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Effect of Exogenous Plant Debris and Microbial Agents on Phytoremediation of Copper-Contaminated Soil in Shanghai

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Abstract: Bioaugmentation is an important measure for improving the efficiency of phytoremediation. The objective was to identify the role of exogenous plant debris with different processing and microbial agents for soil characteristics, copper bioavailability and phytoextraction. The experimental design consisted of four blocks, which were divided into 48 plots. Each plot was planted with *Fraxinus chinensis* and *Salix matsudana* × *alba*, which was added to plant composts, woody chips and effective microorganisms (EM) agents, using an orthogonal experimental design. The results showed that the order of bioaugmentation materials on the Cu phytoextraction of two woody species was plant composts > wood chips > EM agents. The best performance of *F. chinensis* was in the T15 treatment (30% plant composts + 7.5% wood chips + 40 mL·m⁻²), with phytoextraction of 33.66 mg·m⁻², as well as 4.32 mg·m⁻² in the T16 treatment (30% plant composts + 15% wood chips) of *S. matsudana* × *alba*. Cu was accumulated mainly in the roots of the two woody plants. The phytoextraction of the above-ground parts was promoted by bioaugmentation, due to the improvement in the physical soil characteristics and Cu bioavailability. The phytoextraction performance of *F. chinensis* was promoted by the improvement in the Cu concentration after treatments, while for *S. matsudana* × *alba*, it was the dry biomass. Thus, targeted strengthening measures should be applied, to improve the efficiency of phytoremediation.



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Keywords: phytoextraction; plant composts; organic fertilizers; copper; orthogonal design

1. Introduction

Soil pollution is a great concern, as it poses threats to human beings in many industrial countries in the world. In China, copper concentration has exceeded the background values in almost all urban soils, ranging from 23.3 to 1226.3 mg·kg⁻¹ [1,2]. It poses a major risk to plants, animals and humans, and has become an urgent health problem in urban ecosystems [3]. To solve this problem, more and more attention has been paid to the technical research of finding innovative solutions.

Phytoremediation, and more specifically phytoextraction, using fast-growing woody species is an efficient, nonharmful and potentially low-cost technique in comparison to traditional methods [4,5]. Phytoextraction has become a hot spot in the field of phytoremediation of woody plants, because of its well-developed roots, rapid growth and large biomass [6,7]. The efficiency of phytoextraction depends on the capacity of woody species to accumulate and transfer heavy metals in their above-ground tissues. The actual efficiency of phytoextraction depends on the establishment and survival of woody species on contaminated sites [8], as well as their convenient management techniques in the field. As woody species commonly used for ornamental purposes, *Fraxinus chinensis* and *Salix matsudana* × *alba* have good performances in soil phytoremediation, can quickly adapt to the urban environment, and are capable of enduring abiotic stresses [9].

Phytoextraction efficiency is also influenced by cultivation conditions and management measures in the field [10,11]. Bioaugmentation measures, such as fertilizer, organic-matter conditioner and microbial agents are intensive methods which are used for the

enhancement of phytoextraction [12]. In general, plant debris is also used for soil remediation, common as a fertilizer [13,14]. However, the role of organic matter in the bioavailability and phytoremediation of heavy metals is still divergent [12]. On the one hand, organic matter can reduce the bioavailability of heavy metals in the soil by direct adsorption, chelation, and reduction conditions formed by decomposition [15], thus inhibiting the absorption and accumulation of heavy metals by plants [16]. On the other hand, most organic matter can promote desorption of heavy metals from the soil surface by releasing a large amount of water-soluble organic matter, and improve its bioavailability [17]. Most studies show that the water-soluble organic matter of manure, straw and sludge can inhibit the adsorption of heavy metals in soil, and improve its effectiveness and mobility [18]. It has also been found that organic matter such as plant composts, mushrooms, and food waste can improve the phytoextraction efficiency used for the micro-cuttings planting of *S. matsudana* × *alba* [19]. Moreover, microorganisms can also improve phytoremediation efficiency by changing the bioavailability of metals through secreted metal chelates [20], biosurfactants [21] and the redox reaction [22]. In addition, they can promote plant growth and survivability. Effective microorganisms (EM) can achieve the purpose of microbial remediation of soil polluted by low-concentration heavy metals [23], which are a mixed microbial group in an appropriate proportion composed of more than 80 species of microorganisms, including photosynthetic bacteria, lactic acid bacteria, actinomycetes and filamentous bacteria, etc. [24]. Meanwhile, the addition of EM agents to the soil can also improve the soil microbial-community structure [25] and the soil nutrients, by accelerating the decomposition and transformation of organic matter [26] and promoting plant photosynthesis and metabolism [27]. Therefore, exploring the influence of the mechanism of organic matter and microorganisms on phytoextraction is useful for improving the efficiency of fast-growing woody species.

The objective of this study is to explore the role of exogenous plant debris and microbial agents on the phytoextraction of Cu in a field trial, and to explain their accumulation mechanism using correlation analyses for soil characteristics, rhizosphere microbial diversity, and phytoremediation ability. The optimum strengthening measures for the remediation of Cu pollution by *F. chinensis* and *S. matsudana* × *alba* was explored through the application of plant debris-derived products and microbial agents. This will facilitate the rapid promotion of Cu phytoremediation in urban brownfield, as well as providing solutions for the recycling of organic waste.

