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Effect of Commercial Amendments on Immobilization of Arsenic, Copper, and Zinc in Contaminated Soil: Comprehensive Assessing to Plant Uptake Combined with a Microbial Community Approach

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Abstract: Identifying the proper chemical and biological materials as soil amendments is a great concern because they replace soil properties and subsequently change the soil quality. Hence, this study was conducted to evaluate the effects of a diverse range of soil amendments including bentonite (B), talc (T), activated carbon (AC), and cornstarch (CS) in form of sole and composite on the immobilization and bioavailability of As, Cu, and Zn. The amendments were characterized by SEM, FT-IR, and XRF, and applied at 2% (w/w) in the experimental pots with an Asteraceae (i.e., lettuce) for 45 days to monitor plant growth parameters and soil microbial community. Soil pH from 6.1 ± 0.02 significantly increased in the amended soils with the maximum value found for TAC (7.4 ± 0.04). The results showed that soil amendments reduced easily in an exchangeable fractionation of As, Cu, and Zn with the maximum values found for BAC by 66.4%, AC by 84.2%, and T by 89.7% respectively. Adding B, T, AC, and their composites induced dry biomass of lettuce >40 wt.%, while CS and its composites did not affect the dry biomass of the plant. The average content of Cu and Zn in plant tissues decreased >45 wt.% in B, AC, and their composites amended soils; meanwhile, AC and its composites mitigated As uptake by >30 wt.% in lettuce. The results of Biolog Ecoplate showed that the amending soils improved the microbial community, especially for composites (e.g., TCS). The results demonstrated that adding composites amendments provided an efficient method for the immobilization of metals and metalloids, and also induced plant growth parameters and microbial community.

Keywords: heavy metals; soil amendments; immobilization; microbial community

1. Introduction

The contamination of soil by potentially toxic metal(loid)s is becoming a major global concern which may pose a high risk for environmental and human health. Mining activities are known as one of the most influential anthropogenic activities leading to land resource degradation, habitat destruction, and soil and water contamination with heavy metal(loid)s [1]. Literature reviews showed that an inefficient processing procedure of mining ores in Korea caused an abundance of heavy metal(loid)s residues in mine tailings which may leach into to the farmland soils around the mining sites [2,3]. Recently, the general shortage of arable land in Korea was considered as the reason for increasing the cultivation of farmland around mining sites that could make a significant contribution to Korean food production, but that may later cause human health problems [4]. Both essential and non-essential metals can be toxic to living organisms at excessive concentrations and may adversely affect soil quality, human health, and ecosystems.

Over the past decades, varieties of physical, chemical, and biological techniques have been employed for remediating heavy metal(loid)s contaminated soils, such as soil

excavation, soil washing, solidification/stabilization, phytoremediation, etc. [5–9]. Immobilization technology for the remediation of heavy metals in contaminated soils has been popular for its effectiveness and low cost. Immobilization technology using soil amendments can decrease the heavy metals fractions or change their redox states, and thus effectively decrease the mobility, bioavailability, and toxicity of the heavy metals with an improvement in soil properties, thus inducing plant growth parameters [10]. Many studies have been reported on the application of different soil amendments to immobilize heavy metals in contaminated soils such as calcareous materials, phosphates, clay minerals, and so on [1,11,12]. Indeed, choosing an appropriate soil amendment is a big challenge because it changes soil properties, which can result in adverse impacts on soil quality and microorganisms.

Bentonite clays and activated carbon as cost-effective amendments have attracted much attention for the immobilization of heavy metals in soil because of their ready availability, isomorphic substitution, permanent negative charges at surfaces as well as their environmental compatibility [13–18]. Besides, talc as a composite silicate with the chemical formula $Mg_3Si_4O_{10}(OH)_2$ seems to be a practical and potential amendment to soil contamination [19]. Cornstarch, as a biopolymer, has been known as a soil conditioning agent, in that it reduces the frequency of irrigation, erosion, and evaporation of water in the soil, and also improves soil quality by lowering soil degradation [20].

Besides, soil microbes play an essential role in an agricultural ecosystem as an active component. Microorganisms react to soil heavy metals and microbial metabolisms can mediate pollutants' speciation change [21]. Soil microbial activities are related to the type of content of pollutants, and the physicochemical characteristics of soil regulate the chemical behavior of pollutants in soil and thus pollutants' bioavailability. Soil microbial activities are recognized as markers of any disturbance occurring in the soil ecosystem. However, soil microbial activities are difficult to gauge using a single monitoring approach. Therefore, the purpose of this study was to examine the effect of varying types of soil amendments, including activated carbon, bentonite, talc, cornstarch, and a combination of them on mitigating soil heavy metal mobility and plant bioavailability. Using this approach, this study examined the hypothesis that soil microbial activity would vary with changes in soil properties after adding the amendment and explored the effect of the amendments on the soil environment.

2. Materials and Methods

2.1. Soil Samples and Amendments Preparation

Contaminated soil was sampled from surface soil (<20 cm from depth) of a farmland site nearby the vicinity of the abandoned Indae mine (35°42′37″ N and 127°21′22″ S) located in the Republic of Korea. About 50 kg of contaminated soil was collected, stored in plastic bags, and transferred to the laboratory for future experiments. After that, soil samples were dried and sieved to have particle size fractions <2 mm.

Two groups of organic and inorganic amendments were considered for the current study. Bentonite (B) and talc (T), as inorganic soil amendments, with activated carbon (AC) and cornstarch (CS), as organic amendments, were purchased from Daejung Chemical Company. Soil amendments were applied solely (i.e., B, T, AC, and CS) and in the form of composites (i.e., BAC, BCS, TAC, and TCS) for soil remediation. Composites of the amendments were prepared with equally mixing (1/1 wt./wt.) a pair of amendments together. It is hypothesized that combining amendments may provide more binding sites for stabilizing heavy metal(loid)s in soil. Hence, the composites of soil amendments were prepared for further study on the effect of multiplex amendments on the soil quality.

2.2. Greenhouse Experiment

A leaching pot experiment was carried out in a greenhouse in the Sejong University, located in Seoul, the Republic of Korea, to explore the effect of the aforementioned amendments and their composites on heavy metal immobilization and plant growth. A mixture

of washed sand and contaminated soil with a ratio of 1/3 (*w/w*) was added to each pot. Soil amendments (solus and composites) were added to the experimental pots at rates of 2 wt.%., and thoroughly mixed to obtain homogeneity. Before planting, the soil was incubated in the dark for 8 weeks and left to be equilibrated. The average temperature in the greenhouse was 23 °C with a humidity of 65%. A control soil sample was also prepared following the same procedure without adding amendments. All treatments were performed in three replicates. After the equilibration finished, two lettuce seedlings from unpolluted soils were translocated in each experimental pot and watered three times a week (100 mld^{-1}) with distilled water (DW). Forty-five days after planting, the lettuce was harvested, and soil was sampled for further analysis. The harvested plants were separated into groups of root and shoot samples. Plant samples were rinsed with DW, oven-dried (45 °C), ground, and stored in a plastic bag at room temperature before analysis. Moreover, soil samples were collected and stored in a refrigerator at −70 °C before analysis.

2.3. Samples Analysis

The pH of the soil and amendments was measured in a solid to solution ratio of 1:5 using a pH meter (Orion Star^{TM} A211 pH Benchtop Meter, Waltham, MA, USA). The total concentration of As, Cu, and Zn in soil and amendment samples was measured by flame atomic absorption spectrometry (AAS, AA240, Varian, Australia), after the digestion of samples using an aqua regia method [22]. The sequential extraction method for As was measured based on [23], while for Cu and Zn, the method of [24] was applied. These methods are demonstrated in Table 1.

Table 1. The procedure of sequential extraction methods.

