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SOIL HEAVY METALS CONTENT VARIATION DEPENDING ON THE DISTANCE FROM POLLUTION SOURCE AND UPTAKE BY THE *TRIFOLIUM PRATENSE* L. SPECIES HARVESTED FROM COPȘA MICĂ AREA, CENTRAL ROMANIA

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Abstract

Heavy metals pollution has always been a significant problem for human, animal, and plant health. Copșa Mică, Romania, stands out as one of the most polluted areas with heavy metals, mainly due to emissions from two factories (Carbosin and Sometra), which have been operating in the past for about 60 years. In 2023, soil and plant (Trifolium pratense L.) samples were collected from the polluted meadows in the researched area to analyze and determine cadmium, lead, zinc, and copper concentrations. Cd recorded the lowest concentration in both soil and plant samples with values of 5.17 mg·kg^{-1}dry weight (d.w.) and 0.52 mg·kg^{-1}d.w., respectively. The highest content in both soil and plant samples was recorded by Zn, with values of 334.5 mg·kg^{-1}d.w. (soil) and 69.67 mg·kg^{-1}d.w. (plant). Soil heavy metals content variation depending on the distance from the pollution source showed significant values only for Cd and Zn, while the values for Pb and Cu were insignificant. The results obtained from this study will be used to raise awareness of the population living in the affected areas on the risks that arise from using the meadows for grazing and harvesting hay.

***Key words**: accumulation, heavy metals, meadow, pollution, Trifolium pratense L.*

INTRODUCTION

Worldwide, the *Fabaceae* family is one of the most economically important plant families, comprising more than 700 genera and about 20.000 species (Kaurinovic et al., 2012; Schrire et al., 2005). Red clover (*Trifolium pratense* L.) is one of the common species of this family, mostly used as a source of fodder in animal feed. In addition to its use as feed, Savirant et al. (2007) demonstrated that the aerial parts of this plant, especially the leaves, contain high amounts of isoflavones, which can enhance the use of the plant in the food and pharmaceutical industries. Pollution of soil, water, and air with heavy metals from industrial activities, agriculture, and transport affects the whole world, and finding effective and cheap solutions is a challenge for mankind (Borozan et al., 2021). Heavy metals are a serious threat to human life because, through the food chain, they can enter the body causing a range of health problems.

Ali et al. (2012), Bidar et al. (2007), and Liu et al. (2018) cited by Pescatore et al. (2022) reported that forage plants, including red clover, can be successfully used in phytoremediation of polluted soils due to their increased adaptability, rapid growth, high biomass production and resistance to pollution.

Liu et al. (2018) demonstrated that clover plants have high resistance to lead (Pb) pollution.

In a study by Bidar et al. (2009) on the bioaccumulation of heavy metals and their transfer in *T. repens* and *L. perenne* plants grown on polluted soil showed that metals accumulated mainly in the roots as follows: Cd>Zn>Pb, and their transfer to the aerial parts was limited.

Memić et al. (2023) reported high concentrations of Cu and Pb in clover roots, while more Cd and Zn accumulated in leaves, they demonstrated that heavy metals content depended on area and season.

A study by Kujawska et al. (2018) on the effects and transfer of heavy metals in red clover grown on substrates produced from mixtures of different materials showed that the plants accumulated high contents of Cu, Zn, and Ni, but did not present a risk in the use of the plants as feed.

Studies have shown that heavy metals soil pollution has an inhibitory effect on the development of clover plants (Stravinskienė & Račaitė, 2014). Cajak et al. (2023) reported high concentrations of heavy metals in *T. pratense* cultivated in different locations in western Poland, concluding that this species of plant can be utilized as a bioindicator of contamination with these types of metals in urban areas. Heavy metals like Zn and Cu accumulate mainly in roots and leaves and less in the shoots.

Different levels of soil heavy metals (Zn and Cu) contamination affect the leaf surface area of red clover (Sakal et al., 2019).

Heavy metals negatively affect plant development, producing biochemical and physiological changes in their vegetative organs (Asati et al., 2016).

Meng et al. (2022) demonstrated that *T. pratense* plants had a positive response to low concentrations of Pb (500 mg/kg), but double or higher concentrations of Pb are toxic and damage metabolic processes in the plant.

In a study by Malizia et al. (2012), using herbaceous and leguminous plants (*T. pratense*) to monitor heavy metal content in soil, reported higher concentrations in roots compared to leaves, but the difference between the two species was insignificant. However, soil heavy metals concentrations were directly proportional to the levels in plants.