2. Materials and Methods

2.1. Site and Stand Description

The experimental site was located in the grounds of the Chenshan Botanical Garden (31°4'39" N, 121°11'12" E) in Shanghai, China. Shanghai lies in the northern subtropical zone, and has a subtropical monsoon climate. Its average annual temperature is 17.8 °C, with an annual precipitation of 1457.9 mm, of which 60% is concentrated in the rainy season from May to September, and an average annual evaporation of 1257 mm [8]. The average humidity is 64.7%, and the average wind speed is 4.6 m·s⁻¹. An appropriate amount of CuCl₂ dry powder was used to spike the soil, and was mechanically incorporated into the first 25 to 30 cm of soil and left to equilibrate for four months, to act as simulated copper-contaminated soil. More details about the large experimental design will be shown; it was created based on a previous experiment in 2016 by Vincent et al. (2018) [8] and Shang et al. (2020) [28].

Four Cu-spiked uniform blocks (each 10 m × 10 m) were redesigned to carry out this study. The Cu concentration of block 1–4 were 652.8, 634.0, 639.7 and 619.3 mg·kg⁻¹, respectively. Each block was randomly divided into 12 plots of 8 m² (Figure 1). In each subplots plant composts, wood chips, and effective microorganisms (EM) agents were mixed into the 0–45 cm-deep soil on March 2021. Sixteen different treatments were set up using L16(4³) orthogonal experimental design (Table 1), and are illustrated in Figure 1. Wood chips and plant composts were derived from garden waste. The characteristics of the soils, plant composts and wood chips are presented in Table 2. The EM agents holding the number

of active bacteria ≥ 10 billion·mL⁻¹ were derived from Jiangxi Tianyi Biotechnology Co., Ltd. EM agents were diluted to 10 L with water, using different treatments, before being put in the soil.

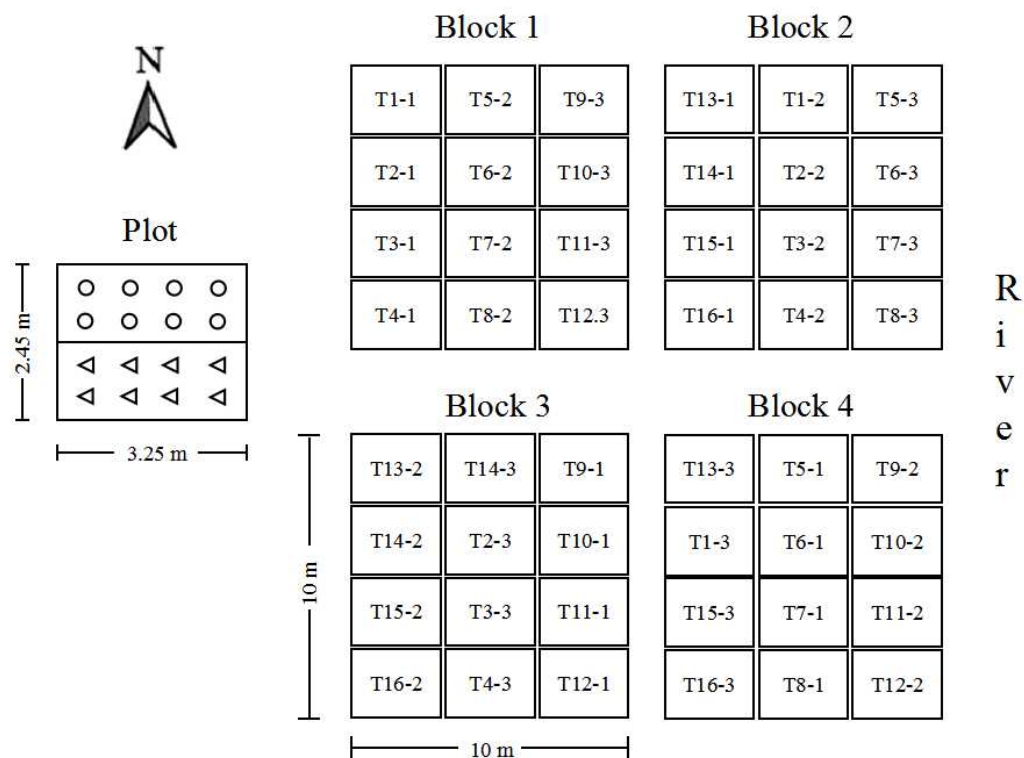


Figure 1. Experimental design of different bioaugmentation measures and planting modes. Note: the circle represents *F. chinensis*; the triangle represents *S. matsudana* × *alba*.

Table 1. Treatments of orthogonal array test.

Treatments	Factors		
	Plant Composts (%vol)	Wood Chips (%vol)	EM Agents (mL·m ⁻²)
T1	0	0	0
T2	0	3.75	40
T3	0	7.5	20
T4	0	15	10
T5	7.5	0	40
T6	7.5	3.75	0
T7	7.5	7.5	10
T8	7.5	15	20
T9	15	0	20
T10	15	3.75	10
T11	15	7.5	0
T12	15	15	40
T13	30	0	10
T14	30	3.75	20
T15	30	7.5	40
T16	30	15	0

Table 2. Characteristics of the soils, plant composts and wood chips.

Parameters	Soils	Plant Composts	Wood Chips
PS (cm)	/	0.3–1.0	2.0–3.0
BD (g·cm ^{−3})	1.31	0.40	0.38
pH	8.20	7.57	7.85
SOM (g·kg ^{−1})	24.2	69.89	109.20
TN (g·kg ^{−1})	2.36	2.37	2.45
TP (g·kg ^{−1})	1.62	1.74	1.75

Note: PS, BD, SOM, TN and TP are the abbreviations for particle size, bulk density, soil organic matter, total nitrogen, and total phosphorus, respectively.

