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A Comparative Study on Poaceae and Leguminosae Forage Crops for Aided Phytostabilization in Trace-Element-Contaminated Soil

Min-Suk Kim [1,*], Hyun-Gi Min [2], Sang-Hwan Lee [3] and Jeong-Gyu Kim [1,2]

[1] O-Jeong Eco Resilience Institute, Korea University, Seoul 02841, Korea; lemonkim@korea.ac.kr
[2] Division of Environmental Science and Ecological Engineering, College of Life Science and Biotechnology, Korea University, Seoul 02841, Korea; daiamin@korea.ac.kr
[3] Institute of Mine Reclamation Technology, Mine Reclamation Corporation of Korea, Wonju-si 26464, Korea; soillsf@mireco.or.kr
* Correspondence: adoniss86@korea.ac.kr; Tel.: +82-10-7163-1316

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Abstract: When applying an aided phytostabilization in trace-element-contaminated agricultural soil, the cultivation of forage crops instead of edible crops can reduce the trace elements transfer to humans while minimizing the income loss of farmers. The objectives of this study were to compare the effect of the type of forage crops at the "family" level (Poaceae and Leguminosae) on aided phytostabilization using physical (water stable aggregation), chemical (Mehlich-3 extraction), and biological assessments (dehydrogenase activity). Pig manure and acid mine drainage sludge were used as soil amendments, and four plant species (*Loliummultiflorum* Lam. var. *italicum* and *Secalecereale* L. [Poaceae representatives], *Viciavillosa* Roth, and *Trifoliumpratense* L. [Leguminosae representatives]) were cultivated after amendment treatments. Chemical assessment showed that the reduction in bioavailability of trace elements was partly observed in legume crops. The positive effects of plant cultivation were determined through physical assessment. The effectiveness of pig manure as an organic amendment was determined by biological assessment. In some treatments, the synergistic effect of the incorporation of chemical stabilization with both plant families was observed but it was difficult to identify a clear distinction between the two families. The translocation of trace elements from root to shoot was low in all plants, indicating that the cultivation of the plants used in this study is safe with regards to the spread of trace elements into the environment. The results suggest that forage crop cultivation in contaminated agricultural soil could ameliorate soil quality after chemical stabilization.

Keywords: amendment treatment; dehydrogenase activity; environmental restoration; gentle remediation option; phytoremediation; water stable aggregates

1. Introduction

Agricultural soil pollution from trace elements, including arsenic and heavy metals, not only causes adverse environmental effects, but also poses risks to human health through contaminated food crops [1,2]. Among the many soil remediation technologies, chemical stabilization has been considered to be suitable for agricultural soils than soil flushing or soil washing in the aspects of minimization of soil structure destruction and nutrient loss [3,4]. The main mechanism of stabilization is that the input of several amendments or stabilizers decreases the mobility or bioavailability of trace elements, thereby reducing the transition of trace elements from soil to plant. Consequently, there has been considerable research focus on chemical stabilization because of its efficiency, economic feasibility, and ability to prevent secondary pollution [5,6].

Despite its many advantages, chemical stabilization may disrupt soil environments and decrease soil quality, leading to adverse effects for plant growth and agricultural productivity [7,8]. Furthermore, a transition period is needed for the stabilization of trace elements after chemical stabilization in the field [7,9]. Therefore, issues may arise if food crops are cultivated directly after chemical stabilization, such as a loss of income and health risks caused by a decline in crop productivity and the uptake of trace elements, respectively.

Gentle soil remediation options (GRO) are new approaches to the effective of restoration of trace-element-contaminated areas and may present viable remedies to overcome previous issues involved with soil remediation. Furthermore, GRO are less invasive to soil structure, functions, and ecosystem services, and have a much lower ecological footprint than conventional soil remediation techniques [10,11]. In particular, GRO are based on low-cost and sustainable tools such as phytotechnologies and ecological restorations using plants for the remediation of widespread contamination and the (phyto)management of brownfields [12–14].

Phytostabilization is a type of phytomanagement that involves simple plant cultivation and the settlement of vegetation cap in trace-element-contaminated areas. This remediation strategy serves to remove the bioavailable fraction of trace elements and prevent the dispersal of trace elements by dust, surface water, and ground water [14,15], and is regarded as a GRO technique. The subsequent cultivation of plants alongside chemical stabilization work could be considered as aided phytostabilization [16] because it serves to stabilize the surface soil, reduce excessive soil erosion, and ameliorate soil physicochemical properties, thereby simultaneously improving the environmental healthy and sustainability of agricultural soil [17,18].

