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# BIOACCUMULATION OF CADMIUM, LEAD, ZINC AND COPPER IN RED FESCUE (*FESTUCA RUBRA* L.) GROWN IN POLLUTED MEADOWS IN COPŞA MICĂ

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***Abstract***

*Studies on the influence of heavy metals are essential because they have a negative impact on human and animal health, the environment, and ecosystems. This work aims to assess the heavy metals content in soil and plant (Festuca rubra L.) samples collected from permanent grasslands in a heavily polluted area during 2023–2024. The total content and DTPA extractable forms of cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) in soil and plant were analyzed. The mean value of total Cd content in soil was 5.50 mg/kg dry weight (DW), while the mean values for Pb, Zn and Cu were 201.1 mg/kg DW, 368.7 mg/kg DW and 46.9 mg/kg DW, respectively. In the plant the mean Cd content had the lowest value (0.47 mg/kg DW), the second lowest mean value was recorded for Pb, while the Zn content was the highest (47.6 mg/kg DW). The values of the correlation coefficients showed that heavy metals uptake by Festuca rubra varies according to the type of metal and its form in the soil. The results of this research showed that soils in the Copşa Mică area continue to have a high content of heavy metals, which may have a negative impact on the quality of human and animal life through their accumulation in the food chain.*

***Key words****: bioaccumulation, Festuca rubra L., heavy metals, meadows, pollution.*

## INTRODUCTION

Mining, metal smelting, and agricultural practices are among the main anthropogenic activities that contribute to environmental contamination with heavy metals. Exposure to heavy metals, even in small quantities, through ingestion of contaminated food and water or inhalation of polluted air can lead to numerous health problems (Osman et al., 2019; Briffa et al., 2020; Budi et al., 2022).

Cd is a toxic heavy metal that is non-biodegradable, posing long-term risks to ecosystems and human health. Cd accumulation in plants causes oxidative stress and affects physiological processes (Suhani et al., 2021; Bouida et al., 2022).

Another microelement that can have negative effects in high amounts is Cu. Many studies have shown that Cu assimilation in high quantities can have numerous toxic effects on plants, such as growth inhibition observed through low seed germination rate, disruption of photosynthesis, oxidative stress, or nutrient imbalance (Adrees et al., 2015; Rehman et al., 2019; Mir et al., 2021).

Kuziemska et al. (2021) reported that the use of organic amendaments, especially cattle manure, reduces the bioavailability of Cu in soil. Also, the application of bacterial siderophores also promoted plant growth and improved phytoremediation by reducing metal toxicity (Grobelak et al., 2017).

Tan et al. (2020) reported that Zn plays an important role in plant metabolism.

However, Zn phytotoxicity can affect normal development, photosynthesis and nutrient uptake, but some plants are tolerant to high concentrations and have potential use in phytoremediation (Broadley et al., 2007; Kaur et al., 2021).

*Festuca rubra* L. is a perennial grass, widespread in various habitats around the world, used in the phytoremediation of contaminated soils due to its well-developed root system, large biomass and high capacity to grow in unfavorable environmental conditions (Gajić et al., 2020).

Phytoremediation is an ecological process of remediation of soils or water polluted with heavy metals. The phytoextraction process refers to the absorption and transfer of contaminants into the aerial parts of plants, while phytostabilization is the immobilization of contaminants in the roots of plants (Hrynkiewicz et al., 2018).

Pusz et al. (2021) analyzed the absorption capacity of heavy metals by red fescue (*Festuca rubra* L.) and reported that it accumulated higher amounts in roots compared to shoots.

Lago-Vila et al. (2015) demonstrated that red fescue can be grown on soils contaminated with heavy metals (Cd, Co, Ni) due to its high phytostabilization capacity.

Niesiobędzka (2023) demonstrated that the concentration of heavy metals in red fescue varies depending on the ecosystem, with the highest concentrations being found in plants grown in urban areas and along roads where the Pb content was above limits.

Red fescue is a species with high potential in phytoremediation of polluted soils, having the ability to retain high amounts of heavy metals in its roots (Gajić et al., 2016).