The tested plants were *Salix matsudana* × *alba* and *Fraxinus chinensis*, with good performance in the previous test and which were ideal woody species for the phytoremediation of heavy metal Cu pollution in urban soil [9]. In each plot, eight *S. matsudana* × *alba* cuttings with a length of approximately 25 cm and eight one-year-old *F. chinensis* seedlings were planted on April 2021, with a total of 768 individuals. During the growing season, weeding was manually performed twice, in June and September. Irrigation was only carried out at the beginning of the experiment.

2.2. Soil Sampling and Analysis

To evaluate the soil characteristics after the materials addition, a mixed sample from three points per plot of 0–30 cm-soil layer were taken from soil samplers (φ5 cm) along the diagonal line, on June 2021. Soil samples were air-dried, crushed, sieved (0.149 mm mesh) and stored in sealed plastic bags [29]. Soil cores were also collected (using a 200 cm³ cylinder) from each plot, for determination of BD and maximum water-holding capacity [30]. SOM was determined by the potassium dichromate oxidation-heating method [31]. Hydrolysable nitrogen was determined by the alkaline-hydrolysis diffusion method [32]. Cation exchange capacity was determined by the ammonium chloride–ammonium acetate exchange method [33]. Soil pH and electrical conductivity were determined using a pH meter (Hach, Loveland, CO, USA) with a measuring range of −2.00 to 14.00, and an electrical conductivity instrument (Leici Company, Shanghai China) with a measuring range of 0.0 to 10.0 ec in the supernatant of 2:5 and 1:5 soil and water mixtures, respectively [34,35]. Water-soluble Cu was measured using inductively coupled plasma-mass spectrometry (ICP-MS) on the supernatant of 1:12.5 soil-to-water ratio. The suspension reacted on a reciprocal shaker for 2 h, was centrifuged (1400 × g) for 10 min, filtered with a nylon membrane (0.45 mm), and stored in 0.2% trace metal grade HNO₃ [36].

2.3. Rhizosphere Soil Sampling and Microorganism Determination

Rhizosphere soil, i.e., the soil adhering to plant roots, was collected by cutting the fine roots of *F. chinensis* randomly from each plot in June 2021. Each sample was put in an aseptic bag and kept frozen until transportation to the laboratory. Each sample was transferred to sterile 50 mL centrifuge tubes containing 20 mL of sterile 10 mM PBS solution, placed in a full temperature shaker at 120 rpm·min^{−1}, and shaken at room temperature for 20 min [37]. The root systems were removed from the 50 mL centrifuge tubes using sterile tweezers, and the remaining suspension was centrifuged at high speed (6000 × g, 4 °C) for 20 min, to collect the rhizosphere soil for DNA extraction and 16S ribosomal RNA gene amplicon sequencing.

Genomic DNA was extracted using the OMEGA Soil DNA Kit (D5625-01). The quantity and quality of the extracted DNA was examined using an Eppendorf RS232G UV-Vis spectrophotometer. The V3-V4 region of 16S rRNA genes were PCR amplified from bacteria compatible with the primers 338F (ACTCTACGGGAGGCAGCAG) and 806R (GGAC-TACHVGGGTWTCTAAT) [38]. The PCR amplification products were sequenced using illumina MiSeq at Shanghai Meiji Biomedical Technology Co., Ltd. These sequence data were filtered and analyzed using the quantitative analysis of microbial ecology (QIIME) [39].

OTU clustering was carried out for non-repetitive sequences employing a 97% similarity. Based on the silver database, the RDP classifier Bayesian algorithm (with a default confidence threshold of 0.7) was used to annotate the selected OTU representative sequences, and a subsequent sequencing-data analysis was carried out. The alpha diversity index of the rhizosphere microorganisms under different intensive treatments was counted; the Shannon index was used to reflect the microbial-community diversity, the Chao index was used to reflect the microbial-community richness, and the Shannoneven index was used to reflect the microbial-community uniformity. Boxplots were produced for the soil microbial diversity index, using the software R.

2.4. Plant Sampling and Cu Concentration Analysis

In order to remove the maximum contaminants, all plants were coppiced at the end of the growth season. To evaluate the yield, the above-ground biomass (shoots and leaves) of all plants was weighed in the field, using an electronic scale (Xiangshan Inc. model ACS-JC21D). Portions of the plants were oven-dried at 80 °C (to a constant mass) before being reweighed, to determine the average dry biomass. The below-ground biomass of all plants was the average root system estimated by the above-ground parts.

Dry plant tissues from each sample were ground with a stainless-steel grinder until all particles passed through a 0.149 mm nylon sieve, to determine the Cu concentration. Approximately 0.5 g of these samples were digested with a mixture of concentrated HNO₃ (10 mL), 30% H₂O₂ (1 mL), and concentrated HCl (2 mL) in Teflon tubes. These digested solutions had a constant volume of 25 mL with deionized water, and 10 mL of the suspensions were filtered through a nylon membrane (Magna-0.45 mm). The Cu concentration of these digested solutions were analyzed, using ICP-MS (Agilent ICPMS 7700) [40]. The translocation factor refers to the efficiency of the plant in translocating the accumulated metal from its roots to the shoots [41].

2.5. Statistical Analysis

All collected data were analyzed using EXCEL 2010 and SPSS 22.0 software. These random factor bioaugmentation materials, as well as the plant species, were compared. The mixed-model ANOVA (Tukey HSD and T-test) was carried out, to contrast the levels of the independent variables, and differences were deemed significant when $p < 0.05$. The relationships among soil characteristics, growth, and Cu accumulation in the plant tissue were analyzed using the Pearson correlation coefficient. A comprehensive assessment of the most appropriate measures was determined through a range analysis. In the range analysis, K_i ($i = 1, 2, 3, 4$) represented the sum of measured values of each factor at the same level, which distinguished the worst level and the best level of each influencing factor. R represented the range between different levels of the same factor. The primary and secondary factors were measured by the magnitude of the range (R), which indicated that this factor had a greater impact on the test results. On the other hand, it indicated that the influence of this factor on the test was relatively small [42].

