Egyptian Journal of Soil Science

http://ejss.journals.ekb.eg/

Additive-Mediated Phytoextraction of Copper-Contaminated Soils Using *Medicago lupulina* L.

Hasmik Vardumyan[1], **Abhishek Singh** [1], **Vishnu D. Rajput**[2], **Tatiana Minkina**[2],
Hassan Ragab El-Ramady[3*] and **Karen Ghazaryan**[1*]

[1] *Faculty of Biology, Yerevan State University, Yerevan 0025, Armenia*
[2]*Academy of Biology and Biotechnology, Southern Federal University, Rostov-on-Don, Russia*
[3] *Soil and Water Dept., Faculty of Agriculture, Kafrelsheikh University, Egypt*

PHYTOREMEDIATION is an acceptable, economical, and eco-friendly way to remediate metal-contaminated soils, especially in areas near industrial zones. Copper (Cu) industries generate effluent that contains various Cu pollutants. Discharging these pollutants into the environment can adversely impact soil and biota through the food chain. In this study, researchers used Medicago lupulina for phytoremediation in soil samples collected from the area surrounding the zangezur copper and molybdenum combine (ZCMC) plant in southeast Armenia. The experiment was conducted ex-situ in pots, and the concentration of Cu heavy metals was analyzed before and after applying phytoremediation using different additives, including ammonium nitrate, citric acid, malic acid, and EDTA. The results showed that these additives increased the phytoextraction process of Cu from the soil while also improving the biomass, root growth, and chlorophyll content of M. lupulina. With bioconcentration factor of root (BCF_{root})>1 and translocation factor (TF)>1 values, Medicago lupulina can be considered a good hyperaccumulator plant with a better capacity for phytoextraction of Cu metal.

Keywords: copper, phytoremediation, ammonium nitrate, citric acid, malic acid, EDTA, *Medicago lupulina,* Armenia.

1. Introduction

Many mining and industrial operations have shut down in the last several decades. There are societal and economic consequences, risks to human health and the environment, and contaminated soil and water from these brownfields (Fig 1) (Schädler et al., 2011; Zanchi et al., 2021). Soil contamination by potentially toxic elements (PTEs) is prevalent in abandoned locations (Gallego et al., 2015). The soil plays a vital role in the farming and cattle-raising industries. When these operations are disrupted, it can lead to harmful substances accumulating and magnifying in the environment. This can negatively impact crop production in agricultural lands and pose a risk to food security (Mani et al., 2016; Antonkiewicz et al., 2018; de Pádua et al., 2021). Inorganic elements known as metals, which might include elements with hazardous potential, have atomic densities ($g \cdot cm^{-3}$) many times greater than water ($1 g \cdot cm^{-3}$). Metals can be categorized as heavy and light metals and semi-metals (Rashid et al., 2023). Based on physical, physiological, and chemical properties, metals have been classified under several sub-groups, namely: transition metals: e.g., chromium, manganese, iron, cobalt, nickel, copper , and molybdenum (Mo); post-transition metals: e.g., aluminum, zinc, cadmium,

---

*Corresponding author e-mail: ramady2000@gmail.com

Received: 28/01/2024; Accepted: 10/02/2024
DOI: 10.21608/EJSS.2024.266169.1714

mercury, and lead; alkaline earth metals: e.g., calcium (Ca), magnesium , beryllium, and barium (; alkali metals: e.g., lithium , sodium, potassium, and cesium; and metalloids, which are also referred to as semi-metals because of their metallic and non-metallic properties: e.g., boron , silicon , arsenic, and antimony (Pourret and Hursthouse, 2019). Environmental contaminants include heavy metals and metalloids (HMs). Because HMs can have a deleterious effect on crop health and production when present in soil at excessive levels, they are also considered agricultural soil pollutants (Fig 2) (Maksymiec, 2007). HMs can persist in the soil for a long time if plants cannot absorb or drain them (Maksymiec, 2007; Wuana and Okieimen, 2011; Elgharably and Mohamed, 2016). Agricultural soils may contain hazardous metals like Cadmium, Lead, Chromium, Arsenic, Mercury, Nickel, Copper, and Zinc. High levels of these metals can negatively affect plant growth and development (Wuana and Okieimen, 2011; Ghori et al., 2019). The health of plants can be severely compromised by pollutants like Cd, Pb, As, Hg, and Cr (Ali et al., 2019). Several minerals are crucial to plant development and yield. Cu, Zn, Fe, Mn, Mo, Ni, Mg, Ca, and B are among the examples (Chen et al., 2022).

These elements can improve ion homeostasis, pigment biosynthesis, photosynthesis, respiration, enzyme activity, gene regulation, sugar metabolism, nitrogen fixation, and other cellular processes in plants at low concentrations (Bashir et al., 2016). Plants require certain components for their growth and development. However, when these components are present in excessive amounts, they can have an adverse effect on the growth and development of plants (Maksymiec, 2007; Ghori et al., 2019). Conversely, if the concentration of these components drops below certain threshold values, it can result in mineral deficiency symptoms in plants (Welch, 2002; Niste et al., 2014). Among the most valuable metals from an economic perspective, Cu also ranks highly as a key resource for future industrialization (Jiang et al., 2021). Cu current applications include the electrical, building, transportation, and information technology (IT) sectors (Seck et al., 2020). Electric car manufacture and power generation also rely on Cu (Blundy et al., 2021). The rising demand for copper products over the last decade has led to a steady growth in the annual global mining of copper (Fig 1) (Izydorczyk et al., 2021). Previous experiments were found that heavy metal(loid) contamination of soil was a major environmental impact of the increased copper mining activities, which were mostly disregarded (Tepanosyan et al., 2018; Kumar et al., 2020; Seck et al., 2020; Blundy et al., 2021; Jiang et al., 2021; Reyes et al., 2021; Shi et al., 2023). The topsoil near copper mines is found to collect many heavy metals (loid)s, including lead, cadmium, arsenate, zinc, chromium, manganese, and nickel, according to previous research (Shen et al., 2017; Chen et al., 2019). Due to their long half-lives in soils, this metal(loid)s poses a threat to human health via soil contamination and the food chain (Shen et al., 2017; Chen et al., 2019). Hence, it is crucial to investigate the possible ecological and health hazards posed by heavy metals (loids) in soils close to Cu mines and assess their contamination levels.

In recent decades, scientists have researched effective methods for cleaning up polluted grounds (Bashir et al., 2016). The many benefits of phytoremediation methods—including their reliance on solar energy, low cost, and little impact on the environment—have garnered a lot of attention in recent years (Singh et al., 2016). Heavy metal phytoaccumulation was influenced by both internal factors—the soil and plants in the area—and external factors—the soil environment (Rafati et al., 2011; El-Zemrany et al., 2016). According to Keller et al. (2005), heavy metal bioaccumulation is more effective in the plant shoot system compared to the root system. Usman et al. (2012) identified the first hyperaccumulators in the *Brassicaceae* and *Fabaceae* families.

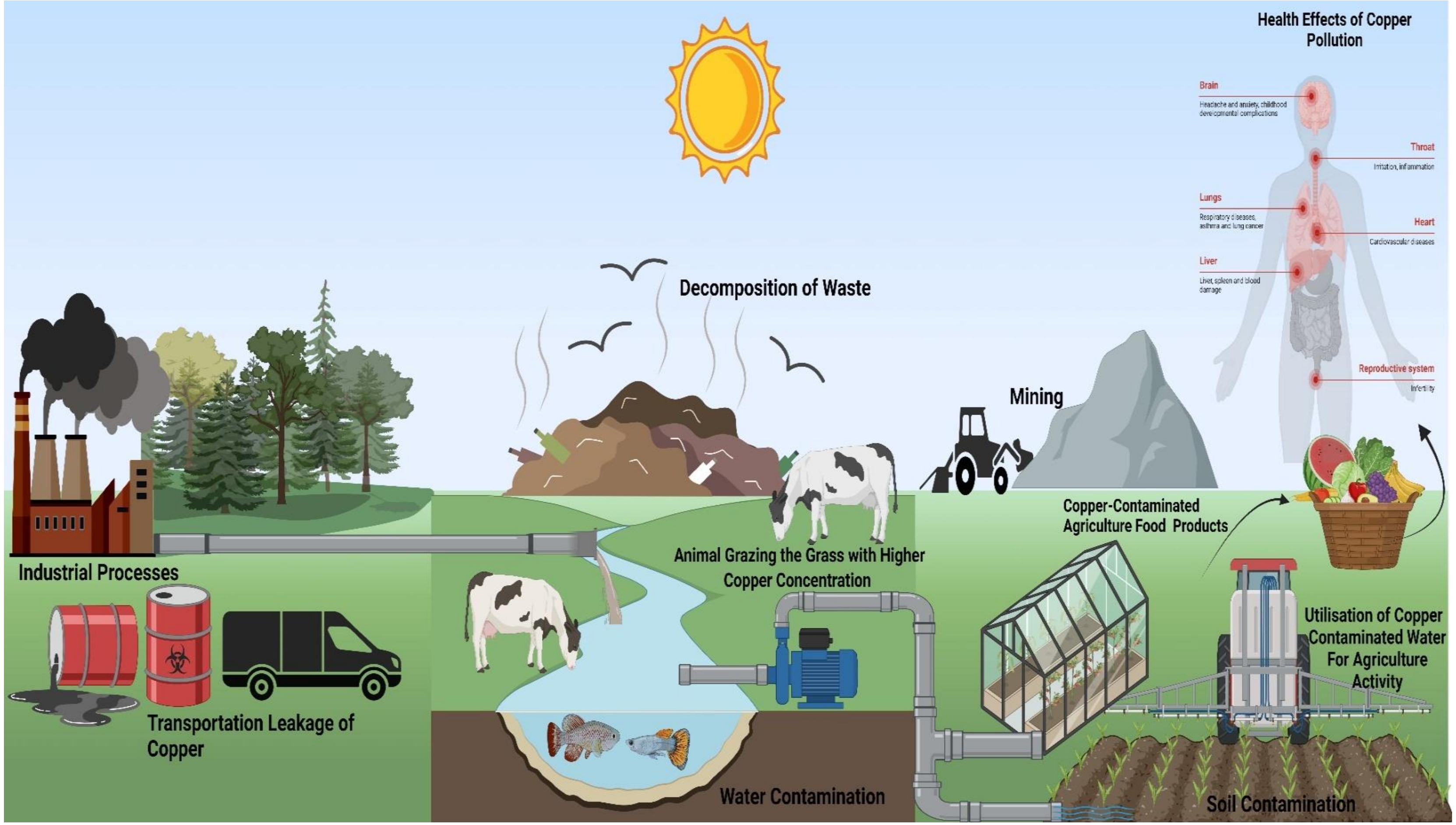

Fig. 1. Different copper sources contaminated the environment and ecologically resulted in Cu content flow in food chain that affected human health.