Phytostabilization, as a means of phytoremediation, has been studied within various research disciplines, with more recent studies focusing on identifying suitable plant species. Mahieu et al. [17] evaluated the suitability of the legume, *Anthyllisvulneraria*, through the examination of its survival, growth, and tolerance in mine sites. Korzeniowska et al. [15] examined the productivity and uptake of trace elements of several cultivated energy crops, such as willow (*Salix viminalis*), reed canary grass (*Phalarisarundinacea*), and maize (*Zea mays*), in a microplot experiment in trace-element-polluted areas to evaluated the suitability of these crops. Also, Domínguez et al. [14] identified the potential for phytoremediation using Holm oak (*Quercus ilex* subsp. *ballota*), by considering the results of biomass, Cd content, and chlorophyll content of oak cultivated in Cd spiked soil. Galende et al. [19] observed the beneficial effects of incorporating organic amendments in Pb/Zn contaminated mine soil for the cultivation of *Festucarubra*. Furthermore, Kim et al. [20] also found that the cultivation and incorporation of legume green manure crops in heavy-metal-contaminated agricultural soil also increased soil fertility. However, most of the previous studies evaluated the efficiency of remediation using chemical assessments only and reported on the basis of the uptake amount of heavy metals, and the biomass of plants; however, they did not consider the soil indicators that have practical importance in agriculture and crop productivity.

In this study, pig manure (PM) and acid mine drainage sludge (AMDS) were used as soil amendments for the stabilization of trace elements. Four conventional forage crop species were selected for assessment of phytostabilization potential, based the possibility of trace elements transfer to humans via the food chain and in consideration of farm income, i.e., that the land could not remain fallow. Additionally, in order to compare the effects on phytostabilization, two Poaceae forage crops (*Loliummultiflorum* Lam. var. *italicum* and *Secalecereale* L.) and two Leguminosae forage crops (*Viciavillosa* Roth and *Trifoliumpratense* L.) were selected. The objectives of this study were: (1) to compare the effect of the type of forage crop at the family level on aided phytostabilization using physical, chemical, and biological assessments, and (2) to provide significant, useful information for GRO practices.

2. Materials and Methods

2.1. Experimental Set-Up

Trace-element-contaminated soil was collected from the Eugene mining (Au) area (37°02′43.99″ N, 128°14′16.87″ E) in Danyang-gun, Chungbuk Province, South Korea. The soil sampling site was agricultural land adjacent to an abandoned mining area. The soil sample was air-dried and passed through a 2 mm sieve because the definition of soil in agriculture in general means particles less than 2 mm.

The two types of soil amendments examined in this study: PM and AMDS were obtained from a commercial fertilizer vendor (Danong, South Korea) and an acid mine drainage chemical purification facility from the Hamtae coal mine in Taebaek-si, Gangwon Province, South Korea, respectively. Toxicity characteristic leaching procedures tests and phytotoxicity tests revealed that the plants did not extract trace elements or absorb heavy metals from AMDS, respectively [21]. Both amendments were air-dried, ground, and sieved to less than 0.5 mm before being stored in polyethylene bottles. The amendments were applied to each soil at a 2% *w*/*w* ratio and were mixed thoroughly for homogeneity. The samples were equilibrated for 2 weeks, during which the soil moisture was maintained at approximately 60% of the soils' water holding capacities and the samples were thoroughly mixed at weekly intervals.

Seeds from four plant species were prepared: *L. multiflorum* and *S. cereale* (Poaceae family representatives) and *T. pratense* and *V. villosa* (Leguminosae family representatives). Each type of seed was planted directly into the mixed soils (5 seeds per pot, 3 pots per treatment). The pots used in this experiment had a 4 kg capacity, 30 cm diameter, and 30 cm height. The trials were conducted under controlled greenhouse conditions (temperature: 15–25 °C; relative humidity: 60–70%). After seed germination, 1–2 seedlings were removed to ensure that a consistent number of seedlings (i.e., 3) remained per pot.

2.2. Soil and Amendment Characteristics

Soil pH, electrical conductivity (EC), organic matter, and trace element bioavailability, were determined. The pH and EC of soils were measured in a 1:5 solid:water suspension using a combination pH-EC meter (Thermo Orion 920A, Thermo Fisher Scientific, Waltham, MA, USA) [22]. The weight loss on ignition was conducted to determine both the water at 105 °C and carbon content at 550 °C, over 24 h [23]. The concentrations of the total trace elements (As, Cd, Cu, Pb, and Zn) were determined by digesting samples with aqua regia (Sigma-Aldrich, St. Louis, MO, USA) according to ISO 11466:1995 [24] and using an inductively coupled plasma optical emission spectrometer (ICP-OES, 730 Series, Agilent, Santa Clara, CA, USA). The bioavailable trace element concentrations were evaluated using Mehlich-3 solution [25]. For physical properties, water-stable aggregation (WSA) was determined according to NIAST [22]. For biological properties, the soil enzyme activity was assessed using dehydrogenase activity, as proposed by Friedel et al. [26] using a spectrophotometer (Shimadzu, UV-1650PC, Tokyo, Japan) for detection of triphenylformazan (TPF). The point of zero charge of AMDS was determined followed by Mall et al. [27]. The accuracy of the analytical data of trace elements was assessed using certified reference material (NIST 2711a, Montana II Soil). The detection limits of As, Cd, Pb, and Zn were 0.001, 0.001, 0.001, and 0.003 mg L^{-1}, respectively.

