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Assisted Phytostabilization of Acidic Polymetallic Mine Tailings Using Marble Waste and Native Plant *Citrullus colocynthis*: Effect on Soil, Plant, and Metal Uptake

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Abstract

This study aimed to assess the effectiveness of the phytostabilization ability of the native Moroccan plant *Citrullus colocynthis* on the neutralization of soil acidity and stabilization of metallic trace elements (MTEs) assisted by marble waste. Mine tailings (MT) collected from an abandoned polymetallic site were mixed with powdered marble (PM) using different mixing ratios (%): ($MTPM_{25}$), ($MTPM_{50}$), ($MTPM_{75}$), and agricultural soil (AS) acting as the control. Seeds of *C. colocynthis* were selected and planted under greenhouse conditions. Growth parameters were measured, and the metal concentrations in substrates and plant tissues were analyzed. The greenhouse results revealed that the growth parameters of *C. colocynthis* in the experimental treatments were significantly ($p \leq 0.05$) lower than those in agricultural soil. The growth of *C. colocynthis* significantly ($p \leq 0.05$) decreased as the ratio of powdered marble increased. The greatest increase in plant biomass was observed in the $MTPM_{25}$, followed by $MTPM_{50}$ and $MTPM_{75}$. Some phytotoxic effects on plants were observed in $MPTM_{75}$. The concentrations of Zn, Cu, Pb, and Cd in *C. colocynthis* roots were significantly ($p \leq 0.05$) higher than those of shoots, with maximum values of 112.98 mg kg^{-1}, 201.3 mg kg^{-1}, 201.2 mg kg^{-1}, and 40.03 mg kg^{-1}, respectively. This is a typical characteristic of accumulator plants and maybe one of the tolerance mechanisms. Plants in the experimental treatments were characterized by TF<1 and BCF<1, which indicate that *C. colocynthis* could be useful for phytostabilization strategies. Our study demonstrates that the phytostabilization by *C. colocynthis* assisted by powdered marble could represent a successful and environmentally friendly strategy to remediate acidic polymetallic sites.

Keywords
Acidic mine tailings; heavy metals; assisted phytostabilization; *C. colocynthis*; marble waste; remediation

1. Introduction

Mining is a globally important economic activity that provides the raw materials for several essential human activities [1]. However, mining is a high-risk activity, not only for the workers (miners, engineers, etc.) associated with this industry but also for the local population and the environment surrounding the mining areas [2]. Tailings are the most environmentally damaging residue from mining activities. Tailings are a by-product of no commercial value, which are generated in huge volumes and stored untreated in the open air [3]. Mine tailings usually contain high levels of heavy metals, have low nutritional value, and are subject to erosion, especially in arid and semi-arid environments. They persist in the environment, contaminate the food chain, and cause various health problems [4].

Sustainable remediation of mine tailings can be difficult to implement due to the high cost of current remediation techniques. Several physical, chemical, and biological methods have been proposed to remediate mine tailings. These include leaching or washing, chemical stabilization with mineral and organic amendments, alkaline hydrolysis, photolysis, bioremediation, and phytoremediation [4-6]. Phytoremediation is a promising and highly researched methodology for the improvement of polymetallic contaminated soils [7]. Several mechanisms of phytoremediation have been identified, such as phytoextraction, phytodegradation, rhizofiltration, and phytostabilization [8]. The most viable phytoremediation mechanism for a particular situation will depend on the physiology of the plant species, the response of the plant species to the treatments, the specific heavy metals, soil structure, and microorganisms [8].

Assisted phytostabilization is a profitable technique that uses plants to reduce the mobility of metallic trace elements (MTEs) and their transfer to the surrounding environment [9, 10]. The key element of a successful phytostabilization program is the optimal choice of the plant species [11].

Metallophytes are generally considered the best candidates because they are well adapted to the environmental conditions of the target site, better in terms of survival, and are characterized by high tolerance to hotspot contamination [12]. Due to their specific genetic and morphological characteristics, metallophytes can develop a biological mechanism to survive and reproduce on polymetallic soils without being affected by the high concentrations of metals.

The native plant used in this study is *Citrullus colocynthis* (L.) Schrader. This species was selected following a meticulous botanical survey on polymetallic mining sites [13]. *C. colocynthis* is a perennial metallophyte with an enhanced ability to survive in xeric and contaminated soils. This plant can accumulate a significant metal load in its tissue and can be easily manipulated in laboratory conditions. *C. colocynthis* grows rapidly, has a short vegetative cycle, and readily produces seeds. It can also be used in several pharmacological activities and medical applications [14]. Most research on *C. colocynthis* has focused on its chemical constituents [15], antioxidant properties [16], anti-inflammatory activity [17], and pharmacological effects [18]. Its potential use as a metal-accumulator plant and its effectiveness in the phytostabilization of contaminated soils has been relatively less well-studied.