3. Results

3.1. Changes in Soil Characteristics

The soil characteristics were obviously changed by different bioaugmentation measures, as shown in Table 3. With the increase of plant compost, maximum water-holding capacity (MWHC), soil organic matter (SOM), hydrolysable nitrogen (HN) concentration and cation-exchange capacity (CEC), were increased. Meanwhile, the bulk density (BD) was decreased and the water-soluble copper (WS-Cu) concentration had a peak in the 15% plant composts. When the addition of plant composts was 30%, the BD of T13, T14, T15 and T16 were 0.77, 0.89, 0.84 and 0.84 g·cm⁻³, respectively, which was significantly lower than the non-compost treatments ($p < 0.05$). The MWHC of four treatments (T13, T14, T15 and T16) added to 30% plant composts was significantly higher than that of the other treatments, and the maximum value was 814.32 g·kg⁻¹ in T13. The SOM, HN concentration and CEC

of the treatment group with 30% plant composts (T13–T16) were highest, with average values of $36.19 \text{ g}\cdot\text{kg}^{-1}$, $141.19 \text{ mg}\cdot\text{kg}^{-1}$ and $17.31 \text{ cmol}(+)\cdot\text{kg}^{-1}$, respectively. There was no obvious regularity or significant difference in pH and electrical conductivity (EC) among the different bioaugmentation measures. The soil WS-Cu concentration in the T9 and T11 treatments were 2.58 and $2.56 \text{ mg}\cdot\text{kg}^{-1}$, respectively, which was significantly higher than that of T1.

Table 3. Soil physiochemical characteristics in different treatments.

Treatments	BD ($\text{g}\cdot\text{cm}^{-3}$)	MWHC ($\text{g}\cdot\text{kg}^{-1}$)	SOM ($\text{g}\cdot\text{kg}^{-1}$)	HN ($\text{mg}\cdot\text{kg}^{-1}$)	CEC ($\text{cmol}(+)\cdot\text{kg}^{-1}$)	EC ($\mu\text{S}\cdot\text{cm}^{-1}$)	pH	WS-Cu ($\text{mg}\cdot\text{kg}^{-1}$)
T1	1.21 ± 0.07 a	437.40 ± 28.68 f	24.54 ± 2.60 c	111.53 ± 16.52 cd	12.48 ± 0.02 f	140.57 ± 12.14 b	8.08 ± 0.04 abcd	1.38 ± 0.07 bcd
T2	1.05 ± 0.07 abc	528.14 ± 53.95 def	33.24 ± 7.18 bc	109.79 ± 23.04 cd	11.98 ± 0.33 f	149.60 ± 7.61 ab	8.09 ± 0.05 abcd	1.79 ± 0.08 abcd
T3	1.06 ± 0.09 abc	497.35 ± 59.23 ef	31.83 ± 4.79 bc	124.79 ± 20.55 bcd	14.72 ± 1.20 cdef	153.57 ± 7.83 ab	8.05 ± 0.07 abcd	1.96 ± 0.44 abcd
T4	1.05 ± 0.04 abc	524.69 ± 39.71 def	30.02 ± 5.82 bc	107.21 ± 30.57 cd	14.29 ± 1.56 def	155.70 ± 7.75 ab	8.12 ± 0.10 abcd	1.29 ± 0.51 cd
T5	1.13 ± 0.00 ab	489.68 ± 3.17 ef	31.50 ± 3.02 bc	117.01 ± 13.58 bcd	16.12 ± 0.88 abcde	154.73 ± 8.86 ab	7.94 ± 0.13 cd	1.50 ± 0.07 abcd
T6	1.02 ± 0.11 abcd	548.64 ± 70.62 cdef	27.95 ± 2.63 bc	100.38 ± 11.95 d	14.43 ± 0.54 def	139.43 ± 6.99 b	8.15 ± 0.02 ab	1.50 ± 0.63 abcd
T7	0.92 ± 0.06 cde	630.13 ± 50.67 bcde	38.48 ± 3.43 ab	134.45 ± 8.31 bcd	15.56 ± 0.10 abcde	155.03 ± 5.41 ab	8.20 ± 0.02 a	2.50 ± 0.14 ab
T8	1.12 ± 0.04 ab	481.39 ± 37.33 ef	33.16 ± 2.16 bc	127.72 ± 15.07 bcd	15.58 ± 1.46 abcde	153.63 ± 8.23 ab	8.13 ± 0.02 abc	2.12 ± 0.45 abcd
T9	1.05 ± 0.03 abc	531.46 ± 19.81 def	45.71 ± 1.11 a	192.89 ± 14.62 a	17.48 ± 0.22 abc	167.00 ± 10.48 a	7.93 ± 0.07 d	2.58 ± 0.76 a
T10	0.95 ± 0.04 bcde	615.15 ± 25.45 bcde	33.39 ± 0.97 bc	119.32 ± 3.27 bcd	13.65 ± 0.50 ef	152.90 ± 7.54 ab	8.08 ± 0.07 abcd	2.46 ± 0.30 abc
T11	0.98 ± 0.12 bcd	588.66 ± 93.14 bcdef	29.63 ± 2.04 bc	114.19 ± 12.96 bcd	16.15 ± 0.07 abcde	152.60 ± 4.48 ab	8.15 ± 0.07 ab	2.56 ± 0.11 a
T12	1.08 ± 0.10 abc	504.64 ± 71.29 ef	29.04 ± 6.89 bc	113.37 ± 23.85 bcd	15.47 ± 1.13 bcde	149.07 ± 12.71 ab	8.17 ± 0.07 ab	2.00 ± 0.36 abcd
T13	0.77 ± 0.07 e	814.32 ± 78.91 a	36.00 ± 2.85 abc	132.13 ± 9.54 bcd	15.63 ± 0.69 abcde	155.77 ± 8.94 ab	8.04 ± 0.03 abcd	1.42 ± 0.15 abcd
T14	0.89 ± 0.10 cde	688.07 ± 87.69 abcd	38.48 ± 4.53 ab	154.42 ± 24.39 abc	17.09 ± 1.32 abcd	159.30 ± 8.80 ab	8.00 ± 0.03 bcd	1.81 ± 0.32 abcd
T15	0.84 ± 0.06 de	709.82 ± 85.97 abc	31.25 ± 2.41 bc	115.36 ± 10.50 bcd	18.07 ± 2.34 ab	148.93 ± 5.39 ab	8.05 ± 0.05 abcd	1.25 ± 0.14 d
T16	0.84 ± 0.02 de	741.86 ± 31.75 ab	39.01 ± 5.02 ab	162.86 ± 13.84 ab	18.46 ± 0.57 a	161.93 ± 4.97 ab	7.99 ± 0.05 bcd	1.50 ± 0.07 abcd