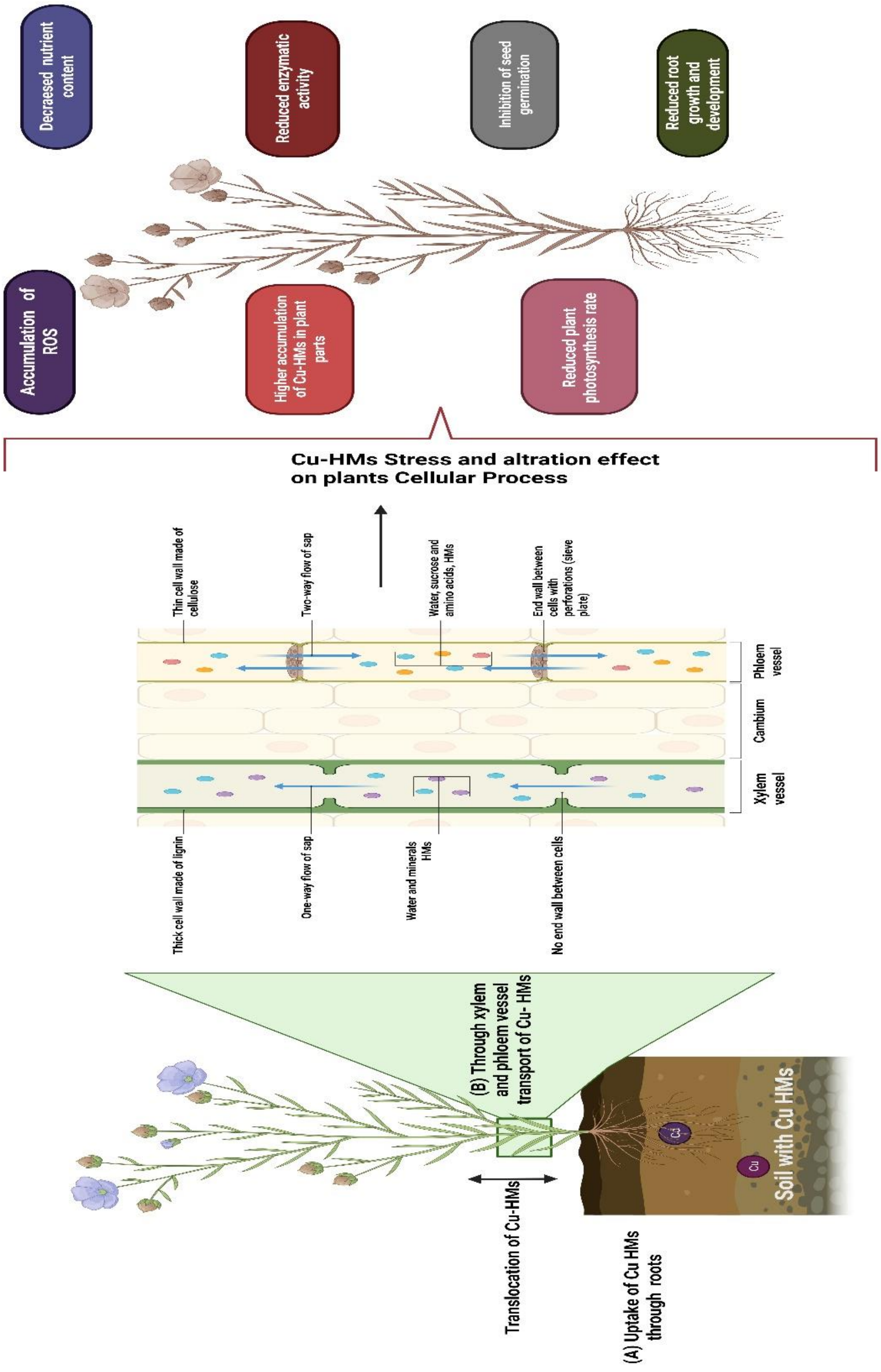

Fig. 2. Negative impact of Cu HMs on plant growth and development.

Accordingly, plants that may hyperaccumulate HMs can be appropriately classified (Rastegari Mehr et al., 2021). Since halophyte plants exist naturally throughout the Mediterranean, their potential utility in heavy metal cleanup is especially intriguing in this region. Tolerance mechanisms to soil salinity are not always specific to sodium and may include other toxic cations; thus, these plant species' drought and salt resistance can help them withstand toxic heavy metal concentrations (Ullah et al., 2020; Marcon et al., 2021; Zulfiqar and Ashraf, 2022). Heavy metal pollution in soils across the Mediterranean is a pressing concern, and naturally occurring indigenous halophytes are considered a possible solution. Some species, including *Atriplex* and *Portulaca oleracea*, have shown promising results. *P. oleracea*, a widespread annual weed, is one of the most ubiquitous plants in the globe (D’Andrea et al., 2014). The legume *Medicago lupulina* originates from central Asia and Europe and is known by several other names, including hop clover, nonesuch, black medick, and legume (*Fabaceae*) (Amer et al., 2013). This weedy plant prefers hot, dry spots in grass and wasteland, including the edges of highways and railroads. It might also be a problem in fields and gardens. Because it grows so much faster than weak grass, black medic is a common sign of insufficient soil nitrogen in lawns. It grows on turf and looks a lot like white clover. Certain plants may survive in mild winters and serve as perennials, even though it is categorized as a cool-season summer annual. Without human interference, black medic may quickly spread by seed and establish extensive colonies. The Medicago genus consists of long-lived plants that can draw heavy metals out of the ground and synthesize biomaterials. Researchers have discovered that M. *lupulina* may thrive in soils with high levels of heavy metal contamination and hyperaccumulate these toxic substances in its tissues. In addition to phytoremediation, one of the *'Ex-situ'* methods involves adding different substances to the soil to reduce or neutralize the effects of contaminants (Ying, 2018; Alori et al., 2022; Hussain et al., 2022). The present investigation aimed to evaluate the phytoremediation efficiency of *M. lupulina* L. which grows in copper contamination in soil collected by adding some additives such as ammonium nitrate, citric acid, malic acid, and EDTA. The bioaccumulation of copper in contaminated reduced translocation in the soil and the plant sections (shoot and root) reduced by using these additives. The objective of this study was to determine the effectiveness of *Medicago lupulina* L. in phytoremediation of copper-contaminated soil. To achieve this, soil with copper contamination was treated with various additives including ammonium nitrate, citric acid, malic acid, and EDTA. The results showed that the bioaccumulation of copper in the contaminated soil was reduced, and the translocation of copper in the root of the plant was also reduced by using these additives.

2. Materials and methods

2.1. Soil characteristics

The polluted soil (mountain Cambisol) used in the phytoremediation trials was gathered 300 meters from the Zangezur Copper and Molybdenum Combine (ZCMC) plant in southeast Armenia (Fig 3) (coordinates N 39,1551° and E 46,1409°). Our earlier research indicates that this area's soil is polluted with different kinds of heavy metals, including copper (Ghazaryan et al., 2018). About 850 kilograms of soil were taken from various locations inside a 10 m by 10 m plot, mixed well, and then put into 12-liter pots to be used for *Ex-situ* research. Similar procedures were used to collect 90 kg of uncontaminated soil from the unaffected territory 4 km from the mining's location (Fig 3) (coordinates N 39,2169° and E 46.2327°). The average annual temperature of the study site is +2 to +3 ^{O}C, and average per year rainfall, respectively: 600-700 mm..Table 1 show the data for a few physicochemical properties of the soil samples. In comparison to uncontaminated soiluncontaminated soil, the contaminated soil employed in phytoremediation trials had a Cu total concentration that was more than 35.6 times greater (Table 1). The direct cause of the contamination is human economic activity, mainly mining. The investigated soil has a high bioavailable copper level—more than 123.1 times higher than the uncontaminated soil value.

2.2. Soil analysis

The amount of organic material in the soil was assessed using the I. V. Tyurin method (Orlov et al. 2005). A pH-meter (Agilent Technologies, 3200P) was used to measure the pH of extracts of the soil samples (1:2.5, soil/water). The distribution of the sizes of soil particles was ascertained following Gee and Bauder (1986).

Table 1.Physicochemical characteristics of collected uncontaminated and contaminated soils from Zangezur Copper and Molybdenum Combine (ZCMC) plant in southeast Armenia (mean ± SD).

Soil characteristics	Uncontaminated soil	Contaminated soil
pH	7.83 ± 0.24	7.89 ± 0.29
Organic matter, g/kg	59.6 ± 4.1	41.2 ± 3.7
Sand, g/kg	320 ± 35	395 ± 35
Silt, g/kg	385 ± 35	390 ± 35
Clay, g/kg	295 ± 25	215 ± 20
Cu_{total}, mg/kg	29.5 ± 2.1	1050.6 ± 51.1
$Cu_{bioavailable}$, mg/kg	1.4 ± 0.5	172.3 ± 11.8

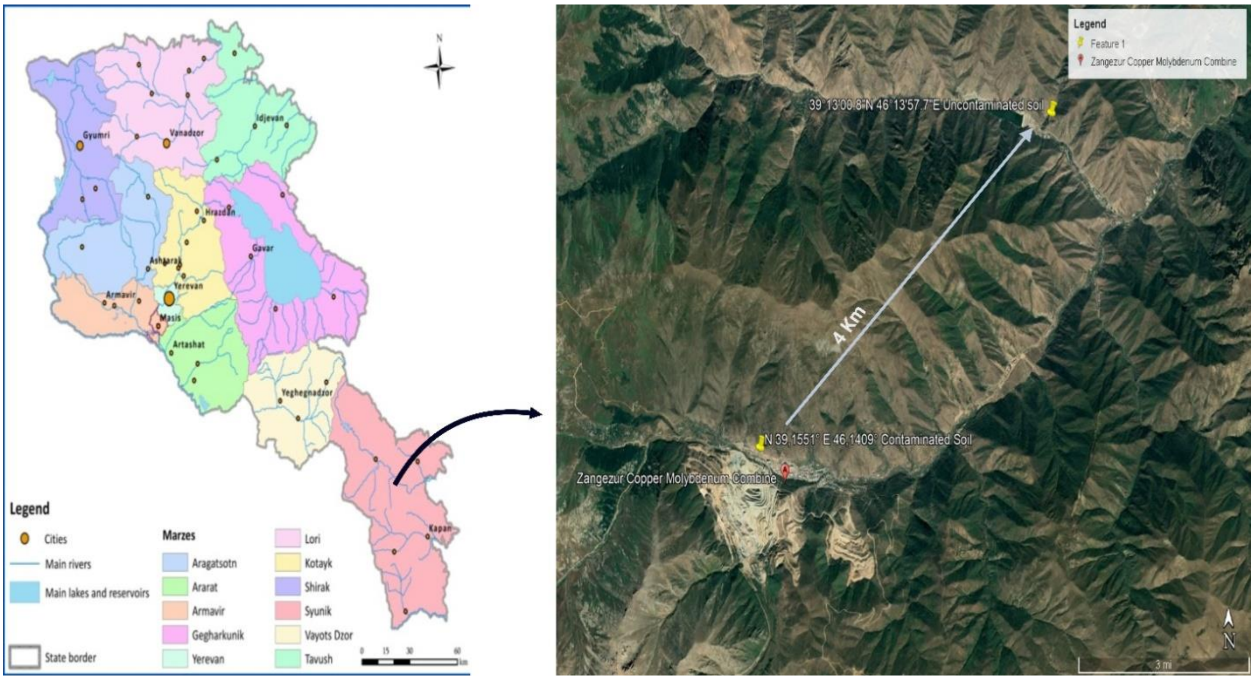

Fig. 3. Location of study and collection area of soil.