Mitrović et al. (2008) reported higher Zn concentrations in the roots of *Festuca rubra* compared to the aerial parts indicating a poor mobility of this metal in the plant. On the other hand, the phenomenon of wilting of young leaves was observed, being associated with Cu deficiency.

Wyszkowska et al. (2022) demonstrated that *Festuca rubra* exhibits higher resistance to Cd concentrations compared to Co, and the accumulation factor indicated that this plant has a high potential in the phytoremediation process for lands contaminated with these metals.

The study aims to assess the bioaccumulation of heavy metals (Cd, Pb, Zn, and Cu) by *Festuca rubra* plants from soil of permanent meadows affected by industrial pollution in the Copșa Mică area.

## MATERIALS AND METHODS

This study was conducted in the most affected area by industrial pollution in Romania, Copșa Mică, during 2023-2024.

This area became known worldwide for its high pollution levels due to the two factories (Carbosin and Sometra) that operated in the past for approximately 60 years. Plant (aerial part) and soil samples were collected from the studied area to determine the content of heavy metals.

*Festuca rubra* plant samples harvested were oven dried then milled and treated with nitric acid in a microwave digestion system (Ethos Easy Microwave Dygestion System from Milestone).

Microwave digestion was performed using 10 mL mixture of HNO3 + H2O2 (9 mL HNO3 and 1 mL H2O2) at 210°C for 20 min, method developed in–house.

The measurement of heavy metals content was performed by atomic absorption spectrometry (Flame GBC 932AA to determine Zn and Cu content and Graphite furnace GBC SavanatAAZ to determine Cd and Pb content).

Soil samples were collected from the topsoil (0-20 cm). A soil sample was composed of 13 sub-samples, air-dried at room temperature, crushed, and sifted through a 0.2 mm to remove stones and plant waste.

For the determination of heavy metals content was used atomic absorption spectrometry, after the extraction by the aqua regia-microwave digestion method (Ethos Easy Microwave Dygestion System from Milestone).

Microwave digestion was performed using 10 mL of aqua regia (7.5 mL HCl and 2.5 mL HNO3) at 140°C for 30 min, method developed according to SR ISO 11466:1999.

(DTPA)–extractable heavy metals were extracted from soil (10 g) with 20 ml of extracting solution (0.05 M DTPA (dietilentriaminopentacetic acid), 0.01 M $CaCl_2$ and 0.1 M tetraethylammonium adjusted to pH 7.3), according to SR ISO 14870:2002.

Statistical data processing was performed using Microsoft Excel 2010.

## RESULTS AND DISCUSSIONS

Based on the values of the statistical parameters that characterize the central tendency and variability of the total content of Cd, Pb, Zn and Cu in soil (Table 1), it was observed that the contents varied from 0.17 mg/kg DW to 17.74 mg/kg DW for Cd, between 13-692 mg/kg DW for Pb, while the content of Zn and Cu had values ranging between 42-993 mg/kg DW, respectively 12-161 mg/kg DW.

Regarding the median results, the highest value 223 mg/kg DW was recorded for Zn and the second highest in the case of the Pb content (118 mg/kg DW). Cu had a median value of 29 mg/kg DW, while the lowest value was observed for Cd (3.24 mg/kg).

Analyzing the values obtained for the geometric mean, they varied from 3.13 mg/kg DW for Cd to 255.9 mg/kg DW for Zn content. The geometric mean values for Pb and Cu contents were 126.5 mg/kg DW and 36.8 mg/kg DW, respectively.