Combining the use of soil amendments (chemical immobilization) with plant cover as part of an assisted phytostabilization regime will lead to more effective remediation [19]. Immobilization techniques often use inorganic amendments to facilitate the removal of metals from the soil solution through adsorption, complexation, or precipitation reactions, thereby making the metals unavailable for uptake by humans or plants and preventing their leaching into groundwater [20]. One such inorganic amendment is marble waste. Marble waste is rich in carbonates, which strongly influence the soil pH, as well as the accumulation and mobility of metals. Several studies have proven its efficiency in the restoration of contaminated soils [19, 21-25]. The use of marble waste is commonly used for phytoremediation, and especially phytostabilization.

The current study aims to propose sustainable solutions for the remediation of polymetallic abandoned mine sites using assisted phytostabilization by *C. colocynthis* and marble waste. Their effects on the neutralization of soil acidity and the uptake of metals were evaluated.

2. Materials and Methods

2.1 Soil Sampling and Characterization

Mine tailings, an acidic polymetallic waste, were directly collected (15-25 cm in depth) from the residues of an abandoned mine site located approximately 35 km northwest of Marrakech, in the Jebilet Mountains (Figure 1). The powdered marble was recovered from a processing unit and used in the experiment without any prior treatment. An agricultural soil (clean soil) was collected from a 0-10-cm-deep layer in the garden of Cadi Ayyad University (Marrakech-Morocco) and used as a reference. The soil had not received manure or pesticide applications.

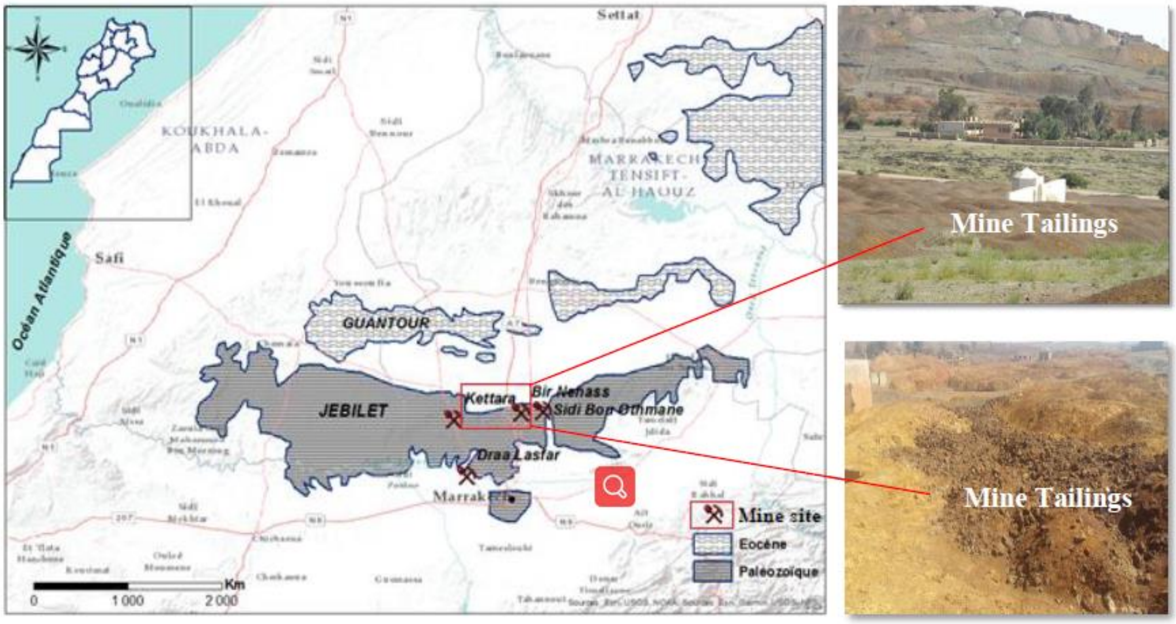

Figure 1 Location of the abandoned mine site [13].

The soil samples were air-dried in ambient conditions and passed through a 2-mm mesh sieve. The physical and chemical properties of the soil were determined according to the standard procedures published in the Official Methods of Soil Analysis [26]. The soil texture was determined using the Robinson pipette method combined with sieving. The calcium carbonate ($CaCO_3$) equivalent was determined using a Bernard calcimeter. Electrical conductivity (1:5 w/v soil water suspensions) and pH (1:2.5 w/v soil water) were measured using a multiparameter probe-type LF 92 WTW. Total organic carbon was determined by the oxidation of the organic matter using potassium dichromate [27], available P (P_2O_5) by the Olsen method [28], and total Kjeldahl nitrogen was determined using the Kjeldahl method [29].

