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Can Soil Improvers (Biochar, Compost, Insect Frass, Lime, and Zeolite) Achieve Phytostabilization of Potentially Toxic Elements in Heavily Contaminated Soil with the Use of Purslane (*Portulaca oleracea*)?

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Abstract: In soil with extremely high contents of Cd (101.87), Pb (26,526.44), and Zn (17,652.63 mg kg^{-1}), we aimed to test the phytostabilization capacity of purslane (*Portulaca oleracea*) with the use of various soil improvers, both organic (biochar, compost, insect frass) and inorganic (lime and zeolite). Thus, in a 60-day pot experiment, we amended this heavily contaminated soil with the five materials at two rates, 2% and 4%, resulting in 11 treatments (control plus five materials × two rates) replicated 10 times. We found that soil extractions of Cd with DTPA (diethylenetriaminepentaacetic acid) were not affected by any of the amendments, as there was no recorded significant reduction in soil Cd. In the case of Pb, there were even significant increases in its extractability with added biochar, and so was the case for compost at 4%. The reason may be the formation of organometallic complexes with organic substances of low molecular weight eluted by the organic amendments. Similarly, Zn extractability increased significantly compared to the control in the compost and frass treatments. As for purslane shoots, Cd decreased from 61 μg kg^{-1} fresh weight (FW) at control to 39 at biochar 4%, but the reduction was non-significant. As for Pb, it decreased with biochar but not significantly, while it exhibited a significant decrease in all other treatments. However, in all cases the content of Pb in purslane was well above the European regulation limit of 0.100 mg kg^{-1} FW (fresh weight) for vegetables and fresh herbs, while Cd fell below the regulation limit of 50 μg kg^{-1} FW at biochar 4%, compost 4%, and frass 2% and 4% (with control being 62.5 μg kg^{-1} FW). We conclude that in heavily contaminated soils, although biochar, along with compost and frass, was not entirely unsuccessful, the tested amendments did not reduce satisfactorily toxic elements to sufficiently low levels both in soil and in the test plant (here, purslane) in order to achieve phytostabilization. However, further research is necessary to identify exact mechanisms and to elucidate the role of different biochars.

Keywords: heavy metals; phytomanagement; soil pollution; toxic elements

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1. Introduction

Various anthropogenic activities have increased the release of potentially toxic elements (PTEs) in the environment over the years. This has led to the eventual deposition of PTEs in surface soils, a process that contributes to the diminishing of soil health and the deterioration of a range of ecosystem functions [1]. In some cases, such enrichment has resulted in the dramatic elevation of PTE contents in soil; one such well-documented case is that of Lavrio, southern Greece, where mining activities are known to have been conducted for the last four millennia [2]. Silver- and Pb-rich ore minerals in the area have resulted in an increase, among other PTEs, in Cd, Pb, and Zn to levels rarely observed in other contaminated areas around the globe, even inside the town of Lavrio itself [3]. Contaminated soils may also cause an increase in the uptake of PTEs by cultivated plants, and if such crops are edible, they create a considerable risk to human health [4].

Thus, it is critical to manage such heavily PTE-contaminated soils. There are various ways to do that. Phytoremediation is a process where plants grown in a contaminated area improve the situation after many cycles of cultivation [5]. One variation of phytoremediation is phytostabilization; this process involves a cultivated plant that aims to reduce PTE availability and uptake rather than to reduce total soil PTE content. Phytostabilization requires the use of PTE-tolerant species; such plants use predominantly two strategies to overcome the soil-induced PTE stress: (a) They control PTE uptake by achieving PTE retention inside the cell wall by the carboxylic reactive groups and the pectin polysaccharides (especially the polygalacturonic and uronic acids); and (b) they develop retention mechanisms, such as the complexation of PTEs with organic compounds, and subsequently transport the complexed metals to non-physiologically-active plant cell compartments, e.g., the vacuole [6]. Also, in such tolerant plants, the production of reactive oxygen species (ROS; $O_2^{\bullet -}$, $^{\bullet}OH$, H_2O_2, and $^1O_2$) is much more well controlled during the metabolic processes in chloroplasts, mitochondria, and peroxisomes. However, PTE presence in plant cells will eventually lead to increased ROS production, and when the antioxidant potential of cells is surpassed, there is a risk for DNA, proteins, lipids, and carbohydrates [7]. Toxicity can also lead to reduced photosynthesis, reduced biomass production, and reduced uptake of essential nutrients [8].