Methods	Target Phase	Reagents
Wenzel et al., 2001 for As fractionation		
Fraction I. Exchangeable	Easily exchangeable	0.05 M $(NH_4)_2SO_4$; 4 h
Fraction II. Bound to carbonates	Phosphate competes with adsorbed As	0.05 M $(NH_4)H_2PO_4$; 16 h
Fraction III. Bound to Fe and Mn oxides	Oxalate competes with adsorbed As on amorphous Fe oxides	0.2 M NH_4-oxalate buffer, pH 3.25 in dark; 4 h
Fraction IV. Bound to organic matter	Associated with crystalline Fe oxides	0.2 M NH_4-oxalate buffer + 0.1 M ascorbic acid, pH 3.25; 30 min/96 °C
Fraction V. Residual	Bound to silicates	HCl/HNO_3 3/1 *v/v*
Tessier et al., 1979 for Cu and Zn fractionation		
Fraction I. Exchangeable	Adsorbed metals	1 g sample, 0.5 M $MgCl_2$; 20 min
Fraction II. Bound to carbonates	Specially adsorbed metals as well as carbonate-bound fractions	1 M NaOAc, pH 5.0 with HOAc; 5 h
Fraction III. Bound to Fe and Mn oxides	Extraction of reducible Fe and Mn oxides	0.04 M $NH_2 \cdot OH \cdot HCl$ in 25% (*v/v*) HOAC; 6 h/96 °C
Fraction IV. Bound to organic matter	Bound to various forms of organic matter and sulfide minerals	0.02 M HNO_3 30% H_2O_2, pH 2.0 with HNO_3, 3.2 M NH_4OAc in 20% (*v/v*) HNO_3; 3 h/85 °C
Fraction V. Residual	Detrital silicate minerals, resistant sulfides, and a small quantity of refractory organic material	HCl/HNO_3 3/1 *v/v*

Total concentrations of heavy metal(loid)s in plant tissues were measured using AAS after digestion of plant tissue samples (1 g) in the solution of 5 mL HNO_3 (70% *w/w*) for 1 h at 120 °C and a subsequent addition of 1 mL H_2O_2.

The specific surface area (SSA) of the amendments was measured by N_2 adsorption using the Micrometrics BET method by Tristar 3000, UK [25]. The morphology of each amendment as well as the elemental composition were examined using a scanning electron microscopy (SEM-EDS) Coxem S-100 production microscope (COXEM, Daejeon, Korea). Fourier transform infrared spectroscopy (FT-IR) was used to determine functional groups

of the amendments. The chemical composition of inorganic amendments (B and T) was determined using X-ray fluorescence (micro-XRF, M4 Tornado, Bruker).

2.4. Microbial Metabolic Activity

Biolog EcoPlate™ (Biolog, Inc., Hayward, CA, USA) was used to identify the metabolic characteristics of microbial communities present in the soil samples based on the utilized carbon source, before and after soil amending. Biolog EcoPlate™ is a quick, effective, and low-cost method to detect the dynamic variation of microbial metabolic and community diversity in soil [26]. Biolog EcoPlate™ is composed of 31 carbon sources (10 carbohydrates, 4 polymers, 9 carbonxylic and ketonic acids, 6 amino acids, and 2 amines/amides) and a control. It contains three replicates of the carbon source and control which became a total of 96 wells. A multichannel pipette was applied in the microplate to dispense the rhizosphere soil diluent into the 96 wells (150 µL per hole) under aseptic conditions. A redox dye (tetrazolium violet) was added to each well, which turned purple when the carbon source used by the microbial communities was present in the sample. For this, 5 g soil was suspended in 0.85% sterile organic salt (NaCl) solution (1:9 *v/v*) and then rotated for 30 min at 150 rpm. After settling down the suspension for 10 min, 1 mL extractant was diluted in 9 mL of tetrazolium violet solution. About 0.125 µL of the diluted solution was added to the plate wells. Plates were incubated at 28 °C for 5 days in a dark place. To identify metabolic diversity, the well absorbance was read automatically every day by the computerized MicroStation™ system [27].

The capability of microorganisms to utilize different carbon sources in microbial communities was calculated by average well-color development (AWCD) [28]. Samples with larger variation were considered as higher carbon source utilization capability and indicated to higher abundance of microorganisms [29]. AWCD was calculated as follows [28]:

$$AWCD = \sum (C_i - R)/31 \tag{1}$$

where C_i is the absorbance value of each reaction well at 590 nm in each carbon source well at time t and R is the absorbance value of the control well at time t.

SAWCD (Substrate Average Well-Color Development) was calculated with the same equation:

$$SAWCD = \sum (C_i - R)/(number\ of\ substrate) \tag{2}$$

where C_i is the absorbance value of substrate within the substrate category [30].

2.5. Statistical Analysis

The experimental data were calculated from triple replicates experiments. The data were statistically analyzed by one–way analysis of variance (ANOVA) and Tukey's test ($p < 0.05$) via SPSS version 20.0. Origin software version 9.1 was applied to figure out the data. The heatmap for monitoring microbial metabolism activities was plotted using a web tool for visualizing clustering of multivariate data https://biit.cs.ut.ee/clustvis/.

3. Results and Discussions

3.1. Physicochemical Properties of Initial Soil and Amendments

The initial soil texture was of loamy sand with slightly acidic (pH 5.9 ± 0.01), and contaminated with total concentrations of As, Cu, and Zn at 39.5 ± 1.3, 33.6 ± 1.5, and 108.5 ± 1.3 mg/kg, respectively (Table 2). These levels are much higher than the average in the world [31]. The soil was rich in organic matter content (2.9 wt.%) compared to the average value (2–3 wt.%) of the cultivable soils in Korea [32]. However, the soil was nutrient-poor with only 0.15 mg/kg of T-N.

Table 2. Physicochemical properties of the initial soil.

Sample	pH	As (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	T-N (mg/kg)	T-P (mg/kg)	T-K (mg/kg)	OM (%)	Texture
Soil	5.9 ± 0.01	39.45 ± 0.38	33.60 ± 0.52	108.5 ± 0.91	0.15	56.8	880.1	2.9	Loamy sand

T-N: total Nitrogen, T-P: total Phosphorus, T-K: total Potassium, OM: organic matter contents.

As shown in Table 3, soil amendments had alkaline pH with average values of 7.8 ± 0.04, 8.8 ± 0.02, 7.2 ± 0.04, 8.2 ± 0.01 for B, T, AC, and CS, respectively. A small amount of heavy metal(loid)s was found in the soil amendments (Table 3). Thus, from a heavy metal(loid)s contamination viewpoint, these amendments are safe to apply for soil treatment. Among soil amendments, a high SSA was found for AC with an average value of 1082 m^2/g, which was a good match with previous studies [33,34]. In contrast, a small value of SSA was found for T (1.8 m^2/g) and CS (0.15 m^2/g). The results of XRF analysis showed that SiO_2 and Al_2O_3 are the major components of bentonite with average contents of 67.8 wt.% and 15.8 wt.%, respectively (Table 4). Meanwhile, the major components of talc included SiO_2, MgO, and CaO with the average contents of 35.3 wt.%, 34.9 wt.%, and 26.9 wt.%, respectively.

Table 3. Physicochemical properties of amendments.

Sample	pH	As (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	SSA(m^2/g)
B	7.8 ± 0.04	0.56 ± 0.01	33.20 ± 0.05	35.22 ± 0.04	60.56
T	8.8 ± 0.02	0.69 ± 0.02	1.40 ± 0.01	29.50 ± 0.06	1.80
AC	7.2 ± 0.04	9.06 ± 0.01	4.6 ± 0.03	1.12 ± 0.02	1081.9
CS	8.2 ± 0.01	0.44 ± 0.005	0.01 ± 0.002	0.58 ± 0.006	0.15

B: bentonite, T: talc, AC: activated carbon, CS: cornstarch, SSA: specific surface area.

Table 4. Chemical composition (wt.%) of inorganic amendments using XRF.

| Sample	SiO$_2$	Al$_2$O$_3$	Fe$_2$O$_3$	K$_2$O	MgO	CaO	TiO$_2$	MnO	SO$_3$	P$_2$O$_5$
B	67.8	15.8	6.94	2.60	2.13	2.12	0.66	0.25	0.1	0.07
T	35.3	0.65	1.59	0.03	34.9	26.9	0.1	0.06	0.02	0.05

B = bentonite, T = talc.