2.3. Trace Elements in Plants

After 3 months, whole plants were harvested and washed in distilled water. The plants were weighed (fresh weight) and then dried in a fan-forced oven at 60 °C for 48 h. The dried samples were digested with concentrated HNO_3 (9 mL) and H_2O_2 (1 mL), using a commercially available sand digester (Sungwon Science, Yongin-si, South Korea) [22]. The digested suspension was filtered through Whatman No. 42 filter papers, and trace element concentrations in the filtered solution were determined using an inductively coupled plasma mass spectrometer (ICP-MS, 7700x, Agilent, Santa Clara, CA, USA). The accuracy of trace elements determination was assessed using certified reference material (BCR-402, white clover for As and CRM-281, rye grass for Cd, Pb, and Zn). And translocation factors (TF) were calculated by dividing trace elements concentration in the plant shoot by its concentration in the plant root [28].

2.4. Statistical Analysis

All measurements were taken in triplicate for each soil, amendment, and plant sample. A one-way analysis of variance (ANOVA) was used to compare the means of different treatments and plants, and data were analyzed using two-way ANOVA with the soil amendments (PM and AMDS) and plant families (Poaceae and Leguminosae) as fixed factors. Where significant *p*-values ($p < 0.05$) were obtained, differences between means were evaluated using Tukey's test. Data analyses were conducted using SAS software (SAS 9.4, Cary, NC, USA).

3. Results and Discussion

3.1. Basic Properties of Soil and Amendments

The basic characteristics and total trace element concentrations of the soil and amendments were determined (Table 1). The pH of the soil was alkaline and the concentrations of As, Pb, and Zn in the soil (642.0, 3018.4, and 1930.1 mg kg^{-1}, respectively) were much higher than the precautionary levels in the Korean soil regulations (25, 200, and 300 mg kg^{-1}, respectively) [29]. The pH of the amendments PM and AMDS was also high, i.e., 7.8 and 8.4, respectively. Despite having high concentrations of Cd and Zn, the low amount of treatment (2%), the leaching properties, and the low bioaccumulation properties of heavy metal from AMDS [21], make AMDS a reasonable soil amendment option.

Table 1. chemical characteristics and total trace elements concentrations of the soil and amendments ($n = 3$).

	Soil	PM [1]	AMDS [2]
pH	8.5	7.8	8.4
EC [3]	0.6	9.8	3.8
LOI [4]	3.4	58.5	20.0
As [5]	642.0	1.7	- [6]
Cd [5]	8.9	2.4	30.0
Pb [5]	3018.4	4.6	6.0
Zn [5]	1930.1	209.1	966.0

[1] Pig manure; [2] Acid mine drainage sludge; [3] Electrical conductivity (ds m^{-1}); [4] Loss on ignition (%); [5] Total trace elements concentrations (mg kg^{-1}); [6] Not detected.

3.2. Effects of Amendment Treatments and Plant Cultivation on Soil Chemical Properties

The amendment treatments and plant cultivation affected the soil pH and EC, with some significant changes (Figure 1); the direction of change varied according to the type of amendment and the plant species. According to Kim et al. [21], who used the same AMDS, when the amount of AMDS concentration increased to 1%, 3%, and 5%, the alkaline soil pH decreased from 8.2 to 7.7. The surface charge of a soil particle depends on the pH of the surrounding soil. If the soil pH is higher than the point of zero charge (pH_{pzc}), H^+ will escape from the surface of soil particles and the surface of the particle will be negative, inversely, if the soil pH is lower than pH_{pzc}, it will be positively charged [30]. In this study, the pH of AMDS (8.4) was similar to that of the soil (8.5), and the pH_{pzc} of AMDS was determined as 7.55. Therefore, not only PM but also AMDS significantly decreased soil pH, from 8.51 to 8.46 ($p < 0.05$).