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 = 17)

| Variable | Minimum | Maximum | Median | Geometric mean | Arithmetic mean | Standard deviation | Coefficient of variation |
|---|---|---|---|---|---|---|---|
| | | | | mg/kg DW | | | |
| $Cd_{soil}$ | 0.17 | 17.74 | 3.24 | 3.13 | 5.50 | 5.50 | 100% |
| $Pb_{soil}$ | 13 | 692 | 118 | 126.5 | 201.1 | 188 | 93.5% |
| $Zn_{soil}$ | 42 | 993 | 223 | 255.9 | 368.7 | 297.8 | 80.8% |
| $Cu_{soil}$ | 12 | 161 | 29 | 36.8 | 46.9 | 38 | 81% |

DW - dry weight

Regarding the arithmetic mean (Table 1), the heavy metals contents followed the order: Zn>Pb>Cu>Cd. The highest content was recorded by Zn at 368.7 mg/kg DW followed by the Pb content (201.1 mg/kg DW). Cu content had the third highest value (46.9 mg/kg DW) while the lowest content (5.50 mg/kg DW) was recorded for Cd.

According to the Order of the Ministry no. 756/1997 for the approval of the Regulation on the assessment of environmental pollution, the mean values for the Cd and Pb contents exceeded the alert threshold of 3 mg/kg, respectively 50 mg/kg and the intervention threshold of 5 mg/kg and 100 mg/kg for sensitive land use while, the mean values for the Zn and Cu contents remained within normal limits, below the alert thresholds of 300 mg/kg and, respectively, 200 mg/kg.

Muntean et al. (2010) obtained similar results regarding the Cd and Pb content in soil in Copșa Mică area.

Following the results for standard deviation, Cd had the lowest value (5.50 mg/kg DW), while Zn recorded the highest (297.8 mg/kg DW). The standard deviation for Pb was 188 mg/kg DW and for Cu, it was 38 mg/kg DW. A large standard deviation for Pb and Cd indicates that pollution is not evenly distributed.

Analyzing the values obtained for the coefficient of variation (Table 1), it is observed that Cd obtained the highest value (100%) followed by Pb which recorded the second highest result (93.5%). In comparison, the content of Cu and Zn recorded values of 81% and 80.8%, respectively.

The high values of the coefficients of variation indicate an uneven distribution of pollution in the 17 analyzed points suggesting non-uniform pollution.

The studied area is characterized both by a wide variability of soil types and the degree of heavy metal contamination.

Shabbir et al. (2020) reported that the main soil characteristics that influence the mobility and availability of Cu in the soil and uptake by plants are pH and organic matter.

Intrinsic soil factors and environmental variables such as pH, soil organic matter and topographic moisture index influence the spatial variation of heavy metals in soil (Wu et al., 2020).

Analyzing the values of statistical parameters that characterize the central tendency and variability of Cd, Pb, Zn and Cu content in soil – DTPA-extractable forms (Table 2), the order of heavy metals was as follows: Zn>Pb>Cu>Cd except for the values obtained for the coefficient of variation where the metals were ranked as: Pb>Cd>Zn>Cu.

Following the minimum and maximum values for the extractable forms, Cd content ranged from 0.08 mg/kg DW to 15.44 mg/kg DW, Pb varied between 2.7 mg/kg DW and 301 mg/kg

DW, Zn ranged from 2.6 mg/kg DW to 326.8 mg/kg DW, while Cu were between 1.13 mg/kg DW to 23.41 mg/kg DW.
Regarding the median values, Cd had the lowest value (2.28 mg/kg DW), while Zn had the highest (63.2 mg/kg DW). Pb had a median value of 58.8 mg/kg DW, whereas Cu obtained 4.83 mg/kg DW.

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 = 17)

| Variable | Minimum | Maximum | Median | Geometric mean | Arithmetic mean | Standard deviation | Coefficient of variation |
|---|---|---|---|---|---|---|---|
| | | | mg/kg DW | | | | |
| $Cd_{DTPA}$ | 0.08 | 15.44 | 2.28 | 2.31 | 4.27 | 4.54 | 106.3% |
| $Pb_{DTPA}$ | 2.7 | 301 | 58.8 | 39 | 73 | 79.2 | 108.5% |
| $Zn_{DTPA}$ | 2.6 | 326.8 | 63.2 | 51.5 | 105.2 | 111.2 | 105.7% |
| $Cu_{DTPA}$ | 1.13 | 23.41 | 4.83 | 5.22 | 6.90 | 6.22 | 90.1% |