To determine the amount of copper the samples were left to air-dry at room temperature (20–22 °C) and soil samples were processed to pass through a 0.15 mm nylon mesh. To find the amount of total copper, the soil materials were treated with a mixture of HNO_3 + $HClO_4$ + HF (5:1:1, V:V: V) (Page, 1982). An atomic absorption spectrometer (AAS, PG990, PG Instruments Ltd, UK) was used to measure the amount of copper.

Acetic acid was utilized to determine the amount of biologically available copper (exchangeable, water- and acid-soluble forms) in soil samples: 1 g of ground soil was placed in a 50-ml tube, combined with 40 ml of 0.11M CH_3COOH, and the mixture was kept at 20–22 °C for 16 hours (He et al., 2013). Following the suspension was filtered with the help of filter paper, the amount of copper in the filtrates was determined using AAS.

2.3. Plant growth circumstances, chemical amendments applied, and analysis

The two-month-long trial was conducted in *ex-situ* settings between April and June of 2021. Eleven distinct schemes were used to grow the *M. lupulina* plant species, with each scheme being used in five test patterns. Table 2 provides a scheme description.

Equal amounts of ammonium nitrate were given to the plants twice a year: once at the end of May, when the plants started to grow quickly, and again in the middle of June. A week after applying the second part of chemical amendments, the plants were sampled at the end of their rapid growth period. The plants were properly cleaned twice with distilled water after being thoroughly flowing in running water. The plant's above-ground and below-ground sections were divided and weighed separately. To achieve a constant weight, both the shoots and the roots were dried at 70 °C. After that, dried roots and shoot were weighed and crushed. Following that, the samples (0.1 g of roots or shoots in 10 ml of acid mixture) were processed for 200 min at 150 °C in a mixture of HNO_3 and HClO4 (4:1, V: V) (Žemberyová et al., 2006). Using AAS, the copper concentration of the resultant solutions was determined.

2.4. Phytoremediation potential of plants

Bioconcentration factor of root (BCF root) and translocation factor (TF) were used to assess *M. lupulina* phytoremediation potential (Ghazaryan et al., 2022). The following formula was used to determine the bioconcentration factor of the root, which was used to evaluate the roots' capacity to collect copper:

$$BCF_{root} = Cu_{root}/Cu_{soil} \qquad (1)$$

where Cu soil is the concentration of bioavailable copper in the soil and Cu root is the concentration of copper in the collected plant roots. The following formula was used to determine the translocation factor:

$$TF = Cu_{above\text{-}ground}/Cu_{root} \qquad (2)$$

where Cu root denotes the concentration of copper in the collected plant's roots and Cu above-ground denotes the concentration of copper in the above-ground part of the collected plant. When using the phytostabilization approach to remediate soils, the crops need to have a high BCF root value but a low TF value. On the other hand, when using the phytoextraction method, relatively high values of both BCF root and TF are required simultaneously (Sajad et al., 2020).

2.5. Statistics

Five replicates were used to measure the amounts of copper in the soil, the roots, and parts of plants above ground. Further assessment was conducted using Duncan's multiple-range tests. SPSS software, version 15, was used to conduct the statistical analysis. One-way ANOVA is used to present all the data as an average with standard error (SE). Fisher's least significant difference (LSD) test was used to establish statistical significance (Rajput et al., 2021). When $p < 0.05$, differences were deemed significant. The Pearson correlation coefficient was computed for the correlation analysis of a few investigated parameters.

Table 2. Description of experimental schemes implemented under *ex-situ* conditions.

Experimental scheme number	Description of an experimental scheme
T_0	Uncontaminated soil
T_1	Contaminated soil
T_2	Contaminated soil + NH_4NO_3 (0.05 g/kg soil)
T_3	Contaminated soil + NH_4NO_3 (0.05 g/kg soil) + citric acid (5 mM/kg soil)
T_4	Contaminated soil + NH_4NO_3 (0.05 g/kg soil) + malic acid (5 mM/kg soil)
T_5	Contaminated soil + NH_4NO_3 (0.05 g/kg soil) + citric acid (2.5 mM/kg soil) + malic acid (2.5 mM/kg soil)
T_6	Contaminated soil + NH_4NO_3 (0.05 g/kg soil) + EDTA (1 mM/kg soil)
T_7	Contaminated soil + NH_4NO_3 (0.05 g/kg soil) + EDTA (2 mM/kg soil)
T_8	Contaminated soil + NH_4NO_3 (0.05 g/kg soil) + EDTA (3 mM/kg soil)
T_9	Contaminated soil + NH_4NO_3 (0.05 g/kg soil) + EDTA (4 mM/kg soil)
T_{10}	Contaminated soil + NH_4NO_3 (0.05 g/kg soil) + EDTA (5 mM/kg soil)

3. Results

3.1. Variations in the above-ground and root masses of M. lupulina cultivated according to several experimental schemes

After three and a half months of growth, the dry masses of the plant's roots and shoot were measured using eleven different schemes to discover the effects of soil pollution by copper on *M. lupulina* (Fig. 4). In comparison to the control, the dry masses of the above-ground plant parts grown under all other schemes decreased. Contrary to that, the dry masses of the root grown by all schemes, excepted third scheme, increased compared with the control. Roots' dry masses of *M. lupulina* grown under different experimental schemes' conditions compared with control essentially increased in the first and fifth schemes (148.9% and 145.6%; respectively) and the maximum decrease of the dry mass in roots of the plant is determined in third scheme (80.2%). At the same time *M. lupulina* grown under conditions of scheme three and four have a maximum decrease in dry mass of shoot (40.4% and 41.3%; respectively) and the minimum above-ground plant parts dry mass decreasing visible in the fifth scheme (86.7%) (Table 3). Scheme five also stands out with copper content in root (965.2 ± 46.0 mg/kg), which is the maximum accumulated level of copper in roots in all eleven schemes, compared with control its content of copper higher 8.2 times. Although the third scheme has the lowest dry mass of root (5.2 grams) in all of the eleven grown schemes of *M. lupulina*, it takes second place for high copper content in roots (889.0 ± 39.3 mg/kg), which is 7.6 times higher than control's root copper content (Table 4).

In first and second schemes determined the lowest copper content both in the root (290.0 ± 13.8 mg/kg and 329.7 ± 15.3 mg/kg, respectively) and above-ground dry mass (93.0 ± 5.6 mg/kg and 96.5 ± 4.9 mg/kg, respectively). In the eighth scheme the highest copper content (889.0 ± 39.3 mg/kg) in shoot of *M. lupulina* from all eleven growth schemes, it contains 55.5 times higher level of copper compared with control.

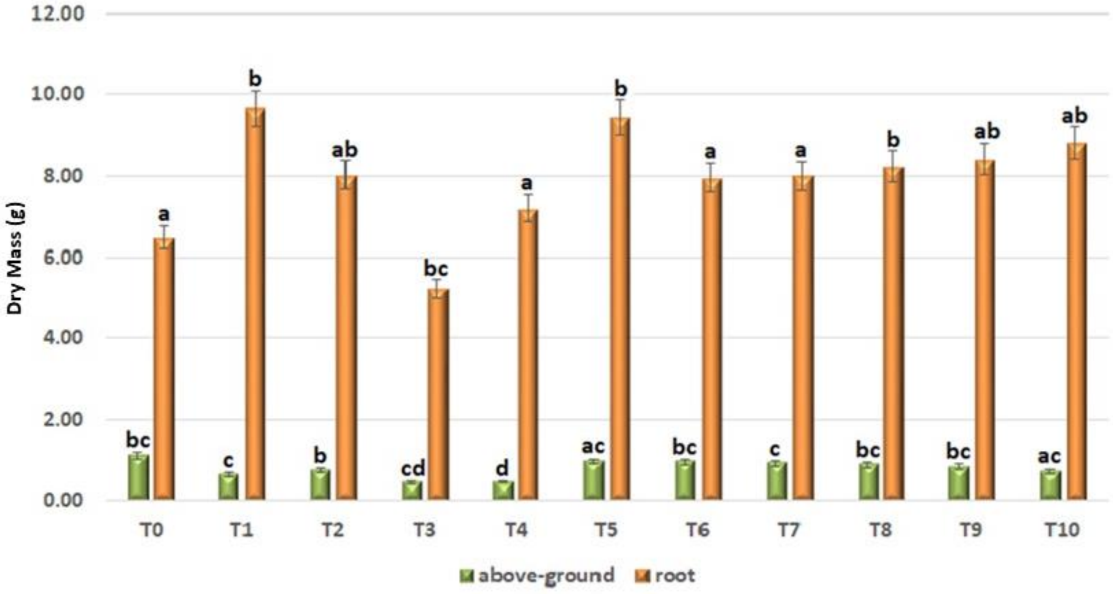

Fig. 4. Dry mass of above-ground part and root of *M lupulina* grown by different schemes (in grams).
Statistically significant difference at p ≤ 0.05 indicated by different letters.

Table 3. Changes in root and above-ground part dry masses of *Medicago lupulina* grown by different experimental schemes (see table 2), compared with control (%).

Experimental scheme number	Above-ground part	Root
T_1	58.3	148.9
T_2	68.4	123.8
T_3	40.4	80.2
T_4	41.3	111.1
T_5	86.7	145.6
T_6	86.1	122.7
T_7	82.4	123.4
T_8	78.7	127.1
T_9	75.2	129.5
T_{10}	63.8	136.0

Scheme eighth also does not show too much decrease in above-ground plant parts' dry mass (0.88 grams), compared with control its shoot weight is 78.7%. In addition, plants grown under conditions of scheme eight has 127.1% of roots' dry mass and 4.3 times higher accumulated copper content in roots compared with control. In the case of complete observation for both shoot and roots, other EDTA schemes demonstrate less effectiveness in dry mass or in copper accumulation. The last one proves that the optimum concentration of EDTA has an eighth scheme. The uncontaminated and contaminated soil characteristics indicate that the total copper content in contaminated soil is about 35 times and bioavailable copper content is about 123 times higher than in control. Considering that and to understand the impact of additives for all nine schemes (T_2-T_{10}) it is crucial to compare dry mass values and copper content of root and shoot of plant with contaminated soil's results (T_1).

Roots' dry masses of *M. lupulina* grown under different experimental schemes' conditions with additives compared with contaminated soil without any additives decrease their value. Instead of that, the shoot of plants dry mass increases, besides scheme three and four that have lowest above-ground dry mass. As opposed to that scheme five, that includes both citric and malic acids together, but in lower concentration, expresses the highest value of the above-ground part of plant.