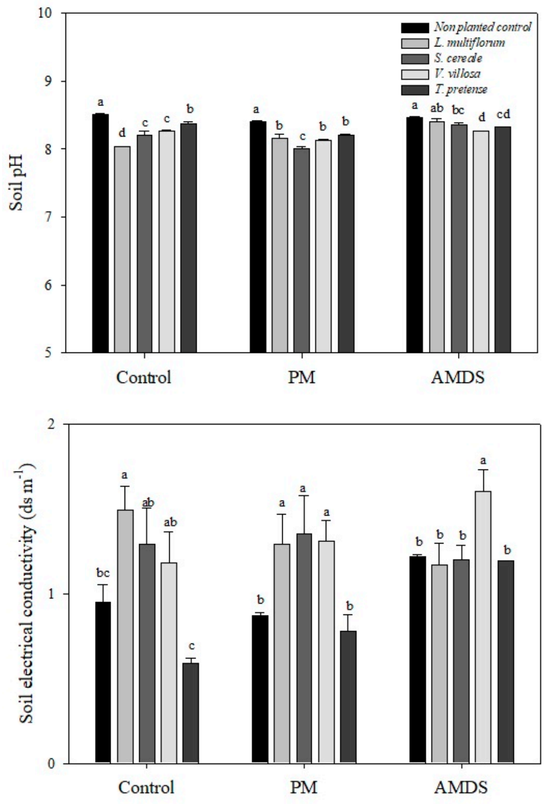

Figure 1. Changes in soil pH and electrical conductivity with different types of amendments treatments and plant species (*Loliummultiflorum* and *Secalecereale* (Poaceae family representatives) and *Trifoliumpratense* and *Viciavillosa* (Leguminosaefamily representatives)). Different letters indicate significant differences among the NPC (nonplanted control) and the four plant species with same treatment, using Tukey's test Error bars represent ± standard deviation of three replicates ($n = 3$).

Cultivation of all four plant species significantly decreased the pH of the alkaline soil, e.g., from 8.51 to 8.04, 8.21, 8.27, and 8.37 for *L. multiflorum*, *S. cereale*, *V. villosa*, and *T. pretense*, respectively, and this tendency was similar in the treatments involving soil amendments, except in the case of *L. multiflorum* in AMDS treatment. Root exudates may affect the solubility and plant uptake of soil nutrients including trace elements, by indirectly affecting microbial activity, physical properties of rhizosphere soil, and root growth pattern of plants, or directly affecting acidification, chelation, precipitation, and oxidation-reduction reactions [31,32].

In addition, plant cultivation changes the soil pH due to the release of CO_2 by root respiration, the imbalance caused by the absorption of cation and anion, the input of nitrogen, and the release of various types of organic compounds including carbohydrates, amino acids, aliphatic acids, aromatic acids, miscellaneous phenolics, fatty acids, and sterols [33–35]. Organic ligands are especially important compositions for metal chelation, the results of which are known as phytometallophores [36]. This study confirmed changes in soil pH resulting from plant cultivation over a three-month period. It is considered that the plants decreased the soil pH so as to increase the nutrient availability in the alkaline soil. The results of the two-way ANOVA showed that not only both amendment types and plant family types but also the interacting effects between them caused significant changes in soil pH (Table 2), therefore, clear explanations, in this regard, cannot be drawn from the present results alone, and further investigation is needed. Although an increase in EC was observed after treatment with amendments and plant cultivations, the EC level was not high enough to inhibit plant growth in general over 4 ds m^{-1} [37].

The Mehlich-3 solution has previously been used to test the availability of nutrients for plants in soil [21,38] and many recent studies have shown that this solution also reflects the bioavailability of soil trace elements [25,39–41]. Figure 2 shows the changes in the bioavailable concentrations of trace elements in contaminated soil after the application of chemical stabilization and plant cultivation treatments.

Table 2. Interaction of amendment and plant family on soil characteristics (soil pH, heavy metals and metalloids concentration, water-stable aggregation, and dehydrogenase activity) analyzed by a two-way ANOVA.

	Amendment		**Plant**		**Amendment × Plant**	
			F value (probability)			
Soil pH	51.49	(<0.0001)	101.62	(<0.0001)	26.00	(<0.0001)
As [1] (mg kg^{-1})	1947.08	(<0.0001)	14.45	(<0.0001)	3.03	(0.0299)
Cd [1] (mg kg^{-1})	285.52	(<0.0001)	21.46	(<0.0001)	19.84	(<0.0001)
Pb [1] (mg kg^{-1})	252.21	(<0.0001)	63.27	(<0.0001)	3.95	(0.0093)
Zn [1] (mg kg^{-1})	21.07	(<0.0001)	85.27	(<0.0001)	7.24	(0.0002)
Water-stable aggregation (%)	10.54	(0.0003)	15.45	(<0.0001)	0.55	(0.7015)
Dehydrogenase (μg TPF g^{-1})	30.73	(<0.0001)	7.05	(0.0026)	2.47	(0.0616)

[1] Mehlich-3 extractable trace elements.