Analyses of major and trace elements were conducted by X-ray fluorescence spectrometry (Olympus, UK) and atomic absorption spectrophotometer (AAS) (Shimadzu AA-6300), respectively. Heavy metal concentrations were determined after the complete digestion of a 0.50 g dry sample of mine tailings at 500^{o}C. The sample was digested in 7 mL nitric acid (HNO_3) (65% w/w), 2 mL hydrofluoric acid (HF) (40% w/w), and 1 mL perchloric acid ($HClO_4$) (60% w/w) in a Teflon beaker. The sample solutions were filtered using a 0.45 mm cellulose nitrate filter, and the volume was adjusted to 10 mL with 0.1 M HNO_3. The total metal concentrations were analyzed in triplicate and calculated on a dry weight basis (mg kg^{-1} dw). The physical and mineral properties of powdered marble were determined using the methods outlined in [25, 30].

2.2 Plant Species

2.2.1 Botanical Description

The native Moroccan plant *Citrullus colocynthis* (L.) Schrader was used in this study (Figure 2). *C. colocynthis* is a creeping, herbaceous perennial plant bristling with hair (Table 1). The plant has rough, angular stems with tendrils and large, alternate, deeply cut leaves. It is characterized by a stout, perennial, long taproot, with occasional rooting at the nodes on thick branches. Yellow flowers with five somewhat welded petals appear in the axils of the leaves in summer.

Figure 2 *Citrullus colocynthis (L.)* Schrader.

Table 1 Intrinsic characteristics of *Citrullus colocynthis* (L.).

Family	Cucurbitaceae
Common name	Coloquinte
Life cycle	Perennial
Stem Height	A creeping stem that can reach 3 m
Root system	Pivoting
Cotyledon type	Dicotyledons
Bioclimate conditions	Arid and semi-arid areas

The spherical fruits measure 5 to 10 cm in diameter with a fleshy pulp. The young fruits are green and light-yellow, becoming completely yellow when ripe. Dry fruits can remain on the plant for a long time. The light, spongy, orange-yellow flesh is very bitter and toxic. The numerous edible seeds are ovoid and flattened, varying in color from orange to blackish brown. The fruit's bitter taste is due to the chemicals colocynthin and colocynthetin. *C. colocynthis* is a widespread species in arid and Saharan climate regions and has a wide range of pharmacological properties that promote its use in several medical applications [14].

2.2.2 Seed Sampling, Treatment, and Germination

C. colocynthis seeds were collected in 2018 from an abandoned mining area located about 33 km from Marrakech City. This region is characterized by a Mediterranean climate (arid to semi-arid bioclimate) with a low and irregular annual rainfall of approximately 300 mm and a mean temperature of 11.5°C in January and 36.8°C in July (ONEM, 1997). In the laboratory, healthy seeds were separated from infertile seeds and disinfected with sodium hypochlorite 5% (NaClO) for 10 min and then rinsed thrice with distilled water. The seeds were mechanically scarified and soaked in 200 mL of water at room temperature for 24 h to break seed dormancy. Then, the seeds were directly sown into pots. During the experiment, germination percentage, germination rate, germination index, and germination stress were controlled and reported.

2.3 Greenhouse Experiment

Growth experiment treatments (Figure 3a) were conducted to test the growth of *C. colocynthis* in mine tailing amended with powdered marble. A total of 3 kg of materials (Mine tailing + powdered marble) at predetermined proportions (Table 2) were thoroughly mixed and placed in the pots. Each treatment was prepared in triplicate. Each pot was sown with five *C. colocynthis* seeds (Figure 3b) and allowed to grow for 4 months from 1st April to 30th July under semi-controlled greenhouse conditions.

Figure 3 Growth experimental treatments (a) and sowing seeds (b) of *C. Colocynthis* (S1-S5: seeds).

Table 2 Proportions of the experimental materials.

Experimental treatments	Total of 3 kg per pot (w/w)
AS	Agricultural Soil
MT	100% Mine Tailings
$MTPM_{25}$	75% Mine Tailings + 25% Powdered Marble
$MTPM_{50}$	50% Mine Tailings + 50% Powdered Marble
$MTPM_{75}$	25% Mine Tailings + 75% Powdered Marble

2.4 Analysis of Plant Material

2.4.1 Physiological Parameters

Four months after planting the seeds, seedlings were removed from the soil. They were washed three times with tap water and then with distilled water to remove all visible soil particles. The length and the fresh and dry weights of the plant tissues in each treatment were measured as follows:

Plant length (PL): Shoot and root lengths of the plants were measured manually using a graduated ruler.

