SOIL HEAVY METALS CONTENT AND BIOACCUMULATION BY THE BLACK MEDICK (*MEDICAGO LUPULINA* L.) PLANTS HARVESTED FROM THE POLLUTED GRASSLANDS IN COPȘA MICĂ AREA

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

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

Environmental contamination with heavy metals resulting from anthropogenic activities affects the quality of human life. Heavy metals enter the human body mainly by ingesting contaminated food or water. Due to the extraction of non-ferrous ores for about half a century, Copșa Mică has been one of the most polluted cities in Europe. In this study, soil and plant samples were collected from polluted grasslands and analyzed for cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) content. The mean values (mg/kg dry weight) obtained for heavy metals total content in soil were as follows: Cd = 5.07 mg/kg D.W., Pb = 183.3 mg/kg D.W., Zn = 330.8 mg/kg D.W., and Cu = 40.3 mg/kg. Analyzing the heavy metals content in the plant, Cd recorded a mean value of 1.19 mg/kg D.W., Pb had a value of 1.75 mg/kg D.W., while Zn and Cu content recorded values of 67.8 mg/kg D.W., respectively 5.47 mg/kg D.W. According to Ministerial Order 756/1997, the values obtained for Cd, Pb, and Zn concentrations in the soil exceeded the permitted limits while Cu remained within normal values.

Key words: bioaccumulation, heavy metals, Medicago lupulina, pollution

INTRODUCTION

Pollution with heavy metals represents a major environmental problem and affects the quality of human and animal life. Studies showed that Cd is a toxic element for both humans and animals and, once absorbed, has a low rate of elimination from the body, leading to chronic diseases including lung, kidney, or prostate cancer (Wang et al., 2013; Genchi et al., 2020).

Food is the primary source of Cd intake, mostly stored in the kidneys (Jarup et al., 2009). Pb is easily absorbed by plants and causes several imbalances, such as growth retardation, inhibition of photosynthesis, and chlorosis.

The accumulation of this element in the plant is facilitated by certain physico-chemical parameters of the soil: soil type, pH, and cation exchange capacity (Sharma et al., 2005). Pb accumulates in the organs of the human body and causes fertility disorders, fatigue, increased blood pressure, encephalopathy, or death (Charkiewicz et al., 2020).

Cu is a necessary element for the normal development of plants, participating in various physiological processes, but in high amounts, it becomes toxic and can inhibit plant growth (Yruela, 2005). Soil type influences the bioavailability of Cu for plants.

Gharbi et al. (2021) reported higher amounts of Cu extracted from sandy clay loam soil than from clay loam soil. Cu plays an important role in the proper functioning of the body. Together with iron, it helps to create red blood cells and maintain healthy blood vessels, nerves, and immune system (Rajeswari et al., 2014).

Studies have shown that high amounts of Cu in the body cause inflammation and tissue damage, and negatively affect the nervous system, cardiovascular systems, hepatic function, and adrenal function (Ashish et al., 2013).

Another micronutrient essential for plant growth is Zn. Studies have shown that excess Zn is more common in acid soils. However, the phytotoxicity of Zn causes numerous physiological and biochemical imbalances in plants such as chlorosis, necrosis, growth inhibition, reduced productivity, etc. (Balafrej et al., 2020; Kaur et al., 2021).

Peralta et al. (2001) reported in a study on the effects of heavy metals on alfalfa plant that root and shoot elongation was reduced as heavy metals concentrations were increased.

Medicago lupulina L. (black medick) is a perennial legume from the *Fabaceae* family. It is mainly used for animal fodder and as a pasture crop due to its high nutrient content and self-reproduction potential (Malysheva et al., 2022; Wilson, 2005). Also, Matanzas et al. (2021) claim that this species can translocate As and Pb. Kong et al. (2015) demonstrated that black medick plants accumulated more Cu in roots compared to the shoots.

Also, black medick is an indicator plant for the presence of Ni and Mn in soil (Ahatović Hajro et al., 2024).