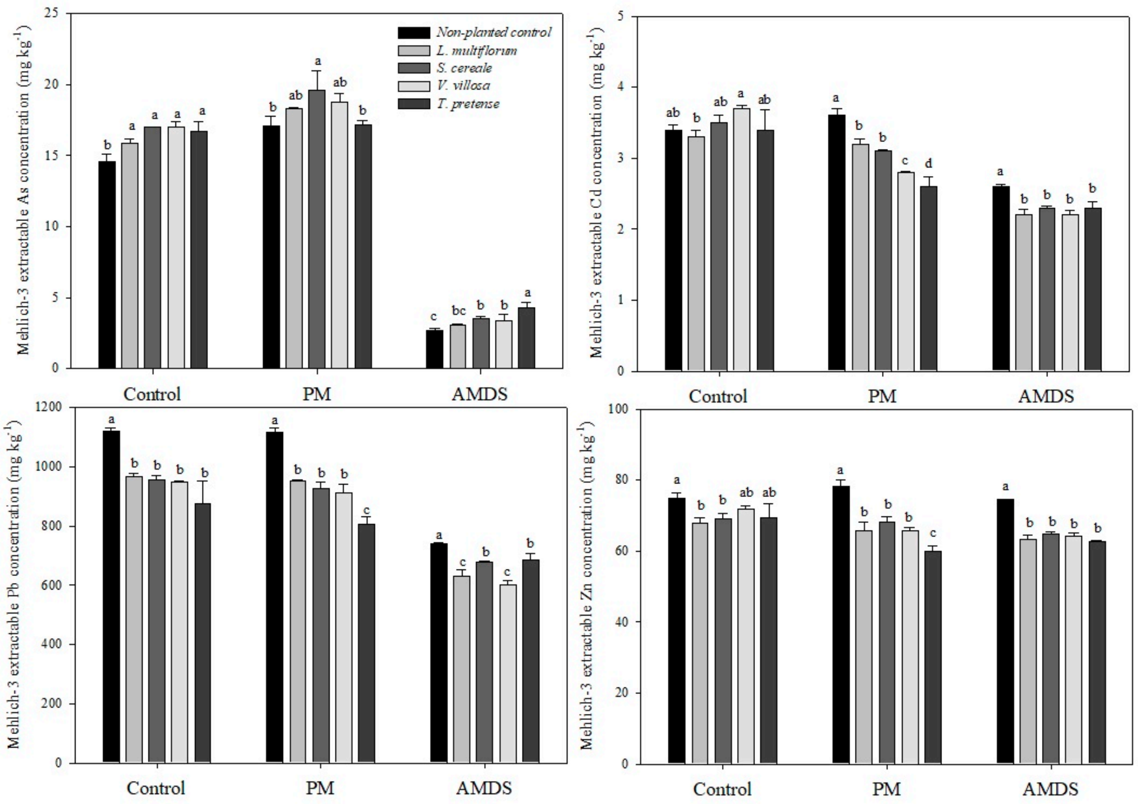

Figure 2. Mehlich-3 solution extractable trace elements concentrations with different types of cultivated plants (*Loliummultiflorum* and *Secalecereale* (Poaceae family representatives) and *Trifoliumpratense* and *Viciavillosa* (Leguminosaefamily representatives)) and amendment treatments (Control; PM, pig manure; AMDS, acid mine drainage sludge). Different letters indicate significant differences among the NPC (nonplanted control) and the four plant species with same treatment, using Tukey's test. Error bars represent ± standard deviation of three replicates (n = 3).

The bioavailability of As increased significantly when soil was treated with PM, in all noncultivated and cultivated treatments except for *T. pratense*; however, significantly decreased in all treatments with AMDS ($p < 0.05$). In toxic soil conditions like upland soils, As mainly exists in arsenate forms, i.e., in the anionic species of $H_2AsO_4^{-}$ and $HAsO_4^{2-}$, and is easily adsorbed onto metals oxides and the clay particle surface [42]. The AMDS from a coal mine purification facility, the main process of which involves the addition of Ca and Fe to acid mine drainage to cause coagulation and precipitation, also contained a large amount of metals with oxides that may have caused efficient As stabilization [21]. In the PM treated soil, the sorption effect of trace elements onto organic matter was negligible, but the effect of dissolved organic carbon was high. Dissolved organic carbon, extracted from PM during aging, may compete with As for adsorption sites on mineral surfaces, hence increasing its bioavailability [42]. In addition, various metal oxides, including Ca, Mg, Fe, Al, and Mn, may easily form As-metal-organic complexes, which prevent As adsorption or precipitation onto metal oxides [43,44].