Fresh material (FM): Immediately after cutting, the weight of the roots and aerial parts were weighed using a precision balance.

Dry material (DM): The weight of both the roots and aerial parts were weighed using a precision balance after drying them in an oven at 70°C for 48 h.

2.4.2 Heavy Metal Analysis

The dried biomass was grounded to a powder in a porcelain mortar and sieved to obtain a size of less than 2 mm. About 1-2 g of plant tissue was burnt to ash in a muffle furnace at 450°C for 4 h and digested in an aqua regia solution (a 1:3 mixture of nitric acid [HNO_3] concentrated with hydrochloric acid [HCl]) overnight at room temperature. The mixture was then boiled for 2 h, according to the standard method NF ISO 11466 (AFNOR, 1995). After cooling, the digested samples were filtered through a 0.45 mm cellulose nitrate filter and adjusted to a volume of 10 mL with 0.1 M HNO_3. The concentrations of total metals in the shoots, stems, and roots were determined by Atomic Absorption Spectrophotometer AAS (Shimadzu AA-6300).

2.5 Data Analysis

The translocation factor (TF) indicates the efficiency of the plant in translocating the accumulated metals from its roots to shoots. It is calculated as follows [4, 31]:

$$TF = [Metal]_{\boldsymbol{shoot}}/[Metal]_{\boldsymbol{root}}$$

The bioconcentration factor (BCF) indicates the efficiency of a plant species in accumulating a metal into its tissues from the surrounding environment. It is calculated as follows [4, 32]:

$$BCF = [Metal]_{\boldsymbol{harvested\ tissue}}/[Metal]_{\boldsymbol{soil}}$$

The data of the different treatments were analyzed by calculating the mean values and standard deviation. The differences between the means of the groups were evaluated using analysis of variance (ANOVA) followed by pairwise comparisons using the Student-Newman-Keuls test as appropriate. P-values of <0.05 indicated statistically significant differences. All the statistical evaluations were performed using the SPSS software (Statistical Package for Social Sciences) (IBM SPSS Statistics 21.0 software, IBM, Chicago, IL, USA).

3. Results and Discussion

3.1 Physicochemical Properties of Materials

The major physicochemical properties of the experimental materials are presented (Table 3). The mine tailings were highly acidic, with a pH of around 2.61 ± 0.09 and high conductivity of 1384 ± 1.71 mS m^{-1}. These residues had relatively low levels of C, P, and N, which effectively promote plant growth and development. Soils with these properties are unsuitable for the establishment of plant cover.

Table 3 General properties of experimental materials.

Element	Unit	MT	PM	AS
pH	---	2.61 ±0.09	8.95 ±0.05	7.21 ±0.06
EC	mS m^{-1}	1384 ±101.2	128 ±1.30	369 ±1.56
$CaCO_3$	%	4.60 ±0.33	92.11 ±1.08	5.03 ±0.45
N	g kg^{-1}	0.3 ±0.05	1.7 ±0.07	12 ±1.05
C	g kg^{-1}	3.5 ±0.21	---	229 ±2.04
P	mg kg^{-1}	1197 ±12.01	490 ±6.02	1185 ±10.09
K	mg kg^{-1}	96.4 ±0.96	990 ±7.21	2560 ±13.06
Na	mg kg^{-1}	8.40 ±0.08	1536 ±9.32	93 ±1.04
S	g kg^{-1}	1.25 ±1.06	0.38 ±0.03	0.56 ±0.03
Fe	g kg^{-1}	23.39 ±2.72	6.76 ±1.02	20.2 ±0.09
Cu	mg kg^{-1}	1670 ±12.17	16 ±7.02	91 ±4.12
Zn	mg kg^{-1}	610 ±4.36	20 ±0.08	100 ±1.22
Pb	mg kg^{-1}	560 ±15.72	---	73 ±6.01
Cd	mg kg^{-1}	78.41 ±0.88	----	---

The presence of toxic levels of metallic trace elements in the soil may also significantly affect plant growth. Mine tailing samples were characterized by high levels of Cu, Zn, Pb, and Cd, which exceeded the limits established by the European Directive 86/278/EEC (Council of the European Communities Directive (86/278/EEC) 1986) on the protection of the environment. Therefore, they pose a potentially serious risk for the environment and public health.

