – 41°03'06″N 44°36'55″E, Shnogh – 41°08'52″N 44°50'16″E, Teghut – 41°07'05″N, 44°50'45″E), Armavir marz near the Araks river (Hushakert – 40°04'52"N, 43°55'35"E), and its genetic prototype is inbred maize (*Zea mays ssp. mays var. B73*) (González-Muñoz et al., 2004).

The ripe of *Zea mays L.* kernels were dried in a fume hood until air-dry at room temperature. For ashing, the plant material was placed in a muffle furnace using pre-calcined porcelain cups at a temperature of + 400°C for up to one hour. Then the samples of the dry residue (ash) were placed in a desiccator for further measurements.

Soil samples under dry weather conditions were taken by the envelope method from the depth of growth of the root system of the studied plant. Point sampling was carried out with non-metal instruments. A pooled sample of soil was prepared by mixing at least five incremental samples taken from the same sample site. After the samples were placed in dark glass containers and transported at a temperature of +4°C for laboratory (instrumental) measurements for 24 hours. After cleaning from the remnants of the root system, insects, and other solid components, the soil was ground in a foot with a pestle and sifted through a sieve with a diameter of not more than 1 mm.

The prepared samples (plants and soils) were placed in special plastic tubes "XRF Sample Cups" with a diameter of 32 mm, on the bottom of which a special polypropylene film was inserted in advance. A special seal was inserted on top of the sample and the sample was pressed with a lid to the desired state. The study of the sample was carried out by directing X-rays directly onto the sample for a total of up to 210 seconds using a portable analyzer "Thermo Scientific ™Niton ™XRF Portable Analyzer" (USA).

The potential biochemical mobility of HMs from soil to plant (K_m) was calculated by formula

$$K_m = \frac{C_p}{C_s} \quad (1)$$

where C_p is the content of HMs in the ash of *Zea mays L.* from a certain region of growth, mg/kg; C_s is HMs content in the corresponding growing soil, mg/kg (Perelman, Kasimov, 1999);

The index of pollution (I_{pol}) was calculated by formula

$$I_{pol} = (K_{m1} \cdot K_{m2} \cdot \ldots \cdot K_{mn})^{1/n}, \quad (2)$$

and the value of the span of pollution (SP) is defined as the ratio $\frac{K_m}{I_{pol}}$, and when SP < 0.1 the pollution satisfy to insignificant, if 0.11 < SP < 0.2 is range of slight pollution, 0.21 <SP <4 is range of moderate pollution, 4.1 < SP <8 is range of severe pollution, SP> 8.1 is range of excessive pollution; n is the number of HMs.

To quantitatively determine the indicators of the antioxidant system, the fifth leaf of *Zea mays L.* was used on the third day of its growth, up to 10 cm long from the base of the leaf according to the method (Sukiasyan, 2019). The obtained single biological material was used to determine the concentration of malondialdehyde (MDA) in the presence of 2-thiobarbituric acid (Hodges et al., 1999); the concentration of Ferric reducing ability of plasma (FRAP) (Benzie, Strain, 1999); concentration of polyphenols (Gálvez et al., 2004); concentration of flavonoids (Chang et al., 2004). Concentrations of all biochemical parameters are presented in the appropriate units and are reduced to the fresh weight of the biological material.

All experiments data had 10 biological and up to 5 technical replicates and were statistically processed. The results were processed taking into account the Student's t-criterion. The observed differences are statistically significant, since at a significance level of $p < 0.05$, the calculated values of the criterion were greater than the critical one (Korosov, Gorbach, 2017).

3. Results and discussion

The dominance of mountainous terrain in Armenia and the presence of increased fracturing as one of the water-control criteria allow are forcing to adapt most of the coastal river territories for agricultural use (Vardanyan, 2019). That is why the level of hazardous HMs content in the soil was estimated on the basis of the ecotoxic principle, comparing the concentration effects of different chemical elements in soil and plants. In this case, the use of maximum permissible additives of chemical elements is appropriate (Vodyanskiy, 2012).

To assess the intensity of HMs migration in the chain of soil-plant, the total contamination was identified for a separate group according to the HMs hazard, which is the index of contamination. At first, the potential biochemical mobility of HMs from soil to plant (K_m) was determined, then on the basis of which the pollution index (I_{pol}) was calculated (Table 1). According to our results, in Hushakert the value of Ipol in a case of highly hazardous HMs (Se, Sb, Cd) is distinguished up to three times increased compared to other regions. For the class of moderately hazardous HMs (V, Hg, Ni, Cu, Cr, As, Ba), the value of Ipol was almost the same, and the value of the Ipol index for slightly hazardous HM (Mo, Pb, Zn, Co) had the greatest value in Teghut, and the smallest is in Shnogh.