Phytostabilization can be assisted with the use of soil improvers that tend to decrease PTE mobility in soil by their physico-chemical stabilization. One of the most widely used PTE-stabilizing amendments is biochar. It is produced by the pyrolysis of organic feedstock at temperatures ranging from 300 to 900 °C under conditions of absence of oxygen. Biochar characteristics are influenced predominantly by the pyrolysis conditions (temperature and pyrolysis time) and the feedstock [9–12]. In recent years, it has been evident in the literature that biochar improves soil structure and reduces the availability of PTEs and organic pollutants while accelerating the attenuation of Cr(VI) by chemically reducing it to its inert Cr(III) species [11,13–15]. Biochar addition also improves nutrient uptake by plants, thereby increasing the biomass yield of cultivated plants [16] and contributing to the C sequestration. Biochar binds PTEs and renders them less available for plants with a variety of mechanisms, including the electrostatic attraction of the hydrated free metal ions onto negative-charged sites (mainly carboxylic), the retention of metals onto reactive C-chain groups, as well as physical entrapment into their porous structure [17]. Similar are the effects of the addition of non-charred organic materials, such as compost. Compost differs from biochar, among other characteristics, in the fact that added C is much less long-lived in soil, as microbial decomposition results in an expected mineralization rate of ca. 10% within the first two years of application [18]. Thus, apart from being used as a means of PTE retention with similar mechanisms as those of biochar, compost improves soil physico-chemical properties and the availability of nutrients to plants, thus boosting their vigor [19,20]. Also, compost is much more beneficial for the soil microbiome [21]. A third organic material that can act as an immobilization agent to soil PTEs is insect frass, a material derived from the waste of farmed insects (i.e., insect manure). Frass has recently been investigated regarding its ability to provide nutrients to plants and has proven to be highly effective, especially concerning N [22]. However, it is an organic material that has not been thoroughly investigated in heavily contaminated soils for its ability to stabilize toxic elements. All these three organic materials function in a way to improve soil conditions while providing additional retention capacity to soil for the immobilization of PTEs. Apart from these, there are also inorganic materials frequently used for their high ion exchange capacity, which increases the total soil cation exchange capacity (CEC) and, thus, the overall PTE retention ability of soil.

One such material is zeolite, frequently used as a mineral soil improver for soil decontamination [23–25]. Zeolite is a tectosilicate primary aluminosilicate mineral with extensive isomorphic substitution of Si with Al in the silicon tetrahedra, which results in an exceptionally high CEC, often of ca. 200 $\text{cmol}_c\ \text{kg}^{-1}$. This feature, along with its unique crystallochemical configuration of the presence of an extensive network of wide-diameter pores, makes zeolite a potent sorbent [26,27]. Metal selectivity for retention depends on the nature of the metal, its concentration, the presence of antagonistic metals and, to a lesser degree, soil pH (the developed negative charge is highly non-variable, i.e., it is mostly non-dependent on solution pH) [26,27]. An alternative way to increase PTE retention in soil without increasing its ion exchange capacity is to add materials that contribute surfaces known for their ability to bind PTEs, such as carbonates. The calcium salt of this material, calcium carbonate, $CaCO_3$, is the most efficient liming material for acidic soils [28,29]. The retention of PTEs by $CaCO_3$ follows the reaction

$$M^{2+}{}_{(aq)} + CaCO_{3(S)} \rightarrow Ca^{2+}{}_{(aq)} + MCO_{3(S)}$$

where M is a metallic PTE, while subscript "S" denotes the solid phase and "aq" the liquid [30]. Retention of PTEs onto such liming materials has been observed for metallic elements Cd, Zn, Ni, Cu, and Pb, as well as for metalloid As [31,32]. Elements with ionic radii (given in picometers; pm) similar to that of Ca^{2+} (114 pm) tend to be strongly retained (i.e., Cd^{2+} (109 pm), Pb (133 pm), and Sr (132 pm)), while those with smaller ionic radii develop weaker bonds (e.g., Fe (75–69 pm), Cu (87 pm), Zn (88 pm), and Ni (83 pm)) [30,33].

Purslane (*Portulaca oleracea* L.) has been cultivated as an edible vegetable herb and for pharmaceutical uses [34,35]. It is a species well known for its ability to tolerate abiotic stresses and grow in soils burdened with anthropogenic activities [36–38]. Purslane has been investigated as a crop for PTE-elevated soils [35,39,40]. Subpiramaniyam [35] and Wei et al. [40] reported the species' phytostabilizing ability: in soil with high contents of Cd (10), Pb (1000), Cu (400), and Zn (1000; units in mg kg^{-1}), purslane was found to have no adverse effects. This was concurred by Javed et al. [41]. However, most investigations come from experiments where soils have been spiked with PTEs just prior to the experimentation; however, obtained results from such tests can hardly represent real conditions regarding soils where PTE deposition is the outcome of many centuries or even millennia. Such soils exhibit different behavior regarding PTEs, which tend to be less readily available due to the "ageing process". This process causes a gradual deposition of PTEs in residual soil pools, where they can become seemingly immobile and, thus, less available to plants. Hence, there is a need for experimental tests that would study the ability of purslane as a potential PTE phytostabilization species in relation to important soil improvers. The aim of this work was to investigate the soil extractability of Cd, Pb, and Zn in soil heavily contaminated with these PTEs and their uptake by purslane in the presence of amended biochar, compost, frass, zeolite, and lime consisting of $CaCO_3$. To this effect, the root-to-shoot translocation factor was also investigated to further test the phytostabilization potential of purslane. This work is a timely contribution that could be of benefit to a range of soil end-users, including soil scientists, farmers, and environmental policymakers alike.

2. Materials and Methods

2.1. Soil and Stabilizing Materials

2.1.1. Soil

The soil was obtained from an olive grove inside the town of Lavrio at 37.719311° N, 24.044154° E; it contained 79.6% sand and 8.8% clay, with pH (1:2.5 H_2O) 8.0, electrical conductivity of 192 µS cm^{-1}, and organic C 1.1%. Lavrio has undergone mining exploitation since the 2nd millennium BC [42] and is renowned for its extremely high PTE contents: (aqua regia; soil digested in a 1-to-20 ratio with 3:1 conc. $HCl:HNO_3$ at 130 °C for 5 h); total Cd was 101.87, Pb 26,526.44, and Zn 17,652.63 (values in mg kg^{-1}). All analyses were performed as per Rowell [43]. The soil was left to air dry before it passed through a 5 mm sieve ready for the pot experiment. Likewise, the measurements of Cd, Pb, and Zn in the amendments were performed using the same methods and analyses as described above.