Scanning electron microscopy was conducted to compare the morphology of B, T, AC, and CS. The different morphology of amendments was shown in Figure 1. Bentonite tended to aggregate and formed large size particles as white squares in Figure 1a [35]. Figure 1b reveals that the particle size of talc was very heterogeneous and angular. Figure 1c shows the morphology of AC was an angular and irregular material, and also had micro pores [36]. Cornstarch in this research had a sphere shape, and homogeneous size particle distribution as shown in Figure 1d [37].

Figure 2 showed the FT-IR spectrum of the bentonite samples. The absorption band at 3619 cm^{-1} is belong to structural OH groups of the bentonite. The band at 691 cm^{-1} is due to the deformation and bending modes of the Si-O bond. The very strong absorption band at 991 cm^{-1} is due to Si-O bending vibration [38]. The band at 3629 cm^{-1} is responsible for free uncompleted hydroxyls, and the band at 1654 cm^{-1} is responsible for bending H-O-H vibration in water.

The FT-IR spectrum for talc powder is shown in Figure 2, where wave numbers of 3675; 1427; 1004; 876; and 667 cm^{-1} dominate. The siloxane group (Si-O-Si) stretching vibrational bands is for peaks at 476 and 1040 cm^{-1}, respectively, while the band at 667 cm^{-1} reflects the Si-O-Mg bond [39]. The bands 3676 cm^{-1} are ascribed to the vibrations of hydroxyl groups linked to Si (Si-OH) and Mg (MgOH), respectively [40]. The talc used in this study was not pure as confirmed in XRF results with including CaO as Table 4. The results from FT-IR showed the same results when the appearance of bands of 1427; 875; and 712 cm^{-1} referred to calcite [41,42].
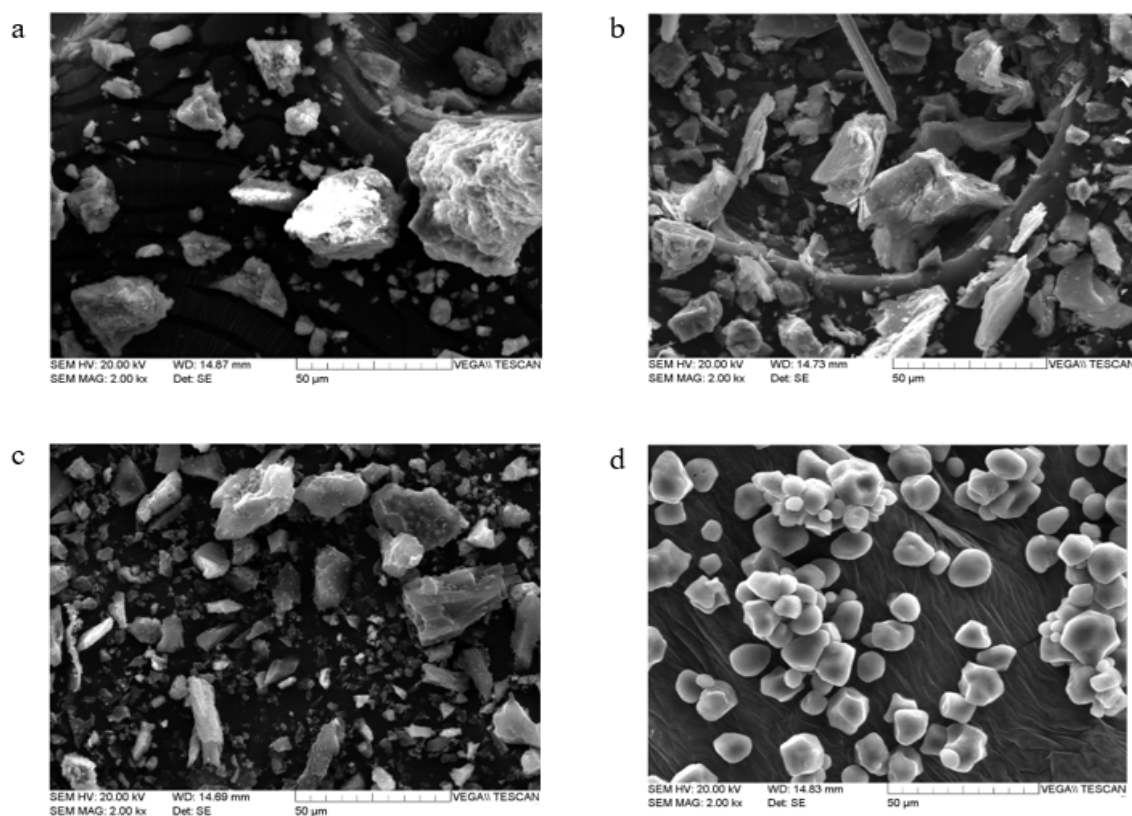
Figure 1. Scanning electron microscopy (SEM) images of (**a**) bentonite, (**b**) talc, (**c**) activated carbon, and (**d**) cornstarch.
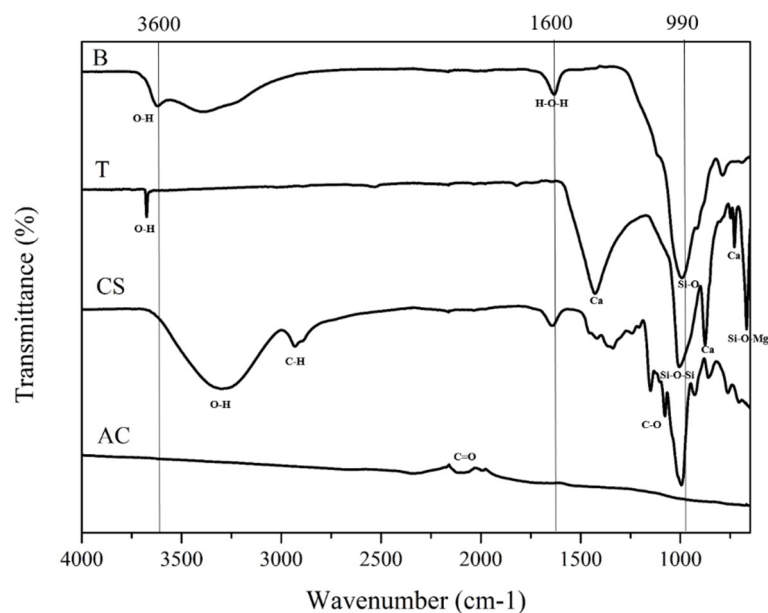
Figure 2. Fourier transform infrared spectroscopy (FT-IR) of various amendments applied to the soil (B: bentonite, T: talc, AC: activated carbon, CS: cornstarch).

FT-IR spectra of AC could be attributed to the antisymmetric stretching vibration of the nonsymmetric vibration absorption of C = O (at 1996 to 2085 cm^{-1}) [43]. The absorbance peak at 2328 cm^{-1} implied the presence of uncommon CO_2, which might result from the measuring conditions [44].

The FT-IR spectra of cornstarch are described in Figure 2. In the spectrum absorbance of CS, the band at 3293 cm^{-1} and 2931 cm^{-1} were allocated to the O–H stretching and the

C–H stretching vibrations, respectively. Meanwhile, the bands at 1149, 1077, and 993 cm^{-1} were characteristic of the C–O stretching vibrations [45,46].

Generally, the results of amendment characterization reveal that they offer the potential application of heavy metal immobilization in soil due to their capacity to adsorb and co-precipitate contaminants.