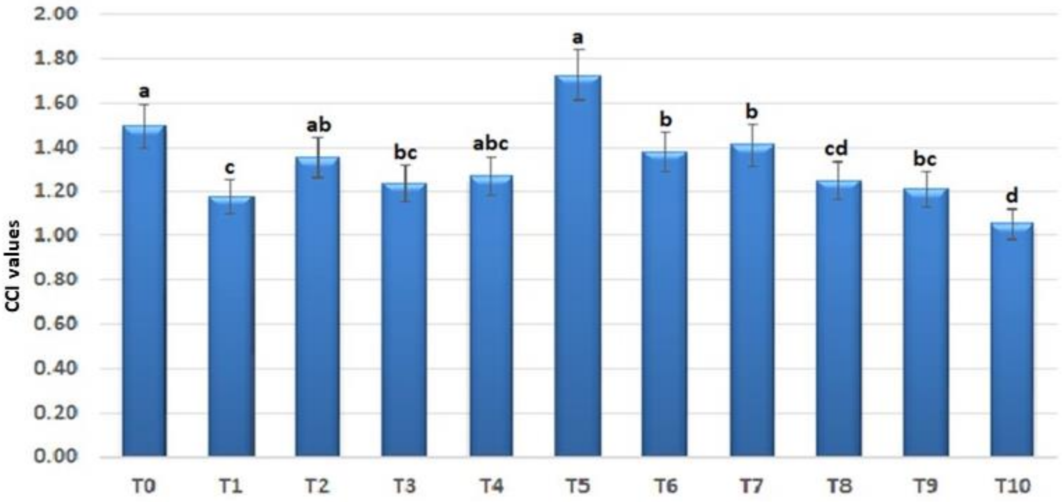

Fig. 5. CCI values of Medicago lupulina plant species in different schemes of experiment. Statistically significant difference at $p \leq 0.05$ indicated by different letters.

3.2. Bioaccumulation of copper in root and shoot of M. lupulina grown by different experimental schemes

The outcomes of the correlation analysis (Table 4) have likewise supported, there is a very strong correlation between dosages of EDTA used in different schemes and changes of both above-ground part and root dry mass ($r = -0,959$ and $r = 0.959$; $p \leq 0.05$, respectively). At the same time there is a strong correlation between dosages of EDTA used in different schemes and copper content in above-ground part and root ($r = 0.778$ and $r = -0.772$; $p \leq 0.05$, respectively). Very strong negative ($r = -0.991$; $p \leq 0.05$) correlation between mass of root and mass of above-ground part for different experimental schemes grown with EDTA also exists (Table 5). Correlation analysis results have also shown a moderate negative correlation ($r = -0.654$; $p \leq 0.05$) between copper above-ground content and mass of the above-ground part of the plant, when between copper above-ground content and dry mass of root there is a moderate positive correlation ($r = 0.671$; $p \leq 0.05$).

A similar situation is visible for root copper content, the correlation between the latter and the mass of the root is a strong negative ($r = -0.875$; $p \leq 0.05$), and between the mass of the above-ground part very strong positive ($r = 0.915$; $p \leq 0.05$).

3.3. Chlorophyll content index of M. lupulina plant in different schemes of experiment

Soil contamination with copper affects also on chlorophyl content index value. In contaminated soil (scheme 1) it CCI value decreases, compared with control it is 78.7%. The highest value of CCI present in scheme five (CCI = 1.73). When it observed with mentioned above results of scheme five, ammonium nitrate with citric and malic acid has positive impact on total growth and copper accumulation of *M. lupulina* (Fig. 5). The lowest value of CCI expressed in plants grown with scheme ten. The latter and th lowest result of above-ground plant part dry mass compared with other schemes with EDTA argue the toxic effect of high level EDTA on plant growth.

Table 4. Copper content in roots and shoot (dry mass) of M. *lupulina* grown by different experimental schemes.

Scheme number	**Root (mg/kg)**	**Above-ground part (mg/kg)**	**Increase in root copper content compared with control (by a factor of)**	**Increase in above-ground part copper content compared with control (by a factor of)**
T0	117.5±7.2a	22.7±1.8a	-	-
T1	290.00±13.8ab	93.0±5.6b	2.5	4.1
T2	329.7±15.3b	96.5±4.9abc	2.8	4.3
T3	889.0±39.3c	665.9±26.1cd	7.6	29.3
T4	545.0±23.1bc	218.2±12.0bcd	4.6	9.6
T5	965.2±46.0c	455.2±16.7bcd	8.2	20.1
T6	519.0±19.8bc	204.7±10.1abc	4.4	9.0
T7	484.8±15.9ab	493.3±21.3c	4.1	21.7
T8	504.5±24.2bc	1259.0±76.9cd	4.3	55.5
T9	490.5±18.8abc	953.2±59.7cd	4.2	42.0
T10	357.6±16.0abc	1029.4±48.3d	3.0	45.3

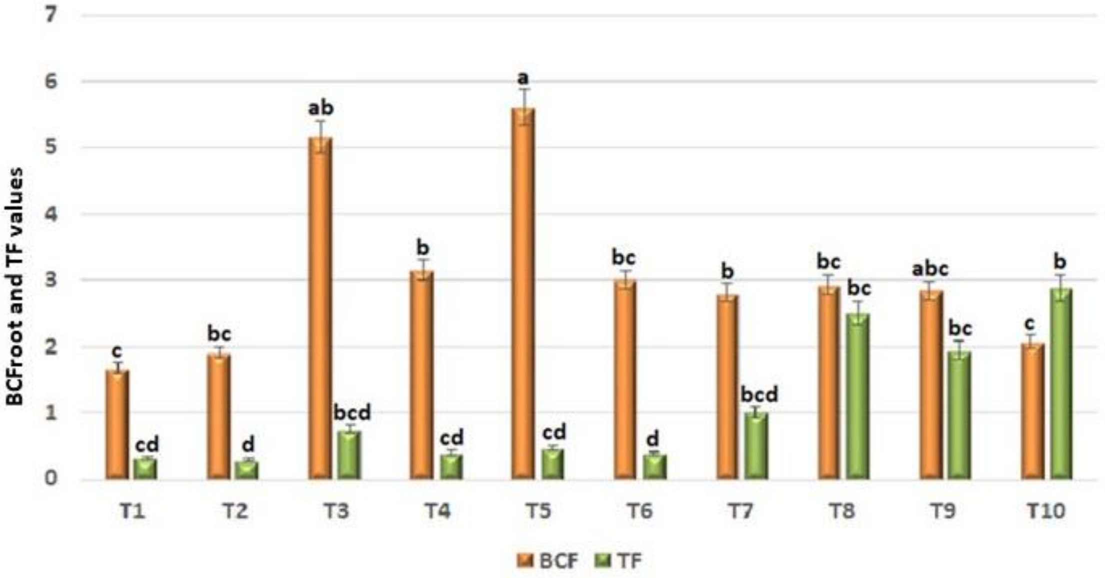

Fig. 6. BCF$_{root}$ and TF values of *Medicago lupulina* plant species in different schemes of experiment. Statistically significant difference at $p \leq 0.05$ indicated by different letters.

3.4. Phytoremediation potential of M. lupulina

BCF root and TF in all contaminated soils' schemes increase values compared with control, besides scheme two TF value (TF = 0.29). It is the lowest TF value, but the BCF root value is not enough (BCF root = 1.91) for the high effectiveness of phytostabilization, because as mentioned above for the phytostabilization approach to remediate soils, high BCF root value and a low TF value of plant is needed. The highest BCF root value is demonstrated with schemes three and five (5.16 and 5.6, respectively), at the same time TF value of those schemes are significantly low (0.75 and 0.47, respectively) (Fig. 6). At the same time for the phytoextraction method, relatively high values of both BCF root and TF are required. Schemes eighth and ten have relatively high values of BCF root and TF: scheme eighth BCF root = 2.93 and TF = 2.5; scheme eighth BCF root = 2.08 and TF = 2.88.

Table 5. Correlation analysis of some studied criteria with EDTA.

	Mass of shoots	**Mass of root**	**Cu$_{\text{above-ground}}$**	**Cu$_{\text{root}}$**	**EDTA**
Mass of shoots	1				
Mass of root	-0.991	1			
Cu$_{\text{above-ground}}$	-0.654	0.671	1		
Cu$_{\text{root}}$	0.915	-0.875	-0.363	1	
EDTA	-0.959	0.959	0.778	-0.772	1

4. Discussion

4.1 Copper toxic effects on plant growth and biomass

Heavy metal ion pollution is a major problem all over the world, thus it is no surprise that phytoremediation developments for cleaning up contaminated soils using plants have received a lot of attention in this area (Krämer, 2005). Metal stress adaption, ecological compensatory mechanisms, exposure to metal combinations, and the accessibility of metals for plant absorption might vary in the field (Lock et al., 2003). Investigating the responses of native plants to HM stress requires field investigations. To choose the best plants for phytoremediation procedures, studies classifying species with the capacity to accumulate HM are crucial (Álvarez et al., 2003). According to this study, *M. lupulina* with various additions such as citric acid, ammonium nitrate, malic acid, and EDTA can collect a significant amount of Cu metal. Similar findings were seen in earlier research on M. *lupulina* plants (Amer et al., 2013; Bauddh et al., 2013). According to the studies cited above, phytoremediation might take advantage of the plants found in toxic soils.

Plants have a major difficulty when heavy metals in the environment enter their systems through their roots and leaves; this interferes with their physiological and metabolic functions. Kopittke and Menzies, (2006) found that copper has a greater impact on *Vigna unguiculata* root mass production than on shoot and leaf mass production (Fig 4 and Table 3-4) (Amin et al., 2021). The reason behind this is that damaged roots absorb fewer nutrients, which leads to stunted shoot growth, rather than the toxic metal themselves. According to Rouphael et al. (2008) heavy metals have phytotoxic effects because they interfere with nutrient transport and absorption. The suppression of growth, which resulted in several morphological alterations throughout development, was associated with heavy metal stress and seed germination. The elongation of plant roots showed a pronounced sensitivity to soil heavy metal overload. Heavy metals may mainly cause the roots to grow shorter because they prevent the plant from absorbing water and mineral nutrients. Root cell division, cell elongation, and the cell cycle were all reduced because of mineral deficiency, which in turn affected the rate of water and mineral absorption (Adrees et al., 2015). According to Barbosa et al. (2013), heavy metal toxicity interfered with plant metabolic processes, resulting in shorter seedlings, and altered root growth and development (Fig 3). According to Rajjak Shaikh et al. (2013), plants experienced a decrease in height due to the increased transportation rate of heavy metals towards the shoot area, which directly impacted sensitive plant components like leaves. Which, in consequently, disrupted photosynthesis and the cellular metabolism of the shoot, causing the plants to grow shorter. Heavy metal poisoning also disrupted hydrolytic enzyme activities, which in turn blocked food from reaching the growing embryo, which shortened the seedlings and stunted the plants growth (Tang and Gao, 2010; Li et al., 2012; Adrees et al., 2015). High plant biomass, defined here as the sum of fresh and dried plant weights, is essential for abundant plant production. Plant growth performance was the primary determinant of biomass (Adrees et al., 2015). The plants showed clear signs of stunted development when exposed to high levels of metal toxicity. Under heavy metal stress, plants biomass decreased due to reduced metabolic activities, poor photosynthetic responses, and decreased absorption of vital mineral nutrients (Fig 4 and Table 3-4) ((Li et al., 2012).