2.1.2. Biochar

Plant residues of the species *Pinus halepensis* were placed in a pyrolysis chamber (flame curtain pyrolysis kiln) at 600 °C for 1 h. After pyrolysis, the charred material was cooled with water and left to stand for air drying for 96 h. Its physico-chemical characteristics are shown in Table 1 and Table S1 (Supplementary material). All analyses and procedures were performed as per Kalderis et al. [44]. The charred material was further pestle-and-mortared and passed through a 2 mm sieve ready for use in the experiment.

Table 1. Cadmium, Pb, and Zn content (mg kg^{-1}) in the test soil and the organic and inorganic materials used as soil improvers. "BDL" stands for "below detection limit".

	Cd (mg kg^{-1})	**Pb (mg kg^{-1})**	**Zn (mg kg^{-1})**
Soil	101.87	26,526.44	17,652.63
Biochar	BDL	BDL	BDL
Compost	BDL	14.70	202.96
Lime	BDL	BDL	BDL
Frass	BDL	BDL	70.24
Zeolite	BDL	53.75	BDL

2.1.3. Compost

The compost was commercially obtained from a local composting company; its chemical analysis is shown in Tables 1 and S2 (Supplementary material). The material was also fully analyzed for its contents in nutrients and PTEs. The compost was applied moist, but its addition was calculated based on its dry matter. To estimate its dry matter, 10 g portions of wet compost were placed in pre-weighed porcelain crucibles and heated in an oven at 105 °C for 24 h, after which they were weighed again. Dry matter was calculated as 100 × [(wet weight) − (dry weight)]/(wet weight). The compost passed, as before (i.e., moist), a 2 mm sieve ready for use in the experiment.

2.1.4. Insect Frass

Insect frass was obtained from the Laboratory of Entomology, University of Thessaly, and derived from the insect *Tenebrio molitor*. The material was fully analyzed for its contents in nutrients and PTEs (Tables 1 and S3 in the Supplementary material). Frass was almost dry and was directly added to the soil for the experiment.

2.1.5. Lime ($CaCO_3$)

The liming material was marble dust obtained commercially from a marble quarry in the vicinity of the Soil Science Laboratory. It is comprised of $CaCO_3$, and its analysis for any PTE impurities is shown in Table 1. The lime passed through a 2 mm sieve to separate any larger particles.

2.1.6. Zeolite

Zeolite was obtained commercially and was a clinoptilolite with the formula $(Na,K,Ca)_{2–3}Al_3(Al,Si)_2Si_{13}O_{36}\cdot 12H_2O$. Its ion exchange capacity equaled 150 cmol$_c$ kg^{-1}, while its analysis concerning PTE impurities is shown in Table 1.

2.2. Experimental Design and Analyses

A weight of 1 kg of the processed soil was thoroughly mixed with the 5 stabilizing soil improvers at two rates, 2% and 4% *w*/*w*; this resulted in 11 treatments (control plus 5 amendments × 2 rates), which were replicated 10 times—thus 110 pots. The mixtures were then placed into 110 plastic pots of a capacity of 2 L, fertilized at a rate of 300 kg N ha^{-1}, 188 kg of P_2O_5 ha^{-1}, and 212 kg of K_2O ha^{-1}, and watered to 2/3 of the water holding capacity until seeds of purslane (*Portulaca oleracea*) were planted in the pots on 21 October 2020 (considered as Day 0). The greenhouse where pots were placed did not control light and temperature. The pots were frequently watered according to their needs, and their positions were changed regularly to compensate for any differences in conditions. The experiment lasted until 21 December 2020 (for 61 days). On the harvest day, plant height was measured, and the aerial plant part was immediately harvested and separated into leaves and stems. Aerial plant parts were recorded for their fresh weight. Subsequently, 3 cores of soil were obtained and made into one composite sample, which was taken for air-drying and sieving through a 2 mm sieve. After that, roots were also sampled by washing the adhered soil. All plant parts (leaves, stems, and roots) were placed in a 70 °C oven until no further weight loss. Biomass was recorded, and they were milled into fine powder.

Then, 1 g of the combined aerial biomass and roots was dry-ashed at 500 °C for 4 h and extracted with 10 mL of 20% HCl. In soils, there was an extraction with DTPA-$CaCl_2$ (a ratio of 1:2 soil:solution shaken for 2 h). All extracts of plants and soils were analyzed for Cd, Pb, and Zn with atomic absorption spectrophotometry (Perkin Elmer A3300, Shelton, CT, USA).

2.3. Secondary Indices

Based on the primary data, the following index was calculated:

Root-to-shoot translocation (translocation factor, TF) = (Concentration in aerial biomass)/(concentration in roots) [45].

Values well below 1.0 indicate that the test plant is an excluder, i.e., suitable for phytostabilization [46].

Also, we calculated PTE concentrations on the basis of fresh weight in order to be able to make comparisons with legal limits in regulations, which report values in units of concentration based on fresh rather than the usual dry weight.

2.4. Statistical Analysis and Data Quality Control

In each batch of extraction, we used blanks as well as reference materials of soil and plant. The percentage of recovery of the three metals ranged between 92% and 108%. All analyses were performed in triplicates, and the coefficient of variation was <15%. All data were analyzed for one-way ANOVA at the level of 95% ($p < 0.05$) after testing for the canonical distribution of data, and then we performed a Duncan's post hoc analysis.