AMDS significantly reduced the bioavailability of Cd in all nonplanted and planted treatment plots. However, PM significantly reduced that of Cd only in *V. villosa* and *T. pretense* crops. A liming effect (which would cause a decrease in Cd mobility) is not expected in the case of alkaline experimental soil because Cd is easily immobilized in high soil pH. However, Cd stabilization could be explained by Cd sorption onto metal oxide sorption sites in AMDS, as was reported in a previous study [21]. In alkaline conditions, the available Cd fraction might be stabilized by precipitation, or chemisorption with carbonate that originated from the PM [45], but a notable effect of PM on Cd stabilization was not exhibited. When treated with PM, the Cd stabilization effects were observed only in the leguminous crops *V. villosa* and *T. pratense*; which have distinctive rhizosphere development and leguminous bacteria, and similar results were found for Zn. The combination of legume species and the PM treatment resulted in a synergistic effect of Cd and Zn heavy metal stabilization. Dary et al. [46] evaluated the applicability of the legume plant, *Lupinusluteus*, for metal phytostabilization. However, neither study could clarify the specific reaction pathway and mechanism; therefore, further research is required to explain this synergistic effect. In the case of Pb, only AMDS significantly reduced the bioavailability of this heavy metal from all of the treatment plots, indicating that the Pb sorption on AMDS surfaces was the main mechanism of Pb stabilization. As with the pH results, the effects of amendments and plants on the bioavailability of trace elements are very complicated and the interactions between the two variables were also found to be significant (Table 2); hence, further investigations are needed. From these bioavailability tests that use conventional chemical assessment, it was shown that only the AMDS amendment was effective in reducing the bioavailability of trace elements. It was difficult to confirm and demonstrate the effect of the PM amendment and plant cultivation treatments on aided phytostabilization through chemical assessment.

3.3. Effects of Amendment Treatments and Plant Cultivation on Soil Physical and Biological Properties

Soil micro/macro structures and the stability of soil porosity are highly dependent on their resistance to water [47,48], and the effects of plant cultivation and amendment treatments on WSA were determined (Figure 3). Both amendments significantly increased WSA in the nonplanted control (NPC) from 40%, to 48% and 47% for PM and AMDS, respectively ($p < 0.05$). Organic matter and metal oxides provided various sorption sites and acted as binding agents, facilitating soil aggregate formation by flocculation, cementation, and the arrangement of soil particles [49,50]. The formation of WSA was higher under the combination of plant cultivation and amendment treatments than when only treated with an amendment (Figure 3). When comparing control soils, without any amendment treatment, the cultivation of plants increased the WSA from 40%, for the NPC treatment, to 50–58% for the different cultivated plants.

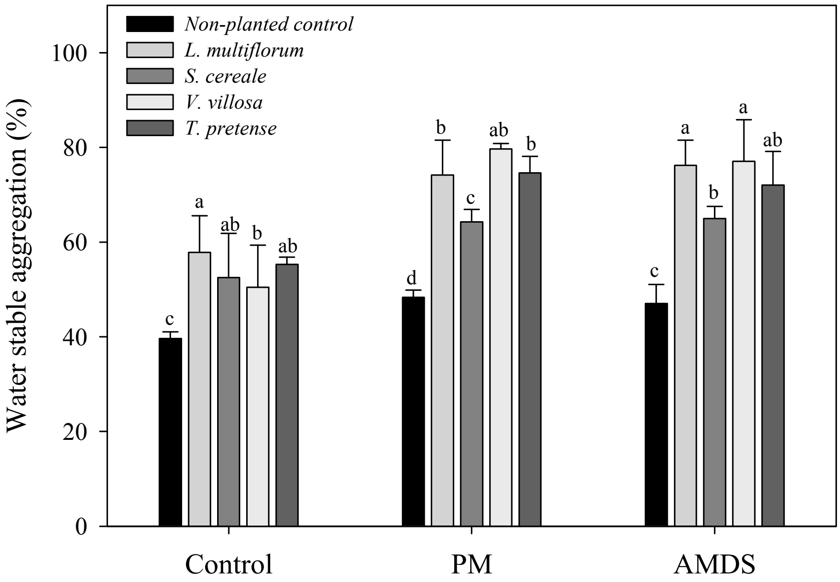

Figure 3. Water stable aggregation of the soils with different types of cultivated plants (*Loliummultiflorum* and *Secalecereale* (Poaceae family representatives) and *Trifoliumpratense* and *Viciavillosa* [Leguminosae family representatives]) and amendment treatments (Control; PM, pig manure; AMDS, acid mine drainage sludge). Different letters indicate significant differences among the NPC (nonplanted control) and the four plant species with same treatment, using Tukey's test. Error bars represent ± standard deviation of three replicates (n = 3).

