*Analele Universităţii din Craiova, seria Agricultură – Montanologie – Cadastru (Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series) Vol. 55/1/2025*

# STUDY REGARDING THE ASSESSMENT OF HEAVY METALS CONTENT IN *FESTUCA RUBRA* L. GROWN ON ARTIFICIALLY CONTAMINATED SOIL

**Bogdan Ștefan OPREA, Dumitru-Marian MOTELICĂ, Nicoleta Olimpia VRÎNCEANU, Vera CARABULEA, Georgiana Iuliana PLOPEANU, Mihaela COSTEA, Veronica TĂNASE**

*National Research and Development Institute for Soil Science, Agrochemistry and Environment, 61 Mărăşti Blvd, District 1, 011464, Bucharest, Romania*

*author email: bogdan.oprea@icpa.ro*

**Corresponding author email: vera.carabulea@icpa.ro**

**Abstract**

*This study aims to evaluate the heavy metals concentration in perennial grass species (Festuca rubra L.) grown on artificially contaminated soil with various doses of cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu). The experiment was set up in pots and included a control variant and five experimental variants. The concentrations used ranged from 3 to 15 mg•kg$^{-1}$ for Cd, 50 to 250 mg•kg$^{-1}$ for Pb, 300 to 1500 mg•kg$^{-1}$ for Zn, and 100 to 500 mg•kg$^{-1}$ for Cu. The soil used in the experiment had a loamy texture, moderate fertility with medium levels contents of nitrogen and organic matter and the pH was slightly alkaline (7.94). The concentrations of heavy metals were within normal limits for alert and intervention thresholds for sensitive land use according to Ministerial Order No. 756/1997. The results obtained from the analysis of the regression equations showed a very significant correlation between the total content and DTPA—extractable form of heavy metals in the soil, and their accumulation in Festuca rubra L. plants, which shows the potential of this species to extract and accumulate heavy metals from the soil depending on the type of metal, level of contamination, and the physico-chemical characteristics of the soil.*

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

## INTRODUCTION

Soil contamination is a major global problem for ecosystems health and food safety. According to Panagos et al. (2013), it is estimated that over 2.5 million sites are polluted globally, with Europe contributing significantly to this statistic.

The main cause of soil pollution globally is high anthropogenic activity, along with industrialization, agriculture, and poor waste management (FAO 2021).

The industrial and commercial sectors, along with municipal waste, contribute significantly to environmental contamination at the European level (Panagos et al., 2013).

Globally, significant pollutants include heavy metals (such as Cd, Pb, Zn, and Cu), polycyclic aromatic hydrocarbons (PAHs), solvents, and petroleum residues (FAO, 2021).

Heavy metals have a negative effect on plant metabolism and health by causing oxidative stress, affecting photosynthesis, respiration, or cell division (Morkunas et al., 2018; Ghori et al., 2019; Riyazuddin et al., 2022).

Metals such as Cu and Zn are essential for plant development in small amounts, but in high amounts they become toxic. Metals such as Cd and Pb are toxic even in low concentrations (Nagajyoti et al., 2010; Alengebawy et al., 2021).

According to Dubey et al. (2018), high concentrations of heavy metals in plants cause oxidative stress and damage cells.

Rashid et al. (2018) reported that heavy metal pollution of agricultural soils leads to decreased crop productivity and has a negative effect on the soil microbiome.

Phytoremediation is an environmentally friendly and low-cost method used to decontaminate soil affected by heavy metal pollution by using plants to extract or stabilize pollutants.

The phytostabilization process consists of immobilizing pollutants and preventing their migration into the environment (Laghlimi et al., 2015; Sarwar et al., 2017; Lavanya et al., 2024; Zhakypbek et al., 2024).

Pusz et al. (2021) reported that *Festuca rubra* can accumulate Zn and, in some cases, Cd and Cu. *Festuca rubra* tolerates and has a high potential for accumulating heavy metals (Sladkovska et al., 2022).

This study aims to evaluate the heavy metals concentration in perennial grass species (*Festuca rubra* L.) grown on artificially contaminated soil with different doses of Cd, Pb, Zn, Cu.