3. Results

3.1. Potentially Toxic Elements in Soil

The studied soil was heavily contaminated with PTEs; pseudo-total Cd was 101.87, Pb = 26,526, and Zn = 17,653 (units, mg kg^{-1}; Table 1). As for the extractability of the studied metals, Cd was 7.06 mg kg^{-1} in the control, and it increased significantly to 8.50 mg kg^{-1} at frass 4%, while the rest of the treatments had no significant differences with the control. The lowest Cd values were recorded in the two biochar treatments and at zeolite 4% (ranging from 6.51 to 6.77 mg kg^{-1}), significantly lower compared to those in the two frass treatments and zeolite 2% (Figure 1). DTPA-extractable Pb in the control was 607.35 mg kg^{-1}, and it increased at biochar 2% (667), compost 4% (677.43), and zeolite 4% (666.43; units in mg kg^{-1}), while that of frass 4% was significantly lower than the control (517.32 mg kg^{-1}; Figure 1). Zinc was 254.13 mg kg^{-1} in the control and increased significantly in the compost and the frass treatments and at zeolite 2%, with the highest being that of frass 4% (389.29 mg kg^{-1}).

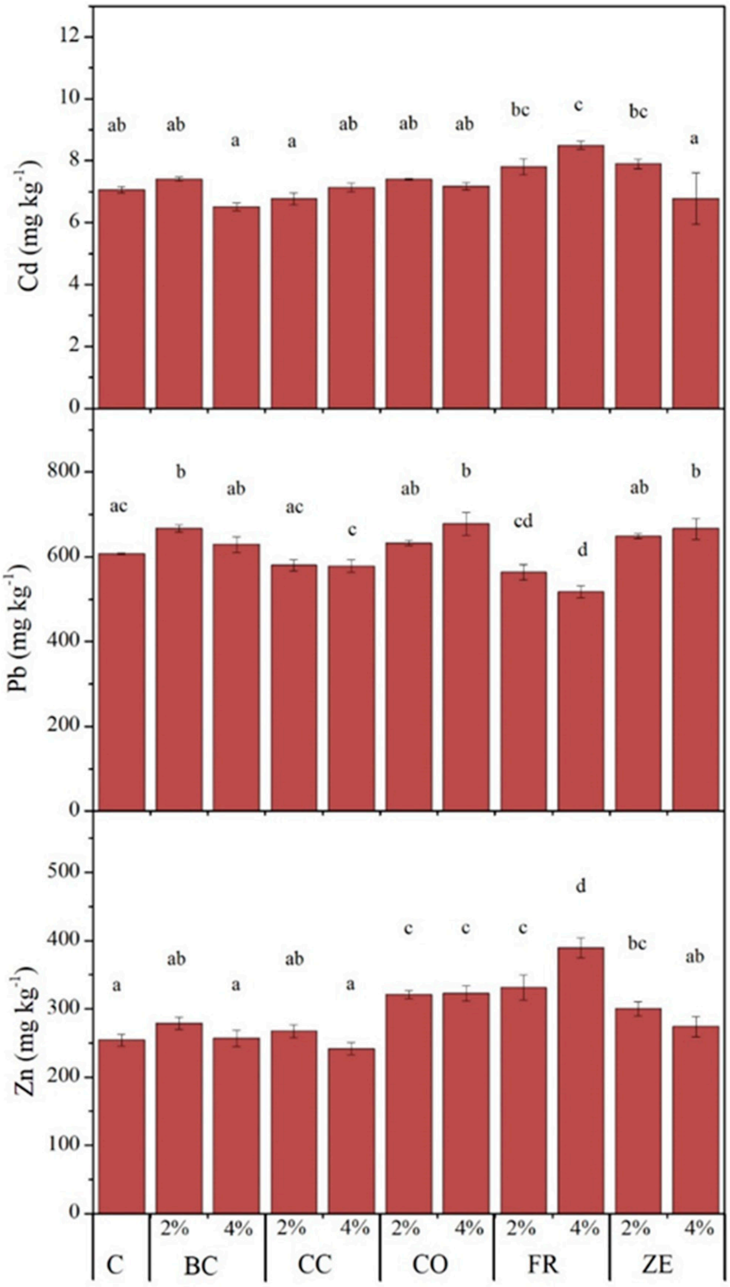

Figure 1. Concentration of Cd, Pb, and Zn (mg kg^{-1} soil) in soil extracted with DTPA in the treatments of the 5 immobilizing agents (C, unamended control; BC, biochar; CC, calcium carbonate as lime; CO, compost; FR, frass; ZE, zeolite) added in soil at 2% and 4% w/w. Different letters indicate significant differences at $p < 0.05$.

3.2. *Growth of Purslane and Its Contents in Potentially Toxic Elements*

Purslane height was 20.24 cm in the control and did not differ from any of the treatments. The only recorded differences were between height at biochar 2% (24.55 cm) and that of frass 4% (14.58 cm) (Table 2). Leaf dry weight was 0.088 g at control and had an increasing trend in the treatments, with that at frass 2% (0.216 g) exhibiting a significant difference from it. Stem dry weight at control was 0.384 g and increased significantly at compost 2% (0.544 g), while it decreased significantly at frass 4% (0.188 g). As for root dry biomass, it was 0.124 g at control and increased significantly at the two compost treatments (compost 2% = 0.200 g; compost 4% = 0.224 g), while there were no significant differences among the control and all other treatments except for the compost (Table 2).