MATERIALS AND METHODS

Soil and plant samples (*Medicago lupulina* L.) were collected from polluted grasslands belonging to the Copșa Mică area and neighboring localities (Axente Sever, Micăsasa, Târnava) to determine the heavy metals content. Each soil sample was a mixture of 13 sub-samples collected from 0-20 cm depth. The samples were air-dried at room temperature, crushed, and sieved. Heavy metals were determined by atomic absorption spectrometry after the extraction using the aqua regia-microwave digestion method. 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. Black medick plant samples (aerial part) were oven dried then milled and treated with nitric acid in a microwave digestion system. To determine the heavy metals content was used atomic absorption spectrometry (Flame GBC 932AA or Graphite furnace GBC SavanatAAZ). The statistical processing of the data was done using Microsoft Excel 2010.

Figure 1. Soil and plant samples (original photo)

RESULTS AND DISCUSSIONS

Analyzing 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 can be observed that the minimum and maximum values ranged for Cd content between 1.22 and 16.92 mg/kg D.W., for Pb between 42 and 599 mg/kg D.W., while the Zn and Cu contents obtained results between 97 and 930 mg/kg D.W., respectively 13 and 108 mg/kg D.W. In terms of arithmetic mean value, Zn content recorded the highest result (330.8 mg/kg D.W.), while Cd content had the lowest value (5.07 mg/kg D.W.), Pb content recorded a value of 183.3 mg/kg D.W., and Cu 40.3 mg/kg D.W. However, Cd content obtained the highest value for the coefficient of variation (88%), while Zn had the lowest (73.4%). Regarding the geometric mean, the values ranged from 3.79 mg/kg D.W. for Cd to 266.6 mg/kg D.W. for Zn. Cu and Pb contents had values of 32.3 mg/kg D.W., respectively, 141.4 mg/kg D.W. Following these results it was determined that Cd, Pb contents exceeded the alert and intervention thresholds for sensitive land use. However, Zn content exceeded only the alert threshold, while Cu was within normal limits (Ministerial Order 756/1997).

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=12)

Variable	Minimum	Maximum	Median	Geometric mean	Arithmetic mean	Standard deviation	Coefficient of variation
	- -			mg/kg D.W.- -			
Cd_{soil}	1.22	16.92	3.43	3.79	5.07	4.46	88.0%
Pb_{soil}	42	599	115	141.4	183.3	153.9	84.0%
Zn_{soil}	97	930	224.5	266.5	330.8	242.9	73.4%
Cu_{soil}	13	108	29	32.2	40.3	31.6	78.4%

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=12)

Variable	Minimum	Maximum	Median	Geometric mean	Arithmetic mean	Standard deviation	Coefficient of variation
	- -			mg/kg D.W.- -			
Cd_{DTPA}	0.83	9.11	2.30	2.53	3.36	2.72	81%
Pb_{DTPA}	14	175	39.5	49.5	70	58.8	84%
Zn_{DTPA}	8	249	52	47.2	68.3	66.1	96.8%
Cu_{DTPA}	1.59	15.10	4.31	4.46	5.37	3.78	70.4%

D.W. - Dry Weight

Following the minimum and maximum values of soil heavy metal contents (DTPA- extractable forms), according to Table 2, it can be seen that they varied as follows: Cd (0.83-9.11 mg/kg D.W.), Pb (14-175 mg/kg D.W.), Zn (8-249 mg/kg D.W.) and Cu (1.59-15.10 mg/kg D.W.). Analyzing the arithmetic mean, the obtained results ranged from 3.36 mg/kg D.W. for Cd to 70 mg/kg D.W. for Pb. The Zn content had a value of 68.3 mg/kg D.W. while for Cu the result was 5.37 mg/kg D.W. In terms of coefficient of variation, the lowest result was obtained for Cu

(70.4%) while the highest concentration was obtained for Zn (96.8%). Cd and Pb had values of 81%, respectively 84%. Analyzing the results for the standard deviation it can be observed that they ranged from 2.72 mg/kg D.W. for Cd to 66.1 mg/kg D.W. for Pb. In the case of the geometric mean, Pb (49.5 mg/kg D.W.) recorded the highest result compared to Cd content which had the lowest value (2.53 mg/kg D.W.). Median values ranged from 2.30 mg/kg D.W. for Cd to 39.5 mg/kg d.w for Pb.