Table 1. Heavy metal pollution index (I_{pol}) in different soil-climatic regions of Armenia

Hazard class of heavy metals	Teghut	Shnogh	Odzun	Hushakert
Highly	3.89 ± 0.36	2.80 ± 0.12	3.83 ± 0.16	6.84 ± 0.37
Moderately	0.53± 0.04	0.45 ± 0.03	0.68 ± 0.06	0.66 ± 0.05
Low	3.09 ± 0.16	1.31 ± 0.07	2.57 ± 0.13	2.21 ± 0.09

The soil has a certain degree of dynamism, primarily due to its moisture content. At the same time, the root system of plants absorbs not only moisture from the horizons of its maximum distribution in the soil but also various pollutants dissolved in it. Plants use soil moisture due to transpiration processes, which is provided by hydration and turgor of the plant cell. This is an important factor in the migration of HMs in biota (Sukiasyan et al., 2015). *Zea mays L.* is a widespread household plant in almost all regions of Armenia. In particular, it is intensively cultivated in the coastal areas of the Debet, Shnogh, and Araks rivers. Although the growth of maize is interrelated with soil and climatic conditions, the plant itself is not demanding on the soil conditions of growth. By calculating the potential biochemical mobility of HMs from soil to plant (K_m), received data was found that Cd, Mo, Zn dominant position when absorbed from the soil by maize samples (Figure 1).