Powdered marble had an alkaline pH of 8.95, a high content of carbonates ($CaCO_3$), and low contents of metallic trace elements (Table 3). The high carbonate content plays a key role in the pH variation and gives the amendment strong acid-neutralizing properties. The agricultural soil had a neutral pH (7.21 ± 0.06), EC $\leq$ 369 mS m^{-1}, and low concentrations of metallic trace elements that did not exceed the AFNOR reference values (NFU44-041, 1985). In contrast, powdered marble was rich in essential elements, which are important for plant growth.

3.2 Effect of Powdered Marble on pH, EC, and MTEs Content

The results of pH, EC, and MTEs concentrations in the experimental treatments are presented in Table 4.

Table 4 pH and MTEs contents of mine tailings mixed with different proportions of powdered marble.

Element	Unit	MT	$MTPM_{25}$	$MTPM_{50}$	$MTPM_{75}$
pH	---	2.61 ±0.09^{c}	7.1 ±0.1^{b}	7.2 ±0.2^{b}	7.97 ±0.1^{a}
EC	mS m^{-1}	1384 ±101.2^{a}	483 ±0.6^{b}	457 ±2.5^{b}	439 ±1.6^{b}
Cu	mg kg^{-1}	1670 ±12.17^{a}	1381 ±6.08^{b}	1186 ±7.81^{c}	1106 ±8.72^{d}
Zn	mg kg^{-1}	610 ±4.36^{a}	350 ±0.89^{b}	240 ±13.11^{c}	130 ±13.11^{d}
Pb	mg kg^{-1}	560 ±15.72^{a}	388.22 ±12.9^{b}	190 ±14^{c}	68.50 ±3.70^{d}
Cd	mg kg^{-1}	78,41 ±2.57^{a}	75.18 ±0.88^{a}	60.01 ±2.86^{b}	33.50 ±1.35^{c}

Results are expressed in mg kg^{-1} of M.S. ± ES. Different letters (a-d) indicate significant differences between the studied treatments (Student-Newman-Keuls test, $p \le 0.05$, n = 3).

The pH values increased from 2.61 in MT to 7.1, 7.2, and 7.97 in treatments amended with powdered marble $MTPM_{25}$, $MTPM_{50}$, and $MTPM_{75}$, respectively (Table 4). In contrast, the EC value decreased from 1384 mS m^{-1} in MT to 483, 457, and 439 mS m^{-1} in $MTPM_{25}$, $MTPM_{50}$ and $MTPM_{75}$, respectively (Table 4). When moist mine tailings are exposed to air, the oxidation and hydrolysis mechanisms of sulfides are activated, resulting in the formation of sulfuric acid. Therefore, the pH values and the solubility of the MTEs increase [33-35]. The application of powdered marble to mine tailings rich in mineral sulfides causes an increase in the pH and a decrease in the EC of the soil.

The concentrations of Cu, Zn, Pb, and Cd in the mine tailings were high (Table 4). After the application of powdered marble, the concentrations of these elements decreased. The addition of powdered marble significantly ($p \le 0.05$) decreased the concentrations of metals in soil, suggesting that the carbonates played an active role in reducing the solubility of metals; thereby, greatly decreasing the spread of metals in the environment. The carbonate materials, which constitute a major part of powdered marble, can reduce metal solubility by the formation of stable metal chelates [24, 36].

3.3 Effect of Powdered Marble on Seed Germination and Plant Growth

No *C. colocynthis* seeds germinated in mine tailings (MT) (Figure 4). This result was expected due to the high acidity and the high metal content in these residues (Table 4). In contrast, maximum germination rates were observed in the agricultural soil. One week after seed sowing, germination rates in treatments amended with powdered marble $MTPM_{25}$, $MTPM_{50}$, and $MTPM_{75}$ were increased (Figure 4). Delayed germination was understandable as dormancy persisted in *C. colocynthis* seeds. Changes in seedling height (cm) of *C. colocynthis* were affected by different proportions of powdered marble.

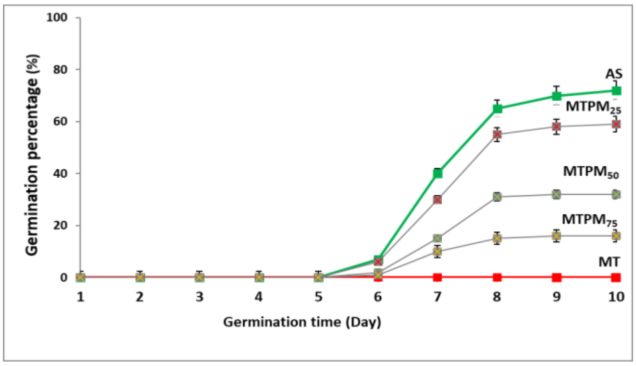

Figure 4 Germination (%) of *C. colocynthis* seeds in the experimental treatments.