Note: Values are mean $\pm$ SE. Different letters in a row indicate a significant difference, $p < 0.05$.

3.2. Microbial Diversity in Rhizosphere Soil

A total of 2,902,193 sequences were obtained from all the 48 samples after removal of the index and primer sequences, quality-control filter and rarefaction. The average length of the sequence was 418 bp, which was consistent with the length of the 16S rDNA V3 + V4 region. The data of 48 samples were flattened, and each sample contained 48,366 effective sequences. In line with the OTU, taxon with 16S rDNA similarity $\geq 97\%$, 8307 OTUs were obtained after clustering, and 46 phyla, 155 classes, 364 orders, 592 families, 1119 genera and 2367 species were identified. According to the microbial diversity indexes of the different treatments, microbial agents were the primary factor affecting rhizosphere microbial diversity, so they were grouped according to the dosage of effective microorganism (EM) agents. This showed that the value of Shannon, Chao and Shannoneven was $\text{EM20} > \text{EM10} > \text{EM0} > \text{EM40}$, respectively, through the analysis of the differences between the index groups (Figure 2). The three indexes in the treatment with $20 \text{ mL}\cdot\text{m}^{-2}$ EM agents were significantly different from those in the treatment with $40 \text{ mL}\cdot\text{m}^{-2}$ EM agents ($p < 0.05$).

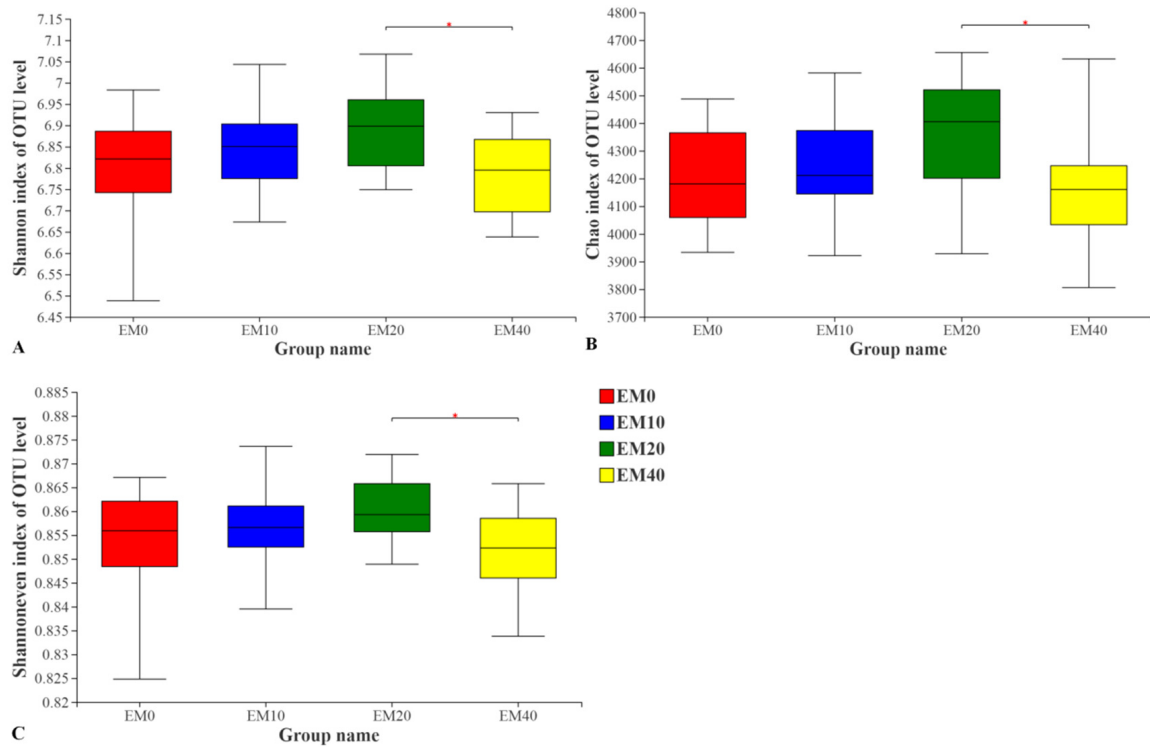


Figure 2. Alpha diversity index statistics of rhizosphere microorganism of *F. chinensis* in different treatments. (A) Shannon index of OTU level; (B) Chao index of OTU level; (C) Shannoneven index of OTU level. Note: $p < 0.05$ is marked as asterisk.

