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Accumulation of heavy metals in vegetables grown in contaminated soils

ENKELEDA SHKURTA[1*], FRAN GJOKA[1], LIRI MIHO[1], MARCO CONTIN[2], ELIAN KASA[1]

[1]Agricultural University of Tirana, Kodër Kamëz, Tirana, Albania

[2]University of Udine, Italy

*Corresponding author; E-mail: ledi.mera@yahoo.com

Abstract

Heavy metals are known for their *adverse* health *effects*. This study analysed total contents *of heavy metals* in common vegetables grown in contaminated soils from industrial and agricultural areas in *northwestern Albania*. The aim was to evaluate the human *health risk* of these metals *through* the *consumption* of contaminated vegetables. The concentrations of Cd, Cr, Ni, Cu, Zn and Pb. in vegetable samples collected from industrial, agricultural and reference areas were measured in HNO_3-H_2O_2 extract by using Atomic Absorption Spectrometer Varian SpectraA-200. The obtained results *showed that all heavy metals* were present in investigated plant samples and the highest concentrations (mg/kg) of Cd (0.236), Pb (0.949), Ni (3.67) and Cu (17.96) were found in samples from industrial area, Cr (2.84) and Zn (65.83) in samples from reference area, indicating different sources of metal contamination in vegetables. The Ni and clay in industrial area, Ni and Cu in agricultural area, and Cr and Ni in reference area were strongly correlated (r=0.979**; r=0.963**, r=0.955*, respectively; p<<0.05), suggesting a common origin of the paired metals. The Pb concentration in all plant samples from industrial and agricultural areas, Cd concentration in 80% of samples from industrial area, and Zn concentration in 40% and 80% of plant samples respectively from industrial and reference areas exceed the safe limit set by the Codex Alimentarius Commission for human consumption. *In other cases* concentrations of heavy metals were below the safe limits. For this *reason*, regular monitoring of heavy metals in vegetables grown in contaminated areas is important to ensure food safety and quality.

Keywords: Contaminated soil, health risk, heavy metal, vegetable.

1. Introduction

Although it has been a long time since many plants do not operate, the consequences of impacts on the pollution environment are still apparent. In the areas surrounding mines and plants, low biodiversity is distinguished. Beside the effect in natural recourses from pollution, another worrying problem is the deployment of new settlements that is becoming a serious risk to the health of income families. Stocks of waste rich in various metals pose a potential risk to the pollution of drinking water reserves, the accumulation of these metals in plants planted by this farmers and their use for personal consumption or selling purposes poses a risk to health people.

Heavy metals are ubiquitous and chemically stable, so that they can be present in all biotic and abiotic components of the environment. Way within food chain for different metals has different starting points. So, for Cd starts from soil between plants roots, for Pb from dust in air, and for As and Hg in polluted water. During transformation from a part of chain in another, some heavy metals can be collected on the top of chain that Is the man. Presence of heavy metals on the soil in high levels constitutes a serious threat for human health and ecosystem in all, so using of contaminated soils with heavy metals for agricultural aims contents a great threat for human health, because metals can be transformed and collected in the human body from the food chain [15]. The main sources of heavy metals in soil includes natural phenomena related to parental materials, human activities through atmospheric deposition, waste disposal, t raffic emission, use of chemicasl in agriculture, as well as use in sludge farming that derive from urban waste water treatment plant [16; 17]. Also heavy metals can be found natyrally in the soil environment from pedogenic alterations processes of parental materials at levels considered as traces (≤1000 mg kg^{-1}) and rarely toxic [8, 13]. Because of disorder and acceleration of the natyrally slowly geochemical cycle of metals from humans, most rural and urban areas can possibly collect one or more heavy metals above the values set as background sufficient to cause risks to human health, plants, animals, ecosystems or other environments [4].

The amount of heavy metals entering in the soil and their mobility depend on the metals origin. So is calculated that anthropogenic emissions in atmosphere, for some heavy metals are one or three times higher than their natural flows [18], cited by Wuana and Okieimen [14]. Heavy metals on the soils from antropogene recourses have a tendency to be mobile, so are more biodisponible than pedogenic or litogenic sources [12, 19], cited by Wuana and Okieimen [14]. Some studies in Albania have indentify high levels of heavy metals in the soils near industrials sites, in agricultural soils where agriculture chemicals and irrigation with polluted water are used intensively, and in natural soils contaminated with these metals [7, 10].