Regarding the results obtained from the amended treatments, the highest increase in plant biomass was observed in treatment $MTPM_{25}$, followed by treatment $MTPM_{50}$ and treatment $MTPM_{75}$ (Figures 5a and 5b). Some phytotoxic effects such as stunting, curling of young shoots, death of leaf tip, chlorosis, and inhibition of root growth were observed on plants when using 75% of powdered marble, possibly due to the compact texture and high level of soluble salts in this treatment. The application of powdered marble significantly ($p \leq 0.05$) enhanced the seed germination rate of *C. colocynthis* and could be used as an amendment in low concentrations (25%).

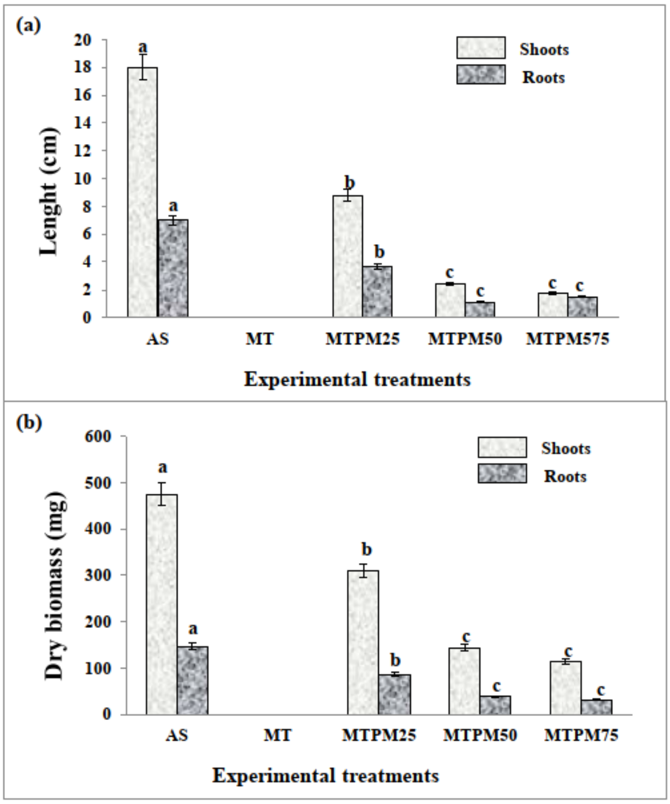

Figure 5 Length (a) and dry biomass (b) of the shoots and roots of *C. colocynthis* at the end of the experiment in the experimental treatments. Different letters (a-c) denote statistically significant differences (Student-Newman-Keuls test, p≤0.05) between the investigated treatments.

In the treatments using powdered marble, *C. colocynthis* plants were able to grow, although the weight of dry biomass, the length of the leaves, and roots were significantly ($p \leq 0.05$) lower than those recorded in plants grown in the agricultural soil (Figure 5a and Figure 5b). The capacity of agricultural soil to supply the essential macronutrients (N, P, K) could explain the different relative growth of the plants. The lack of essential elements could be the major cause of limited plant growth in the amended treatments. Therefore, fertilizer amendment should be supplemented to the powdered marble, as it is recommended in most soil rehabilitation projects [24, 37].

3.4 Effects of Powdered Marble on Metal Accumulation in Plants

In *C. colocynthis* plants growing in the treatments $MTPM_{25}$, $MTPM_{50}$, and $MTPM_{75}$, the concentrations of MTEs (Zn, Cu, Pb, and Cd) were significantly higher ($p \leq 0.05$) than those growing in agricultural soil (Figure 6). The highest levels of MTEs in plant tissues were obtained in the treatment $MTPM_{25}$, followed by the treatments $MTPM_{50}$ and $MTPM_{75}$ (Figure 6). The application of powdered marble significantly ($p \leq 0.05$) limited the accumulation of MTEs by *C. colocynthis* tissues. The increase in soil pH resulted in a significant decrease in metal content in the shoots and roots. Some physical and chemical processes, including precipitation, absorption, complexation, redox reaction, and ion exchange, occur with changes in soil pH, which can influence the uptake of metals from soil; thus, reducing their uptake by plants at a higher soil pH [14, 21-23].