Lambrechts et al. (2014) showed that *Trifolium repens* has a lower tolerance to heavy metal pollution compared to *Lolium perenne*. They demonstrated that exposure of white clover to Cd resulted in decreased biomass production and inhibition of root growth.

According to Sotiriou et al. (2023) in a study regarding growing white clover on heavy metals contaminated soil using zeolite, they demonstrated that the plants accumulated heavy metals in the dry biomass above normal consumption limits. They indicated that this species accumulated higher amounts of Cd and Zn compared to Pb.

This study aims to assess the accumulation of Cd, Pb, Cu, and Zn by *T. pratense* species harvested from polluted natural meadows in Copșa Mică area and soil heavy metals content variation depending on the distance from the pollution source.

MATERIALS AND METHODS

Soil and plant samples (*T. pratense*) were collected in 2023, from 5 localities affected by pollution (Copșa Mică, Axente Sever, Micăsasa, Târnava, and Valea Viilor) in Sibiu County, to analyze the heavy metals content (Cd, Cu, Pb, and Zn). Soil samples were collected from the first soil layer (0-20 cm). A soil sample was composed of 13 sub-samples, dried at room temperature, grounded, and sieved to remove stones and plant waste. Atomic absorption spectrometry, after the extraction by the aqua regia - microwave digestion method was used to determine heavy metals content from soil samples. DTPA- extractable heavy metals were exctracted from soil (10 g) with 20 ml of extracting solution (0.05 M DTPA, 0.01 M $CaCl_2$ and 0.1 M tetraethylammonium adjusted to pH 7.3), according to SR ISO 14870:2002. Red clover plant samples (aerial part) collected were oven dried then milled and treated with nitric acid in a microwave digestion system. Atomic absorption spectrometry (Flame GBC 932AA or Graphite furnace GBC SavanatAAZ) method was used to determine the heavy metals content. The statistical processing of the data was done using Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

Analyzing the values of statistical parameters that characterize the central tendency and the variability of the soil heavy metal total contents (Table 1) for the minimum and maximum values, they varied as follows: Cd (0.38 $mg \cdot kg^{-1}$ d.w. - 16.9 $mg \cdot kg^{-1}$ d.w.), Pb (25 $mg \cdot kg^{-1}$ d.w. - 599 $mg \cdot kg^{-1}$ d.w.), Zn (89 $mg \cdot kg^{-1}$ d.w. - 930 $mg \cdot kg^{-1}$ d.w. and Cu (15 $mg \cdot kg^{-1}$ d.w. - 161 $mg \cdot kg^{-1}$ d.w.).

In terms of average soil metal concentration, the lowest value was obtained for Cd (5.15 $mg \cdot kg^{-1}$ d.w.) while Zn had the highest value (334.5 $mg \cdot kg^{-1}$ d.w.). Also, Pb recorded an average value of 177.4 $mg \cdot kg^{-1}$ d.w. and 53.7 $mg \cdot kg^{-1}$ d.w. for Cu. Biasoli et al. (2006) obtained similar results for heavy metals contents in urban samples. The coefficient of variation of the heavy metals contents in the soil samples analyzed ranged from 68.3% for Zn to 84.7% for Cd. The values recorded for Pb and Cu contents were 80.4% and 75.8% respectively. The standard deviation ranged from 4.38 mg·kg^{-1} d.w. for Cd to 228.3 mg·kg^{-1} d.w. for Zn. Analyzing the median and geometric mean, Cd content recorded the lowest value for both statistical parameters, Pb had the highest value (132 mg·kg^{-1}d.w.) for the median, while Zn obtained the highest value for the geometric mean (277.9 mg·kg^{-1}d.w.). The heavy metals' total content in the soil was in the following order: Zn>Pb>Cu>Cd. Cd and Pb content in soil exceeded alert and intervention thresholds for sensitive land use. Zn content exceeded only the alert threshold, while Cu was within the limits (Ministerial Order 756/1997).

The values of statistical parameters that characterize the central tendency and the variability of the Cd, Pb, Zn, and Cu contents in soil - DTPA-extractable forms were presented in Table 2.