3.3. Cu Concentration in Plant Tissues

In general, the Cu concentration in the below-ground parts of plants was significantly higher than that in above-ground parts, especially in the tissues of *S. matsudana* × *alba* (Figure 3). With the increase in the amount of plant composts added, the Cu concentration in the plant tissues of two woody species showed an upward trend, and 30% plant composts treatments had a significantly higher concentration than that of the others. As shown in Figure 3A, the Cu concentration of the above-ground parts of *F. chinensis* in the T15, T16 and T13 treatments were 37.55, 33.45 and 27.62 mg·kg⁻¹, respectively. The highest concentration of below-ground parts was in T15, which was 85.88 mg·kg⁻¹. As shown in Figure 3B, the Cu concentration of the above-ground parts of *S. matsudana* × *alba* in the T13, T15 and T14 treatments were 14.67, 13.16 and 12.82 mg·kg⁻¹, respectively. The highest concentration of below-ground parts was in T13, which was 139.83 mg·kg⁻¹.

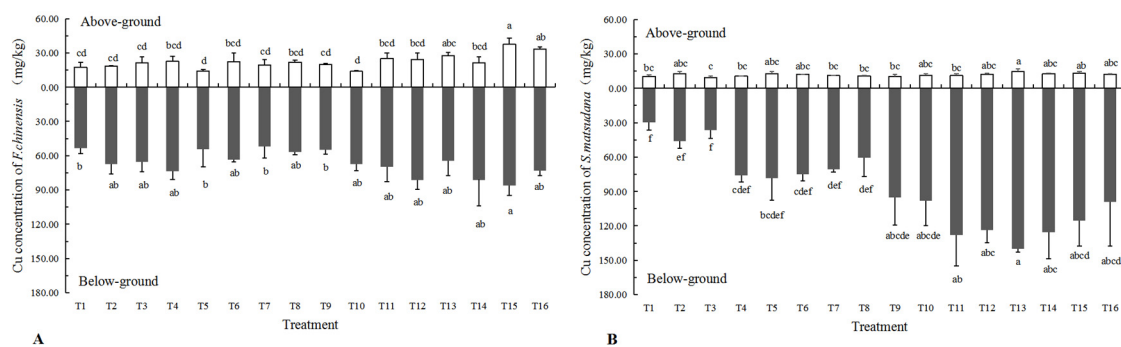


Figure 3. Cu concentrations of above-and below-ground parts of two woody species. (A) Cu concentration of *F. chinensis*; (B) Cu concentration of *S. matsudana* × *alba*. Note: Values are mean ± SE. Different letters in a row indicate a significant difference, $p < 0.05$.

The addition of plant composts had a significant effect on the translocation factor of plants, showing different trends in the two tree species. The translocation factor of *F. chinensis* with 30% plant composts treatments was higher than that of other treatments, and the highest treatment was T16, T15 and T13, which were 0.46, 0.44 and 0.43, respectively. The transfer coefficient of *S. matsudana* $\times$ *alba* decreased significantly when adding plant composts, because the Cu concentration of below-ground parts increased greatly.

3.4. Yield and Cu Quantity Extracted by the Plants

The yield was evaluated at the end of the growing seasons (Figure 4). The biomass and Cu quantity extraction of the above-ground, below-ground and whole plant of two woody species had improved to a certain extent, among different bioaugmentation measures. The performance of *F. chinensis* was better than *S. matsudana* $\times$ *alba*, especially in 30% plant composts treatments.