## MATERIALS AND METHODS

This study was conducted in 2024. The experiment was set up in pots, each containing 10 kg of soil, and included a control variant (unpolluted) and five experimental variants with different concentrations for each heavy metal studied (Cd, Pb, Zn, and Cu) derived from their soluble salts (acetate).

The concentrations used were as follows: for Cd ($v_1$ = 3 mg•kg$^{-1}$, $v_2$ = 6 mg•kg$^{-1}$, $v_3$ = 9 mg•kg$^{-1}$, $v_4$ = 12 mg•kg$^{-1}$, and $v_5$ = 15 mg•kg$^{-1}$); for Pb ($v_1$ = 50 mg•kg$^{-1}$, $v_2$ = 100 mg•kg$^{-1}$, $v_3$ = 150 mg•kg$^{-1}$, $v_4$ = 200 mg•kg$^{-1}$, and $v_5$ = 250 mg•kg$^{-1}$); for Zn ($v_1$ = 300 mg•kg$^{-1}$, $v_2$ = 600 mg•kg$^{-1}$, $v_3$ = 950 mg•kg$^{-1}$, $v_4$= 1200 mg•kg$^{-1}$, and $v_5$= 1500 mg•kg$^{-1}$) and Cu ($v_1$ = 100 mg•kg$^{-1}$, $v_2$ = 200 mg•kg$^{-1}$, $v_3$ = 300 mg•kg$^{-1}$, $v_4$ = 400 mg•kg$^{-1}$, and $v_5$ = 500 mg•kg$^{-1}$).

The concentrations were established considering the alert and intervention threshold values for sensitive land use, as specified in Ministerial Order No. 756/1997.

The soil used in experiment was collected from the first layer (0-20 cm) from Agârbiciu, Sibiu County.

To obtain an average sample four subsamples were taken from the collected soil, which was air-dried, ground, sieved to remove plant debris and homogenize the material. The soil sample was analyzed to determine its chemical and physical characteristics (pH, organic matter, available phosphorus (PAL), available potassium (KAL), coarse sand, fine sand, silt, clay, and texture) and heavy metals content.

Soil pH was determined potentiometrically in a 1:2.5 (g/v) soil-water suspension.

PAL and KAL were extracted with ammonium acetate lactate at pH 3.75, according to Romanian standard STAS 7184/19-82, based on the Egner-Riehm-Domingo procedure (1960).

The K content was measured by flame photometry, while the P content was determined by UV-Vis spectrophotometry.

Organic matter content was measured on 0.2 mm ground soil samples using the dichromate oxidation method, followed by titration with ferrous ammonium sulfate, according to Walkley and Black (1934).

The Kjeldahl method was used to determine total nitrogen (Nt).

The heavy metals content of the soil was determined by atomic absorption spectrometry after extraction by the aqua regia and microwave digestion method.

Microwave digestion was performed using 10 ml of aqua regia (7.5 ml HCl and 2.5 ml $HNO_3$) at 140 °C for 30 minutes, a method developed according to SR ISO 11466:1999.

DTPA- extractable heavy metals were exctracted from soil (10 g) with 20 ml of extracting solution (0.05 M DTPA, 0.01 M CaCl2 and 0.1 M tetraethylammonium adjusted to pH 7.3), according to SR ISO 14870:2002.

The plant species used in the experiment was *Festuca rubra* L., variety Căprioara, from the Research-Development Institute for Grasslands, Brașov.

After a six-week vegetation period, the plants were harvested with scissors, chopped, oven-dried, crushed, and treated with nitric acid in a microwave digestion system.

The heavy metals content in the plant (aerial part) was determined by atomic absorption spectrometry (Flame GBC 932AA for determining the Zn and Cu content and GBC SavanatAAZ graphite furnace for determining the Cd and Pb content).

The statistical processing of the data was done using Microsoft Excel 2010.

## RESULTS AND DISCUSSIONS

Analyzing Table 1.a, regarding the chemical characteristics of the soil used in the experiment, it can be observed that it had a slightly alkaline pH (7.9), which can influence the mobility of heavy metals by reducing their bioavailability, and low organic matter content (2.71%).

According to Rashid et al. (2023), the bioavailability of heavy metals to plants is higher at acidic pH and with low organic matter.