3.2. Effects of Amendments on Soil Properties

The studied soil from farmlands nearby the mining area was slightly acidic (Table 2). As shown in Figure 3, pH of soil significantly ($p < 0.05$) increased in the amended soil especially for T, TAC, and TCS amended soils from 6.1 ± 0.02 to 7.3 ± 0.01, 7.4 ± 0.04, and 7.3 ± 0.04 after planting. The maximum increase value of pH in soil was found for treatment with TAC. Soil pH also increased slightly for the soil applied with AC, CS, and BCS from 6.1 ± 0.02 to 6.6 ± 0.02, 6.5 ± 0.04, and 6.5 ± 0.05 after 45 days from planting, respectively. Moreover, no significant difference in soil pH was found in B and BAC amended soils compared to control soil.
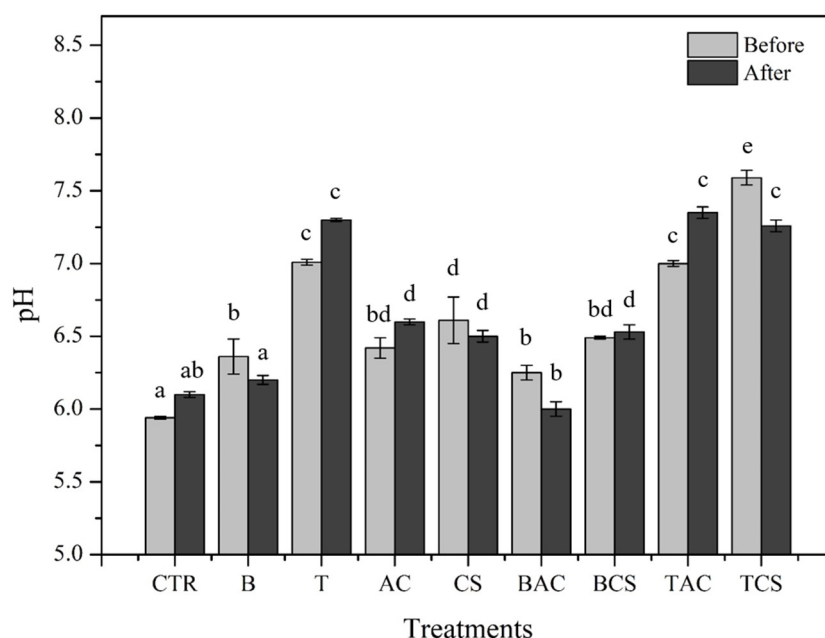
Figure 3. pH of soils at various amendment treatments before and after phytotoxicity test. Means with the same letters are not significantly different from each other under different amendment treatments according to Tukey's test ($p < 0.05$, n = 3) (CTR: control, B: bentonite, T: talc, AC: activated carbon, CS: cornstarch).

Soil pH is an important factor that affects the behavior of metals in soil and directly or indirectly changes their mobility and toxicity to plants and microorganisms. Our results showed high efficiency for increasing soil pH using T and its composites. Indeed, T typically contains calcite as confirmed with the characterization of talc using the FT-IR and XRF results in Figure 2 and Table 4. The calcite and H$^+$ in soil colloids could be replaced with Ca^{2+} and subsequently released into the soil solution and increase soil pH. Besides, Ca^{2+} may further react with HCO$_3$ to form CO$_2$ and H$_2$O which led to an increase in the pH of soil [47].

An increase in soil pH amended with AC and CS is mainly attributed to the existence of the carbonaceous materials and the surface oxygen-containing functional groups such as carboxyl and hydroxyl groups [48].

3.3. Effects of Amendments on As, Cu, and Zn Fractional Distribution in Soil

The chemical formation of metal(loid)s in the treated/non-treated soils after 45 days of planting was explored using a sequential extraction method (Figure 4). In general,

sequential extraction methods still have limitations due to loss and/or gain in the extraction process. They relatively reflect the heavy metal(loid) concentrations in soils existing in chemical forms. Nevertheless, the results from sequential extraction play an important role in assessing the correlation and variation of chemical speciation in soils with amendments.

Based on the sequential extraction results, As was mainly distributed in the organic matter, Fe and Mn oxides, and carbonate fractions with the average values of 50, 25, and 18 wt.%, respectively (Figure 4a). No significant change was found for these fractions of As after amending soil. However, As was related more with exchangeable fraction in the control soil than treated soils. Comparing to the control soil, an exchangeable fraction of As was reduced by 36.8, 25.6, 54.4, 45.6, 66.4, 57.6, 23.2, and 40.8 wt.%, after amending soil with B, T, AC, CS, BAC, BCS, TAC, and TCS, respectively. Arsenic in soils can be associated with several reactive components. Although the total As concentration may manifest the overall level of As in soils, it does not provide information regarding the chemical form or potential mobility and bioavailability of a particular element. Indeed, the bioavailability of heavy metal(loid)s relates to their easily leachable fractions (e.g., exchangeable and carbonate) [10]. A promising result for As immobilization through a reduction in the exchangeable fraction was found in the amended soils especially for AC and its composites.

As it is shown in Figure 4b, Cu was mainly distributed in the un-bioavailable forms of residual (60 wt.%) and organic matter (33 wt.%) fractions. Adding soil amendments showed a significant increase ($p < 0.05$) in the organic matter fractions of Cu with the maximum values for AC (55 wt.%) amended soils. In contrast, a significant decrease in the bioavailable form of an exchangeable fraction of Cu was found after amending soil. Compared to the control soil, the exchangeable fraction of Cu was reduced by 84.2, 79.7, and 74.4 wt.%, after amending soil with AC, BAC, and TAC, respectively. Among soil amendments, AC and its composite were the most effective agents to change the distribution of the Cu in soil.
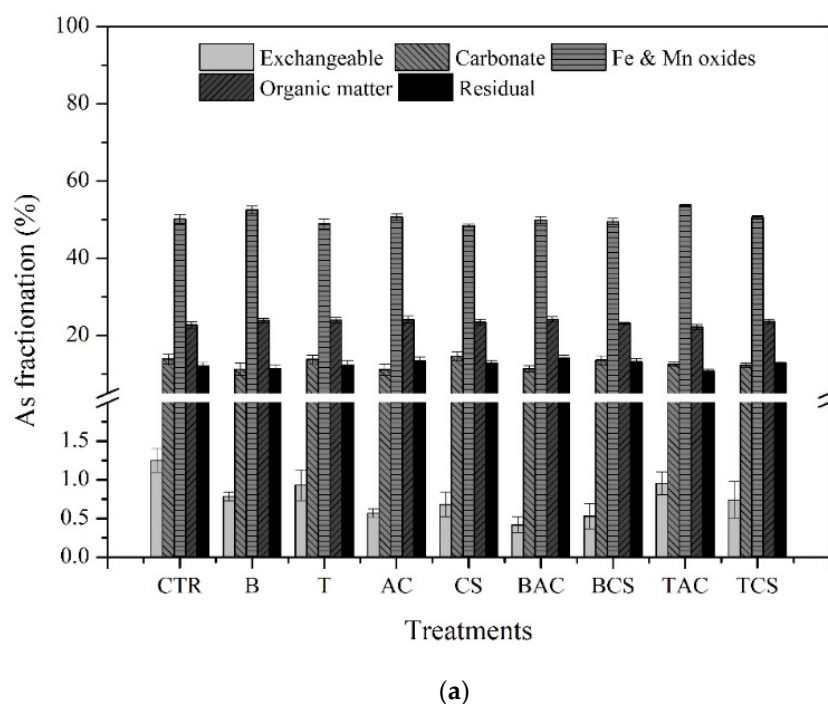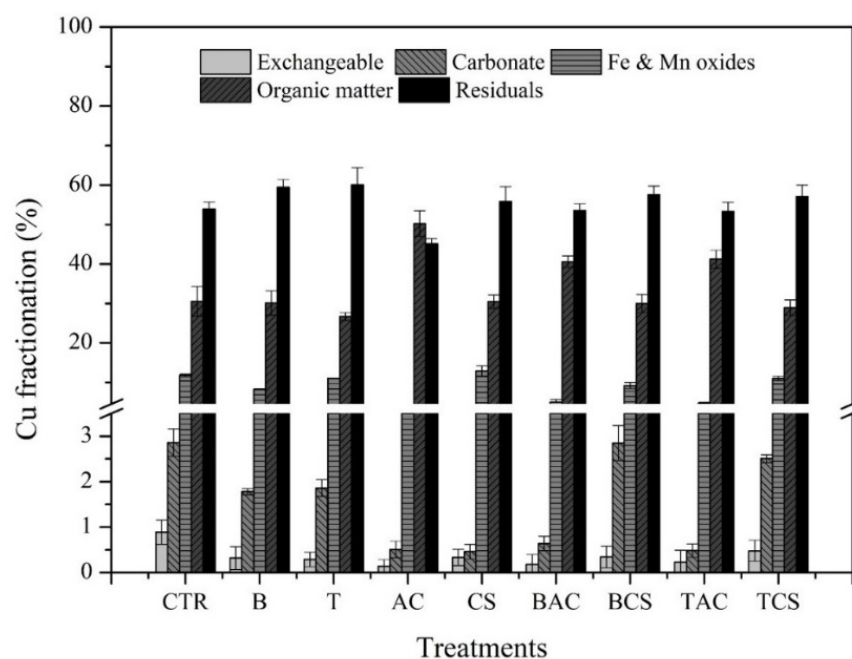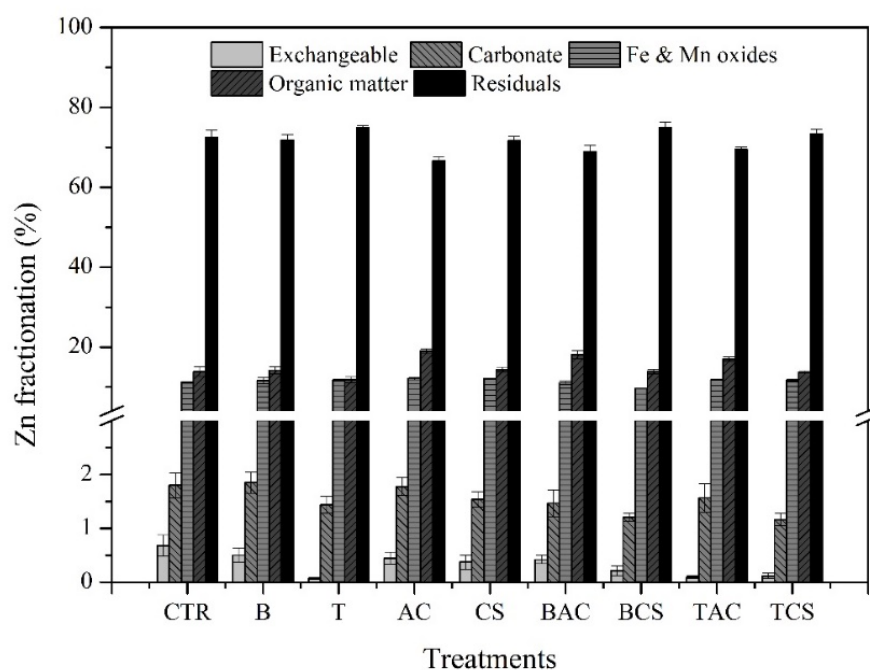