Table 2. Growth parameters (height, dry weight leaf, dry weight stem, and dry weight roots) of the cultivated purslane (*Portulaca oleracea*) in the treatments of unamended control, biochar, lime ($CaCO_3$), compost, frass, and zeolite added at 2% and 4% in a soil heavily contaminated with Cd, Pb, and Zn.

	Height (cm)	**Leaf (g)**	**Stem (g)**	**Root (g)**
Control	20.24 ab	0.088 a	0.384 a	0.124 a
Biochar 2%	24.55 b	0.124 ab	0.396 a	0.112 a
Biochar 4%	22.33 ab	0.052 a	0.336 a	0.086 a
Lime 2%	20.96 ab	0.080 a	0.256 ac	0.076 a
Lime 4%	23.47 ab	0.084 a	0.400 a	0.106 a
Compost 2%	23.40 ab	0.112 a	0.544 b	0.200 b
Compost 4%	22.75 ab	0.072 a	0.500 ab	0.224 b
Frass 2%	21.24 ab	0.216 b	0.492 ab	0.124 a
Frass 4%	14.58 a	0.056 a	0.188 c	0.058 a
Zeolite 2%	22.26 ab	0.104 a	0.376 a	0.088 a
Zeolite 4%	23.90 ab	0.124 ab	0.428 ab	0.114 a
Significance	***	*	***	***

* Significant at $p < 0.05$; *** Significant at $p < 0.001$. Different letters within each column indicate significant differences at $p < 0.05$.

As for the metal contents in plants, Cd in aerial purslane (whole aerial part was analyzed) was 1.21 mg kg^{-1} dry weight (DW), and there were no significant differences with the rest of the treatments, except for zeolite 4%, which was significantly higher than the control (2.54 mg kg^{-1}). This treatment was, in fact, significantly higher than any other treatment (Figure 2). Aerial Pb was 53.73 mg kg^{-1} at control and showed a decreasing trend in all other treatments, with all other treatments except for biochar having significantly lower Pb concentrations; the CC 2% (lime at 2%) had the lowest Pb content among treatments (26.37 mg kg^{-1}; Figure 2). Zinc was 180.17 mg kg^{-1} at control, and the rest of the treatments exhibited no significant differences, except from frass 4% (93.13 mg kg^{-1}; Figure 1). As for PTEs in roots, Cd content at control was 10.72 mg kg^{-1}, and no significant differences among any treatments were recorded (Figure 2). Root Pb was 215.03 mg kg^{-1} at control and not significantly different from any other treatment. However, the highest Pb content (366.31 mg kg^{-1}) was recorded at zeolite 2%, significantly higher compared to the two frass and the two lime treatments (Figure 2). As for Zn content in roots, at control, it was 344.34 mg kg^{-1}; the control exhibited no significant differences with any of the other treatments, although the highest Zn content was recorded at frass 4% (599.90 mg kg^{-1}), a value which was significantly different from frass 2% (176.24 mg kg^{-1}; Figure 2).

Metal concentration in purslane aerial biomass, expressed in fresh weight, indicated that Cd at control was higher than the limit of the EU 2012 Directive and China (60.5 vs. 50 μg kg^{-1} FW) but lower than the Australia 2011 (100 μg kg^{-1} FW) and the Codex Alimentarius (200 μg kg^{-1} FW) limits. Biochar 4%, compost 4%, and the two frass treatments achieved the reduction of Cd FW content below the 50 μg kg^{-1}-threshold (Table 3). As for Pb, its FW level was 2.69 mg kg^{-1} at control (compared to the unanimous limit of 0.10 mg kg^{-1} FW in all recorded regulations), and it was not reduced below the regulation limit in any of the treatments. As for Zn, it had at least a two-fold difference in any treatment (i.e., lower) than the limit for China of 20 mg kg^{-1} FW (Table 3).