Table 3. Values of statistical parameters that characterize the central tendency and the variability of the cadmium, lead, zinc, and copper contents in the black medick (*Medicago lupulina* L.) (n=12)

Variable	Minimum	Maximum	Median	Geometric mean	Arithmetic mean	Standard deviation	Coefficient of variation
	mg/kg D.W.						
Cd_{plant}	0.09	3.54	0.80	0.72	1.19	1.11	93.3%
Pb_{plant}	0.18	3.86	1.59	1.26	1.75	1.18	67.4%
Zn_{plant}	26	173	53.5	58.1	67.8	44.9	66.2%
Cu_{plant}	1.6	9.5	5.25	4.99	5.47	2.21	40.4%

D.W. - Dry Weight

Concerning the results for the values of the statistical parameters characterizing the central tendency and variability for Cd, Pb, Zn, and Cu contents in black medick plants, according to Table 3, Zn content obtained the highest values (median = 53.5 mg/kg D.W., geometric mean = 58.1 mg/kg D.W.), except for the coefficient of variation (66.2%).

However, Cd obtained the lowest results (median = 0.80 mg/kg D.W., geometric mean = 0.72 mg/kg D.W.), but in terms of the coefficient of variation, it had the highest value (93.3%).

The heavy metal contents in the plant ranged from 0.09 to 3.54 mg/kg D.W. for Cd, from 0.18 to 3.86 mg/kg D.W. for Pb, Zn had values between 26 and 173 mg/kg D.W., while Cu content ranged from 1.6 to 9.5 mg/kg D.W. Amer et al. (2012) reported that *M. lupulina* accumulated high amounts of Pb in roots.

Regarding the arithmetic mean values, Cd content was the lowest (1.19 mg/kg D.W.), and Pb was the second lowest (1.75 mg/kg D.W.). The highest mean value was obtained for Zn (67.8 mg/kg D.W.), and Cu had a concentration of 5.47 mg/kg D.W. Analyzing the standard deviation, the data in Table 3 show that Cd (1.11 mg/kg D.W.) obtained the lowest value, followed by Pb (1.18 mg/kg d.w.) and Cu (2.21 mg/kg D.W.), while Zn had the highest result (44.9 mg/kg D.W.).

Vardumyan et al. (2024) analyzed black medick for the phytoremediation process of Cu-polluted soils using different additives. They demonstrated that *M. lupulina* is a good hyperaccumulator of this metal.

Jian et al (2019) reported that the application of 400 mg/kg of Cu and Zn inhibited black medick plant growth.