Table 1. Physico-chemical characteristics and heavy metals content of the soil (n=4)

a. Chemical characteristics

| pH $H_2O$ | Organic matter (%) | Nt (%) | PAL ($mg•kg^{-1}$) | KAL ($mg•kg^{-1}$) |
|---|---|---|---|---|
| 7.9 | 2.71 | 0.183 | 53 | 186 |

b. Physical characteristics

| Coarse sand (%) 2.0-0.2mm | Fine sand (%) 2.0-0.02mm | Silt (%) 0.02-0.002 mm | Clay (%) <0.002 mm | Texture |
|---|---|---|---|---|
| 12 | 44 | 19.3 | 24.7 | LL |

c. Heavy metals content ($mg•kg^{-1}$)

| Cd | Pb | Zn | Cu |
|---|---|---|---|
| 0.771 | 41.1 | 124 | 27.8 |

Elekes (2014), cited by Laghlimi et al. (2015), demonstrated that the availability of heavy metals to plants is directly influenced by soil reaction (pH) because their solubility and mobility in the soil solution are determined by soil acidity.

Heavy metals, especially Pb and Cr, are immobile at neutral or alkaline pH and become unavailable to plants (Mahmood 2010).

According to Olaniran et al. (2013), soils with low organic matter content are more likely to be contaminated with trace elements, and their bioavailability is higher. Applying compost to land contaminated with heavy metals can improve soil quality by reducing metal mobility (Brown et al., 2003).

The Nt and KAL contents had medium levels with values of 0.183 $mg•kg^{-1}$ and 186 $mg•kg^{-1}$, respectively, while the PAL content was high (53 $mg•kg^{-1}$) (Florea et al., 1987).

In case of physical characteristics (Table 1.b), it can be seen that the soil has a loamy texture (LL), consisting of 12% coarse sand, 44% fine sand, 19.3% silt, and 24.7% clay, providing good water retention capacity and good drainage.

Analyzing Table 1.c, the heavy metals content in the soil had the following values: 0.771 mg•kg$^{-1}$ for Cd, 41.1 mg•kg$^{-1}$ for Pb, 124 mg•kg$^{-1}$ for Zn, and 27.8 mg•kg$^{-1}$ for Cu. According to Ministerial Order No. 756/1997, these did not exceed the normal limits for alert and intervention thresholds for sensitive land use.

Figures 1.a, 1.b show the log-log diagrams for the regression curves that estimate the stochastic dependence between the total Cd content in the soil, the Cd content in the soil – extractable form with DTPA, and the Cd content in *Festuca rubra* L. plants.

Analyzing Figure 1.a, regarding the relationship between total Cd content in soil and Cd content in *Festuca rubra* L., a positive relationship between the two variables can be observed, with a very significant correlation coefficient of 0.9837.

In the case of the relationship between the Cd content – DTPA – mobile forms and the Cd content in *Festuca rubra* L. (Figure 1.b), the correlation coefficient recorded a very significant value of 0.9750, indicating a close positive relationship between the mobile fraction available to the plant and its absorption by the plant, showing that an increase in the Cd content in the soil causes an increase in the plant tissues.