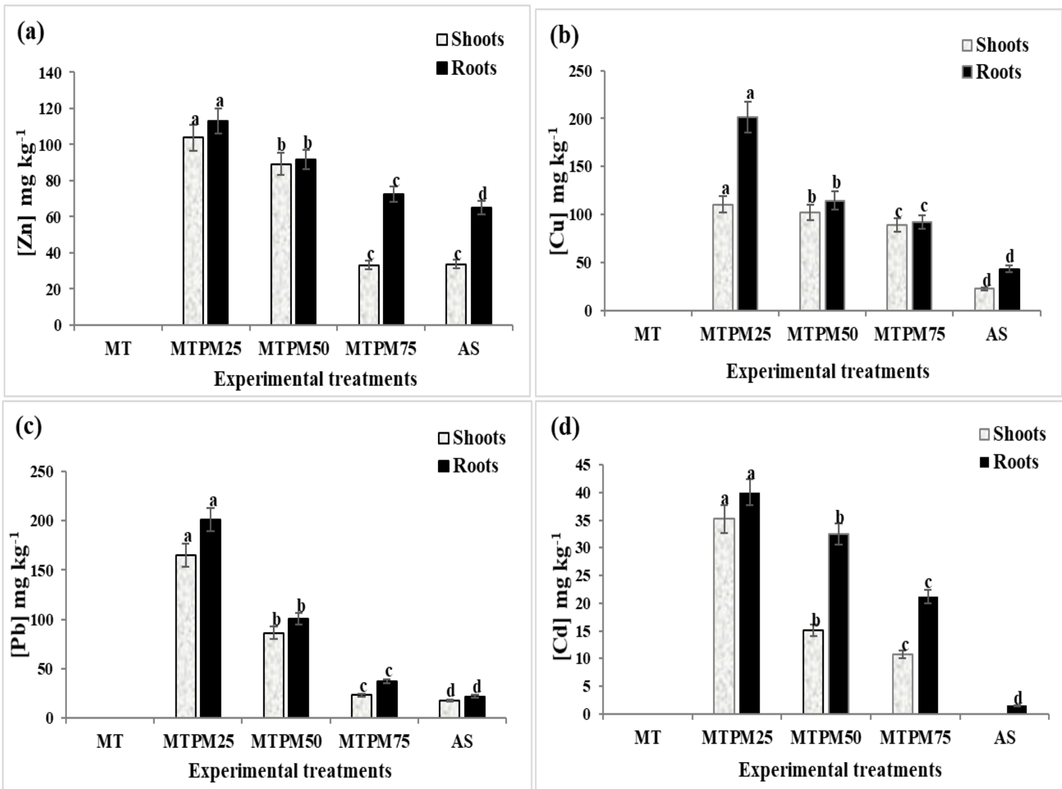

Figure 6 The concentrations of Zn (a), Cu (b), Pb (c), and Cd (d) (mean ± standard deviation) of the leaves and roots of *C. colocynthis* in the experimental treatments. Different letters (a-d) indicate significant differences between the studied treatments (Student-Newman-Keuls test, $p \leq 0.05$, n = 3).

The metal concentrations in *C. colocynthis* plants were higher than those obtained in plants growing in natural soil estimated by Kramer [38] (Zn: 100-300 mg kg^{-1}, Pb: 0.6-28 mg kg^{-1}, Cu: 20-30 mg kg^{-1}, and Cd: 0.1-3 mg kg^{-1} dry weight). *C. colocynthis* plants can tolerate and accumulate significant concentrations of MTEs in their tissues. The concentrations of MTEs in roots were significantly ($p \leq 0.05$) higher than those in shoots ([Shoots]<[Roots]) (Figure 6). This physiological trait is a tolerance mechanism in accumulator plants [39]. *C. colocynthis* is a good candidate for use in the stabilization of acidic polymetallic soils due to its high tolerance and strong accumulation of MTEs in roots. However, it cannot be classified as a hyperaccumulator of MTEs and is therefore suitable for the phytostabilization of soils contaminated with MTEs (Cu, Zn, Pb, and Cd) [40]. Metal concentrations were below the levels stipulated by the US Domestic Animal Metal Toxicity limits [41], which confirms that *C. colocynthis* is a good candidate for phytostabilization and could be used safely without detriment to grazing animals.

3.5 Efficiency of Phytoremediation

Bioconcentration factor (BCF) and translocation factor (TF) were used to assess the accumulation efficiency of *C. colocynthis*, as well as to estimate their phytoremediation potential (phytostabilization and/or phytoextraction) [4].

The experimental treatments of *C. colocynthis* were characterized by TF<1.0 (Figure 7a). A TF of <1.0 indicates that the plants can accumulate metals in their roots and limit the translocation of metals to the shoots. This reduces the mobility of metals and their leaching into groundwater. The plants minimize the transfer of metals into the food chain, ultimately improving human health.