The Cd content recorded the lowest mean value (3.42 mg·kg^{-1} d.w.) while the highest mean content (80.1 mg·kg^{-1} d.w.) was obtained by Zn. Analyzing the heavy metals contents (extractable forms) of the soil following the minimum and maximum values were as follows: Cd (0.23-9.53 mg·kg^{-1} d.w.), Pb (4-260 mg·kg^{-1} d.w.), Zn (7-323 mg·kg^{-1} d.w.), and Cu (2.46-23.41 mg·kg^{-1} d.w.). Median values ranged from 2.28 mg·kg^{-1} d.w. for Cd to 52 mg·kg^{-1} d.w. for Zn. Following the geometric mean values for the content of heavy metals in the soil, it is observed that the lowest value was obtained for Cd (2.41 mg·kg^{-1} d.w.) while Zn had the highest value (49.5 mg·kg^{-1} d.w.), the content of Pb and Cu recorded values of 42.6 mg·kg^{-1} d.w., respectively 6.45 mg·kg^{-1} d.w. Cd recorded the lowest value for the standard deviation (2.93 mg·kg^{-1} d.w.) followed by Cu with a value of 5.65 mg·kg^{-1} d.w. and Pb (72.9 mg·kg^{-1} d.w.). Analysing the values of the coefficient of variation they ranged from 71.8% for Cu to 112.9% for Zn content. The Cd and Pb contents had values of 87.5 mg·kg^{-1} d.w. and 105.5 mg·kg^{-1} d.w., respectively. The heavy metals content in the soil (DTPA-extractable forms) was in the following order: Zn>Pb>Cu>Cd.

Table 1. Values of statistical parameters that characterize the central tendency and the variability of the total cadmium, lead, zinc and copper contents in soil (n = 15)

Variable	Minimum	Maximum	Median	Geometric mean	Arithmetic mean	Standard deviation	Coefficient of variation
	- - - - - - - -	- - - - - - - -	- - - - mg·kg^{-1}d.w.	- - - - - -	- - - - - -	- - - - - -	
Cd_{soil}	0.38	16.92	3.91	3.67	5.17	4.38	84.7%
Pb_{soil}	25	599	132	135.5	177.4	142.7	80.4%
Zn_{soil}	89	930	270	277.9	334.5	228.3	68.3%
Cu_{soil}	15	161	38	42.8	53.7	40.7	75.8%

Table 2. Values of statistical parameters that characterize the central tendency and the variability of the cadmium, lead, zinc, copper contents in soil - DTPA-extractable forms (n = 15)

Variable	Minimum	Maximum	Median	Geometric mean	Arithmetic mean	Standard deviation	Coefficient of variation
	- - - - - - - -	- - - - - - - -	- - - - mg·kg^{-1} d.w.	- - - - - -	- - - - - -	- - - - - -	
Cd_{DTPA}	0.23	9.53	2.28	2.41	3.42	2.93	85.7%
Pb_{DTPA}	4	260	35	42.6	69.1	72.9	105.5%
Zn_{DTPA}	7	323	52	49.5	80.1	90.4	112.9%
Cu_{DTPA}	2.46	23.41	5.73	6.45	7.87	5.65	71.8%

Table 3. Values of statistical parameters that characterize the central tendency and the variability of the cadmium, lead, zinc, and copper contents in the red clover (*Trifolium pratense)* (n = 15)

Variable	Minimum	Maximum	Median	Geometric mean	Arithmetic mean	Standard deviation	Coefficient of variation
	- - -- - - - - - - - -	- - - - - - - - - - - - mg·kg^{-1}d.w. -	- - - - - - - - - -	-- - - - - - - - - - - -	- - - - - -		
$Cd_{red\ clover}$	0.08	1.68	0.38	0.313	0.52	0.509	98.8%
$Pb_{red\ clover}$	0.13	1.68	0.56	0.498	0.67	0.486	73.1%
$Zn_{red\ clover}$	33	169	48	58.24	69.67	48.89	70.2%
$Cu_{redclover}$	6	13.5	8.2	8.28	8.5	2.14	25.2%