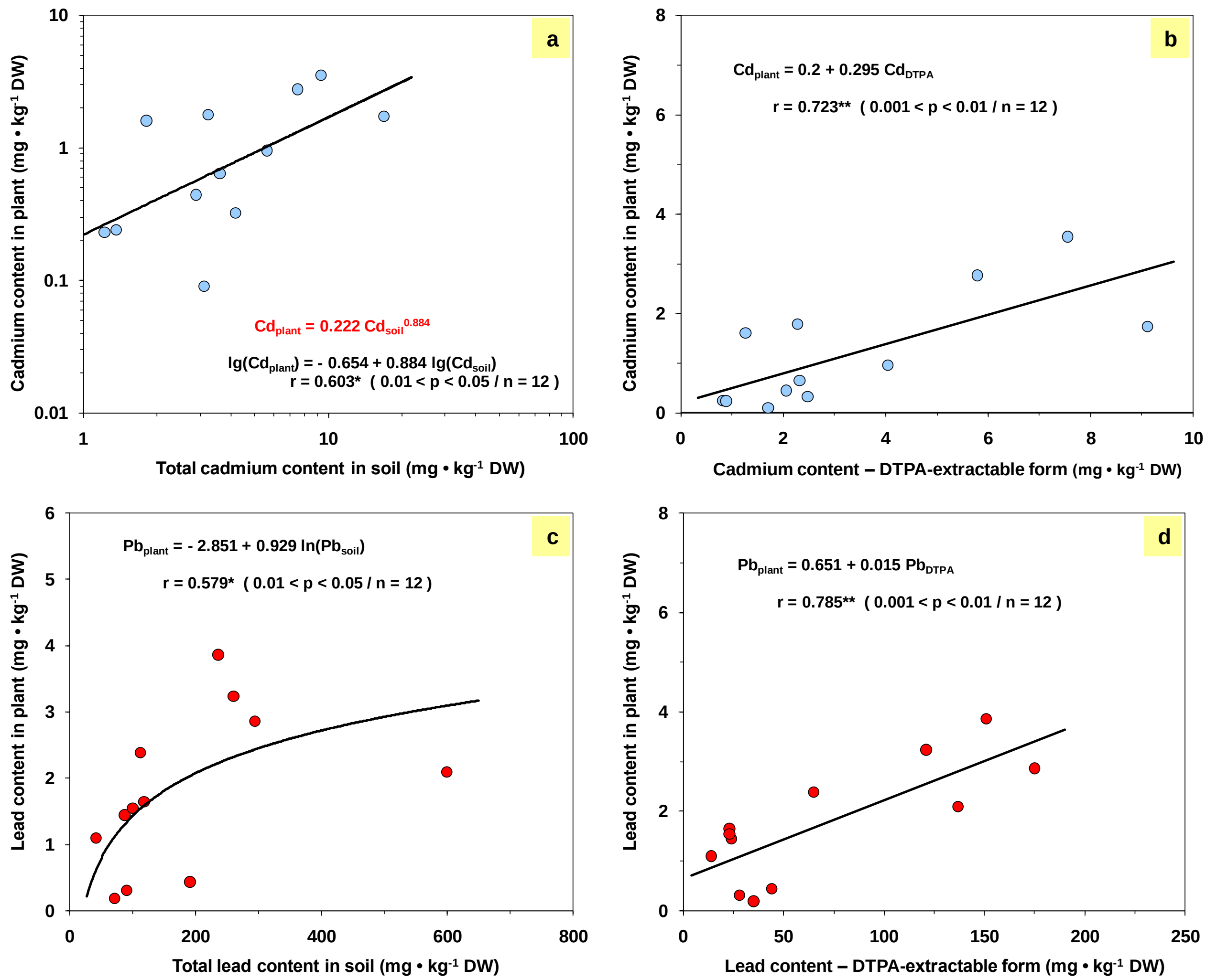

Figure 2. Log-log diagrams for power regression curves that estimate the stochastic dependency between total cadmium content in soil (a), soil cadmium content – DTPA-extractable form (b), total lead content in soil (c), soil lead content – DTPA-extractable form (d) and cadmium/lead contents in *Medicago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

Figure 2 shows logarithmic diagrams for power regression curves that estimate the stochastic dependency between total soil content and DTPA-extractable forms of Cd and Pb and the amounts of those metals in black medick plants.

According to Figures 2a and 2c, the values of linear correlation coefficients for total soil contents (r = 603* for Cd and r = 0.579* for Pb) were significantly different from zero.

However, Figures 2b and 2d showed that the correlation coefficient recorded distinctly significant values for DTPA-extractable forms of Cd (r = 0.723**) and Pb (r=785**), indicating a good correlation between the soil metals contents and metals content in black medick plants.

Analyzing the values of the correlation coefficients for the total Zn and Cu content in soil according to Figures 3a and 3c the result was insignificant in both cases (r = 0.327 for Zn and r = 0.477 for Cu).

Concerning the results obtained for the mobile forms of heavy metals in soil, according to Figures 3b and 3d, the value of Zn content (r = 787**) was distinctly significantly different indicating a good correlation between the Zn content in the plant and the soil content, while the result obtained for Cu (r = 0.499) was insignificant.