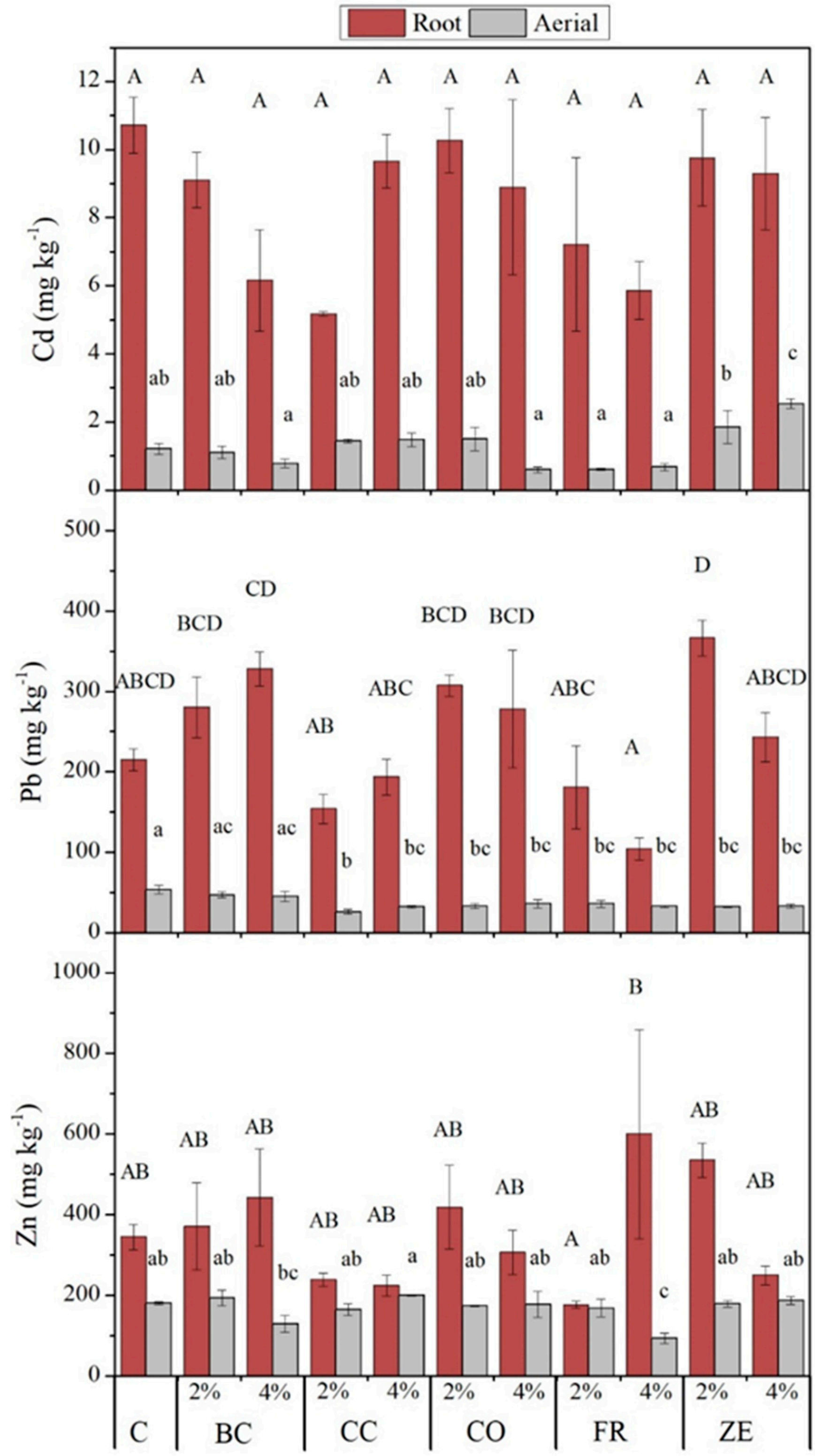

Figure 2. Concentration of Cd, Pb, and Zn (mg kg^{-1} dry weight) in the aerial parts of purslane (*Portulaca oleracea*) in the treatments of the 5 immobilizing agents (C, unamended control; BC, biochar; CC, calcium carbonate as lime; CO, compost; FR, frass; ZE, zeolite) added in soil at 2% and 4% w/w. Different capital letters indicate significant differences among treatments in roots at $p < 0.05$. Different small-case letters indicate significant differences among treatments in aerial parts at $p < 0.05$.

Table 3. Fresh weight (FW) concentration of Cd (in µg kg^{-1}), Pb (mg kg^{-1}), and Zn (mg kg^{-1}) of the cultivated purslane (*Portulaca oleracea*) in the treatments of unamended control biochar, lime, compost, frass, and zeolite added at 2% and 4% in soil heavily contaminated with Cd, Pb, and Zn, as well as regulations and Directives of the European Union [47,48] (EC/1881/2006, EC/629/2008), China [49,50] (GB2762-2012 and according to Li et al., 2012), Australia [51] (Standard 1.4.1, 2011), and Codex Alimentarius [51] (FAO/WHO) (2019). Please notice the difference in units in metal concentrations: Cd in µg kg^{-1}, while Pb and Zn in mg kg^{-1}. Concentrations that are higher than some regulations are presented in italics and bold.

	Cd (µg kg^{-1} FW)	**Pb (mg kg^{-1} FW)**	**Zn (mg kg^{-1} FW)**
Control	***60.5 a***	***2.69 a***	9.01 ab
Biochar 2%	***55.5 a***	***2.36 ab***	9.65 ab
Biochar 4%	***39.2 a***	***2.27 ab***	6.43 ac
Lime 2%	***72.3 a***	***1.32 c***	8.21 ab
Lime 4%	***73.8 a***	***1.63 bc***	9.98 b
Compost 2%	***75.1 a***	***1.66 bc***	8.67 ab
Compost 4%	30.0 a	***1.82 bc***	8.85 ab
Frass 2%	30.6 a	***1.81 bc***	8.41 ab
Frass 4%	33.7 a	***1.65 bc***	4.66 c
Zeolite 2%	***92.5 b***	***1.61 bc***	8.95 ab
Zeolite 4%	***126.9 b***	***1.65 cb***	9.37 ab
Significance	**	*	*
Directive EC/1881/2006 [47]		0.10	
Directive EC/629/2008 [48]	50		
China (GB2762-2012) (2012) [49]	50	0.10	20 ¥
Australia (Standard 1.4.1, 2011) [51]	100	0.10	
Codex Alimentarius (2019) [52]	200	0.10	

* Significant at $p < 0.05$; ** Significant at $p < 0.01$. Different letters within each column indicate significant differences at $p < 0.05$. ¥ According to Li et al. [50].

3.3. Translocation Factor

As for the TF, at control Cd was 0.130 and Zn 0.549, but within treatments in each metal, no significant differences were recorded. Pb TF at control was 0.294, significantly higher than that at biochar 4% (0.136), biochar 2% (0.108), biochar 4% (0.149), frass 2% (0.089), and frass 4% (0.142), while there was no treatment significantly higher than the control (Table 4).