When studying heavy metals in the environment, special importance is given to the knowledge of fate and their movement on the soil, since the metals can be included in the food chain by contaminating it. The fate and movement of heavy metals in the soil depends heavily on chemical form and metal species. The most common heavy metals found in contaminated sites, in descending order of contents are: Pb, Cr, As, Zn, Cd, Cu and Hg [14]. These metals are important as they are able to reduce plant production due to the risk of bioaccumulation and increased concentration in the food chain [20], quoted by Wuana and Okieimen [14].

According to Shiowatana and Buekers [21,22], quoted by Wuana and Okieimen [14], heavy metals in the soil are adsorbed by initial rapid reactions followed by slow adsorption reactions and are therefore redistributed in various chemical forms with different bioavailability, mobility and toxicity. This distribution is believed to be controlled by heavy metal reaction in the soil, such as digestion and minerals precipitation, ions exchange (adsorbation and desorbation), water complexation, immobilization and biological mobilization, and taking from plants [23], quoted by Wuana dhe Okiemen [14]. This study aims to determine the levels of heavy metals in vegetables grown in soils contaminated by different sources.

2. Material and Methods

2.1. Description of study area

For this study are selected three sampling areas with different characteristics which are indexed as: i) Industrial site I, located near the former Industrial complex (Waste disposal point of ex Industrial complex, Shullaz village), ii) Agricultural Site B, located on agricultural soils of Bregu i Matit Plain (Gajush Village), iii) Control Site R, located in extensive agricultural area on the irrigation channel of the area (Pllane village). These areas have some similarities to the geo-climatic conditions, but differ from the history of land use, the heavy metal source on earth, and the level of soil contamination with heavy metals. Site I includes traditional gardens companied with residential homes, it is flat up to easily corrugated and has largely grassy brown meadow (Chromic Cambisols) low quality for agriculture. Site B is mainly rural in nature companied with Residential homes, it is flat and the land is are grey brown meadow (Eutric Cambisols) of high quality for agriculture. Site R is a misture of agricultural lands and roadside trading units, it is flat with a hilly areas in East and the lands are grey brown meadows (Eutric Cambisols) of high quality of agricultural. Simple vegetables cultivated in studding area are tomatoes, lettuce, cabbage, peppers, spinach, eggplant, various spices, etc. These are cultivated during all the year and are use generally for familiar consumption and the rest in local trade.

2.2. Plant sampling

Samples of plants were collected from private gardens used by local farmers to grow vegetables on period May-June 2015 on the three areas of study. Five sampling points were randomly selected for each site, based on existing gardens distribution. From each sampling point were obtained from 3 composite soil samples and 3 leafy vegetables samples (salad, cabbage or spinach) in the same place with soil samples. The sampling sites are presented in Figure. 1.

For this study, a total of 45 sol samples and 45 plant samples were collected. They are taken at the normal stage of consumption. The collected samples were immediately transported to the laboratory for preparation, including washing with potable water and then to distilled water to remove surface impurities and drying in the environment. Then plant material is milled, sieved with a sieve with ø 2 mm and stored in glass containers which are then used to determine the total content of heavy metals.