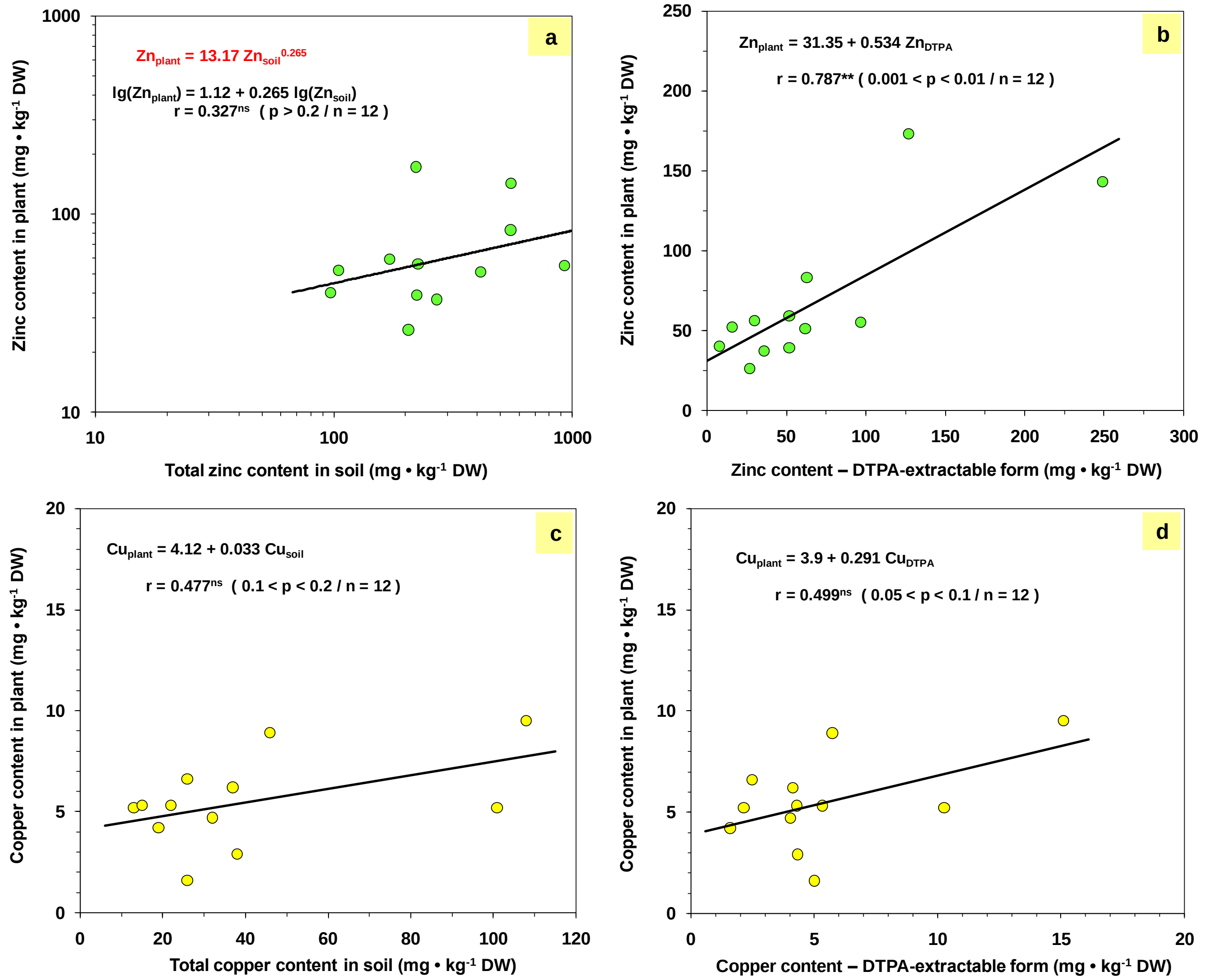

Figure 3. Log-log diagrams for power regression curves that estimate the stochastic dependency between total zinc content in soil (a), soil zinc content – DTPA-extractable form (b), total copper content in soil (c), soil copper content – DTPA-extractable form (d) and zinc/copper contents in *Medicago lupulina* plants

CONCLUSIONS

The results obtained in this study indicate that soil metals concentration influences the metals levels in the black medick plant. The lowest levels were recorded for Cd, with 5.07 mg/kg D.W. in total soil content, 3.36 mg/kg D.W. in extractable form, and 3.36 mg/kg D.W. in the plant.

Pb obtained the highest level for the extractable form (70 mg/kg D.W.) and second highest concentration for total soil content (183.3 mg/kg D.W.). However, according to Ministerial Order 756/1997, Cd and Pb exceeded the alert and intervention thresholds for sensitive land use.

Zn concentration reached the highest value for the total soil content (330.8 mg/kg D.W.) exceeding the alert threshold for sensitive land use (Ministerial Order 756/1997). Also, in black medick plant, Zn content had the highest level (67.8 mg/kg D.W.).

Regarding Cu content, it was within permitted limits, with a total soil content of 40.3 mg/kg D.W., while the concentration for the extractable form was 5.37 mg/kg D.W. In the plant, Cu had the second-highest level (5.47 mg/kg D.W.). The total heavy metals content in the soil ranked as follows: Zn>Pb>Cu>Cd. However, the mobile forms in the soil were ordered as Pb>Zn>Cu>Cd. Within the black medick plants, the metal content followed this order: Zn> Cu> Pb>Cd.