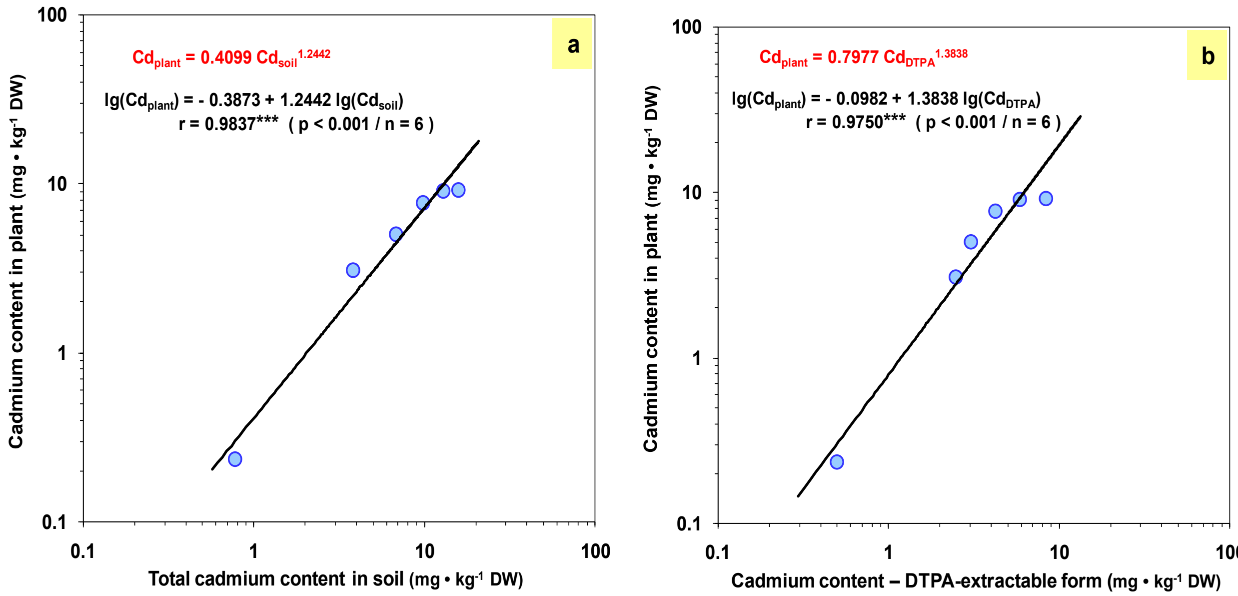


Figure 1. Log-log diagrams for regression curves that estimate the stochastic dependency between the total cadmium content in soil (a), the soil cadmium content – DTPA-extractable form (b) and the cadmium content in the *Festuca rubra* plants.

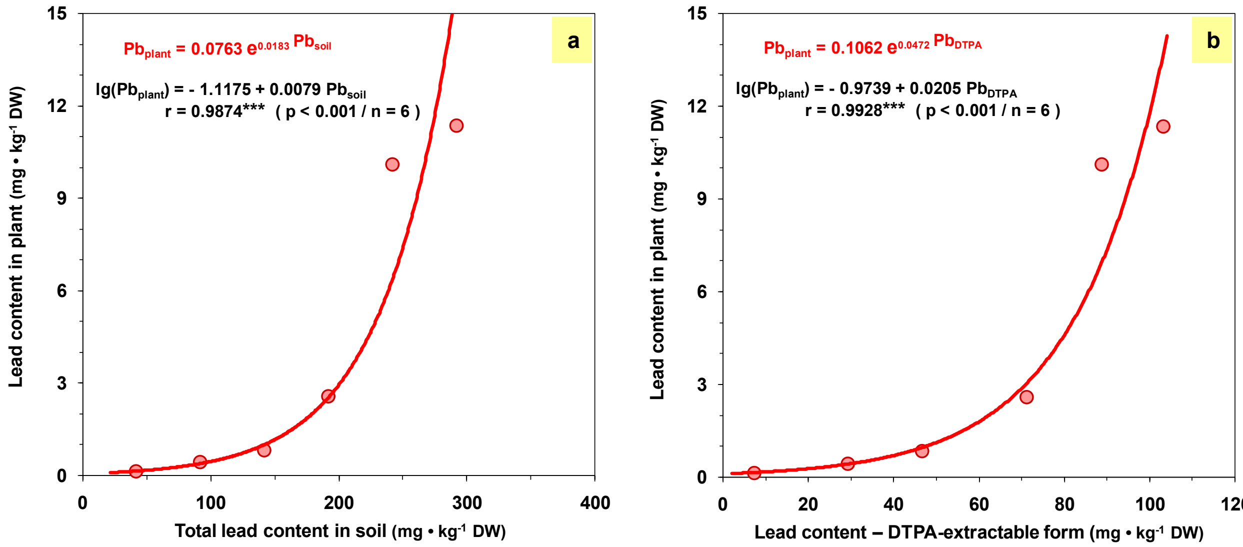


Figure 2. Exponential regression curves that estimate the stochastic dependency between the total lead content in soil (a), the soil lead content – DTPA-extractable form (b) and the lead content in the *Festuca rubra* plants.

Golda and Korzeniowska (2016) compared tolerance to Cd in three species of perennial grasses (*Poa pratensis, Lolium perenne,* and *Festuca rubra*). They reported that *Festuca rubra* is a species suitable for phytostabilization rather than phytoextraction.