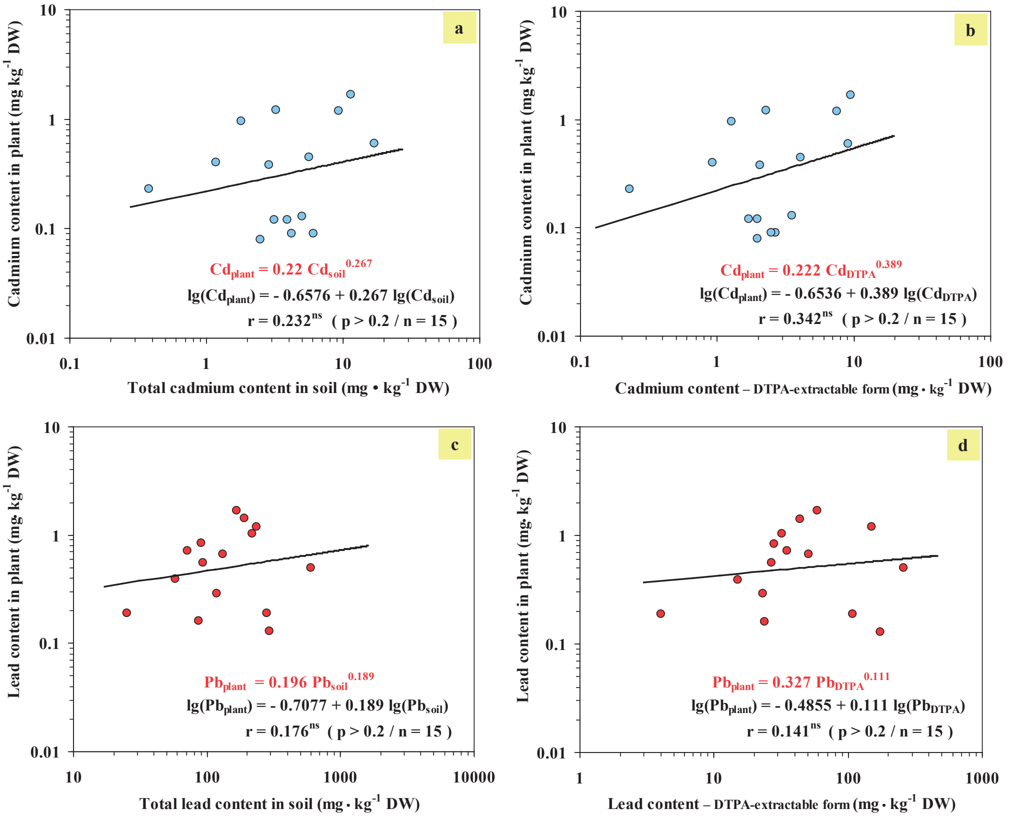


Figure 1. Log-log diagrams for power regression curves that estimate the stochastic dependency between total cadmium content in soil (a), soil cadmium content - DTPA-extractable forms (b), total lead content in soil (c), soil lead content - DTPA-extractable forms (d) and cadmium/lead contents in *Trifolium pratense* plants

Regarding the values of statistical parameters that characterize the central tendency and the variability of the Cd, Pb, Zn, and Cu contents in the red clover (*T. pratense*) (Table 3), it shows that the total Cd content recorded the lowest mean value (0.52 mg·kg^{-1} d.w.), while the Zn content (69.67 mg·kg^{-1} d.w.) was the highest. The mean values of Pb and Cu contents were 0.67 mg·kg^{-1} d.w. and 8.5 mg·kg^{-1} d.w., respectively.

Analyzing the geometric mean values of the heavy metal contents, Cd content obtained the lowest value (0.313 mg·kg^{-1} d.w.) while the highest value was obtained for Zn (58.24 mg·kg^{-1} d.w.). The median values for heavy metals content in red clover plants were: 0.38 mg·kg^{-1} d.w. Cd, 0.56 mg·kg^{-1} d.w. Pb, 8.28 mg·kg^{-1} d.w. Cu and 48 mg·kg^{-1} d.w. Zn, respectively. The standard deviation for heavy metal contents in the plant samples analyzed ranged from 0.486 mg·kg^{-1} d.w. for Pb to 48.09 mg·kg^{-1} d.w. Zn. Liu et al. (2018) demonstrated that the content of heavy metals in plants depends on their centration in the soil.

Pricop et al. (2010) showed that red clover can be successfully used to remediate Pb polluted soils.

The heavy metals contents in the red clover were in the following order: Zn>Cu >Pb>Cd.

The logarithmic diagrams for power regression curves that estimate the stochastic dependency between total contents and DTPA- extractable forms of Cd and Pb in soil, and the contents of those heavy metals in *T. pratense* plants (Figure 1) show that the values obtained were insignificant for both metals. However, in the case of Cd content (Figure 1a, 1b) the correlation coefficient value was higher for the extractable forms (r = 0.342) compared to the total content (r = 0.232). Figure 1c, 1d shows that the correlation coefficient value for Pb content was higher for total forms (r = 0.176).