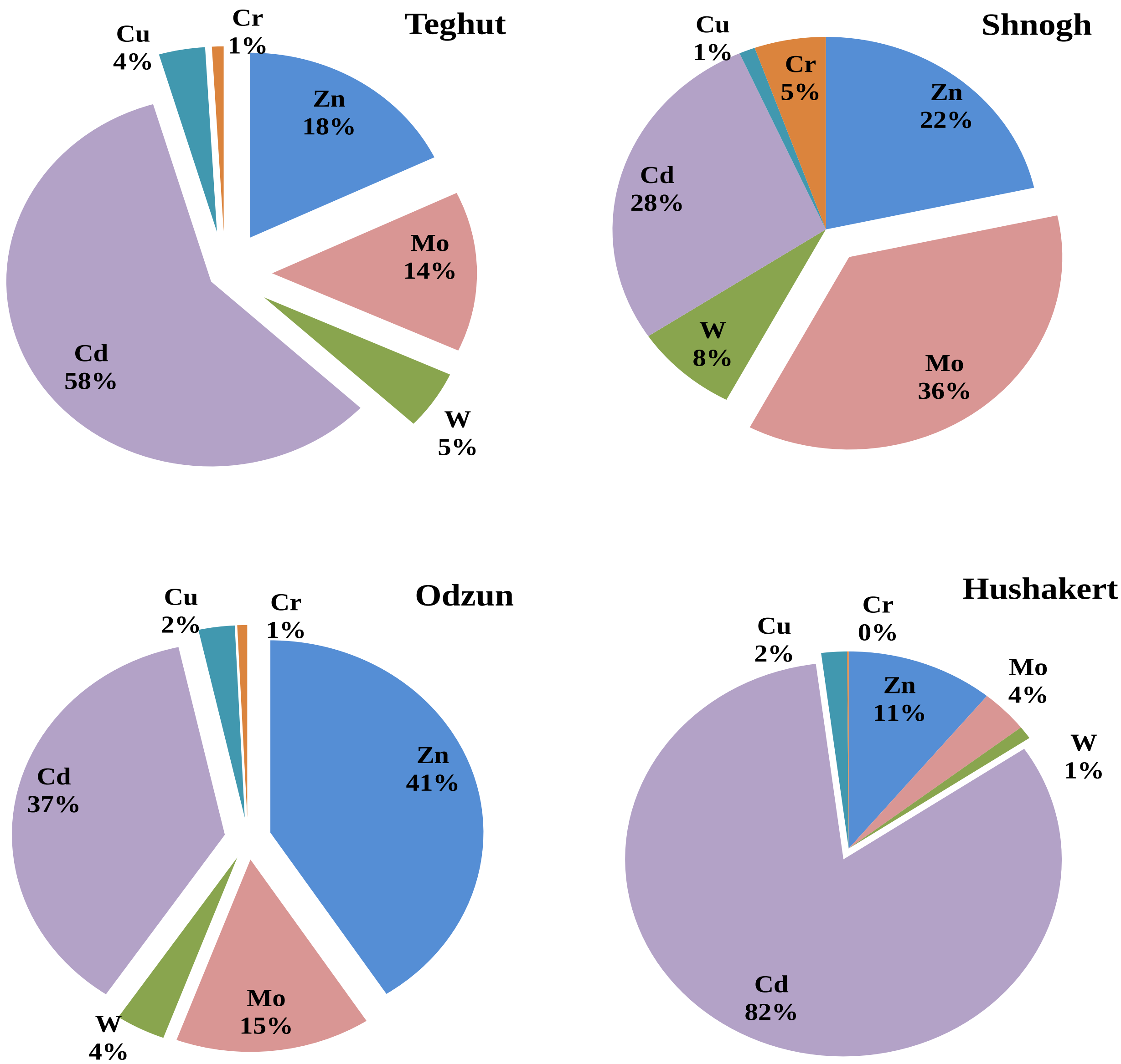

Fig. 1. The potential biochemical mobility of HMs from soil to plant (K_m) in different soil-climatic regions of Armenia

It should be noted that the activation of plant protection mechanisms with an increase in the HMs content in the environment begins already at the level of the root system (Koevoets et al., 2016). This is manifested both by the passive (non-metabolic) transfer of ions into the cell in accordance with the gradient of their concentration and by the active (metabolic) process of absorption by the cell against the concentration gradient (Godbold, Kettner, 1991).

Further, for evaluating the state of the environment was calculating the ratio of SP (Table 2). Among the elements such as Se and Sb from the group of highly hazardous HMs, are distinguished by a wide K_m value, the value range of which ranges from 13 (Shnogh) to 164 (Hushakert).

Table 2. The values of span pollution (SP) in different soil-climatic regions of Armenia

Hazard class of heavy metals		Teghut	Shnogh	Odzun	Hushakert
Highly	Se	0.36	0.87	0.33	0.33
	Sb	0.14	0.24	0.30	030
	Cd	20.14	4.74	10.27	10.27
Moderately	V	0.32	0.67	0.26	0.13
	Hg	3.25	6.10	6.59	4.62
	Ni	0.53	1.05	1.68	3.07
	Cu	9.18	1.24	4.26	5.77
	Cr	2.32	5.54	1.17	0.36
	As	0.58	0.23	0.43	0.65
	Ba	0.15	0.15	0.16	0.40
Low	Mo	6.82	6.64	3.84	2.69
	Pb	0.30	0.07	0.16	0.51
	Zn	8.69	3.95	10.58	8.26
	Co	0.08	0.07	0.04	0.05

According to the content of Se and Sb, the soil-plant system is in a state of moderate contamination. In the case of Cd, it can be stated that in all regions of the study, the chain of soil-plant is in an excessively contaminated state. In the second group as moderately hazardous HMs, according to the value of K_m plant intensively absorbs and accumulates Hg and Cr in Shnogh. A similar situation we can observe in Teghut along with strong absorption of Cu by the plant, and also a similar picture of the migration of Hg, Cu, and Ni from soil to plant we obtained in Odzun and Hushakert. To obtain a reliable picture of the degree of pollution, the SP readings were compared. The moderate pollution is observed in Teghut for Hg and Cr, in Shnogh for Cu and Ni, as well as in Odzun and Hushakert, only for Ni. At the same time, excessive pollution of the studied territories was noted in Teghut by Cu. For such as HMs like Hg and Cr in the Shnogh, also Hg and Cu in both Odzun and Hushakert severe pollution was recorded. Among the elements from the second group, Ba, As, and V are especially distinguished, for which the range of the K_m coefficient does not exceed unity. It means according to the numerical equivalent of the span of pollution ranges from slightly to moderately polluted in some cases. In the group of low-hazard HMs in the chain of soil-plant, there is weak mobility of Co and Pb, and the range of values of the SP ratio is limited from medium to moderate, but the situation is different with Mo and Zn. The values of the K_m coefficient show that *Zea mays L.* is predisposed to the absorption and accumulation of these HMs, reaching its highest Mo value in Teghut (18.80) and Zn in Odzun (43.33), and the lowest Mo value in Hushakert

(7.08) and for Zn in Shnogh (3.95). The contamination by zinc is excessive in all studied areas, except for Shnogh as in the case of the pollution by molybdenum in Teghut, is strong.