Based on the transfer values obtained for Cd content by Pusz et al. (2021), they classified *Festuca rubra* as a plant suitable for phytostabilization.

Figure 2.a, 2.b shows exponential regression curves estimating the relationship between total Pb content in soil (2.a), DTPA-extractable Pb in soil (2.b), and Pb in *Festuca rubra*.

The correlation coefficients were very significant: 0.9874 for the relationship between total soil content and plant content, and 0.9928 for the the relationship between mobile fraction and *Festuca rubra* content.

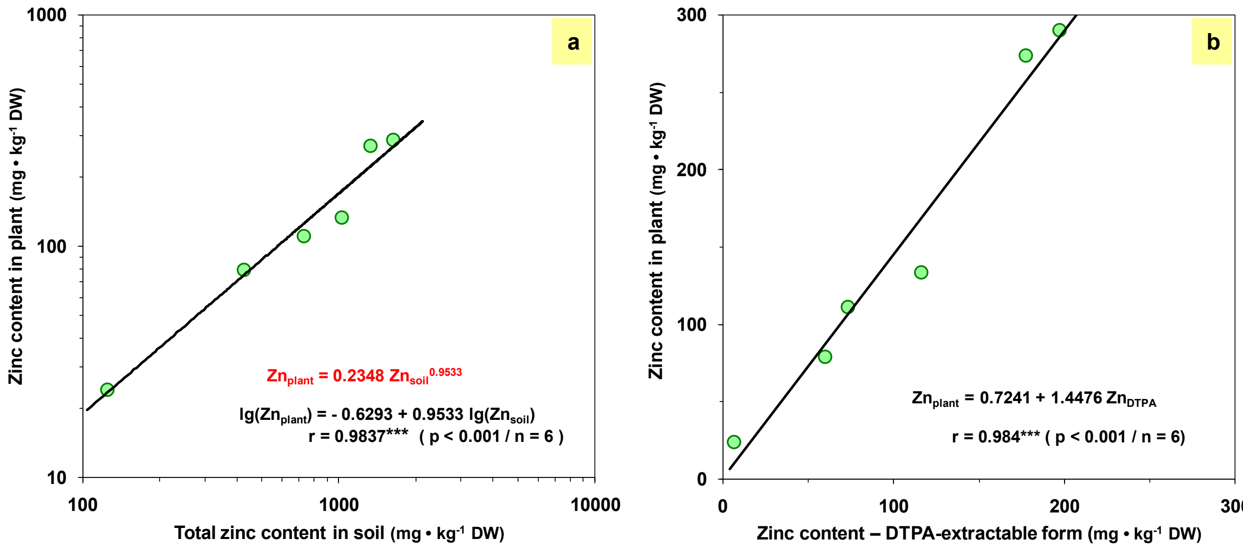


Figure 3. Regression equations that estimate the stochastic dependency between the total zinc content in soil (a), the soil zinc content – DTPA-extractable form (b) and the zinc content in the *Festuca rubra* plants.