In conclusion, the results obtained show that the studied area still presents a high risk of contamination due to the levels of heavy metals above the permissible limit. These results suggest a potential risk of bioaccumulation in the food chain, thus it is necessary to monitor these meadows in the future and to implement remedial measures to reduce soil metal levels.

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

Ahatović Hajro, A., Hasanović, M., Murtić, S., Kalajdžić, A., Pojskić, N., & Durmić-Pašić, A. (2024). Serpentine environment prevails over geographic distribution in shaping the genetic diversity of Medicago lupulina L. Molecular Genetics and Genomics, 299(1), 28.

Amer, N., Chami, Z. A., Bitar, L. A., Mondelli, D., & Dumontet, S. (2012). Evaluation of Atriplex Halimus, Medicago Lupulina and Portulaca Oleracea For Phytoremediation of Ni, Pb, and Zn. International Journal of Phytoremediation, 15(5), 498–512.

Ashish, B., Neeti, K., & Himanshu, K. (2013). Copper toxicity: a comprehensive study. Research Journal of Recent Sciences ISSN, 2277, 2502.

Balafrej, H., Bogusz, D., Triqui, Z. E. A., Guedira, A., Bendaou, N., Smouni, A., & Fahr, M. (2020). Zinc hyperaccumulation in plants: A review. Plants, 9(5), 562.

Charkiewicz, A. E., & Backstrand, J. R. (2020). Lead toxicity and pollution in Poland. International journal of environmental research and public health, 17(12), 4385.

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The effects of cadmium toxicity. International journal of environmental research and public health, 17(11), 3782.

Gharbi, F., Rejeb, S., Ghorbal, M. H., & Morel, J. L. (2005). Plant Response to Copper Toxicity as Affected by Plant Species and Soil Type. Journal of Plant Nutrition, 28(3), 379–392. https://doi.org/10.1081/PLN-200049147

Järup, L., & Åkesson, A. (2009). Current status of cadmium as an environmental health problem. Toxicology and applied pharmacology, 238(3), 201-208.

Jian, L., Bai, X., Zhang, H., Song, X., & Li, Z. (2019). Promotion of growth and metal accumulation of alfalfa by coinoculation with Sinorhizobium and Agrobacterium under copper and zinc stress. PeerJ, 7, e6875.

Kaur, H., & Garg, N. (2021). Zinc toxicity in plants: a review. Planta, 253(6), 129.

Kong, Z., Mohamad, O. A., Deng, Z., Liu, X., Glick, B. R., & Wei, G. (2015). Rhizobial symbiosis effect on the growth, metal uptake, and antioxidant responses of Medicago lupulina under copper stress. Environmental Science and Pollution Research, 22, 12479-12489.

Malysheva, N. Y., Shelenga, T. V., Solovyeva, A. E., & Malyshev, L. L. (2022). Specific features of the biochemical composition of life forms of black medic (Medicago lupulina L.). Ecological genetics, 20(3), 231-242.

Matanzas, N., Afif, E., Díaz, T. E., & Gallego, J. R. (2021). Phytoremediation potential of native herbaceous plant species growing on a paradigmatic brownfield site. Water, Air, & Soil Pollution, 232(7), 290.

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

Peralta, J. R., Gardea-Torresdey, J. L., Tiemann, K. J., Gomez, E., Arteaga, S., Rascon, E., & Parsons, J. G. (2001). Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (Medicago sativa L.). Bulletin of Environmental Contamination & Toxicology, 66(6).

Rajeswari, S., & Swaminathan, S. (2014). Role of copper in health and diseases. Int. J. Cur. Sci, 10, 94-107.

Sharma, P., & Dubey, R. S. (2005). Lead toxicity in plants. Brazilian journal of plant physiology, 17, 35-52.

Vardumyan, H., Singh, A., Rajput, V. D., Minkina, T., El-Ramady, H., & Ghazaryan, K. (2024). Additive-mediated phytoextraction of copper-contaminated soils using Medicago lupulina L. Egyptian Journal of Soil Science, 64(2), 599-618.

Wang, B., & Du, Y. (2013). Cadmium and its neurotoxic effects. Oxidative medicine and cellular longevity, 2013(1), 898034.

Wilson, L. C. (2005). Characteristics of black medic (Medicago lupulina L.) seed dormancy loss in Western Canada.

Yruela, I. (2005). Copper in plants. Brazilian journal of plant physiology, 17, 145-156.