Table 4. Translocation factor (TF = metal concentration in aerial plant over metal concentration in roots; unitless) of the cultivated purslane (*Portulaca oleracea*) in the treatments of unamended control biochar, lime, compost, frass, and zeolite added at 2% and 4% in soil heavily contaminated with Cd, Pb, and Zn.

	Cd	**Pb**	**Zn**
Control	0.130	0.294 ac	0.549
Biochar 2%	0.119	0.174 bc	0.651
Biochar 4%	0.152	0.136 bd	0.408
Lime 2%	0.279	0.178 bc	0.688

Table 4. *Cont.*

	Cd	Pb	Zn
Lime 4%	0.151	0.173 bc	0.939
Compost 2%	0.162	0.108 b	0.500
Compost 4%	0.079	0.149 bd	0.698
Frass 2%	0.125	0.248 acd	0.943
Frass 4%	0.112	0.336 a	0.299
Zeolite 2%	0.221	0.089 b	0.341
Zeolite 4%	0.309	0.142 bc	0.788
Significance	NS	**	NS

** Significant at $p < 0.01$; NS: Non-significance. Different letters within each column indicate significant differences at $p < 0.05$.

4. Discussion

The tested materials were added to this heavily contaminated soil so that PTE adverse effects may be alleviated; the expected effect could be based on two distinct mechanisms: (a) PTEs could be stabilized in soil due to increased retention [53], and (b) plants could become more vigorous and, hence, withstand toxicity more effectively [54]. The former mechanism could be realized due to an increase in cation exchange capacity (induced by the organic amendments and zeolite), an increase in reactive organic groups available for PTE retention (organic amendments), or an increase in carbonate phases known for their ability to sorb metals (in the case of added lime as $CaCO_3$). The latter mechanism could be realized due to added nutrients, the increase in microbial activity, and the improvement in growth conditions in soil (such as increased water retention and better soil aeration). Based on these hypotheses, it was expected that the added soil improvers would decrease PTEs and improve plant growth. Our results, however, showed that the protective effect of the used materials was very conservative or even, in some cases, opposite from what was expected.

Regarding plant growth parameters (height and dry biomass), where enhanced values compared to the control would indicate reduced PTE toxicity, the height of any of the treatments did not exhibit significant differences compared to the control. As for dry weights, only leaves at frass 2% were significantly higher than the control, while the other treatments were not. As for roots, only the two compost additions fared better than the control, but no other treatment did. These results suggest that the concentrations of N added with frass, compost, and biochar were not high enough to lead to significant differences in vegetation growth [55]. In other words, the decomposition of our organic materials was not fast enough to fit the exact timing of plant growth or to fit the time frame of our 60-day pot trial. As for the frass, this was rather surprising because there is evidence indicating a rather fast decomposition potential of this material, possibly associated with the high N content relative to C, giving frass a C/N ratio close to 10—favorable for fast decomposition [56]. Several works show that a reduced decomposition rate may be linked to the reduced selectivity for this material of soil-decomposing microbial biomass, as also suggested by Fabian et al. [55] and Watson et al. [57]. Another side effect related to added organic matter is also possible: added organic matter may have increased PTE availability, as also suggested by Sun et al. [58], possibly due to the evolution of dissolved organic carbon (DOC), which is known to increase metal availability. The formation of such soluble organometallic complexes is likely in neutral-to-alkaline soils where conditions favor the evolution of DOC. This, in turn, could mask or counter-impact any beneficial effects in growth parameters brought by the added organic matter that causes the net result of the added organic matter, bringing conservative positive results or not increasing plant vigor sufficiently. Root growth may have had a beneficial effect from added compost due to improved physical soil conditions and better aeration and water retention.

The extractions of the studied metals with DTPA seem to elucidate the reasons for the very conservative increase in growth parameters with the added materials: Cd extractability increased at frass 4% compared to the control, while it did not decrease in any treatment. Likewise, Pb extractability increased, compared to the control, at biochar 2%, compost 4%, and zeolite 4%, while it decreased at frass 4%. Also, DTPA-extractable Zn increased at compost 2% and 4%, at frass 2% and 4%, and at zeolite 2%. This is a picture of increased rather than decreased PTE mobility in soil. It is likely that this trend for increased extractability was induced by the formation of soluble complexes of the studied metals with DOC as chelating ligands that were also selected by the extraction with DTPA. This concurs with Jalali et al. [59]. It is also likely that the decomposition of added organic matter caused a temporary pH depression, which, in turn, would have caused a surge of increased DTPA extractability [60]. There is evidence that this is likely the reason for the increased DTPA extractability (rather than the initially expected decrease): there was no such decrease in the lime treatment. The inorganic nature of added $CaCO_3$ could not have influenced any changes in organic matter. However, there is another factor to consider, which could explain the increased Pb at zeolite 4%: zeolite was found to contain some impurities of Pb (54 mg kg^{-1}, Table 1). Also, Zn was found in compost (203 mg kg^{-1}) and frass (70 mg kg^{-1})—the four treatments of which (compost and frass at 2% and 4%) were found with increased Zn extractability. Although added Pb and Zn concentrations were low compared to the very high initial contents in soil, it is known that newly introduced metals are much more mobile than those deposited in soils over long periods of time [61]. Thus, these impurities may have caused the recorded increased DTPA extractability of Pb and Zn.