The most of results of investigations in the area of migration processes of HMs transfer, as well as their redistribution in biota, are of important methodological importance for organizing a system for monitoring the state of the environment (Mikheva et al., 2003). Environmental stresses such as drought, salinity, cooling, HMs toxicity, ultraviolet radiation, and pathogenic microorganisms inevitably provoke the generation and activate reactive oxygen species (ROS) in plants due to disruption of cellular homeostasis (Kovalchuk, Kovalchuk, 2008; Misra, Mani, 1991; Sharma, Dubey, 2005). An increased concentration of ROS is extremely dangerous for organisms. They are a group of free radicals that are derived from molecular oxygen. About 1% of the consumption of molecular oxygen by plants is directed to the production of ROS in chloroplasts and mitochondria. Moreover, if the plant organism itself cannot cope with such a situation, then the ROS level exceeds its defense mechanisms. The cell goes into a state of "oxidative stress", causing lipid peroxidation (LPO), protein oxidation, damage to nucleic acids, inhibition of enzymes, which ultimately leads to unambiguous cell death (Chen et al., 2010; Meriga et al., 2004). The use of plants as indicators for changes in the background concentrations of HMs in the soil should be considered the time-factors, which affect the solubility and forms of HMs. In conditions of a significant increase in the level of HMs, the pollutants themselves can change the properties of the soil, affecting the solubility of the metal. However, membrane transfer leads to an increase in the level of HMs in the plant in comparison with their concentrations in the soil. This, in turn, allows plants to be used to detect and identify relevant changes in the environment (Sukiasyan et al., 2016).

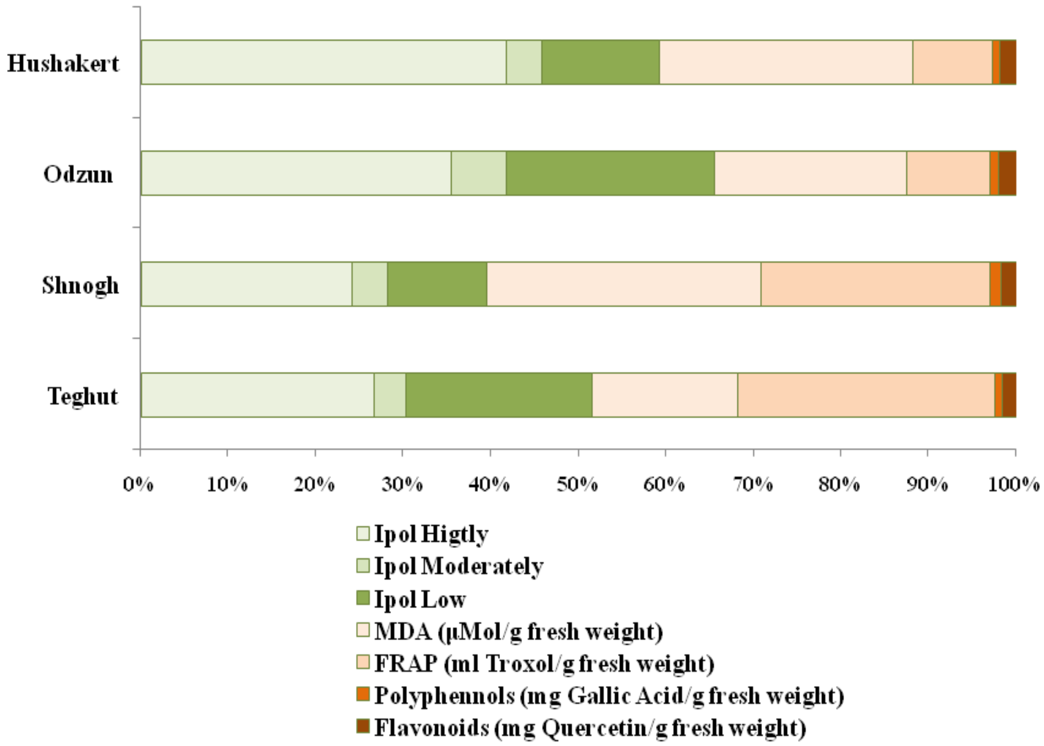

Fig. 2. Comparative analysis between antioxidant indicators of samples of *Zea mays L.* plants and pollution index (Ipol) as grow soil from different soil-climatic regions of Armenia