(**a**)

Figure 4. *Cont.*

(**b**)

(**c**)

Figure 4. Arsenic, Cu, and Zn fractionation by sequential extraction of soil with different treatments after 45 days planting lettuce (Arsenic extracted by Wenzel et al., 2001, Cu and Zn extracted by Tessier et al. 1979, CTR: control, B: bentonite, AC: activated carbon, CS: cornstarch). Data are mean ± standard deviation (n = 3). (**a**) Arsenic fractionation, (**b**) Copper fractionation, (**c**) Zinc fractionation.

Based on the sequential extraction results, Zn was associated mostly with a residual fraction with an average value of 75 wt.% (Figure 4c). Amending soil did not change the share of the residual fraction of Zn. However, a slight increase in the organic matter fractionation of Zn was observed after adding to soil with AC and its composites (from 17 to 20 wt.%). A significant reduce in the easily leachable fractions (i.e., exchangeable and

carbonate) of Zn was found after amending soil amendments. For Zn immobilization, T and its composites showed higher efficiency than other treatments. In detail, T, TAC, and TSC reduced the Zn mobility in the exchangeable phase by 89.7%, 86.7%, and 82.3%.
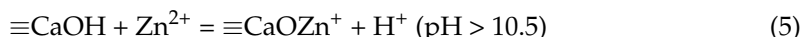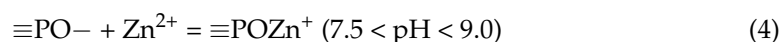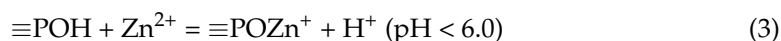
Generally, B and AC showed promising results to immobilize As and Cu in the soil through a reduction in the bioavailable forms, while T and CS were more effective in mitigating the bioavailability of Zn.

Arsenic and Cu are more related to an organic compound, which is why AC showed a more significant effect on As and Cu immobilization. Meanwhile, Zn is related more to inorganic compounds, and T originated from clay minerals with high pH, showing more efficiency to immobilize Zn.

In detail, according to the results of amendment characterization, AC had the highest specific surface area (1082 m^2/g) that shows the potential capacity for heavy metal adsorption. The effect of activated carbon on As, Cu, Zn, and Cd immobilization has been reported in the previous study and also concurred with the results of this study [33,34,49]. In addition, activated carbon includes C = O functional group, which can induce heavy metal immobilization via cation exchange and functional group complexation.

In the case of bentonite, XRF results showed that bentonite had a high percent amount of SiO_2 which is the most abundant component in nature and plays an important role as As storage mineral. Arsenic can be incorporated into mineral structure as impurities and adsorbed onto a mineral surface [50]. Bentonite also had a functional group as O-H can immobilize heavy metals like Cu and Zn via adsorption, functional group complexation, and surface precipitation, as confirmed by other research works [51,52].

In this study, talc had a high chemical composition of MgO (34.9%) and CaO (26.9%) which could increase the pH in soil lead to a corresponding increase in the net negative charge of variably charged colloids in soils and sediments, such as clays, organic matter, and Fe and Al oxides [53]. That is the reason why the soil treated with talc showed the highest pH which led to the increase the heavy metal sorption, especially for Zn. The mechanism of surface complexation on talc surface can be explained as follows [54]:

$$\equiv POH + Zn^{2+} = \equiv POZn^+ + H^+ \text{ (pH} < 6.0) \tag{3}$$

$$\equiv PO- + Zn^{2+} = \equiv POZn^+ \text{ (7.5} < pH < 9.0) \tag{4}$$

$$\equiv CaOH + Zn^{2+} = \equiv CaOZn^+ + H^+ \text{ (pH} > 10.5) \tag{5}$$

In this study, cornstarch also showed a high pH value (8.2) which also has a potential capacity for heavy metal immobilization in soil via adsorption, inner-sphere surface complexation, and precipitation techniques. Besides, the functional group of cornstarch as O-H, C-O also played an important role for Cu, Zn, and Cd adsorption, surface precipitation as other research works confirmed [55,56].