Figure 2 shows the logarithmic plots for power regression curves that estimate the stochastic dependency between the total contents and DTPA- extractable forms of Zn, and Cu in soil and the amounts of those metals in *T. pratense* plants.

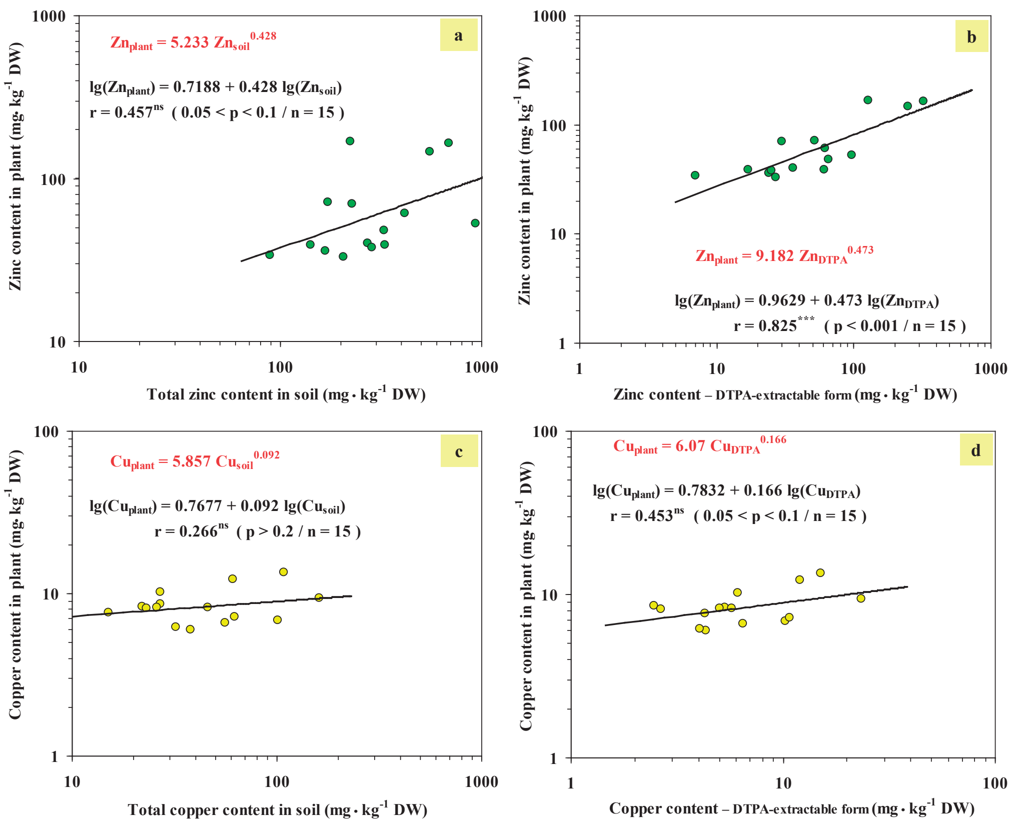
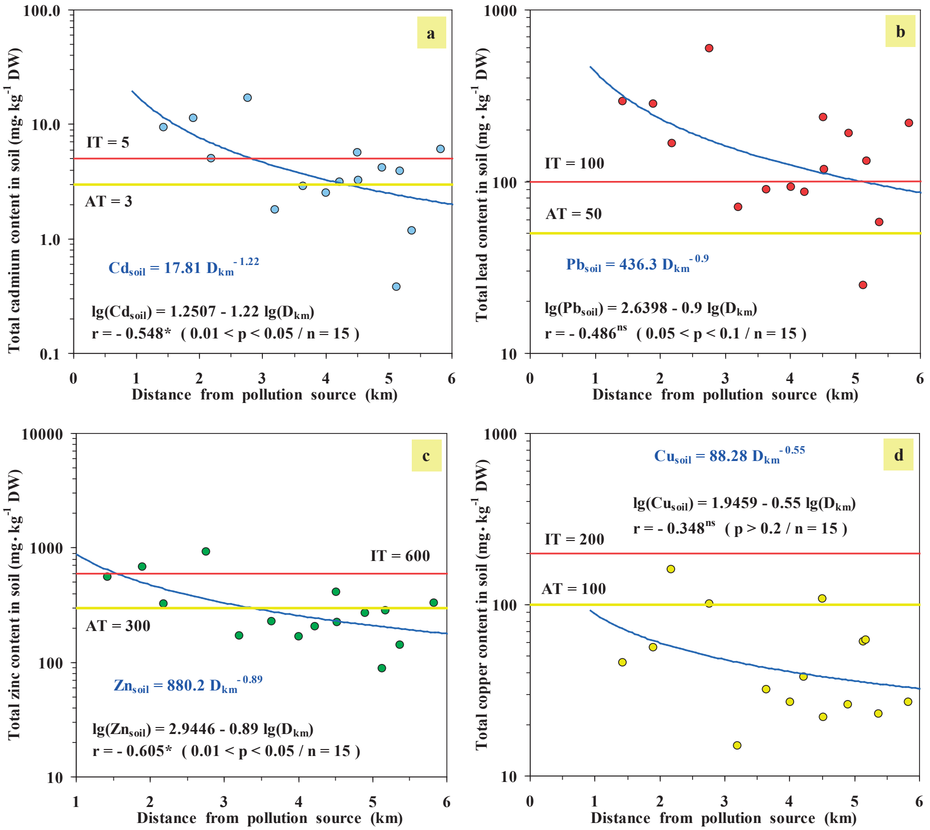