4.2 Adverse impact of copper on chlorophyll levels

HMs accumulation negatively affected the photosynthesis rate, impacting plant growth and development. According to Clijsters et al. (1999), heavy metals directly impact chloroplast function, cell membrane stability, chlorophyll production suppression, and Calvin cycle disruption. Monni et al. (2000) found that Chl concentrations in *Empetrum nigrum* plants were significantly lower in areas close to the Harjavalta copper and nickel smelting factory in southwestern Finland compared to areas further away. High amounts of Cu caused Trigonella foenum-graecum to exhibit narrowing of the leaves, increasing chlorosis, and necrosis (Elleuch et al., 2013). One study found that copper inhibited chlorophyll production in barley leaves (Caspi et al., 1999). As seen in Figure 5, the overall chlorophyll concentration of *M. lupulina plant* was much lower in Cu contaminated soil in compared to uncontaminated and additive treatments. As a result of an increase in reactive oxygen species (ROS) production, metals can cause chloroplast membranes to peroxide, which in turn slows down photosynthetic pigmentation (Malar et al., 2016). Metal interference during photosynthesis, either directly or indirectly, caused ROS to be produced, which in turn altered the structure of pigment protein complexes through degradation and destabilization of proteins in the antenna complex and total distortion of thylakoid membranes, resulting in reduced pigment contents and plant growth (Wodala et al., 2012). Chlorophyll pigment levels dropped because an overabundance of harmful metals hindered the absorption of potassium, calcium, magnesium, and iron, which are crucial components of photosynthetic pigments, and because heavy metals prevented the incorporation of divalent cations (Gopal and Rizvi, 2008). There is a evidence in the literature shows that toxic metals may inhibit chlorophyll production by destroying the photosynthetic architecture at the thylakoid level (Kabata-Pendias and Pendias, 2000).

4.3 Cu-phytoextraction and phytoremediation efficiency

Phytoextraction and phytostabilization are the primary processes of phytoremediation, which utilizes plants' aerial and underground components to mitigate soil heavy metal contamination (Fig 6). The phytoextraction process involves using plant roots to absorb and transport heavy metal contaminants to aboveground components. Subsequently, these pollutants are removed from soil by means of harvesting and treatment (Flathman and Lanza, 1998; Padmavathiamma and Li, 2007; Houben et al., 2013). By allowing heavy metals to be absorbed and stored in the soil via roots, phytostabilization decreases their mobility and effectiveness, making them more stable and harmless in the soil (Flathman and Lanza, 1998; Padmavathiamma and Li, 2007; Houben et al., 2013). In addition, the underground root network can prevent rainfall erosion and leaching and create an ideal rhizosphere environment for heavy metal precipitation (Houben et al., 2013; Li et al., 2022). The plant canopy that forms after phytoremediation can lower the near-surface wind speed and the diffusion of fine particle pollutants. Figure 6 shows that plant species examined *M. lupulin* had BCF and TF values > 1. This indicates that *M. lupulina* species are well-suited for phytoextraction because of their high capacity to transfer Cu from roots. When comparing plant species for their phytoremediation capability and considering the plants' ability to remove heavy metals from substrate, BCF has been demonstrated to be an excellent indicator of metal accumulation capacity (McGrath and Zhao, 2003; Gardea-Torresdey et al., 2005). Yoon et al. (2006) found that the BAC has an additional relevant parameter for comparing heavy metal concentrations in shoots and soil, while Cui et al. (2007) and Li et al. (2007) reported that TF helped assess the ratio of heavy metal concentrations in shoots and roots. According to Fitz and Wenzel (2002) plant species with BCF and TF values < 1 are likely not suitable for phytoextraction and phytoremediation, but plant species with BCF, and TF values > 1 are expected to be effective phytoextractors and suitable for phytoextraction and phytoremediation of soil contaminated with metals. According to Mendez and Maier, (2008), the plant species that met the requirements for phytostabilization had BCF values >1 and TF values < 1 show an excellent phytoextraction and phytoremediation efficiency.

4.4 Limitations of current study and future research directions

The USGS (United States Geological Survey) estimates that there are around 5.6 billion tons of mined copper reserves worldwide (Schnebele et al., 2019; Izydorczyk et al., 2021). The soil contamination status in the area of Cu mines may not be completely represented globally as our study only examined a Cu mining location. Furthermore, there may be inconsistencies in the data because of differences in geological conditions and extraction techniques throughout the several utilized studies, which might affect the accuracy of the results. Though the scientific community acknowledges these differences, they have little impact on the overall evaluation outcomes (Xiao et al., 2020). In health risk evaluations, resident behavior is also crucial. Errors produced by regional variations in human behavior are impossible to be eradicated. We mostly relied on USEPA (U.S. Environmental Protection Agency) rules and generally acknowledged research results for selecting the toxicity criteria to minimize this inaccuracy (Chen et al., 2022). One of the most critical elements affecting the amount of heavy metal(loid) pollution in the soil is the weather in the Cu mining location (Chen et al., 2022). In order to mitigate the negative effects of copper mining, most evaluation and monitoring studies fail to account for climatic factors that can influence the ecosystem. Therefore, future research should delve more into the impact of climate conditions. Furthermore, remediation of heavy metal(loid) polluted areas in the Cu mining region is necessary to lessen the flow of harmful components into the food chain. Future research must focus on monitoring climate using a combination of novel techniques, such as nanoparticles and biochar, to better understand the mitigation of Cu pollution in soil and develop more sustainable methods of phytoremediation.

4.5 Soil pollution management strategies for Remediation

The study's thorough evaluation of pollution and health risks should lead to the establishment of innovative and long-term plans to improve soil health around Cu mines and achieve sustainable mineral economic growth (Kookana, 2010; Schnebele et al., 2019; Kumar et al., 2020). Cu poses serious ecological concerns in the current locations under investigation. Cu presents a significant carcinogenic danger to adults and children in the area (Kopittke and Menzies, 2006; Dalcorso et al., 2010). Priority control pollutants for soils around Cu mining sites is Cu. Consequently, unique approaches are required to manage and restore soil's heavy metal (loid) pollution. Mining wastewater, polluted dusts, and other mining wastes are significant sources of heavy metal(loid)s (particularly Cu, Cd, and As), hence it is important to set stringent regulatory standards regarding Cu mining operations to decrease these inputs (Chen and Li, 2018; Sytar et al., 2019). It is also important to employ different approaches to remediate soil Cu contamination depending on the different levels of pollution. In highly polluted areas, phytoextraction has proven to be an effective and environmentally acceptable method for removing heavy metal(loid)s from soil, including Cu (Zakari et al., 2021; Sári et al., 2024). An outstanding copper hyperaccumulator is *M. lupulina*, which can efficiently draw copper from soil and transfer it to their roots and shoots parts (Amer et al., 2013).

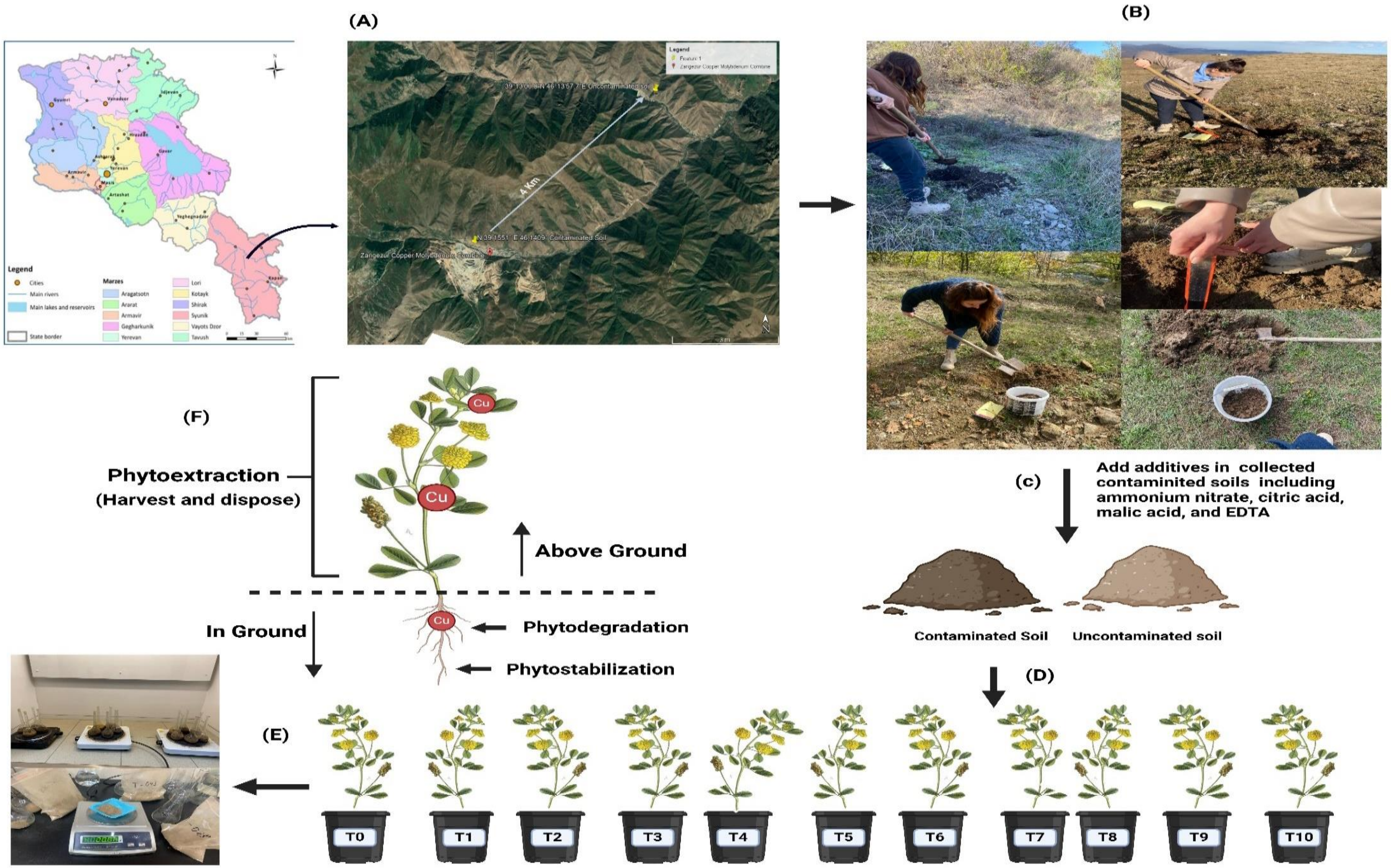

Fig. 7. For the current experiment (Fig A-B), soil samples were collected from the area surrounding the Zangezur Copper and Molybdenum Combine (ZCMC) plant in southeast Armenia. In the collected soil, (Fig C-D) additives such as ammonium nitrate, citric acid, malic acid, and EDTA were added along (Fig F) with *M. lupulina* plant for phytoextraction and phyto-stabilization to address Cu contamination. Further analysis will be conducted in the lab (Fig E).