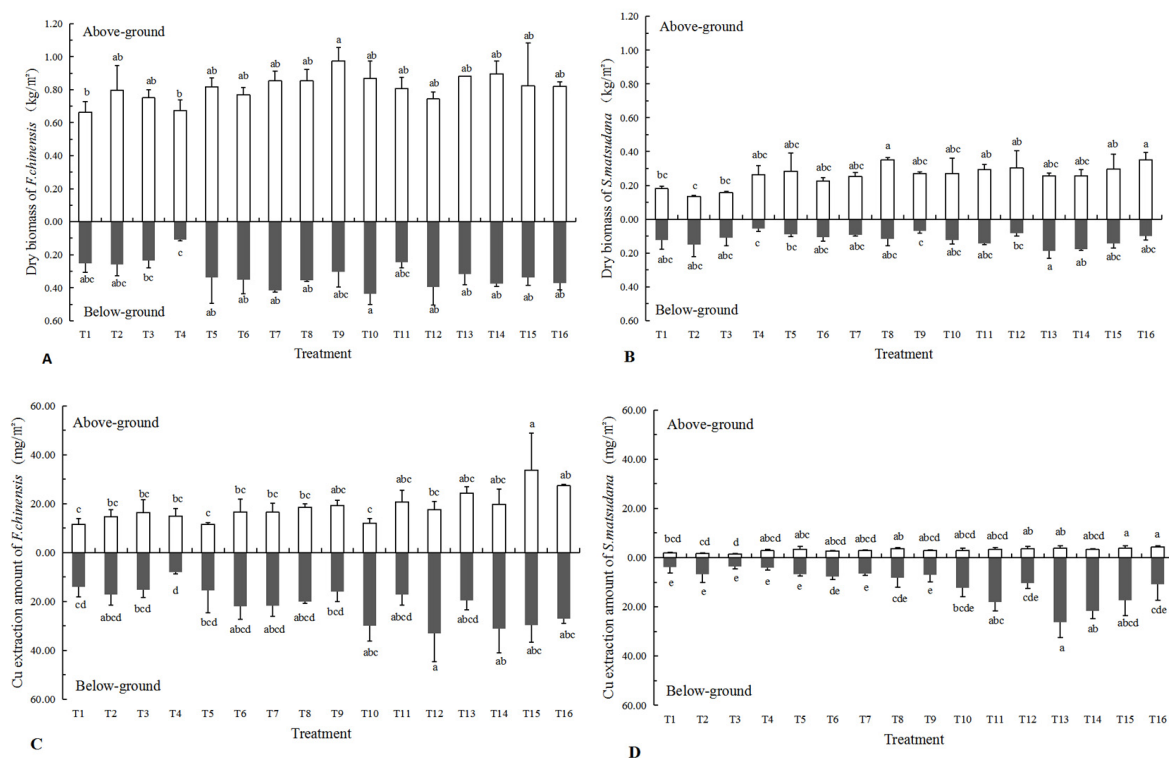


Figure 4. Yields and Cu extraction of above- and below-ground parts of two woody species. (A) Dry biomass of *F. chinensis*; (B) Dry biomass of *S. matsudana* $\times$ *alba*; (C) Cu extraction amount of *F. chinensis*; (D) Cu extraction amount of *S. matsudana* $\times$ *alba*. Note: Values are mean $\pm$ SE. Different letters in a row indicate a significant difference, $p < 0.05$.

The maximum biomass of above-ground parts of *F. chinensis* was in T9, with $0.95 \text{ kg} \cdot \text{m}^{-2}$, with $0.30 \text{ kg} \cdot \text{m}^{-2}$ of below-ground parts in T10. The biomass of *F. chinensis* treated with 7.5%, 15% and 30% plant composts was higher than that of treatments without plant composts (Figure 4A). Meanwhile, the Cu extraction of the above-ground parts of *F. chinensis* in T15 was the highest, reaching $33.66 \text{ mg} \cdot \text{m}^{-2}$, and the differences between the treatments without plant composts and with 7.5% plant composts, were significant. The same trend was indicated in the below-ground parts, except for that of T12 (Figure 4C). Similarly, the maximum biomass of the above-ground parts of *S. matsudana* $\times$ *alba* was in T8 and T16, which reached $0.35 \text{ kg} \cdot \text{m}^{-2}$, while the maximum biomass of below-ground parts was $0.19 \text{ kg} \cdot \text{m}^{-2}$ in T13 (Figure 4B). With the increase in the plant composts addition, the biomass of *S. matsudana* $\times$ *alba* also increased gradually, and reached the maximum value in the 30% plant composts addition. Meanwhile, higher Cu extractions of above-ground parts were in T15 and T16, with 3.86 and $4.32 \text{ mg} \cdot \text{m}^{-2}$, respectively. With the increase in

plant composts addition, the Cu extraction of below-ground parts increased significantly, and reached a peak value of $26.23 \text{ mg}\cdot\text{m}^{-2}$ in T13 (Figure 4D).

3.5. Correlation Analysis

The correlation analysis results of the above-ground indexes and soil characteristics are shown in Table 4. Although there were some differences in the influence of soil on Cu concentration and dry biomass of above-ground parts between the two woody plants, the influence of Cu phytoextraction was consistent. The Cu phytoextraction of the two woody plants was significantly negatively correlated with BD ($R^2 = -0.729$ and -0.706), and significantly positively correlated with MWHC ($R^2 = 0.720$ and 0.692) and Ws-Cu ($R^2 = 0.736$ and 0.647).

Table 4. Correlation analysis between above-ground indexes and soil characteristics.

Index	Cu Concentration		Dry Biomass		Cu Phytoextraction	
	<i>F. chinensis</i>	<i>S. Matsudanas</i> × <i>alba</i>	<i>F. chinensis</i>	<i>S. Matsudanas</i> × <i>alba</i>	<i>F. chinensis</i>	<i>S. Matsudanas</i> × <i>alba</i>
BD	−0.625 **	−0.596 *	−0.480	−0.309	−0.729 **	−0.706 **
MWHC	0.617 *	0.662 **	0.473	0.339	0.720 **	0.692 **
SOM	0.072	0.080	0.832 **	0.252	0.295	0.259
HN	0.115	−0.059	0.710 **	0.305	0.301	0.248
CEC	0.622 *	0.229	0.651 **	0.392	0.251	0.135
EC	0.088	−0.036	−0.440	−0.071	−0.162	−0.046
pH	−0.007	−0.193	0.503 *	0.095	−0.227	−0.047
WS-Cu	−0.374	−0.438	0.527 *	0.727 **	0.736 **	0.647 **
Shannon	0.079	/	0.617 *	/	0.216	/
Chao	0.079	/	0.612 *	/	0.238	/
Shannoneven	0.128	/	0.563 *	/	0.241	/

Note: ** Correlation is significant on 0.01 layer (double tail), * correlation is significant on 0.05 layer (double tail).

There were differences in the phytoextraction performance of the above-ground parts between *F. chinensis* and *S. matsudanas* × *alba*. The Cu extraction of the above-ground parts of *F. chinensis* was significantly positively correlated with the Cu concentration in the plant tissue ($R = 0.949$), while *S. matsudana* × *alba* mainly depended on dry biomass ($R = 0.921$) (Table 5).

Table 5. Correlation analysis between Cu-extraction amount with Cu concentration and dry biomass of above-ground parts of *F. chinensis* and *S. matsudana* × *alba*.