DW–dry weight

Analyzing the results for the geometric mean, the highest value was obtained by Zn (51.5 mg/kg DW), Pb had the second highest value (39 mg/kg DW), while Cd and Cu obtained results of 2.31 mg/kg DW and 5.22 mg/kg DW, respectively.
Following the arithmetic mean values, they ranged between 4.27 mg/kg DW for Cd and 105.2 mg/kg DW Zn.
Pb had an arithmetic mean of 73 mg/kg DW, while Cu recorded 6.90 mg/kg DW.
Regarding the standard deviation, the lowest value was obtained for Cd (4.54 mg/kg DW), followed by Cu with a value of 6.22 mg/kg DW.
Pb had a standard deviation of 70.2 mg/kg DW, while Zn had the highest value (111.2 mg/kg DW).
Analyzing the coefficient of variation, the highest result was obtained by Pb (108.5%) and the lowest by Cu (90.1%). For the other metals, the values were 106.3% for Cd and 105.7% for Zn, respectively.
The high values obtained for the coefficient of variation and standard deviation indicate that pollution does not show a uniform distribution and that there are areas where the accumulation of heavy metals is significant. The median values are not influenced by extremes as is the case for the arithmetic mean. At the same time the geometric mean indicates a possibly asymmetric distribution of pollution.
Table 3 shows the statistical parameters that characterize the central tendency and variability of heavy metals content (Cd, Pb, Zn and Cu) in *Festuca rubra* plants.

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 fescue (*Festuca rubra* L.) (n = 17)

| Variable | Minimum | Maximum | Median | Geometric mean | Arithmetic mean | Standard deviation | Coefficient of variation |
|---|---|---|---|---|---|---|---|
| | | | mg/kg DW | | | | |
| $Cd_{F.rubra}$ | 0.01 | 1.64 | 0.39 | 0.16 | 0.47 | 0.48 | 102.1% |
| $Pb_{F.rubra}$ | 0.09 | 1.92 | 0.35 | 0.36 | 0.62 | 0.61 | 98.4% |
| $Zn_{F.rubra}$ | 7.4 | 89.2 | 50.1 | 36.0 | 47.6 | 29.5 | 62% |
| $Cu_{F.rubra}$ | 1.19 | 7.53 | 3.35 | 3.44 | 3.84 | 1.79 | 46.6% |

DW - dry weight

The ability of *Festuca rubra* to accumulate Cd, Pb, Zn and Cu indicates that it can be used in the phytoremediation of polluted soils (Gajić et al., 2020; Pusz et al., 2021).

Korzeniowska and Stanislawska-Glubiak (2023) demonstrated in a study regarding Zn accumulation in different grass species that *Festuca rubra* compared to *Deschampia caepitosa* is more suitable for phytostabilization than for phytoextraction because it has the ability to retain this element in the roots and limits the translocation to the leaves.

Gołda & Korzeniowska (2016) showed that *Festuca rubra* accumulates high amounts of Cd in the roots and translocates very small amounts to the aerial parts.

Fernández et al. (2017) reported that *Festuca rubra* is an efficient species for phytostabilization of heavy metals especially mercury and arsenic from contaminated areas.

Wyszkowska et al. (2022) investigated the potential use of *Festuca rubra* in phytostabilization of soils contaminated with heavy metals, including Zn and Cu and the results demonstrated that this species efficiently accumulates these metals, being suitable for phytostabilization in contaminated environments.

Based on the minimum and maximum heavy metals content in the plant, Cd levels ranged from 0.01 mg/kg DW to 1.64 mg/kg DW, while Pb varied between 0.09 mg/kg DW and 1.92 mg/kg DW, Zn content ranged from 7.4 mg/kg DW to 89.2 mg/kg DW, while Cu levels were between 1.19 mg/kg DW and 7.53 mg/kg DW.

Analyzing the median values, the highest concentration was obtained by Zn (50.1 mg/kg DW), while Pb had the lowest (0.35 mg/kg DW). The median values for Cd and Cu were 0.39 mg/kg DW and 3.35 mg/kg DW, respectively.