Due to its ability to absorb or immobilize significant amounts of contaminants in the rhizosphere environment, phytostabilization has been extensively utilized for the remediation of sites with moderate levels of contamination (Amer et al., 2013). Soil microbes can bioreduce, biosorb, extracellularly precipitate chemicals, and valence convert metal contaminants (Xing et al., 2020). Some *de-novo* phytostabilization agents like biochar and nano materials may also solidify mobile portions of metal pollutants into precipitated or sorbed fractions in situ after applied in ex-situ method (Zulfiqar et al., 2019; Helaoui et al., 2023). This process decreases the mobility and bioavailability of the pollutants in soil. The government, mining companies, environmental protection organizations, and academic institutions must work together to execute these control and remediation plans effectively. It is also important to establish methods for routinely monitoring and supervising soil contamination in order to ensure the long-term viability of the Cu mining sector.

5. Conclusion and Future Prospective

This research has shown a solar-powered, efficient, eco-friendly, and cost-effective way to remove metals from polluted soil by harnessing plants' inherent ability. According to the Cu-polluted soil remediation findings, *M. lupulina* had considerably greater root growth, and chlorophyll contents at higher Cu concentrations. *M. lupulina* has the greatest concentration of Cu in their roots. All things considered, the data and observations pointed to *M. lupulina* as a species that can accumulate, a short growth cycle, and high resistance to metal stress. The high BCF, and TF values further supported the idea that *M. lupulina* was the best plant species to use for Cu phytoextraction, and it remedied the Cu-contaminated soil more quickly and in more flushes than the others (Fig 7). It was easy to dispose of the gathered plant biomass above the group part since it was biodegradable. It might be used as a raw material for large-scale composting or phytomining, or as an alternative source of biofuel energy. In addition, residents in the impacted regions could reap the benefits of the natural, risk-free strategy by reducing the levels of harmful metals in agricultural land irrigated with untreated water

Further future perspectives towards the studied problem of removing Cu from contaminated soils using *M. lupulina* L. plant through additive-mediated phytoextraction requires further investigation and practical application for sustainable land rehabilitation. To achieve this, long-term investigations are necessary to assess the effectiveness of this approach over multiple growing seasons. Additionally, optimizing additive dosages and application methods can improve phytoextraction efficiency while minimizing adverse effects on soil biota and plant vitality. Furthermore, studying the interactions between *M. lupulina* L. and contaminated soils at the molecular and microbial level can provide insights into enhancing metal uptake pathways and root exudates, improving remediation efficacy. The feasibility and effectiveness of additive-mediated phytoextraction under real-world conditions should also be validated by utilization different other types of additives like nanoparticles and biochar in laboratory experiments to field-scale applications (El-Ramady et al., 2020; Elramady et al., 2021; Singh et al., 2024a, 2024b). Finally, integrating phytoextraction with complementary techniques such as phytostabilization and bioremediation may offer sustainable land rehabilitation efforts synergistic benefits. In conclusion, while the current study provides foundational insights, ongoing research is necessary to fully harness the potential of additive-mediated phytoextraction in addressing copper contamination in soils.

List of abbreviations:

HMs: Havey metal
EDTA: Ethylenediaminetetraacetic acid
Cu: Copper
CCI: Chlorophyll content index
TF: Translocation factor
BCF: Bioconcentration factor of root
USGS: United States Geological Survey

Declarations

Ethics approval and consent to participate

Consent for publication: The article contains no such material that may be unlawful, defamatory, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the agreement.

Availability of data and material: Not applicable.

Competing interests: The authors declare that they have no conflict of interest in the publication.

Funding: Not applicable.

Authors' contributions: Authors HV, AS, VDR, TM, KG, HS write the original draft and HV, AS, VDR, TM, KG, HS edit and finalize the manuscript. All authors read and agree for submission of manuscript to the journal.

Acknowledgments:

YSU internal grant is supported by KG. AS is supported by the 23PostDoc-4D007 grant provided by the Science Committee of the Republic of Armenia. VDR and TM are supported by the Strategic Academic Leadership Program of Southern Federal University, known as "Priority 2030," and the Ministry of Science and Higher Education of the Russian Federation (grant number: FENW-2023-0008).

References

Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F., Farid, M., et al. (2015). The effect of excess copper on growth and physiology of important food crops: a review. *Environ. Sci. Pollut. Res.* 22, 8148–8162. doi:10.1007/S11356-015-4496-5/METRICS.

Ali, H., Khan, E., and Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. *J. Chem.* 2019. doi:10.1155/2019/6730305.

Alori, E. T., Gabasawa, A. I., Elenwo, C. E., and Agbeyegbe, O. O. (2022). Bioremediation techniques as affected by limiting factors in soil environment. *Front. Soil Sci.* 2, 937186. doi:10.3389/FSOIL.2022.937186/BIBTEX.

Álvarez, E., Fernández Marcos, M. L., Vaamonde, C., and Fernández-Sanjurjo, M. J. (2003). Heavy metals in the dump of an abandoned mine in Galicia (NW Spain) and in the spontaneously occurring vegetation. *Sci. Total Environ.* 313, 185–197. doi:10.1016/S0048-9697(03)00261-4.

Amer, N., Chami, Z. Al, Bitar, L. Al, Mondelli, D., and Dumontet, S. (2013). Evaluation of Atriplex halimus, Medicago lupulina and Portulaca oleracea for phytoremediation of Ni, Pb, and Zn. *Int. J. Phytoremediation* 15, 498–512. doi:10.1080/15226514.2012.716102.

Amin, H., Arain, B. A., Jahangir, T. M., Abbasi, A. R., Mangi, J., Abbasi, M. S., et al. (2021). Copper (Cu) tolerance and accumulation potential in four native plant species: a comparative study for effective phytoextraction technique. *Geol. Ecol. Landscapes* 5, 53–64. doi:10.1080/24749508.2019.1700671.

Antonkiewicz, J., Kołodziej, B., Bielińska, E. J., Witkowicz, R., and Tabor, S. (2018). Using Jerusalem Artichoke to Extract HeavyMetals from Municipal SewageSludge Amended Soil. *Polish J. Environ. Stud.* 27, 513–527. doi:10.15244/PJOES/75200.

Barbosa, R. H., Tabaldi, L. A., Miyazaki, F. R., Pilecco, M., Kassab, S. O., and Bigaton, D. (2013). Foliar copper uptake by maize plants: effects on growth and yield. *Ciência Rural* 43, 1561–1568. doi:10.1590/S0103-84782013000900005.

Bashir, K., Rasheed, S., Kobayashi, T., Seki, M., and Nishizawa, N. K. (2016). Regulating subcellular metal homeostasis: The key to crop improvement. *Front. Plant Sci.* 7, 204298. doi:10.3389/FPLS.2016.01192/BIBTEX.

Bauddh, K., Kumar, S., Bhimrao, B., and Singh, R. P. (2013). Bioaccumulation and translocation potential of Na+ and K+ in native weeds grown on industrially contaminated soil. *Artic. Int. J. ChemTech Res.* Available at: https://www.researchgate.net/publication/285705514 [Accessed January 1, 2024].

Blundy, J., Afanasyev, A., Tattitch, B., Sparks, S., Melnik, O., Utkin, I., et al. (2021). The economic potential of metalliferous sub-volcanic brines. *R. Soc. Open Sci.* 8. doi:10.1098/RSOS.202192.

Chen, J., Li, J., Zhang, H., Shi, W., and Liu, Y. (2019). Bacterial heavy-metal and antibiotic resistance genes in a copper tailing dam area in northern China. *Front. Microbiol.* 10, 447913. doi:10.3389/FMICB.2019.01916/BIBTEX.

Chen, L., Zhou, M., Wang, J., Zhang, Z., Duan, C., Wang, X., et al. (2022). A global meta-analysis of heavy metal(loid)s pollution in soils near copper mines: Evaluation of pollution level and probabilistic health risks. *Sci. Total Environ.* 835, 155441. doi:10.1016/J.SCITOTENV.2022.155441.

Chen, W., and Li, H. (2018). Cost-Effectiveness Analysis for Soil Heavy Metal Contamination Treatments. *Water. Air. Soil Pollut.* 229, 1–13. doi:10.1007/S11270-018-3784-3/METRICS.

Clijsters, H., Cuypers, A., and Vangronsveld, J. (1999). Physiological responses to heavy metals in higher plants; defence against oxidative stress. *Zeitschrift fur Naturforsch. - Sect. C J. Biosci.* 54, 730–734. doi:10.1515/ZNC-1999-9-1018/PDF.

Cui, S., Zhou, Q., and Chao, L. (2007). Potential hyperaccumulation of Pb, Zn, Cu and Cd in endurant plants distributed in an old smeltery, northeast China. *Environ. Geol.* 51, 1043–1048. doi:10.1007/S00254-006-0373-3/METRICS.

D'Andrea, R. M., Andreo, C. S., and Lara, M. V. (2014). Deciphering the mechanisms involved in Portulaca oleracea (C4) response to drought: metabolic changes including crassulacean acid-like metabolism induction and reversal upon re-watering. *Physiol. Plant.* 152, 414–430. doi:10.1111/PPL.12194.

Dalcorso, G., Farinati, S., and Furini, A. (2010). Regulatory networks of cadmium stress in plants. *Plant Signal. Behav.* 5, 663–667. doi:10.4161/PSB.5.6.11425.

de Pádua, M. P., Caetano, A. L., Polo, M., Pasqual, M., and Pereira, F. J. (2021). Ecophysiological Responses of Copaifera langsdorffii Grown in Mining Tailings Under Lower Water Availability. *Water. Air. Soil Pollut.* 232, 1–12. doi:10.1007/S11270-021-05037-

Y/METRICS.

El-Ramady, H., El-Henawy, A., Amer, M., Omara, A. E.-D., Elsakhawy, T., Elbasiouny, H., et al. (2020). Agricultural Waste and its Nano-Management: Mini Review. *Egypt. J. Soil Sci.* 60, 349–364. doi:10.21608/EJSS.2020.46807.1397.

El-Zemrany, H. M., El-Shinnawi, M. M., and Faiyad, R. M. N. (2016). Contribution of Organic Amendments to Growth of Faba Bean and Maize Planted in Soil Exposed to Heavy Metals Pollution. *Egypt. J. Soil Sci.* 56, 701–722. doi:10.21608/EJSS.2016.33246.