Soil enzymes play an essential role in aspects of the nutrient cycle and soil biological quality [51]. Furthermore, soil enzyme activity has been used as an indicator to evaluate the effect of many kinds of treatments on soil quality [52]. Among the many types of soil enzymes, dehydrogenase activity (DHA) was chosen, because dehydrogenase is an intracellular enzyme that facilitates the oxidation-reduction reactions of several substrates, and is sensitive, rapid, and inexpensive [53,54]. Figure 4 shows that all crop species significantly increased soil DHA, compared with the control soils. This result demonstrated the effects of the cultivated plant roots on the rhizosphere, which resulted in the increases in soil biological activity. When comparing the amendments with nonplanted cultivation (NPC), the PM and AMDS treatments also increased the DHA, and the degree of increase was more than 3 times higher in the PM (34.1 μg TPF g^{-1}) treatment than in the AMDS (9.4 μg TPF g^{-1}) treatment. These findings are consistent with those of previous studies. Pérez de Mora et al. [55] reported that DHA levels increased with the use of municipal waste compost and biosolid compost. Furthermore, the addition of sewage sludge also increased DHA levels in soils contaminated with several concentrations of Cd [56]. Among the four types of plants cultivated, significant increases in DHA were found only in treatments with *L. multiflorum* and *T. pratense*, when compared with the NPC treatments, in both PM and AMDS treated soils (Figure 4). This result may be explained by differences in the cultivation and rhizosphere effects of the different plant species; however, plant cultivation increased DHA levels regardless of plant species in control soils (Figure 4). The two-way ANOVA revealed that both amendments and plant cultivation had a significant influence on the formation of WSA and DHA and no interaction between two variables was observed (Table 2). These results suggest that the physical (WSA) and biological (DHA) properties rather than chemical properties (soil pH and bioavailability of trace elements) more clearly explain the influence of the various factors.

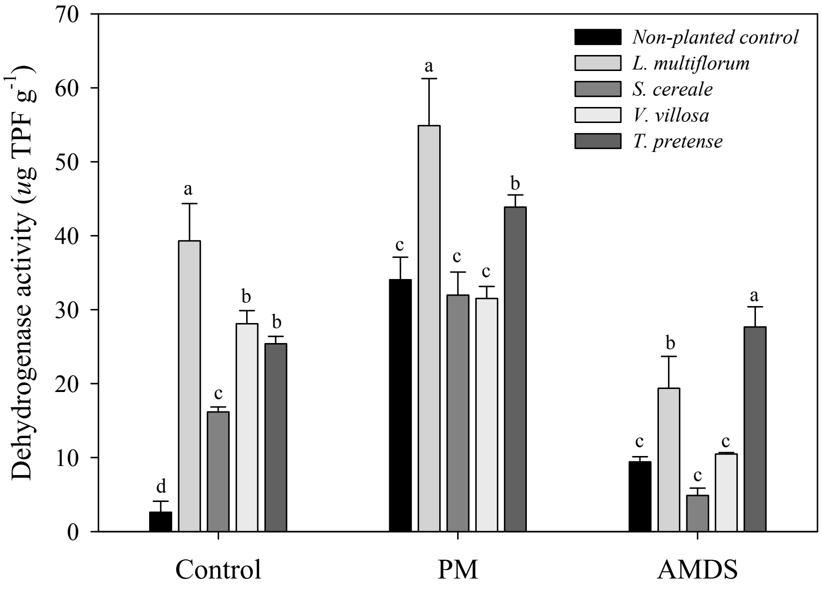

Figure 4. Dehydrogenase activity (i.e., the detection of triphenylformazan (TPF)) in soils with different types of cultivated plants (*Loliummultiflorum* and *Secalecereale* (Poaceae family representatives) and *Trifoliumpratense* and *Viciavillosa* (Leguminosae family representatives)) and amendment treatments (Control; PM, pig manure; AMDS, acid mine drainage sludge). Different letters indicate significant differences among the NPC (nonplanted control) and the four plant species with the same treatment, using Tukey's test. Error bars represent ± standard deviation of three replicates (n = 3).