In our studies, certain correlation patterns have been obtained that make it possible to establish the relationship between biochemical parameters (FRAP, MDA, polyphenols, and flavonoids) and the index of pollution (I_{pol}) determined. Correlation analysis of the concentration of FRAP of plant tissue found that in a region with an increased concentration of hazardous HMs (Hushakert, Odzun), its value is much lower compared to a region with a characteristic high content of Mo, Pb, Zn, Co (Teghut and Odzun) (Figure 2). Plants differ in their ability to both accumulate individual HMs and absorb them, depending on the properties of the soil (Atoyants et al., 2009). But ROS are well known for their dual role: at high concentrations, they cause damage to biomolecules, while at low or moderate concentrations; it acts as secondary intracellular messengers in plant cells (Gichner et al., 1980).

Studies have shown that maintaining a state of high antioxidant activity during the utilization of toxic ROS is associated with increased plant tolerance to environmental stresses (Bagaeva et al., 2013). There is several mechanisms of ROS generation in which HMs are involved. Oxidative-active metals promote the formation of hydroxyl radicals ($OH^{\bullet}$), which are the most aggressive type of ROS (Juknys et al., 2012). Metals with a weak redox capacity, such as cadmium, lead, zinc, nickel, etc., can contain singlet oxygen, which is capable of creating another type of ROS superoxide ($O2^{\bullet -}$) (Sharma et al., 2016). Subsequently, ROS cause nonspecific oxidation of proteins and membrane lipids, DNA damage, and enzyme inhibition, leading to cell death (Schützendübel, Polle, 2016). On the other hand, ROS plays an important role in the plant protection system, because the dangerous effect of ROS is determined by their concentration, and if it exceeds the threshold level for defense mechanisms, oxidative stress will occur.

In the case of the response of the secondary product (MDA) of LPO processes an almost similar picture is observed regardless of soil and climatic conditions. The antioxidant status of the plant fully resists the effects of HMs, regardless of their hazard class. When considering the secondary metabolites of LPO processes (polyphenols and flavonoids) a weak response of these systems to environmental pollution is noted. In fact, the complex system of plant antioxidant defense, the main purpose of which is to reduce the reactivity and/or remove ROS in various organelles (chloroplasts, mitochondria, and peroxisomes) caused by the toxicity of a number of HMs, does not manifest itself in the same way. Their participation in biogeochemical processes, as well as the constant growing anthropogenic pollution of the environment, predetermined the leading place of HMs among pollutants capable of changing the metabolic processes of plant growth and development. There is a large amount of information on the scale and irreversible consequences of HMs biota pollution. Discussions of the current situation ultimately boil down to comparing the concentration changes of HMs in the environment with their accepted maximum permissible concentrations without delimiting the nature of their occurrence.

Analyzing the current situation, we have developed a quality approach for assessing environmental pollution using the example of HMs migration into the chain of soil-plant, taking into account the adaptation of plants under anthropogenic load. A change in its antioxidant status was considered as the main mechanism of adaptation of a plant organism. In response to oxidative stress provoked by anthropogenic stress, in our case, it is HMs in the plant organism that activates the work of non-enzymatic (polyphenols, flavonoids, etc.) and low molecular antioxidants (MDA), which are present in plant tissues and are capable of neutralizing ROS non-enzymatically.

5. Conclusion

Comprehensively analyzing the results obtained, we are inclined to assert that the antioxidant system is that sensitive component of the plant organism, which primarily reacts to changes in the environment caused by uncontrolled changes in micro and macro elements in biota. The excess concentration changes the K_m coefficient for Mo, Zn, and Cr for the studied samples of maize from different soil and climatic conditions are shown. It was found that, depending on the soil conditions, in almost all studied territories, Mo and Zn are strong concentrators, and the remaining HMs are deconcentrators in the range from strong (0.04-0.0025) to weak (0.4-0.25).

Thus, the multidirectional approaches to the study of some geoecological problems of pollutants distribution considered in the article reasonably indicate the specific features of HMs migration in the chain of soil-plant in the general concept of regional geoecology.

6. Acknowledgements

The work was supported by the Science Committee of RA, in the frames of the research project № 15T-2H409.

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