Elgharably, A., and Mohamed, H. M. (2016). Heavy Metals Uptake by Wheat, Bean and Onion and Characterization of Microorganisms in a Long-Term Sewage Wastewater Treated Soil. *Egypt. J. Soil Sci.* 56, 605–620. doi:10.21608/EJSS.2016.3334.

Elleuch, A., Chaâbene, Z., Grubb, D. C., Drira, N., Mejdoub, H., and Khemakhem, B. (2013). Morphological and biochemical behavior of fenugreek (Trigonella foenum-graecum) under copper stress. *Ecotoxicol. Environ. Saf.* 98, 46–53. doi:10.1016/J.ECOENV.2013.09.028.

Elramady, H., Elbasiouny, H. Y., Elbehiry, F., and Zia-ur-Rehman, M. (2021). Nano-Nutrients for Carbon Sequestration: A Short Communication. *Egypt. J. Soil Sci.* 61, 389–398. doi:10.21608/EJSS.2021.107134.1480.

Fitz, W. J., and Wenzel, W. W. (2002). Arsenic transformations in the soil–rhizosphere–plant system: fundamentals and potential application to phytoremediation. *J. Biotechnol.* 99, 259–278. doi:10.1016/S0168-1656(02)00218-3.

Flathman, P. E., and Lanza, G. R. (1998). Phytoremediation: Current Views on an Emerging Green Technology. *J. Soil Contam.* 7, 415–432. doi:10.1080/10588339891334438.

Gallego, J. R., Esquinas, N., Rodríguez-Valdés, E., Menéndez-Aguado, J. M., and Sierra, C. (2015). Comprehensive waste characterization and organic pollution co-occurrence in a Hg and As mining and metallurgy brownfield. *J. Hazard. Mater.* 300, 561–571. doi:10.1016/J.JHAZMAT.2015.07.029.

Gardea-Torresdey, J. L., Peralta-Videa, J. R., De La Rosa, G., and Parsons, J. G. (2005). Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. *Coord. Chem. Rev.* 249, 1797–1810. doi:10.1016/J.CCR.2005.01.001.

Gee, G.W. and J.W. Bauder, 1986. Particle Size Analysis. In: Methods of Soil Analysis, Part A. Klute (ed.). 2 Ed., Vol. 9 nd . Am. Soc. Agron., Madison, WI, pp: 383-411. Available at: https://www.sciepub.com/reference/135479 [Accessed January 10, 2024].

Ghazaryan, K. A., Movsesyan, H. S., Khachatryan, H. E., and Ghazaryan, N. P. (2018). Geochemistry of Potentially Toxic Trace Elements in Soils of Mining Area: A Case Study from Zangezur Copper and Molybdenum Combine, Armenia. *Bull. Environ. Contam. Toxicol.* 101, 732–737. doi:10.1007/S00128-018-2443-0.

Ghazaryan, K. A., Movsesyan, H. S., Minkina, T. M., Nevidomskaya, D. G., and Rajput, V. D. (2022). Phytoremediation of copper-contaminated soil by Artemisia absinthium: comparative effect of chelating agents. *Environ. Geochem. Health* 44, 1203–1215. doi:10.1007/S10653-021-01151-2/METRICS.

Ghori, N. H., Ghori, T., Hayat, M. Q., Imadi, S. R., Gul, A., Altay, V., et al. (2019). Heavy metal stress and responses in plants. *Int. J. Environ. Sci. Technol. 2019 163* 16, 1807–1828. doi:10.1007/S13762-019-02215-8.

Gopal, R., and Rizvi, A. H. (2008). Excess lead alters growth, metabolism and translocation of certain nutrients in radish. *Chemosphere* 70, 1539–1544. doi:10.1016/J.CHEMOSPHERE.2007.08.043.

He, Q., Ren, Y., Mohamed, I., Ali, M., Hassan, W., and Zeng, F. (2013). Assessment of Trace and Heavy Metal Distribution by Four Sequential Extraction Procedures in a Contaminated Soil. *Soil Water Res* 8, 71–76.

Helaoui, S., Boughattas, I., Mkhinini, M., Chebbi, L., Elkribi-Boukhris, S., Alphonse, V., et al. (2023). Biochar amendment alleviates heavy metal phytotoxicity of Medicago sativa grown in polymetallic contaminated soil: Evaluation of metal uptake, plant response and soil properties. *Plant Stress* 10, 100212. doi:10.1016/J.STRESS.2023.100212.

Houben, D., Couder, E., and Sonnet, P. (2013). Leachability of cadmium, lead, and zinc in a long-term spontaneously revegetated slag heap: Implications for phytostabilization. *J. Soils Sediments* 13, 543–554. doi:10.1007/S11368-012-0546-5/METRICS.

Hussain, A., Rehman, F., Rafeeq, H., Waqas, M., Asghar, A., Afsheen, N., et al. (2022). In-situ, Ex-situ, and nano-remediation strategies to treat polluted soil, water, and air – A review. *Chemosphere* 289, 133252. doi:10.1016/J.CHEMOSPHERE.2021.133252.

Izydorczyk, G., Mikula, K., Skrzypczak, D., Moustakas, K., Witek-Krowiak, A., and Chojnacka, K. (2021). Potential environmental pollution from copper metallurgy and methods of management. *Environ. Res.* 197, 111050. doi:10.1016/J.ENVRES.2021.111050.

Jiang, X., Liu, W., Xu, H., Cui, X., Li, J., Chen, J., et al. (2021). Characterizations of heavy metal contamination, microbial community, and resistance genes in a tailing of the largest copper mine in China. *Environ. Pollut.* 280, 116947. doi:10.1016/J.ENVPOL.2021.116947.

Kabata-Pendias, A., and Pendias, H. (2000). Trace Elements in Soils and Plants: Third Edition. *Trace Elem. Soils Plants Third Ed.*, 1–414. doi:10.1201/9781420039900/TRACE-ELEMENTS-

SOILS-PLANTS-ALINA-KABATA-PENDIAS.

Keller, C., Ludwig, C., Davoli, F., and Wochele, J. (2005). Thermal Treatment of Metal-Enriched Biomass Produced from Heavy Metal Phytoextraction. *Environ. Sci. Technol.* 39, 3359–3367. doi:10.1021/ES0484101.

Kookana, R. S. (2010). The role of biochar in modifying the environmental fate, bioavailability, and efficacy of pesticides in soils: a review. *Soil Res.* 48, 627–637. doi:10.1071/SR10007.

Kopittke, P. M., and Menzies, N. W. (2006). Effect of Cu toxicity on growth of cowpea (Vigna unguiculata). *Plant Soil* 279, 287–296. doi:10.1007/S11104-005-1578-Z/METRICS.

Krämer, U. (2005). Phytoremediation: novel approaches to cleaning up polluted soils. *Curr. Opin. Biotechnol.* 16, 133–141. doi:10.1016/J.COPBIO.2005.02.006.

Kumar, A., Tripti, Maleva, M., Kiseleva, I., Maiti, S. K., and Morozova, M. (2020). Toxic metal(loid)s contamination and potential human health risk assessment in the vicinity of century-old copper smelter, Karabash, Russia. *Environ. Geochem. Health* 42, 4113–4124. doi:10.1007/S10653-019-00414-3/METRICS.

Li, H., Yao, J., Min, N., Liu, J., Chen, Z., Zhu, X., et al. (2022). Relationships between microbial activity, enzyme activities and metal(loid) form in NiCu tailings area. *Sci. Total Environ.* 812, 152326. doi:10.1016/J.SCITOTENV.2021.152326.

Li, M. S., Luo, Y. P., and Su, Z. Y. (2007). Heavy metal concentrations in soils and plant accumulation in a restored manganese mineland in Guangxi, South China. *Environ. Pollut.* 147, 168–175. doi:10.1016/J.ENVPOL.2006.08.006.

Li, X., Yang, Y., Zhang, J., Jia, L., Li, Q., Zhang, T., et al. (2012). Zinc induced phytotoxicity mechanism involved in root growth of Triticum aestivum L. *Ecotoxicol. Environ. Saf.* 86, 198–203. doi:10.1016/J.ECOENV.2012.09.021.

Lock, K., Janssens, F., and Janssen, C. R. (2003). Effects of metal contamination on the activity and diversity of springtails in an ancient Pb-Zn mining area at Plombières, Belgium. *Eur. J. Soil Biol.* 39, 25–29. doi:10.1016/S1164-5563(02)00006-7.

Maksymiec, W. (2007). Signaling responses in plants to heavy metal stress. *Acta Physiol. Plant.* 29, 177–187. doi:10.1007/S11738-007-0036-3/METRICS.

Malar, S., Vikram, S. S., Favas, P. J. C., and Perumal, V. (2016). Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [Eichhornia crassipes (Mart.)]. *Bot. Stud.* 55, 1–11. doi:10.1186/S40529-014-0054-6/FIGURES/5.

Mani, D., Kumar, C., and Patel, N. K. (2016). Integrated micro-biochemical approach for phytoremediation of cadmium and lead contaminated soils using Gladiolus grandiflorus L cut flower. *Ecotoxicol. Environ. Saf.* 124, 435–446. doi:10.1016/J.ECOENV.2015.11.016.

Marcon, L., Oliveras, J., and Puntes, V. F. (2021). In situ nanoremediation of soils and groundwaters from the nanoparticle's standpoint: A review. *Sci. Total Environ.* 791, 148324. doi:10.1016/J.SCITOTENV.2021.148324.

McGrath, S. P., and Zhao, F. J. (2003). Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotechnol.* 14, 277–282. doi:10.1016/S0958-1669(03)00060-0.

Mendez, M. O., and Maier, R. M. (2008). Phytostabilization of mine tailings in arid and semiarid environments - An emerging remediation technology. *Environ. Health Perspect.* 116, 278–283. doi:10.1289/EHP.10608/ASSET/B807681A-46D6-4E6F-84EB-D013B82C9F84/ASSETS/GRAPHIC/EHP0116-000278F1.JPG.

Monni, S., Salemaa, M., White, C., Tuittila, E., and Huopalainen, M. (2000). Copper resistance of Calluna vulgaris originating from the pollution gradient of a Cu-Ni smelter, in southwest Finland. *Environ. Pollut.* 109, 211–219. doi:10.1016/S0269-7491(99)00265-1.

Niste, M. G., Stoian, V., Monica, N., Vidican, R., Rotar, I., Pop, R., et al. (2014). Plant Nutrition Affected by Soil Salinity and Response of Rhizobium Regarding the Nutrients Accumulation Ioan Rotar Plant Nutrition Affected by Soil Salinity and Response of Rhizobium Regarding the Nutrients Accumulation. Available at: http://journals.usamvcluj.ro/index.php/promediu [Accessed October 17, 2023].

Padmavathiamma, P. K., and Li, L. Y. (2007). Phytoremediation Technology: Hyper-accumulation Metals in Plants. *Water, Air, Soil Pollut. 2007 1841* 184, 105–126. doi:10.1007/S11270-007-9401-5.