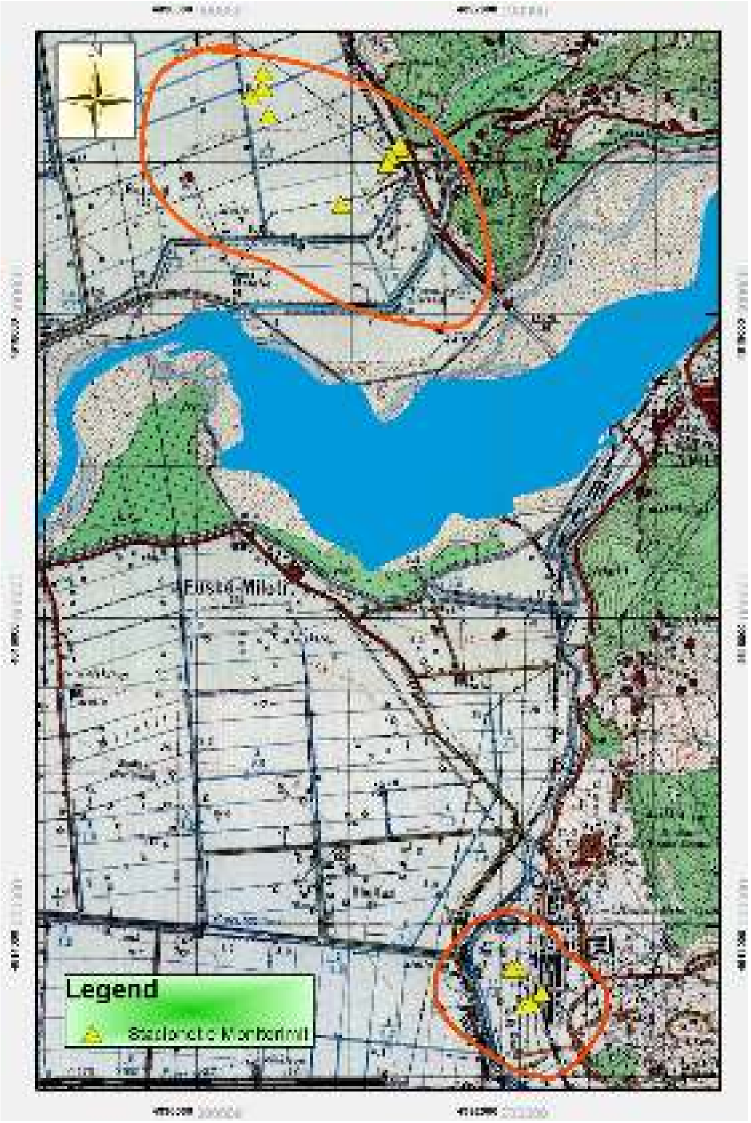

Figure 1. Distribution of sampling sites

2.3. Sample analyses

Plant samples were analyzed for the total content of Cd, Cr, Cu, Zn, Ni, Pb. The samples are prepared in according to ISO 11464, where plant particles ≤ 2mm are used for analyses. The total contents of heavy metals in plants was extracted with Aqua Regia according to DIN ISO 11466. The sample (0.3g) was digested with 8 ml of HNO_3 (69%), and 2 ml H_2O_2 (33%) in digestive system on oven in 180 ºC for 15 minutes, gently homogenizing the sample in the reagent. After cooling the sample was filtered throught a filter Whatman No.42 paper and the filtered part was taken up to a final volume of 50 ml of distilled water. The metal concentrations were determined by atomic absorption spectrometer (AAS) (Spectra A-200), equipped with a GTA 100 (Referenca: EN 16173, Sludge, treated biowaste and soil- Digestion of Nitric Acid soluble fractions of elements).

2.4. Data analyses

The data obtained was analyzed using SPSS 20.0, and results are expressed as average ± standard deviation. Calculated Indices are: (i) Concentrations of heavy metals in plants obtained from AAS - GTA in mg / L are converted to mg / kg of dry weight (DW) using the following formula: mg / kg = (mg L-1 * 0.05) / 0.0003 . The values were compared and valued at FAO/WHO standards for heavy metals in vegetables and plants (Chauhan, 2014 and FAO/WHO 1976); (ii) Transfer factors (Ft) of metals from soils to plant (Alloway, 1995); Ft = content of metal in plant / metal content in soil (mg * kg-1 dry weight). this index is used to assess the risk of metal transfer from soil to plants. the level of metals in plants is assessed by comparing with the safety margins set by FAO / WHO (2001) for heavy metals in food and vegetable. ANOVA and LSD test are used to find the statistical differences in soill and plant parameters between sites and sampling points.

3. Results and Discussion

The results obtained from the analysis of heavy metals in cabbage and lettuce plants are presented in Table 1.