3.4. Effects of Amendments on Plant Growth Index and Phytotoxicity

The study revealed that the response of plant to amendment application varied between amendment types (Figure 5). Amending soil, except for CS and its composites, significantly ($p < 0.05$) induced plant growth index after 45 d planting. Compared with the control samples (19.75 ± 0.25 cm), lettuce height significantly increased in B (24.5 cm ± 0.5 cm), T (23.75 cm ± 0.75 cm), AC (29.5 cm ± 0.7 cm), BAC (25.5 cm ± 0.5 cm), TAC amended soils (26.25 cm ± 0.55 cm). Adding soil amendments meaningfully increased the dry mass of lettuce from 1.73 ± 0.13 g in the control samples to 2.86 ± 0.46, 3.18 ± 0.07, 3.37 ± 0.12, 2.8 ± 0.02, 2.98 ± 0.13 g, in B, T, AC, BAC, and TAC, respectively. Previous studies showed that AC, T, and/or B can induce plant growth parameters mainly due to nutrient supply by these amendments [33]. Noteworthy, no significant change in the plant growth index was found using CS and its composites. This may be attributed to a decrease in soil respiration by the decomposition of CS which later reduces plant growth [57].
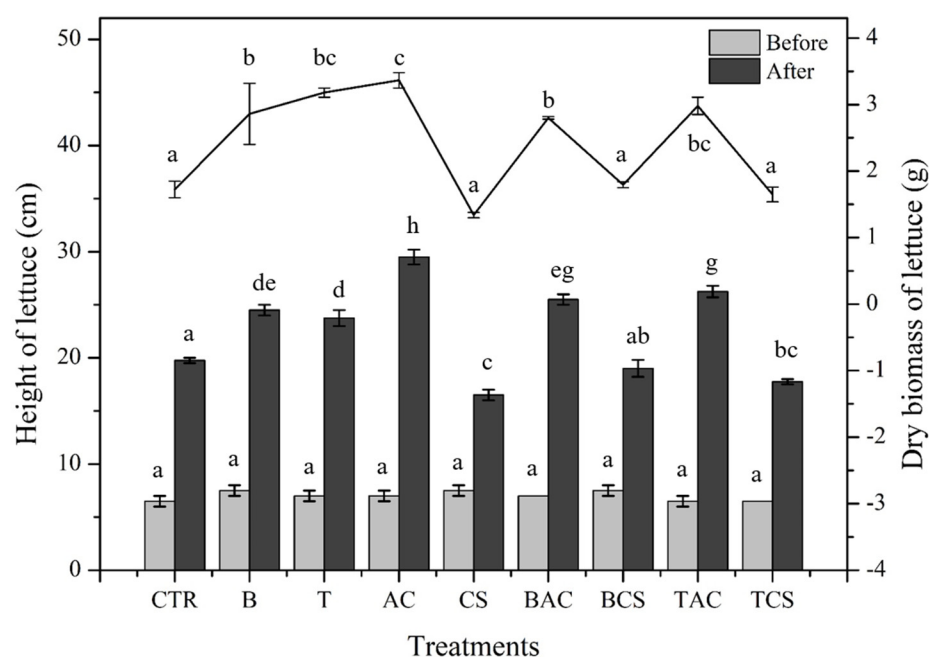
Figure 5. Plant growth at various amendment treatments after 45 days. Means with the same letters are not significantly different from each other under different amendment treatments according to Tukey's test ($p < 0.05$, n = 3) (CTR: control, B: bentonite, AC: activated carbon, CS: cornstarch).

Figure 6 shows concentrations of heavy metal(loid)s in lettuce tissues after 45 days of planting. In Figure 6a, a small amount of As was observed in the plant shoots after amending soil with B (0.075 ± 0.008 mg/kg), AC (0.051 ± 0.007 mg/kg), BAC (0.05 ± 0.005 mg/kg), T (0.107 ± 0.008 mg/kg), and TAC (0.113 ± 0.005 mg/kg) compared with the control samples (0.156 ± 0.005 mg/kg), while the other treatments did not affect As accumulation in roots. Amending soil samples did not affect as uptake by plant shoots after 45 days of planting. Our study concurred with previous work that found an effect of AC and B on reducing the plant uptake of heavy metal contaminated in soil [16,34].

The results also showed that adding amendments reduced Cu uptake by both plant roots and shoots (Figure 6b). Amending soil with AC significantly (<0.05) reduced Cu uptake by plant root (4.76 ± 0.13 vs. 12.08 ± 0.12 mg/kg in control) and shoot (3.78 ± 0.03 vs. 7.28 ± 0.31 mg/kg in control). Although no consistency was found between soil amendments and Zn uptake by root and shoot, the concentration of Zn in plant tissues decreased after amending soil samples (Figure 6c). A great decrease in Zn uptake by root (6.09 ± 0.05 vs. 10.88 ± 0.07 mg/kg in control) and shoot (4.76 ± 0.04 vs. 6.28 ± 0.11 mg/kg in control) was found in the amended soils with BCS. The low level of Zn uptake by plants could be explained by the low Zn mobility in soil when applied CS and its composites, which are presented in Figure 4.

3.5. Effect of Amendments on Metabolic Function of Soil Microbial Communities

The capability of carbon source utilization is a satisfactory factor to describe the metabolic profile of microorganisms and the quality of soil environmental because it reflects the potential of microbes to respond promptly to changes in the environment. Biolog Ecoplate is a rapid, efficient, and inexpensive method used to detect the dynamic variation of microbial metabolism and community diversity in soil. The metabolic activity of microorganisms in the soil was assessed by the AWCD of Biolog Ecoplate; a higher AWCD shows a higher microbial metabolic activity [27–29].

Among these treatments, Figure 7 shows that the AWCD of treatments with B, AC, BAC was stabilized at 1.47, 1.34, and 1.50, respectively, and had no significant difference compared to the control; the AWCD of the control was 1.43 after 120 h incubation time.

Meanwhile, the AWCD of treatments with T, CS, TAC, TCS showed a significant difference compared to the control at 1.66, 1.68, 1.52, 1.70, respectively. Generally, the AWCD was slightly inhibited with the presence of B, AC, and BAC in the soil; meanwhile, T, CS, TAC, and TCS promoted microorganism activity by improving the AWCD index. These results concurred with other research on the effects of B and AC on microbial community. For example, Yuebing Sun showed that bentonite had a negative effect on a microbial community when applied to soil [58]. Wei Que also showed that the microbial community was destroyed when AC was applied to the sediment [33].
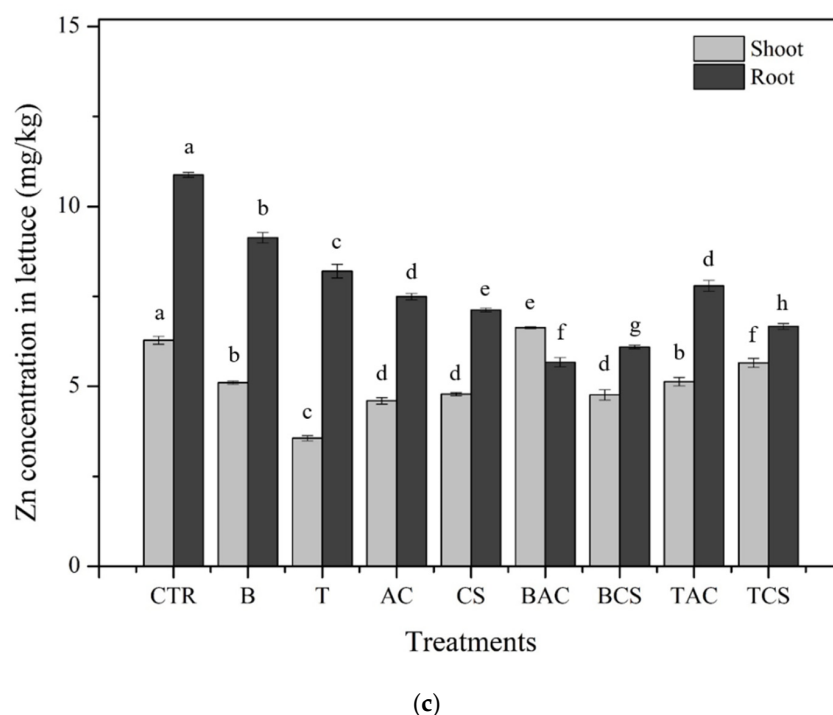

(**a**)

(**b**)