Figure 2. Log-log diagrams for power regression curves that estimate the stochastic dependency between total zinc content in soil (a), soil zinc content - DTPA-extractable forms (b), total copper content in soil (c), soil copper content - DTPA-extractable forms (d) and zinc/copper contents in *Trifolium pratense* plants.



AT - Alert Threshold IT - Intervention Threshold

Figure 3. Total cadmium (a), lead (b), zinc (c) and copper (d) contents in soil depending on the distance from the pollution source - S.C. SOMETRA S.A. (Copşa Mică, 0-20 cm layer)

Analyzing Figure 2a, 2b shows that the correlation coefficient recorded a very significant value in the case of the DTPA-extractable form of Zn in soil ($r = 0.825$) compared to the total content which had an insignificant value. In the case of Cu content (Figure 2c, 2d), the value of the linear correlation coefficient does not differ significantly from zero for both forms of metal and therefore there is no dependence between the two variables considered. However, the correlation coefficient value for the DTPA-extractable form was higher (0.453) compared to total content (0.266).

Figure 3 shows that the Cd and Zn variation contents in soil depending on the distance from the pollution source recorded significant values ($r = -0.548$, respectively $r = -0.605$) compared to Pb and Cu variation contents whose values ($r = -0.486$, respectively $r = -0.348$) were insignificant.

Wang et al. (2018) demonstrated that the length of the roads around the sample site has a great impact on the concentrations of heavy metals. They showed that the 1-2 km range influences the concentrations of heavy metals in the soil in the study area.

Cd and Pb are harmful heavy metals that should be constantly monitored due to their high concentrations in soil with a negative impact on human, animal, and plant health (Chen et al., 2015).

Marrugo-Negrete et al. (2017) demonstrated that heavy metals follow an ascending order depending on the land use (rice>pasture> cotton). They showed that there are significant differences for Cu, Cd and Zn contents in soils cultivated with paddy compared to those cultivated with pasture or cotton.

CONCLUSIONS

Heavy metals contents in soil, plant (aerial part), and soil pollution content variation depending on the distance from the pollution source were analyzed in this study. The Zn content obtained the highest value both in soil (total soil content = 228.3 $mg \cdot kg^{-1}$ d.w.; DTPA-extractable form = 90.4 $mg \cdot kg^{-1}$ d.w.), and in plant (48.99 $mg \cdot kg^{-1}$ d.w.). The lowest content in the soil was obtained by Cd, and the lowest content in the plant was obtained by Pb. However, according to Ministerial Order 756/1997, the Zn, Cd, and Pb content exceeded the alert and intervention thresholds for sensitive land use, only Cu had concentrations within the permitted limits. The heavy metal contents in the soil were as follows: Zn>Pb>Cu>Cd. The heavy metal contents in the plant were in the following order: Zn>Cu>Cd>Pb. Soil pollution content variation depending on the distance from the pollution source recorded significant values only for Cd and Zn. However, in addition to the distance from the pollution source, there are other factors such as precipitation, wind direction, and slope inclination that can influence the degree of metal pollution in certain areas. The value of the linear correlation coefficient obtained for the dependence between the DTPA-extractable form of Zn in the soil and that in the plant differs statistically significantly from zero indicating a strong correlation between the two variables. The results from this study and future data will be used to increase people's awareness of the risks they are exposed to by using meadows for grazing animals or harvesting hay.

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