Table 1. The content of heavy metals in study areas

Sample plants	Pb	Cd	Cr	Ni	Zn	Cu
Industrial Site / Shullaz						
I-1	0.57	0.22	2.327	3.337	47.037	5.287
I-2	0.51	0.41	2.462	3.327	23.017	7.85
I-3	0.425	0.102	2.48	1.647	51.55	22.03
I-4	2.166	0.223	2.54	3.157	63.55	33.34
I-5	1.074	0.225	3.1	6.91	61.22	21.3
Average	0.949	0.236	2.5818	3.6756	49.2748	17.9614
Agriculture Site / Gajush						
B-1	0.82	0.178	2.72	3.97	55.31	14.6
B-2	0.36	0.058	2.99	1.76	37.7	2.7
B-3	0.88	0.1045	3.05	4.67	40.72	16.35
B-4	0.54	0.112	1.9	5.03	53.45	25.47
B-5	0.47	0.0935	3.38	1.98	43.37	4.04
Average	0.614	0.1092	2.808	3.482	46.11	12.632
Reference Site / Pllanë						
R-1	0.198	0.17	3.23	2.6	65.857	2.7
R-2	0.196	0.075	3.32	2.39	63.29	11
R-3	0.255	0.12	2.71	1.54	45.023	9.697
R-4	0.377	0.062	2.37	1.02	74.57	15
R-5	0.176	0.03	2.57	0.877	80.39	8.11
Average	0.2404	0.0914	2.84	1.6854	65.826	9.3014
LSD, $p < 0.05$	0.8	0.12	0.7	2.42	21.01	14.5
Safety Level (Vegetable)[a]	0.3	0.2	5	60	60	40
Normal Level (Plants)[b]	<2.4	0.5-30		20-100	0.02-50	2.5

[a]FAO/WHO, 2007 adopeted by Chauhan (for Cd, Cr, Cu, Zn) and Kachenko & Singh (for Pb); [b]FAO/WHO

The average content of heavy metals can be classified in descending order: Zn > Cu > Ni > Cr > Pb > Cd for vegetables cultivated in industrial and agricultural soils and Zn > Cu > Cr > Ni > Pb > Cd > for vegetables cultivated in reference soils. It is interesting to note that the highest content of Pb, Cd, Ni and Cu were founded in vegetables samples studied in industrial soil and higher content of Zn and Cr were found in the samples were foundin samples of vegetables studied in the reference soil. Also was noticed that the average contents of metals analyzed in vegetables samples in Industrial soils and in the agricultural area were higher than in reference area and much closer to each other. Pb had 3 times higher average contents on industrial soil vegetables than the safety level, while in the agricultural soils the content was 2 time higher. This may be due to the absorption of the leaves of this element in the form of dust coming mainly from the vicinity of the Cu melting plant within the industrial complex.

Alloway 1990 [1] noticed that plant pollution with Pb is generally from outside. The high content of Pb in vegetables samples on agriculture soils can be result of agricultural chemicals application such as organic fertilizers, pesticides and chemical fertilizers. The ANOVA showed that there were no statistically significant differences (F<Fcrit) between the three sampling sites for analyzed Pb, Cr, Ni, Zn, Cu metals, but only for Cd had a statistically significant difference (F>Fcrit) Tables Nr. 2, 3, 4, 5, 6 and 7

Table 2. ANOVA results for Pb in plants

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	1.257719	2	0.62886	3.225847	0.075661	3.885294
Within groups	2.339329	12	0.194944			
Total	3.597048	14				

Table 3. ANOVA results for Cd in plants

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	0.062478	2	0.031239	5.355051	0.021766	3.885294
Within groups	0.070003	12	0.005834			
Total	0.132481	14				

Tabela 4. ANOVA results for Cr in plants

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	0.203682	2	0.101841	0.546741	0.592593	3.885294
Within groups	2.23523	12	0.186269			
Total	2.438912	14				

Tabela 5. ANOVA results for Ni in plants

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	12.03414	2	6.017069	2.692378	0.108162	3.885294
Within groups	26.81823	12	2.234853			
Total	38.85237	14				

Tabela 6. ANOVA results for Z in plants

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	1120.713	2	560.3567	3.329193	0.070769	3.885294
Within groups	2019.793	12	168.3161			
Total	3140.506	14				

Tabela 7. ANOVA results for Cu in plants

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	191.129	2	95.56451	1.191847	0.337182	3.885294
Within groups	962.1824	12	80.18187			
Total	1153.311	14				

LSD test showed that by the content of Pb, Cd.Cr, Zn, and Cu there were statistically significant differences between sampling points on Industrially contaminated soil, the content of Cr, Ni, Zn and Cu had statistically significant differences between points of sampling on agricultural soil, while for the content of Pb, Cr, Ni and Cu there were statistically significant differences between sampling points on the reference soils.