At the plant level, the picture was different from what was exhibited in the DTPA extractions, as Pb and Zn had reduced levels in various treatments compared to the control: Pb decreased significantly in all treatments except for biochar, while Zn decreased at frass 4%. Biochar was expected to play a vital role in reducing metal availability due to the fact that it is an alkaline material which tends to increase soil pH [29]. Also, the variety and abundance of reactive groups (carboxylic, hydroxylic, amines, and phenolic) achieve PTE retention and reduce their bioavailability. Also, electrostatic attraction is possible, and this contributes to the increased PTE retention by biochar [12,15]. However, in our work biochar seems to have been less successful. Chen et al. [15], in their review concerning the role of biochar in metal availability, reported that Pb in alkaline soils may be the least affected by decreased availability. This may be a contributing factor. Also, it is possible that there were issues of non-homogeneity of the soil–biochar mixture in our pots, leading to plant roots being more exposed to soil particles rather than to biochar. It is noteworthy that compost and frass were successful in reducing the Pb levels in plants. Compost is a selective term for a wide range of organic materials and can thus have a variety of effects on soil PTEs. However, due to added ion exchange capacity to soils, it is expected to contribute to decreasing PTE availability [62], as was the case here. Frass has rarely been tested as a material for PTE retention. It has recently been evaluated as a source of macronutrients for soil and plants [22], but its usage as a soil improver in contaminated soils is not proven yet. Thus, our work is a valuable contribution towards this direction. Frass was also successful with Zn at the rate of 4%. As for liming, it proved to be successful in the reduction of Pb levels in purslane shoots, although the soil was alkaline. The mechanisms that led to this are not entirely elucidated [63]. However, it is likely that with added $CaCO_3$, there is an increase in the negative charge of soil colloids, in the formation of hydroxyl metal species, which are non-available to plants, and in the formation of insoluble metal carbonate complexes [64,65]. All three such mechanisms seem plausible in the case of Pb in our work.

Although, concerning the matter of the significance of the reduction of metals in purslane aerial biomass, the trends were not very positive, in terms of comparisons of the fresh weight (FW) concentrations against the maximum legal limits, things were somehow different. Biochar at 4% achieved a reduction of Cd below the 50 μg kg^{-1} FW limit (of EU and China), while in the control, soil Cd was above the limit. This seems to suggest that biochar did have a positive protective role at the rate of 4%. Also, compost at 4% and the two frass treatments (2% and 4%) had this beneficial effect. This means that if Cd was the restrictive element, biochar and compost at 4%, as well as frass, would be successful in making purslane safe for human consumption due to Cd stabilization (Table 3). However, concerning Pb, no treatment proved nearly as successful as it should be in reducing Pb FW levels below the unanimous across countries and organizations limit of 0.10 mg kg^{-1} FW: Pb was nearly in all treatments one order of magnitude higher than the limit.

Concerning TF, the usual index used in phytoremediation tests, the low values well below unity (the threshold which appears to be a requirement in order to categorize a plant as a hyperaccumulator) confirmed the case of placing purslane among the excluder plants. Concerning Cd, TF values across all treatments were very low and by no means significantly different from the control. This finding also confirms the earlier findings of the rather weak beneficial effect of the added soil improvers in reducing Cd phytoavailability, but, all the same, makes purslane a candidate species for cultivation in Cd-contaminated soils, even if severely enriched. Pb TF was higher than that of Cd, and Zn TF was even higher, but not anywhere close to unity. This rather agrees with other findings concerning purslane [66].

5. Conclusions

Biochar was tested among other materials as a potential soil improver that would reduce the mobility and availability of Cd, Pb, and Zn to purslane in soil severely contaminated due to continuous mining activities over millennia, where toxicity effects are expected to be a lot less acute than if soil was spiked just prior to the experimentation. We tested two hypotheses: that metal availability can be reduced due to metal retention in soil with the assistance of the added materials (soil-related stabilization) and that the adverse effects of metals on plants could be reduced due to increased plant vigor and robustness in resisting metal uptake or in withstanding their toxicity (plant-related stabilization). Concerning the former hypothesis, we found evidence that it was not entirely invalid. Metal concentration in the dry aerial biomass decreased in the treatments of lime, compost, frass, and zeolite for Pb and at frass for Zn; also, Cd decreased below the permissible limit of public consumption in terms of FW concentration at biochar, compost, and frass. However, it is difficult to claim a fully successful protective effect of the added materials: DTPA extractions did not decrease compared to the control (with the exception of Pb at frass 4%) but rather increased (Cd at frass 4%; Pb at biochar 2%, compost 4%, and zeolite 4%; and Zn at compost 2% and 4%, frass 2% and 4%, and zeolite 2%). Also, the TF did not seem to decrease with any of the amendments compared to the control. Thus, the former hypothesis cannot be nullified nor confirmed based on the current findings—it needs further exploration. As for the latter hypothesis, no increased plant vitality could be confirmed: plant growth parameters seemed to be non-affected by the added amendments. Thus, the second hypothesis seems to be nullified. We conclude that among the tested amendments, frass at both rates and compost at 4% were the treatments that were successful in decreasing Cd content in purslane below the threshold limits concerning edible plants. We see this work as a step towards identifying the underlying mechanisms and the role of various amendments, some of which are well known, while some are not (like frass). We recognize the necessity for future work concerning the role of biochar, especially when combined (either before pyrolysis or after) with various other materials that would decrease C/N and, thus, its incorporation in the soil matrix. Also, the necessity of further tests of frass, whose complex behavior as a source of organic-borne nutrients and as a potential sink for toxic elements is yet to be elucidated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13112827/s1, Table S1: Characteristics of biochar; Table S2: Characteristics of compost; Table S3: Characteristics of frass.

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