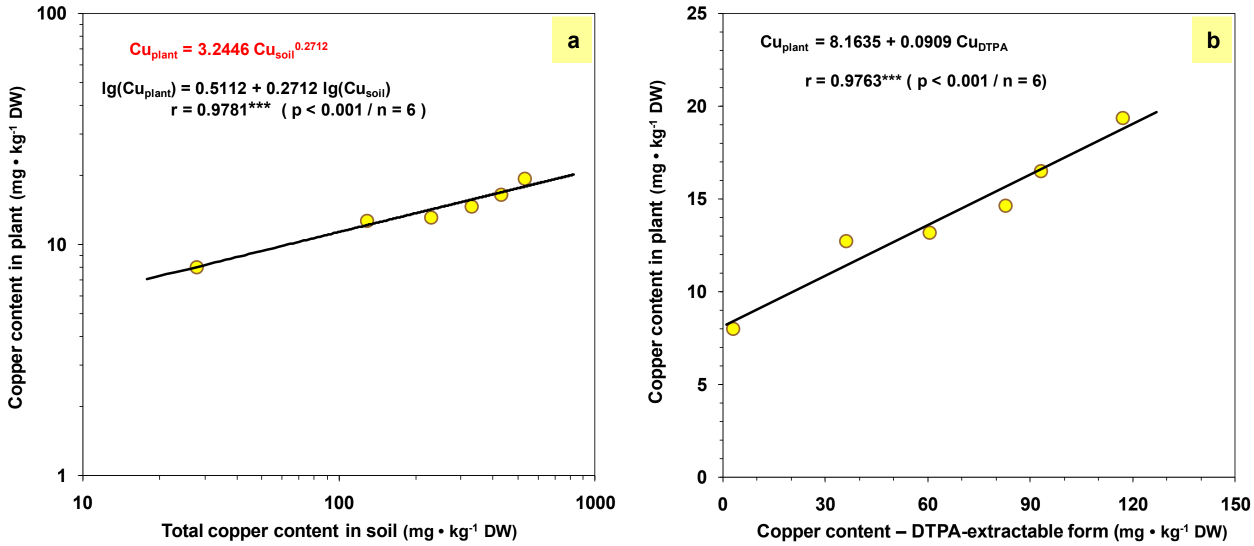


Figure 4. Regression equations that estimate the stochastic dependency between the total copper content in soil (a), the soil copper content – DTPA-extractable form (b) and the copper content in the *Festuca rubra* plants.

Kushwaha et al. (2018) reported that Pb content in plants correlates more closely with the bioavailable fraction than with the total soil content, and that the metal's mobility and soil properties significantly influences its transfer. In a study conducted by Ginn et al. (2008), they reported that Pb can remain immobilized in the roots of *Festuca rubra* and that root-metal interactions can limit translocation.

In Figure 3.a, 3.b regarding Zn content, the regression equations showed strong correlation among total content, mobile form, and content accumulated in the plant. The correlation coefficient values obtained were very significant in both cases: 0.9837 for the relationship between total Zn content and Zn content in the plant and 0.984 for the relationship between mobile Zn content and that accumulated in *Festuca rubra*.

For Cu content (Figure 4a. 4b), the regression equations showed a positive dependence, but lower compared to the Zn content. The correlation coefficient between the total content in the soil and the Cu content in the plant was 0.9781, while in the case of the correlation between the mobile Cu content in the soil and that in *Festuca rubra*, the result was 0.9763.

Gawrylik et al. (2020), in a comparative study on the bioaccumulation of Cu, Pb, and Zn in different grass species, including *Festuca rubra*, demonstrated that these species can accumulate high amounts of Cu and Zn from the soil, which are more readily available, and transfer them to the aerial parts compared to Pb.

## CONCLUSIONS

The research results showed a strong correlation between the variables studied, demonstrating that the increase in heavy metals content in plants is directly correlated with the increase in heavy metals content in the soil.

The correlation coefficients recorded very significant values, which means that the bioaccumulation of the four metals studied (Cd, Pb, Zn, and Cu) in this experiment, can be estimated using the total and mobile content (DTPA) in the soil.

The plant used in this study (*Festuca rubra* L.) has the ability to extract and accumulate heavy metals in its vegetative parts and can be used in phytoremediation processes.

**ACKNOWLEDGEMENTS**

This work was financed by project number PN 23.29.04.01 entitled: "Assessment of heavy metals bioaccumulation in meadows vegetation using the regression analysis to develop a guide of good practices for grazing and animal feedstock in areas affected by industrial pollution".

**REFERENCES**

Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R., & Wang, M.-Q. (2021). Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. *Toxics*, *9*(3), 42.

Brown, S.L., Henry, C.L., Chaney, R., Compton, H. and De Volder, P.S. (2003) Using Municipal Biosolids in Combination with Other Residuals to Restore Metal-Contaminated Mining Areas. Plant Soil, 249, 203-215.

Dubey, S., Shri, M., Gupta, A., Rani, V., & Chakrabarty, D. (2018). Toxicity and detoxification of heavy metals during plant growth and metabolism. Environmental Chemistry Letters, 16, 1169-1192.

Egner H, Riehm H, Domingo WR (1960). Untersuchungen uber die chemische Bodenanalyse als Grundlage fur die Beurteilung de Nahrstoffzustandes der Boden. II. K Lantbr Hogsk Ann 26:199-215.

Elekes, C. C. (2014). Eco-technological solutions for the remediation of polluted soil and heavy metal recovery. *Environmental Risk Assessment of Soil Contamination, InTech, Rijeka*, 309-335.