3.1. Contamination with metals of vegetables

In the industrial and agricultural areas all vegetable samples exceeded the safety level for Pb (0.3 mg/kg) determined by the Codex Commission Alimentarius Commission [6] adapted by Chauhan [2], while only in Industrial samples area are exceeded the safety level for Cd (0.2 mg/kg) (Table1). High content of Pb and Cd in vegetables can harm the health of consummators. Pb and Cd don't have biological essential functions and are very toxically living organisms, including humans. Also is noticed that we had exceeded the safety level and exceeded the normal level of metals inplants for Zn in 4 sampling areas and 2 samples of Industrial areas. The content of the other metals analyzed resulted to be under safety levels. While Cu repository in plants in all analyzed samples of 3 studies areas was many times higher than the normal range of metals in plants. In these study, the correlation analysis was olso applied to identify the role of soil parameters in accumulation of heavy metals in plants. This analysis is done for each area in itself according three main parameters of soils such as pH, organic carbon and clay. The correlations are presented in tables 8,9 and 10.

Table 8. Correlation of the content of metals in plants with Industrial soil parameters.

	pH	Corg	Clay	Pb	Cd	Cr	Ni	Zn	Cu
pH	1								
Corg	-,001	1							
Clay	-,792	-,319	1						
Pb	-,195	,915*	,026	1					
Cd	-,112	,037	,148	-,062	1				
Cr	-,962**	-,038	,880*	,245	-,043	1			
Ni	-,794	-,144	,979**	,194	,243	,873	1		
Zn	-,284	,383	,269	,640	-,716	,471	,291	1	
Cu	-,357	,757	-,053	,789	-,466	,370	,027	,758	1
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

Correlation analysis for industrial zone showed that there was a statistically significant and positive relation of nickel with clay ($r=0.979$, $p\leq 0.01$), Cr with clay ($r=0.880$, $p\leq 0.05$), Pb with organic carbon ($r=0.915$, $p\leq 0.05$), while we had a statistically significant relation but negative of Cr with pH ($r=0.962$, $p\leq 0.01$). The correlation analysis for agricultural area is shown in table No.9. Statistically significant and positive correlations were found between Cu and Ni ($r=0.963$, $p\leq 0.01$) and statistically significant but negative correlations were found between Cr and water pH ($r=0.900$, $p\leq 0.05$).

Table 9. Correlation of the content of metals in plants with Agricultural soil parameters**.**

	pH	Corg	Clay	Pb	Cd	Cr	Ni	Zn	Cu
pH	1								
Corg	,043	1							
Clay	,109	,734	1						
Pb	-,321	-,639	-,058	1					
Cd	-,609	-,337	-,186	,709	1				
Cr	-,900*	-,333	-,216	-,030	-,287	1			
Ni	-,727	-,086	,341	,695	,542	-,685	1		
Zn	-,811	,141	,068	,346	,841	-,677	,588	1	
Cu	-,805	,139	,420	,494	,484	-,838	,963**	,675	1
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

In the reference area there was a statistically significant and positive link between Chrome and Nickel only ($r=0.995$, $p\leq 0.05$) and we didn't have any other connection, the data for the correlation analysis are presented in table no.10.

Table 10. Correlation of the content of metals in plants with reference soil parameters**.**

	pH	Corg	Clay	Pb	Cd	Cr	Ni	Zn	Cu

pH	1								
Corg	-,419	1							
Clay	-,740	,322	1						
Pb	-,516	-,527	,509	1					
Cd	-,374	,564	,630	-,159	1				
Cr	-,035	,845	,304	-,639	,582	1			
Ni	-,201	,807	,541	-,440	,763	,955*	1		
Zn	,818	-,714	-,463	,008	-,589	-,291	-,386	1	
Cu	-,405	-,354	,135	,732	-,647	-,558	-,551	,081	1
*. Correlation is significant at the 0.05 level (2-tailed).									

Results showed that had statistically significant and positive correlations between Cu and Ni pairs for agriculture area and between Ni and Cr for the reference area, suggesting that these metals may have the same origin [11]. To determine if heavy metals come from the soil, correlation have been found between total metal concentrations and available in soil and plants [11] (Table No.11).

Table 11. Correlation coefficients between the concentrations of metals in soil and plant in all three study areas

Heavy metals in soil	Heavy Metasl in Plants					
	Pb	Cd	Cr	Ni	Zn	Cu
Pb_{Total}	0.413					
Cd_{Total}		0.127				
Cr_{Total}			-0.261			
Ni_{Total}				-0.348		
Zn_{Total}					-0.298	
Cu_{Total}						0.365
$Pb_{Available}$	0.517*					
$Cd_{Available}$		0.416				
$Cr_{Available}$			-0.266			
$Ni_{Available}$				-0.147		
$Zn_{Available}$					-0.093	
$Cu_{Available}$						0.4567

* Correlation is significant at the 0.05 level (2-tailed).