Analyzing the arithmetic mean values, Zn had the highest concentration (47.6 mg/kg DW), followed by Cu (3.84 mg/kg DW). The mean values for Cd and Pb were 0.47 mg/kg DW and 0.62 mg/kg DW respectively.

The arithmetic mean significantly higher than the median in the case of Pb and Cd (0.62 mg/kg DW vs. 0.35 mg/kg DW and 0.47 mg/kg DW vs. 0.39 mg/kg DW, respectively), suggests the presence of potential contamination hotspots while for Cu, the small difference between the mean and the median indicates a more uniform distribution.

Regarding the geometric mean, values ranged from 0.16 mg/kg DW for Cd to 36 mg/kg DW for Zn. The geometric mean values for Pb and Cu were 0.36 mg/kg DW and 3.44 mg/kg DW, respectively.

In a study conducted by Gómez et al. (2016) they showed that *Festuca rubra* tolerates high concentrations of Zn but is sensitive to Pb, especially in the mobile form (Pb-EDTA).

However, in a study by Begonia et al. (2005), the addition of EDTA and acetic acid was shown to enhance Pb uptake in tall fescue by increasing its translocation index, leading to higher Pb concentrations in shoots. This suggests that chelate amendments may improve the efficiency of tall fescue for phytoextraction.

In terms of standard deviation, Cd had the lowest value (0.48 mg/kg DW), the second lowest result was for Pb (0.61 mg/kg DW), followed by Cu with a value of 1.79 mg/kg DW, while Zn obtained the highest value (29.5 mg/kg DW).

Following the coefficient of variation, the highest value was obtained by Cd (102.1%) and the lowest value (46.6%) by Zn. Pb had a value of 98.4% while Cu obtained 62%.

The very high values of the coefficient of variation obtained for Cd and Pb (102.1% and 98.4%, respectively) suggest an extremely non-uniform accumulation of these metals in the plant indicating significant differences between harvesting points while the lower values obtained for Zn and Cu (62% and 46.6% respectively) suggest a more balanced accumulation in the sampled plants.

Following the high values obtained for standard deviation and coefficient of variation suggest that some areas are more affected than others.

Analyzing the log-log diagram for the power regression curves (Figure 1a), it can be observed that the correlation coefficient had distinctly significant value ($r = 0.697^{**}$) indicating a significant positive relationship between total Cd concentration in soil (mg/kg DW) and Cd concentration accumulated in the plant (*Festuca rubra*).

Similarly, the correlation coefficient value for soil Cd content DTPA-extractable form was also distinctly significant ($r = 0.659^{**}$) indicates a statistically significant correlation, but slightly weaker than for total Cd.

This relationship may signal a high mobility of Cd in soil and increased bioavailability, which

makes *Festuca rubra* an effective bioindicator of soil Cd contamination.

In a study conducted by Dong et al. (2019), they reported that tall fescue is effective in phytoremediation of Cd. They demonstrated that plants extract Cd from soil and can excrete it through leaves via hydathodes.

However, to reduce the risk of bioaccumulation it is necessary to add soil amendments that reduce Cd mobility, such as biochar, lime or zeolites.