Florea, N., Bălăceanu, V., Răuță, C., Canarache, A., (1987). Methodology for conducting soil studies. Part III—Ecopedological indicators. Bucharest.

Food and Agriculture Organization (FAO). (2021). *Global assessment of soil pollution*. Rome. https://openknowledge.fao.org/server/api/core/bitstreams/8fa95d84-bb05-4c77-b02e-425f70ba6834/content

Gawryluk, A., Wyłupek, T., & Wolański, P. (2020). Assessment of Cu, Pb and Zn content in selected species of grasses and in the soil of the roadside embankment. Ecology and evolution, 10(18), 9841–9852.

Ghori, N., Ghori, T., Hayat, M., Imadi, S., Gul, A., Altay, V., & Ozturk, M. (2019). Heavy metal stress and responses in plants. *International Journal of Environmental Science and Technology*, 16, 1807-1828.

Gołda, S., & Korzeniowska, J. (2016). Comparison of phytoremediation potential of three grass species in soil contaminated with cadmium. Environmental Protection and Natural Resources, 27(1), 8-14.

Laghlimi, M., Baghdad, B., Hadi, H., & Bouabdli, A. (2015). Phytoremediation Mechanisms of Heavy Metal Contaminated Soils: A Review. *Open Journal of Ecology*, 5, 375-388.

Lavanya, M., Viswanath, D., & Sivapullaiah, P. (2024). Phytoremediation: An eco-friendly approach for remediation of heavy metal-contaminated soils-A comprehensive review. Environmental Nanotechnology, Monitoring & Management.

Mahmood, T. (2010) Phytoextraction of Heavy Metals—The Process and Scope for Remediation of Contaminated Soils. Soil & Environment, 29, 91-109.

Ministry Order No. 756 from November 3, 1997 for approval of Regulation concerning environmental pollution assessment published in Official Monitor No. 303/6 November 1997.

Morkunas, I., Woźniak, A., Mai, V. C., Rucińska-Sobkowiak, R., & Jeandet, P. (2018). The Role of Heavy Metals in Plant Response to Biotic Stress. *Molecules*, *23*(9), 2320.

Nagajyoti, P., Lee, K., & Sreekanth, T. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental Chemistry Letters*, 8, 199-216.

Olaniran, A.O., Balgobind, A. and Pillay, B. (2013) Bioavailability of Heavy Metals in Soil: Impact on Microbial Biodegradation of Organic Compounds and Possible Improvement Strategies. International Journal of Molecular Sciences, 14, 10197-10228.

Panagos, P., Van Liedekerke, M., Yigini, Y., & Montanarella, L. (2013). Contaminated sites in Europe: review of the current situation based on data collected through a European network. *Journal of environmental and public health*, *2013*(1), 158764.

Pusz, A., Wiśniewska, M., & Rogalski, D. (2021). Assessment of the accumulation ability of Festuca rubra L. and Alyssum saxatile L. tested on soils contaminated with Zn, Cd, Ni, Pb, Cr, and Cu. Resources, 10(5), 46.

Rashid, A., Schutte, B., Ulery, A., Deyholos, M., Sanogo, S., Lehnhoff, E., & Beck, L. (2023). Heavy Metal Contamination in Agricultural Soil: Environmental Pollutants Affecting Crop Health. Agronomy.

Riyazuddin, R., Nisha, N., Ejaz, B., Khan, M. I. R., Kumar, M., Ramteke, P. W., & Gupta, R. (2022). A Comprehensive Review on the Heavy Metal Toxicity and Sequestration in Plants. *Biomolecules*, *12*(1), 43.

Sarwar, N., Imran, M., Shaheen, M., Ishaque, W., Kamran, M., Matloob, A., Rehim, A., & Hussain, S. (2017). Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. *Chemosphere*, 171, 710-721 .

Zhakypbek, Y., Kossalbayev, B., Belkozhayev, A., Murat, T., Tursbekov, S., Abdalimov, E., Pashkovskiy, P., Kreslavski, V., Kuznetsov, V., & Allakhverdiev, S. (2024). Reducing Heavy Metal Contamination in Soil and Water Using Phytoremediation. Plants, 13.