From analyzed results for the three study areas, we found that there was a statistically significant and positive correlation of available Pb in the soil with the content of Pb in plants (r= 0.517, $p \leq 0.05$), while other metals in the soil extracted with Aqua Regia and Mehlich 3 did not show statistically significant links with respective metals in plants. According to Bunzl et al. [24], quoted by Chojnacka et al. [3], the existence of a important relationship between metal concentrations soil and plants is a prerequisite for the soil–plant transfer factor concept.

3.2. Transfer of heavy metals in vegetables

According Liang et.al [25], one of the main components of human exposure to metals through food chain is the transfer factor of metals from soil to plants. To estimate the risk of transferring heavy metals transformation from soils to plants is calculated transfer factor (Ft) [1].This factor is given as the ratio between the content of metal in plants to the content of metal on the soil (mg/kg dried weight). Interpretation of these parameters is made according to Table No.12 [26].

Table 12. Interpretation of transfer factor of metals in plant.

Transfer factor values	Plant categorization
≥ 1	Accumulator – plant has accumulated metal
~ 1	Indicator- plant is not indicated from metal
≤ 1	Excludes-Plant exludes getting metals

Transfer factors values for heavy metals from soil to plant are reported in Table No 13 together with pH value of the soil.It is noted that Transfer factors values are smaller than one for the six metals analyzed in all three study areas. As apparently from the table, pH is high, and therefore transfer factors values result low, from which we conclude that despite the very high values of metals in the soil the transfer risk is low. Transfer factors values in descending order are as follows: (i) for Industrial soil: Zn > Pb > Cu >Cd > Ni > Cr >: (ii) for agricultural soil: Zn >Cu > Pb > Cd > Cr > Ni: and (iii) for reference soil: Zn >Cu >Cd > Ni > Pb.

Table 13. Metal transfer factors from soil to plant.

Soil	Soil pH	**Pb**	**Cd**	**Cr**	**Ni**	**Zn**	**Cu**
Industrial	7.744	0.0992	0.0387	0.014	0.017	0.24	0.09
Agriculture	7.754	0.0604	0.016	0.015	0.014	0.65	0.33
Reference	7.749	0.01	0.05	0.19	0.013	0.65	0.51

4. Conclusions

Average content (mg/kg) of heavy metals in plants various: Pb from 0.24 to reference soil at 0.949 in industrial soil, Cd from 0.0914 in reference soil at 0.236 to industrial soil, Cr from 2.58 to industrial soil to 2.84 in reference soil, Ni from 1.6854 in reference soil to 3.6756 to industrial soil, Zn from 46.11 to agricultural soil to 65.826 in reference soil, and Cu from 9.3014 in reference soil to 17.9614 on industrial soil. ANOVA showed statistically significant difference ($F<F_{crit}$) between three sampling sites only for Cd.In industrial and agricultural soils Pb exceeded the safety level (0.3 mg/kg) in all analyzed samples, Cd exceeded this level (0.2 mg/kg) on industrial soil, Zn exceeded the safety level (60 mg/kg) and the range normal in 4 samples of reference soil and in 2 samples of industrial soil, Cu in all samples analyzed in all three study areas was many times higher than the normal range of metals in plants. Correlation analyze in industrial soil showed that there was a statistically significant relation of Ni with clay ($r = 0.979$, $p <0.01$); Cr with clay ($r = 0.880$, $p <0.05$); Pb with organic carbon ($r = 0.915$, $p <0.05$); while we had a statistically significant but negative Cr linkage with pH ($r = 0.962$, $p <0.01$). In agricultural soil, statistically significant and positive connections were found between Cu and Ni ($r = 0.963$, $p <0.01$) and statistically significant but negative correlations were found between Ni and pH ($r = 0.900$, $p <0.05$). In the reference soil there was a statistically significant and positive correlation between Cr and Ni ($r = 0.955$, $p <0.05$). The transfer factor values are less than 1 for all analyzed metals in all three study sites, suggesting a low transfer risk to plants. The transfer factor values in descending order are as follows: (i) for the industrial soil: Zn> Pb> Cu> Cd> Ni> Cr; (ii) for the agricultural soil: Zn> Cu> Pb> Cd> Cr> Ni; and (iii) for reference soil: Zn> Cu> Cr> Cd> Ni> Pb.

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