C. colocynthis is characterized by excluder traits and can be used successfully in phytostabilization [42]. Similar results were observed for other plant species (*Medicago sativa* L., *Zygophyllum fabago*, *Helichrysum decumbens, Erica andevalensis*, Tamarix sp., *Lygeum spartum*, Cistus, Cytisus...) in mining areas [25, 43-45]. The mobility of MTEs into *C. colocynthis* tissues from mine tailings was evaluated through the determination of the BCF (Figure 7b). The BCF values of Cu, Zn, and Pb in *C. colocynthis* plants under different treatments were significantly ($P \leq 0.05$) lower than 1 (BCF<1), except for Cd, indicating its lower accumulation of metals in tissues and can be considered as a metal excluder species growing on mine tailings.

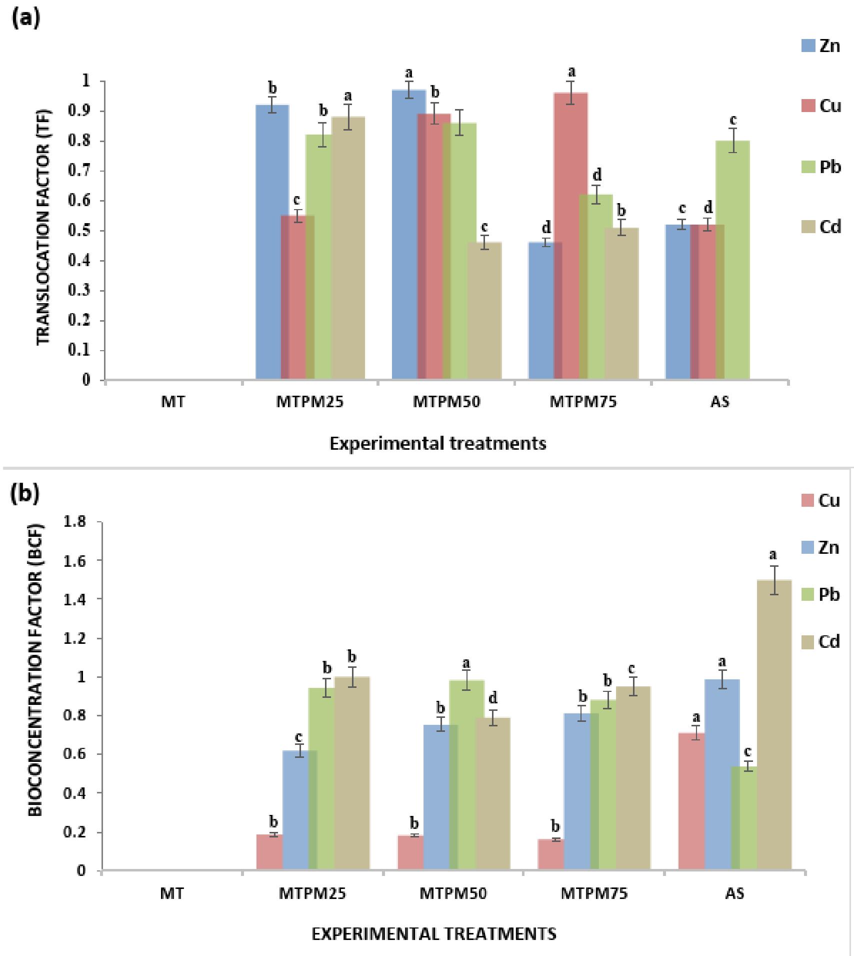

Figure 7 Translocation and bioconcentration factors of Cu, Zn, Pb, and Cd in *C. colocynthis* plants under the experimental treatments. Different letters (a-d) indicate significant differences between the different treatments studied (Student-Newman-Keuls test, $p \leq 0.05$, n = 3).

In general, plants exhibiting TF<1 and BCF<1 are suitable for MTEs phytostabilization programs [4, 39, 46, 47], as these low values indicate that a given species is unable to extract large amounts of metal from the soil and translocate it to the shoots.

4. Conclusions

This study demonstrated that powdered marble can be successfully used in the remediation of a highly acidic polymetallic contaminated soil, correcting soil acidity and reducing the uptake of metals, which facilitates the establishment of *C. colocynthis*. The best plant growth was obtained in $MTPM_{25}$, with 25% of $CaCO_3$ as the liming material. The application of powdered marble led to a decrease in the uptake of Cu, Pb, and Zn, probably as a result of increased soil pH. This could be explained by the formation of metal carbonates, which limit the absorption of metals by *C. colocynthis* roots. Since *C. colocynthis* is a perennial that self-propagates in mine tailings, is well-adapted to the local conditions, and is a metal excluder (TF<1 and BCF<1); it is the best suitable plant for the re-vegetation and phytostabilization of polymetallic mine sites.

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Author Contributions

These authors contributed equally to this work.

Competing Interests

The authors have declared that no competing interests exist.

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