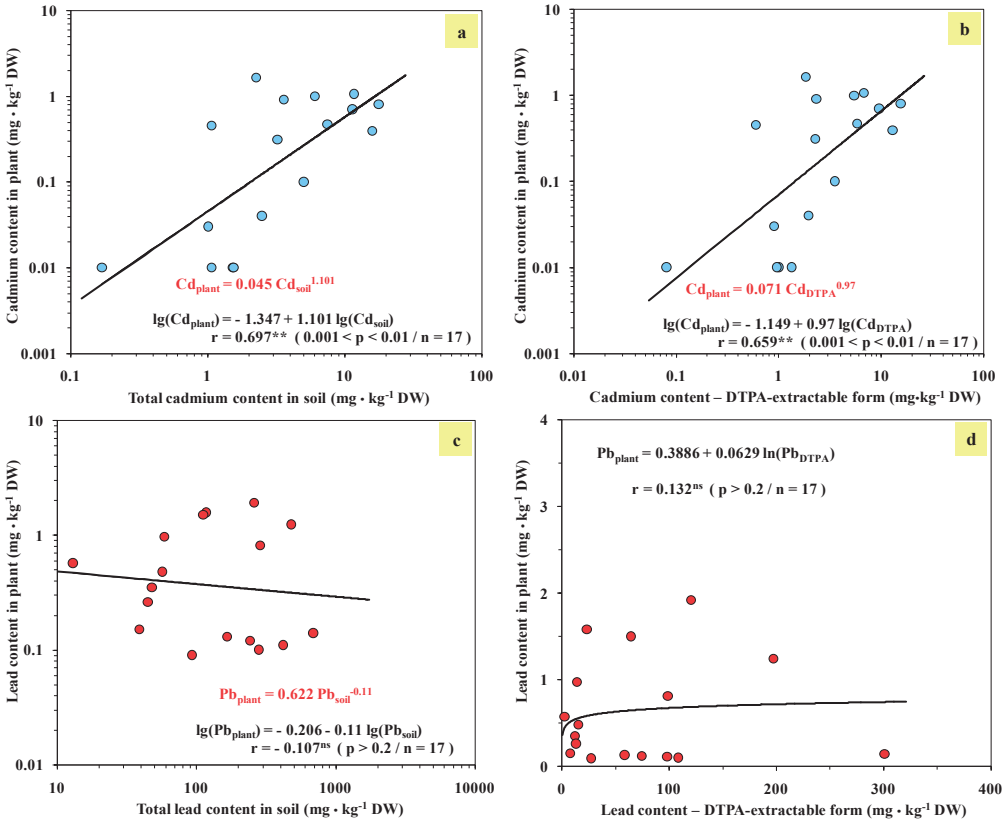


Figure 1. Log-log diagrams for power regression curves that estimate the stochastic dependency between total cadmium content in soil (a), soil cadmium content – DTPA-extractable form (b), total lead content in soil (c), soil lead content – DTPA-extractable form (d) and cadmium/lead contents in *Festuca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

Figures 1c, 1d show the relationship between the total Pb and DTPA-extractable form contents in the soil and Pb accumulated in the plant. A very weak correlation is observed suggesting that these parameters are not suitable to assess the accumulation of Pb in aerial parts of *Festuca rubra* plants.

The absorption and toxicity of Pb depends on both time and concentration and affects plants by reducing the rate of seed germination and impairing the ability to absorb nutrients due to its interference with enzymatic activities (Zulfiqar et al., 2019).

According to log-log diagram (Figure 2a) which presents the power regression curves that estimate the stochastic dependence between the total Zn content in soil and Zn content in *Festuca rubra*, the correlation coefficient had a highly significant value (r = 0.826***) indicating a strong correlation.

A highly significant value (r = 0.885***) of the correlation coefficient is also observed for the Zn DTPA–extractable form indicating that bioavailable of this metal is a determining factor for accumulation in the plant.

Wołejko et al. (2013) investigated the impact

of sewage sludge on the accumulation of heavy metals in soil and grass mixtures (*Lolium perenne*, *Festuca rubra* and *Poa pratensis*). They demonstrated that the application of sewage sludge positively influenced the growth of the grass mixture, with Cd, Zn and Cu being more readily assimilated by plants than Pb and Ni.