Figure 6. *Cont.*

(**c**)
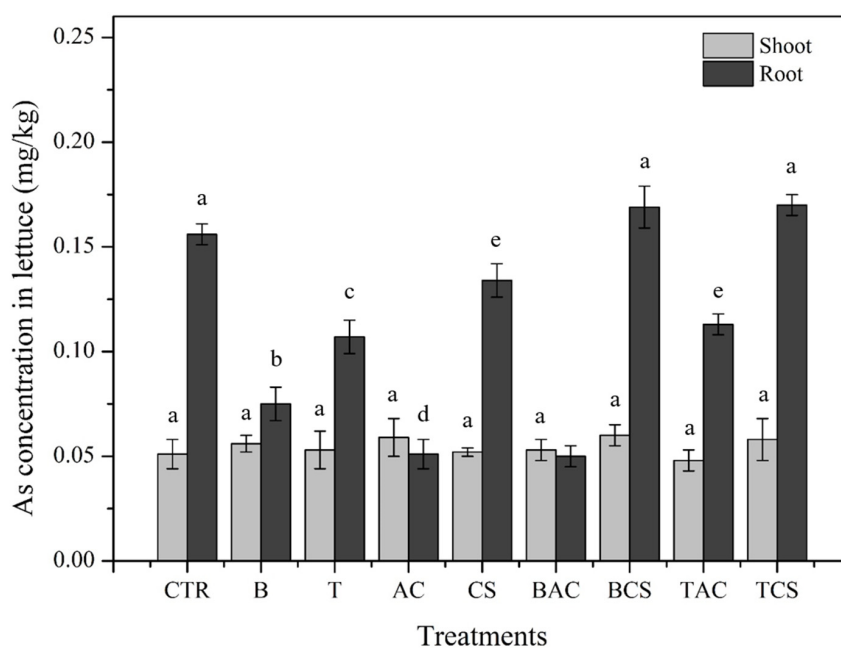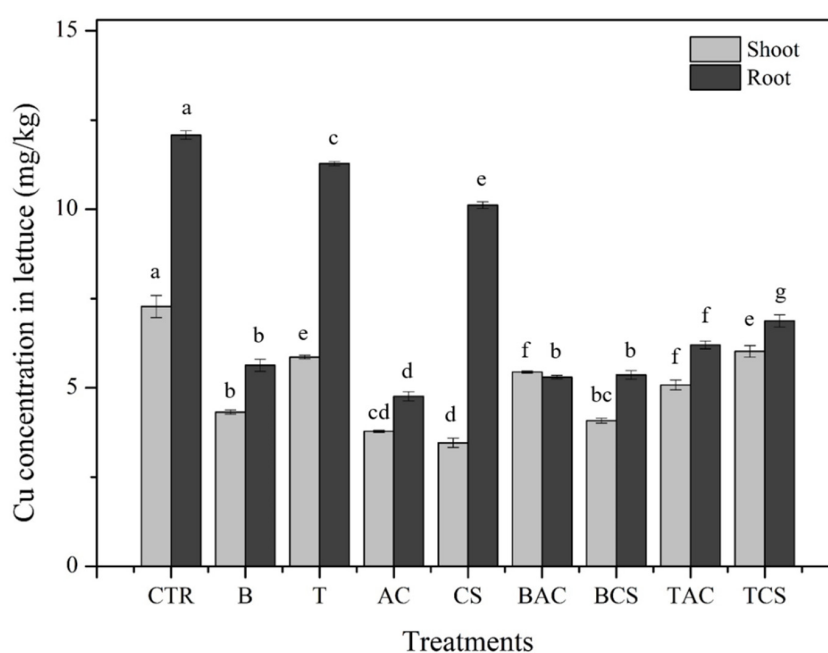
Figure 6. Arsenic, Cu, and Zn concentration uptake by lettuce with the presence of various amendment treatments after 45 days. Means with the same letters are not significantly different from each other under different amendment treatments according to Tukey's test ($p < 0.05$, n = 3) (CTR: control, B: bentonite, AC: activated carbon, CS: cornstarch). (**a**) Arsenic in lettuce tissue, (**b**) Copper in lettuce tissue, (**c**) Zinc in lettuce tissue.
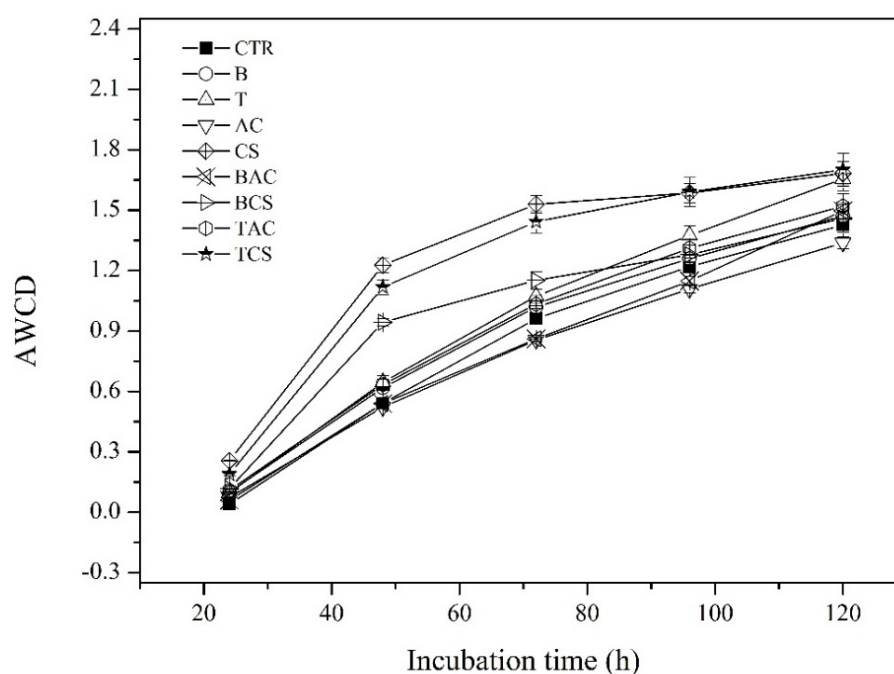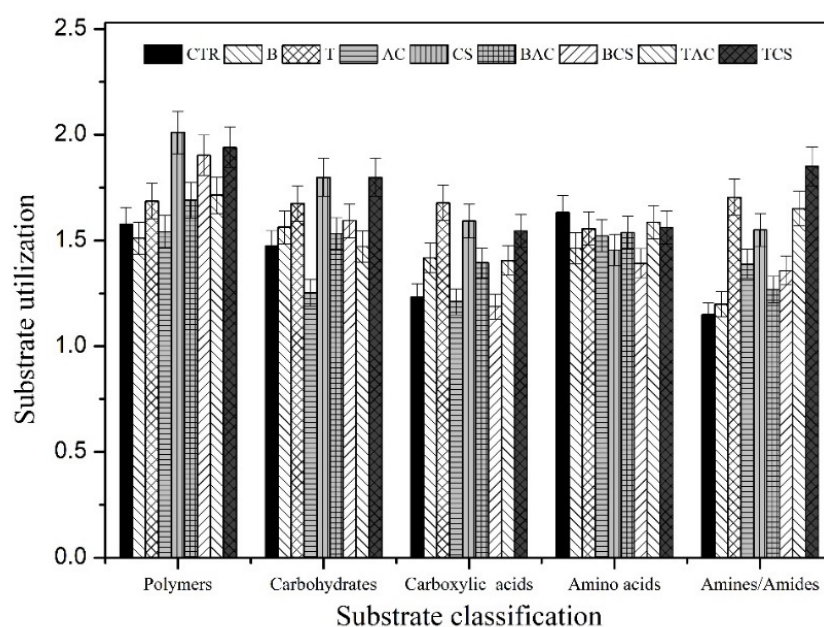

Figure 7. Changes in average well-color development (AWCD) of various amendment treatments with incubation time (CTR: control, B: bentonite, AC: activated carbon, CS: cornstarch). Data are mean ± standard deviation (n = 3).

The SAWCD value is presented in Figure 8. When comparing the distribution of SAWCD for each amendment treatment, we can see that by applying the amendments, the

amino acid SAWCD value decreased compared to the untreated soil, while all of these soils increased the amines/amides SAWCD value up to 0.7 units in TCS; however, the increases of B, AC, and BAC were not significantly different from the control soil. In the polymers, carbohydrates, and carboxylic acid utilization, the control soil showed a lower value of SAWCD than most of the treatments, excepting B, AC, BCS.

Figure 8. Carbon substrate classification of various amendment treatments after 120 h incubation (CTR: control, B: bentonite, AC: activated carbon, CS: cornstarch). Data are mean ± standard deviation (n = 3).

In Figure 9, the heatmap analysis with cluster dendrogram presents in more detail the effect of various amendment treatments on soil microbial community. It can be seen that the groups B, AC, and BAC were similar to the control response to carbon sources utilization compared to T, CS, and TCS. Furthermore, the groups of carbon sources utilized by microbes such as A3, C4, E3, D3, G4, H4, F1, H1, G3, E1, and H2 were the most affected when applying amendments. For the addition of B, AC, and CS, these carbon sources had no significant different change compared to the control; in contrast, T, CS, and TCS increased the carbon source utilization. Other carbon sources had fluctuating changes compared to the control soil when the amendments were added.