Page, A. L. (1982). Methods of soil analysis-Part 2: Chemical and Microbiological properties. (2nd edition). *Am. Soc. Agron. Inc. Publ.Madison, USA* 9, 421-422 (Phosphurus). Available at: https://books.google.com/books/about/Methods_of_Soil_Analysis_Chemical_and_mi.html?id=roAXAQAAIAAJ [Accessed January 10, 2024].

Pourret, O., and Hursthouse, A. (2019). It's Time to Replace the Term "Heavy Metals" with "Potentially Toxic Elements" When Reporting Environmental Research. *Int. J. Environ. Res. Public Health* 16. doi:10.3390/IJERPH16224446.

Rafati, M., Khorasani, N., Moattar, F., Shirvany, A., Moraghebi, F., and Hosseinzadeh, S. (2011). Phytoremediation Potential of Populus Alba and Morus alba for Cadmium, Chromuim and Nickel Absorption from Polluted Soil. *Int. J. Environ. Res.* 5, 961–970. doi:10.22059/IJER.2011.453.

Rajjak Shaikh, I., Rajjak Shaikh, P., Ahmed Shaikh, R., and Abdulla Shaikh, A. (2013). Phytotoxic effects of Heavy metals (Cr, Cd, Mn and Zn) on Wheat (Triticum aestivum L.) Seed Germination and Seedlings growth in Black Cotton Soil of Nanded, India. *Res. J. Chem. Sci.* 3, 14–23. Available at: www.isca.in [Accessed January 2, 2024].

Rajput, V. D., Minkina, T., Fedorenko, A., Chernikova, N., Hassan, T., Mandzhieva, S., et al. (2021). Effects of Zinc Oxide Nanoparticles on Physiological and Anatomical Indices in Spring Barley Tissues. *Nanomater. 2021, Vol. 11, Page 1722* 11, 1722. doi:10.3390/NANO11071722.

Rashid, A., Schutte, B. J., Ulery, A., Deyholos, M. K., Sanogo, S., Lehnhoff, E. A., et al. (2023). Heavy Metal Contamination in Agricultural Soil: Environmental Pollutants Affecting Crop Health. *Agronomy* 13, 1521. doi:10.3390/AGRONOMY13061521/S1.

Rastegari Mehr, M., Shakeri, A., Amjadian, K., Khalilzadeh Poshtegal, M., and Sharifi, R. (2021). Bioavailability, distribution and health risk assessment of arsenic and heavy metals (HMs) in agricultural soils of Kermanshah Province, west of Iran. *J. Environ. Heal. Sci. Eng.* 19, 107–120. doi:10.1007/S40201-020-00585-7/METRICS.

Reyes, A., Cuevas, J., Fuentes, B., Fernández, E., Arce, W., Guerrero, M., et al. (2021). Distribution of potentially toxic elements in soils surrounding abandoned mining waste located in Taltal, Northern Chile. *J. Geochemical Explor.* 220, 106653. doi:10.1016/J.GEXPLO.2020.106653.

Rouphael, Y., Cardarelli, M., Rea, E., and Colla, G. (2008). Grafting of cucumber as a means to minimize copper toxicity. *Environ. Exp. Bot.* 63, 49–58. doi:10.1016/J.ENVEXPBOT.2007.10.015.

Sajad, M. A., Khan, M. S., Bahadur, S., Naeem, A., Ali, H., Batool, F., et al. (2020). Evaluation of chromium phytoremediation potential of some plant species of Dir Lower, Khyber Pakhtunkhwa, Pakistan. *Acta Ecol. Sin.* 40, 158–165. doi:10.1016/J.CHNAES.2019.12.002.

Sári, D., Ferroudj, A., Dávid, S., El-Ramady, H., Abowaly, M., Abdalla, Z. F., et al. (2024). Is Nano-Management a Sustainable Solution for Mitigation of Climate Change under the Water-Energy-Food Nexus? *Egypt. J. Soil Sci.* 64, 0–0. doi:10.21608/EJSS.2023.233939.1656.

Schädler, S., Morio, M., Bartke, S., Rohr-Zänker, R., and Finkel, M. (2011). Designing sustainable and economically attractive brownfield revitalization options using an integrated assessment model. *J. Environ. Manage.* 92, 827–837. doi:10.1016/J.JENVMAN.2010.10.026.

Schnebele, E., Jaiswal, K., Luco, N., and Nassar, N. T. (2019). Natural hazards and mineral commodity supply: Quantifying risk of earthquake disruption to South American copper supply. *Resour. Policy* 63. doi:10.1016/J.RESOURPOL.2019.101430.

Seck, G. S., Hache, E., Bonnet, C., Simoën, M., and Carcanague, S. (2020). Copper at the crossroads: Assessment of the interactions between low-carbon energy transition and supply limitations. *Resour. Conserv. Recycl.* 163, 105072. doi:10.1016/J.RESCONREC.2020.105072.

Shen, Z. J., Xu, D. C., Chen, Y. S., and Zhang, Z. (2017). Heavy metals translocation and accumulation from the rhizosphere soils to the edible parts of the medicinal plant Fengdan (Paeonia ostii) grown on a metal mining area, China. *Ecotoxicol. Environ. Saf.* 143, 19–27. doi:10.1016/J.ECOENV.2017.04.042.

Shi, J., Qian, W., Jin, Z., Zhou, Z., Wang, X., and Yang, X. (2023). Evaluation of soil heavy metals pollution and the phytoremediation potential of copper-nickel mine tailings ponds. *PLoS One* 18, e0277159. doi:10.1371/JOURNAL.PONE.0277159.

Singh, A., Margaryan, G., Harutyunyan, A., S. Movsesyan, H., Khachatryan, H., Rajput, V. D., et al. (2024a). Advancing Agricultural Resilience in Ararat Plain, Armenia: Utilizing Biogenic Nanoparticles and Biochar under Saline Environments to Optimize Food Security and Foster European Trade. *Egypt. J. Soil Sci.* 64, 459–483. doi:10.21608/EJSS.2024.257000.1703.

Singh, A., Rajput, V. D., Varshney, A., Sharma, R., Ghazaryan, K., Minkina, T., et al. (2024b). Revolutionizing Crop Production: Nanoscale Wonders - Current Applications, Advances, and Future Frontiers. *Egypt. J. Soil Sci.* 64, 0–0. doi:10.21608/EJSS.2023.246354.1684.

Singh, S., Parihar, P., Singh, R., Singh, V. P., and Prasad, S. M. (2016). Heavy metal tolerance in plants: Role of transcriptomics, proteomics, metabolomics, and ionomics. *Front. Plant Sci.* 6, 165395. doi:10.3389/FPLS.2015.01143/BIBTEX.

Sytar, O., Kumari, P., Yadav, S., Brestic, M., and Rastogi, A. (2019). Phytohormone Priming: Regulator for Heavy Metal Stress in Plants. *J. Plant Growth Regul.* 38, 739–752. doi:10.1007/S00344-018-9886-8.

Tang, L., and Gao, S. (2010). Effects of Zinc on Growth and Antioxidant Responses in Jatropha curcas Seedlings. Available at: http://www.fspublishers.org [Accessed January 2, 2024].

Tepanosyan, G., Sahakyan, L., Belyaeva, O., Asmaryan, S., and Saghatelyan, A. (2018). Continuous impact of mining activities on soil heavy metals levels and human health. *Sci. Total Environ.* 639, 900–909. doi:10.1016/J.SCITOTENV.2018.05.211.

Ullah, S., Adeel, M., Zain, M., Rizwan, M., Irshad, M. K., Jilani, G., et al. (2020). Physiological and biochemical response of wheat (Triticum aestivum) to TiO2 nanoparticles in phosphorous amended soil: A full life cycle study. *J. Environ. Manage.* 263, 110365. doi:10.1016/J.JENVMAN.2020.110365.

Usman, A. R. A., Lee, S. S., Awad, Y. M., Lim, K. J., Yang, J. E., and Ok, Y. S. (2012). Soil pollution assessment and identification of hyperaccumulating plants in chromated copper arsenate (CCA) contaminated sites, Korea. *Chemosphere* 87, 872–878. doi:10.1016/J.CHEMOSPHERE.2012.01.028.

Welch, R. M. (2002). The impact of mineral nutrients in food crops on global human health. *Plant Soil* 247, 83–90. doi:10.1023/A:1021140122921.

Wodala, B., Eitel, G., Gyula, T. N., Ördög, A., and Horváth, F. (2012). Monitoring moderate Cu and Cd toxicity by chlorophyll fluorescence and P 700 absorbance in pea leaves. *Photosynthetica* 50, 380–386. doi:10.1007/S11099-012-0045-3/METRICS.

Wuana, R. A., and Okieimen, F. E. (2011). Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *ISRN Ecol.* 2011, 1–20. doi:10.5402/2011/402647.

Xiao, X., Zhang, J., Wang, H., Han, X., Ma, J., Ma, Y., et al. (2020). Distribution and health risk assessment of potentially toxic elements in soils around coal industrial areas: A global meta-analysis. *Sci. Total Environ.* 713, 135292. doi:10.1016/J.SCITOTENV.2019.135292.

Ying, G. G. (2018). Remediation and mitigation strategies. *Integr. Anal. Approaches Pestic. Manag.*, 207–217. doi:10.1016/B978-0-12-816155-5.00014-2.

Yoon, J., Cao, X., Zhou, Q., and Ma, L. Q. (2006). Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ.* 368, 456–464. doi:10.1016/J.SCITOTENV.2006.01.016.

Zakari, S., Jiang, X., Zhu, X., Liu, W., Allakonon, M. G. B., Singh, A. K., et al. (2021). Influence of sulfur amendments on heavy metals phytoextraction from agricultural contaminated soils: A meta-analysis. *Environ. Pollut.* 288, 117820. doi:10.1016/J.ENVPOL.2021.117820.

Zanchi, C. S., Batista, É. R., Silva, A. O., Barbosa, M. V., Pinto, F. A., dos Santos, J. V., et al. (2021). Recovering Soils Affected by Iron Mining Tailing Using Herbaceous Species with Mycorrhizal Inoculation. *Water. Air. Soil Pollut.* 232, 1–13. doi:10.1007/S11270-021-05061-Y/FIGURES/6.

Žemberyová, M., Barteková, J., and Hagarová, I. (2006). The utilization of modified BCR three-step sequential extraction procedure for the fractionation of Cd, Cr, Cu, Ni, Pb and Zn in soil reference materials of different origins. *Talanta* 70, 973–978. doi:10.1016/J.TALANTA.2006.05.057.

Zulfiqar, F., and Ashraf, M. (2022). Antioxidants as modulators of arsenic-induced oxidative stress tolerance in plants: An overview. *J. Hazard. Mater.* 427, 127891. doi:10.1016/J.JHAZMAT.2021.127891.

Zulfiqar, U., Farooq, M., Hussain, S., Maqsood, M., Hussain, M., Ishfaq, M., et al. (2019). Lead toxicity in plants: Impacts and remediation. *J. Environ. Manage.* 250, 109557. doi:10.1016/J.JENVMAN.2019.109557.