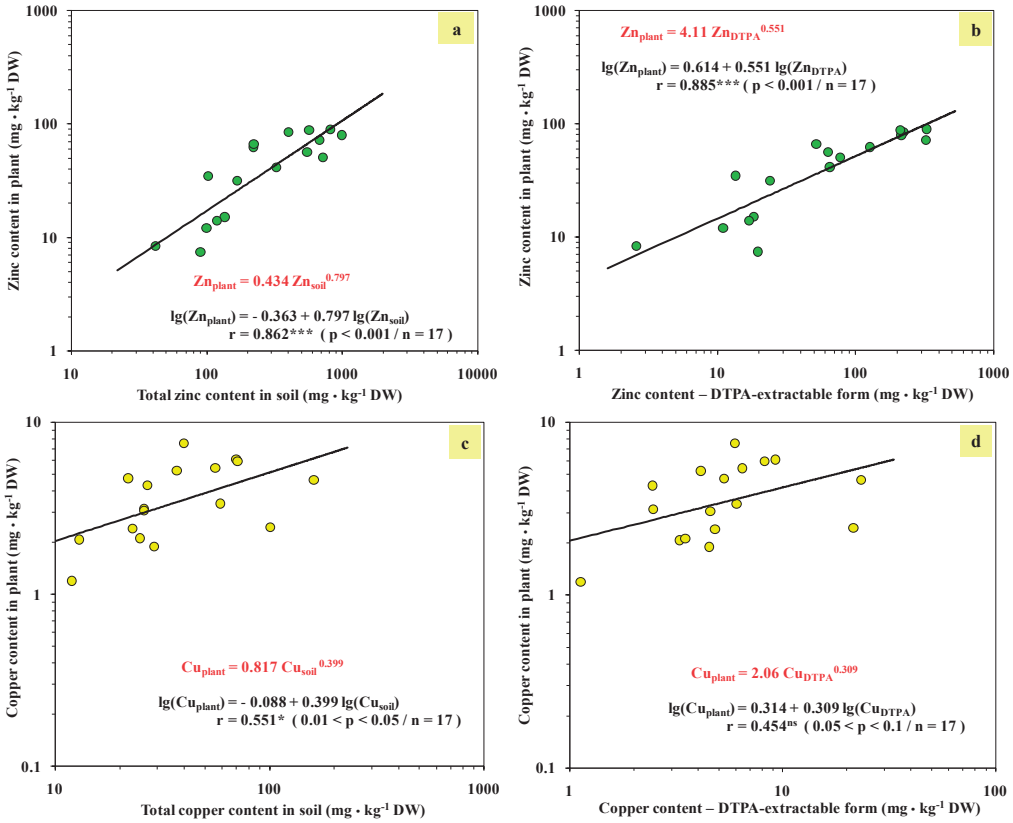


Figure 2. Log-log diagrams for power regression curves that estimate the stochastic dependency between total zinc content in soil (a), soil zinc content – DTPA–extractable form (b), total copper content in soil (c), soil copper content – DTPA–extractable form (d) and zinc/copper contents in *Festuca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

Analyzing the log-log diagram for the power regression curves that estimate the stochastic dependence between total Cu content in the soil and Cu content in plants (Figure 2c), it can be observed that the correlation coefficient value was significant (r = 0.551*). However, in the case of Cu content DTPA-extractable form (Figure 2d), it can be observed that the correlation coefficient value was insignificant (r = 0.454) suggesting a low accumulation rate of bioavailable Cu in the plant.

This could indicate either a higher Cu tolerance or an active uptake-regulating mechanism. Malagoli et al. (2014) reported that *Festuca rubra* accumulates more Cu in its roots than in its aerial parts.

Padmavathiamma and Li. (2009), demonstrated that *Festuca rubra* accumulated high amounts of Cu in the roots indicating that it is a plant that can be successfully used in the phytostabilization process.

## CONCLUSIONS

Cd content in soil recorded the lowest mean value (5.50 mg/kg total content and 4.27 mg/kg DTPA-extractable form) while Zn had the highest mean value (368.7 mg/kg total

content and 105.2 mg/kg DTPA-extractable form).

The bioaccumulation of Cd and Zn in aerial parts of the plant (*Festuca rubra*) can be estimated using the total content and DTPA–extractable forms in soil. However, for Cu and Pb, due to their low mobility within the soil–plant system, the studied parameters are not suitable indicators of bioaccumulation.

The results of this research showed that soils in the Copșa Mică area continue to have a high content of heavy metals, which may have a negative impact on the quality of human and animal life through their accumulation in the food chain. Continuous monitoring of areas at risk of heavy metals contamination is needed to assess the dynamics of heavy metals accumulation in soil and plants. Such research is essential for tracking changes in contamination levels and determining the safety of using pastures for livestock farming or using land for crop growing.

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