A large number of research works have shown the influence of changing soil properties such as pH on soil microbial [59,60]. Most microorganisms had the intracellular pH around neutral, which is why increasing or decreasing pH is sufficient to impose stress and likely affects microbial diversity [61]. In this study, B, AC, and BAC applied to soil slightly changed pH but not significantly different than the control while T, CS, TCS increased the soil pH nearly neutrally. That is the reason why the microbial community in soil treated with T, CS, and TCS was more diverse than B, AC, and BAC. On the other hand, the available Zn content also played an important factor in the microbial community structure [62]. Zn is known as one of the necessary micronutrients for cell metabolism, but Zn content above a specific threshold can be toxic to living organisms, especially soil microbes, and largely affects the functional groups of microbial communities [63]. Our results agree with this, as T and CS showed a higher efficiency of immobilizing Zn compared to B and AC. Furthermore, talc is easily loaded with microorganisms and cornstarch has amylose and amylopectin, which can be fermented by bacteria [64,65]. This might explain the microbial diversity in these amendment treatments.
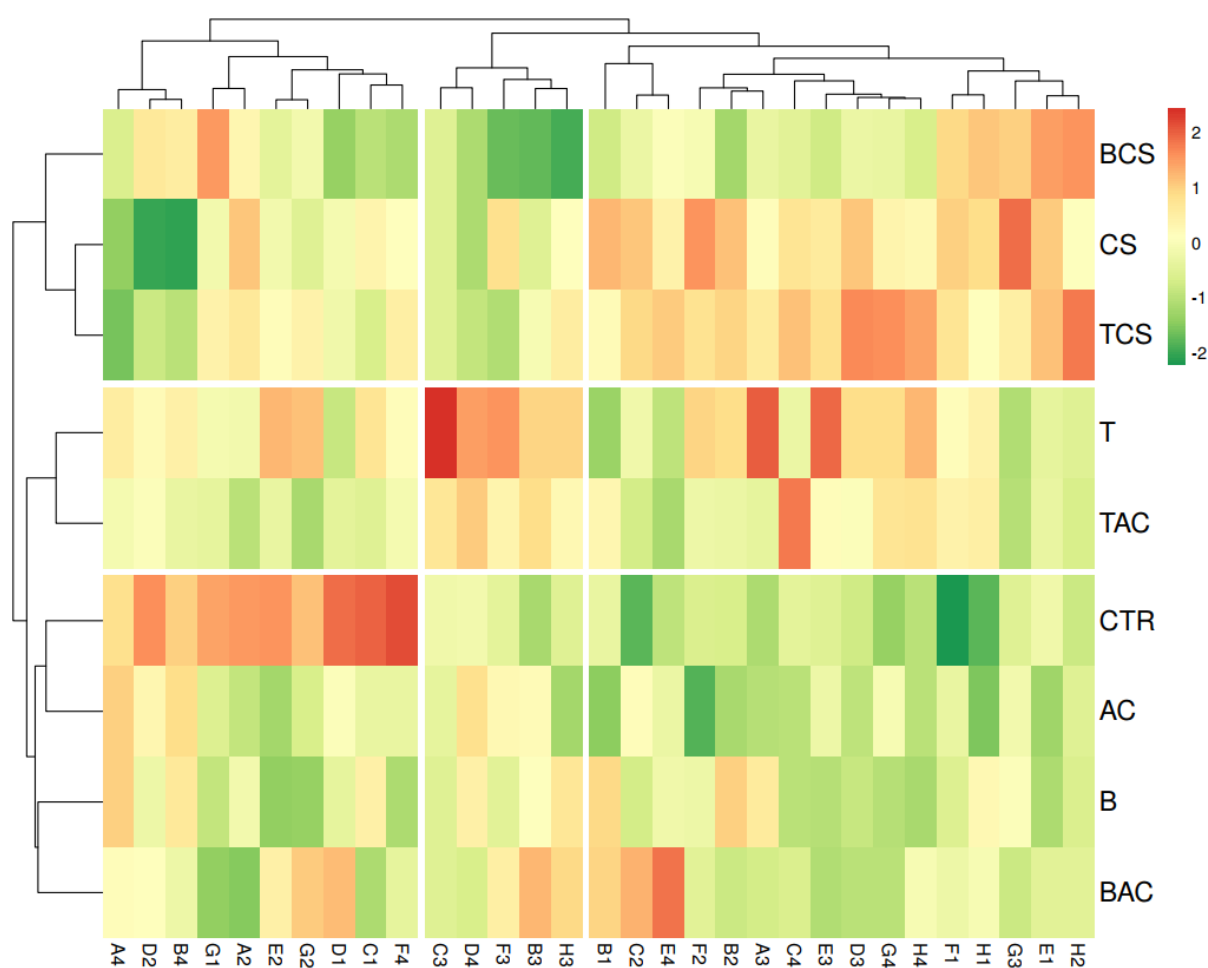
Figure 9. Heatmap of microorganism in various amendment treatments depending on carbon substrates in Biolog EcoPlate. Explanation: A1 to H4 see Table 5. (CTR: control, B: bentonite, AC: activated carbon, CS: cornstarch).

Table 5. Biolog Ecoplate^{TM} carbon source substrate category [66].

Well Number	Carbon Source	Category
B1	Pyruvic acid and methyl ester	Carbohydrates
G1	D-Cellobiose	Carbohydrates
H1	α-D-Lactose	Carbohydrates
A2	β-Methyl-D-glucoside	Carbohydrates
B2	D-Xylose	Carbohydrates
C2	i-Erythritol	Carbohydrates
D2	D-Mannitol	Carbohydrates
E2	N-Acetyl-D-glucosamine	Carbohydrates
G2	Glucose-1-phosphate	Carbohydrates
H2	D,L-α-Glycerol phosphate	Carbohydrates
C1	Tween 40	Polymers
D1	Tween 80	Polymers
E1	α-Cyclodextrin	Polymers
F1	Glycogen	Polymers
F2	D-Glucosaminic acid	Carboxylic and ketonic acids
A3	D-Galactonic acid-γ-lactone	Carboxylic and ketonic acids
B3	D-Galacturonic acid	Carboxylic and ketonic acids
C3	2-Hydroxybenzoic acid	Carboxylic and ketonic acids
D3	4-Hydroxybenzoic acid	Carboxylic and ketonic acids
E3	γ-Hydroxybutyric acid	Carboxylic and ketonic acids

Table 5. *Cont.*

Well Number	Carbon Source	Category
F3	Itaconic acid	Carboxylic and ketonic acids
G3	α-Ketobutyric acid	Carboxylic and ketonic acids
H3	D-Malic acid	Carboxylic and ketonic acids
A4	L-Arginine	Amino acids
B4	L-Asparagine	Amino acids
C4	L-Phenylalanine	Amino acids
D4	L-Serine	Amino acids
E4	L-Threonine	Amino acids
F4	Glycyl-L-glutamic acid	Amino acids
G4	Phenylethylamine	Amines/amides
H4	Putrescine	Amines/amides

4. Conclusions

The remediation of contaminated soil using soil amendments for heavy metals immobilization has been studied for many years; however, there still is a gap in the research to find appropriate amendments for immobilizing metals and metalloids at the same time without negatively affect plant growth and microorganism activities. Therefore, this study was conducted to simultaneously examine the effect of various soil amendments on metals bioavailability and its subsequent influence on the phytotoxicity and microbial diversity in contaminated farmland.

Our results indicated that the soil amendments and their composites significantly affected the soil, plant, and microbial metabolic functions as follows:

(1) Amending soil-induced pH caused a heavy metal(loid)s immobilization.
(2) Among soil amendments, B and AC and their composites show a higher efficiency for As and Cu immobilization in soil with a great reduction in their easily bioavailable fractions.
(3) T, CS, and their composites showed a great effect on Zn immobilization in soil.
(4) The lettuce grew well in B, AC, and their composites amended soils with the minimum amount of As and Cu found in its tissue, while Zn concentration in plant tissue was higher.
(5) No significant change in the microbial communities was found for the B, AC, and their composite amended soils, while T, CS, and their composites induced soil microbial diversity.

Our study suggested that using the composites of amendments has the most beneficial effects on soil, plants, and microbial metabolic functions.

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