Furthermore, these results indicate that plant cultivation may be a useful tool for the amelioration of soil quality, with regards to the biological aspects, and that a synergistic effect is achievable through a combination of organic amendment treatments and plant cultivation. However, it was difficult to clearly identify the effects of differences in plant family between Poaceae and Leguminosae. Nevertheless, regardless of the plant family, it was clear that plant cultivation increased the WSA and DHA levels; further, some plants showed synergistic effects with amendments, indicating that plant cultivation directly after chemical stabilization could ameliorate the physical and biological soil properties.

3.4. Uptake and Translocation of Trace Elements

Table 3 presents the trace element concentrations in four plant species, grown for 3 months in contaminated soils that were treated with PM and AMDS. TF has been used to evaluate the phytoremediation potential of plants, and the TF should be as low as possible, considering that trace elements transfer from soil to living organisms, including plants, insects, and other animals [57,58]. In this experiment, all of the trace elements accumulated predominantly in the roots of the plants (Table 3), with the exception of Zn for *T. pratense* in AMDS treated soil (TF = 1.13). These results demonstrated, via the TF, that the "root trap" of cereals and leafy crops effectively accumulated trace elements; which is an advantageous characteristic for safety reasons, whereby the risk of trace element transfer (i.e., in the consumed aerial plant parts) is reduced [16,59,60]. The four kinds of plants tested, from the Poaceae and Leguminosaefamilies, have been used domestically as nonedible crop plants by human consumption. It is desirable to use plants such as these, because they are no longer seen as exotic species, and, more importantly, they offer a simple and practical means of restoring agricultural land to ensure that edible plants can be cultivated safely.

Table 3. Heavy metals and metalloids (As, Cd, Pb, and Zn) concentrations (mg kg^{-1}) in plants shoot and root of cultivated plants (*Loliummultiflorum* and *Secalecereale* (Poaceae family representatives) and *Trifoliumpratense* and *Viciavillosa* (Leguminosaefamily representatives)) based on dry weights (n = 3).

	Shoot				**Root**			
	As	**Cd**	**Pb**	**Zn**	**As**	**Cd**	**Pb**	**Zn**
L. Multiflorum								
Con [1]	3.29 a	1.94 a	7.1 b	93 a	94.9 a	17.3 a	360 a	315 a
PM	3.12 a	1.45 b	9.8 a	97 a	39.6 b	8.3 b	151 b	150 b
AMDS	2.72 b	1.07 c	8.6 ab	98 a	23.2 c	5.4 c	111 b	122 b
S. cereale								
Con	4.49 a	1.82 a	13.5 a	82 a	73.1 a	11.5 ab	132 a	199 a
PM	2.89 b	0.78 b	5.6 b	42 c	78.6 a	13.9 a	99 b	107 b
AMDS	2.57 b	0.73 b	5.4 b	52 b	30.1 b	10.1 b	92 b	105 b
V. villosa								
Con	2.67 a	3.26 a	6.3 a	89 b	73.5 a	23.6 a	198 a	208 a
PM	3.39 a	3.42 a	5.4 a	172 a	70.2 a	20.1 a	151 a	182 a
AMDS	3.17 a	3.16 a	3.0 b	82 b	32.4 b	14.6 b	126 b	131 b
T. pretense								
Con	5.80 a	0.87 a	2.9 b	92 b	54.3 a	18.3 a	166 ab	207 a
PM	4.63 a	0.50 b	5.4 a	97 b	36.1 b	8.4 b	199 a	177 b
AMDS	1.40 b	1.01 a	4.8 a	129 a	21.4 c	5.5 c	110 b	114 c

Means (n = 3) followed by same letter within a row are not significantly different ($p > 0.05$). [1] Con, control; PM, pig manure; AMDS, acid mine drainage sludge.

4. Conclusions

This study was conducted to investigate the differences in plant family (i.e., Poaceae and Leguminosae) on aided phytostabilization in contaminated agricultural soil, using physical, chemical, and biological assessments. The cultivation of all four species of plants significantly increased the WSA and DHA and showed synergistic effects when treated with trace element immobilization amendments. The reduction in bioavailability of trace elements in soil was partly observed in legume crops, but most of the reduction was dependent on amendment treatment. The translocation of trace elements from root to shoot was low in all plants, indicating that the cultivation of the plant species used in this study is safe with regards to the spread of trace elements into the environment. It was possible to confirm the effect of plant cultivation in most of the results, however, it was difficult to clearly confirm the effect of the difference in plant family. Nevertheless, this study revealed the suitability of combining chemical amendments with plant cultivation for the amelioration of trace-element-contaminated soil. Further investigations are needed to clarify specific mechanisms of the synergistic effects that were observed in this study. Furthermore, the efficiency of soil remediation should be evaluated not only by conventional chemical assessments based on bioavailability, but also by physical and biological assessments, for a true measure of the success of environmental restoration in GRO.

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