Index	Cu-extraction Amount	
	<i>F. chinensis</i>	<i>S. matsudana</i> × <i>alba</i>
Cu concentration	0.949 **	0.520 *
Dry biomass	0.308	0.921 **

Note: ** Correlation is significant on 0.01 layer (double tail), * correlation is significant on 0.05 layer (double tail).

3.6. Comprehensive Assessment by Range Analysis

The results of a range analysis on the Cu extraction of above-ground parts are shown in Table 6. The most important factor for both *F. chinensis* and *S. matsudana* × *alba* was the plant composts, identified by range value. The grade of the three factors was the following: plant composts > wood chips > EM agents. The optimum ratio of Cu extraction of the above-ground parts in *F. chinensis* was 30% plant composts + 7.5% wood chips + 40 $\text{mL}\cdot\text{m}^{-2}$ EM agents, while the above-ground parts of *S. matsudana* × *alba* was 30% plant composts + 15% wood chips + 40 $\text{mL}\cdot\text{m}^{-2}$ EM agents.

Table 6. Range analysis of Cu extraction of above-ground parts of two woody species.

Species		Factors		
		Plant Composts	Wood Chips	EM Agents
<i>F. chinensis</i>	K1	57.54	66.53	76.01
	K2	63.05	63.23	67.80
	K3	69.54	87.12	74.01
	K4	105.06	78.31	77.37
	R value	47.52	23.89	9.57
	Optimal level	30%	7.5%	40 mL·m ⁻²
<i>S. matsudana</i> × <i>alba</i>	K1	7.88	12.07	12.32
	K2	12.75	10.66	12.36
	K3	12.73	11.47	11.32
	K4	15.29	14.45	12.65
	R value	7.41	3.79	1.33
	Optimal level	30%	15%	40 mL·m ⁻²

4. Discussion

4.1. Role of Exogenous Plant Debris and Microbial Agents on Phytoremediation

This study aimed to improve the copper phytoextraction of plant tissues by means of the enhancement of physical, chemical and biological soil characteristics, using plant composts, wood chips, and effective microorganisms (EM) agents. The order of these factors in the enhancement of Cu extraction in the above-ground parts of *F. chinensis* and *S. matsudana* × *alba* was plant composts > wood chips > EM agents. The results of the correlation analysis showed that the phytoextraction amount was improved by the enhancement of physical soil characteristics and the metal bioavailability in *F. chinensis* and *S. matsudana* × *alba*.

As a raw material, plant debris is produced in bark blocks, wood chips, composts, earthworm manure and biochar, to improve urban soil. Generally considered as a physical-structure conditioner, wood chip is widely used as organic mulch, but rarely used for raw-materials mixture. Compost can effectively improve soil characteristics, especially fertility [43,44], through organic matter, nitrogen and phosphorus in the soil [45]. And that compost can provide soil microorganisms with nutrients needed for reproduction, and enhance microbial activity [46]. At the same time, microorganisms can promote the decomposition of organic matter in compost, effectively control nitrogen loss, and play a role in nitrogen fixation and fertilizer conservation [47]. Under the synergetic additions of three bioaugmentation materials, bulk density (BD), maximum water-holding capacity (MWHC), soil organic matter, hydrolysable nitrogen, and cation-exchange capacity changed significantly among the different treatments, especially with plant composts increased. Unexpectedly, plant composts had a greater impact on BD and MWHC than wood chips, due to the large difference in proportion.

The bioavailability of heavy metals directly affects the phytoextraction efficiency of plants, and its effect even exceeds the total concentration of heavy metals [48]. Organic matters such as compost, manure and feedstock are generally used to promote phytoremediation in contaminated soil, as well as for improvement in properties and quality [14,19]. There are differences between the role of heavy-metal bioavailability and phytoextraction efficiency. On one hand, such organic matters containing rich humus can significantly improve the biomass of plants and the bioavailability of heavy metals in the soil, by releasing a large amount of water-soluble organic matter [13,17]. Microorganisms will produce a variety of amino acids and low-molecular-weight organic acids during their growth and metabolism, which can also activate solid heavy metals in the soil [49]. On the other hand, organic matter can also reduce the bioavailability of heavy metals in the soil through direct adsorption, chelating and the reduction conditions formed by decomposition [15], and inhibit the absorption and accumulation of heavy metals by plants [16,17]. A certain concentration of EM agents can effectively improve the diversity, richness and uniformity

of *F. chinensis*. A high concentration of microbial agents may lead to excessive microbial density in the soil, and inhibit the physiological activities of microorganisms. When more than 15% plant compost was used in this experiment, the water-soluble Cu (WS-Cu) concentration reached a peak, due to the absorption of plant composts, which may be greater than the desorption. It was reported that the form of heavy metal in the soil was related to the pH of the soil, and a low pH value can reduce the adsorption rate of heavy metal, and increase the content of available metal [50]. However, no significant correlation between WS-Cu concentration and pH was found in this study, which may be due to the alkaline soil characteristics in this experiment.

The EM agent was the primary factor affecting the microbial diversity index. In the plot where *F. chinensis* was planted, the diversity, richness and uniformity of soil microorganisms were improved by adding a certain concentration of EM agents. EM agents can improve soil microbial-community structure, increase soil microbial quantity and soil enzyme activity. A high concentration of EM agents may lead to excessive microbial density in the soil, and inhibit the physiological activities of microorganisms [51]. It was found that microorganisms can promote plant growth [52,53] and improve the survivability of heavy metal [54,55] through various mechanisms. The same result was found in this experiment: the dry biomass of *F. chinensis* was significantly positively correlated with the microbial diversity indexes. However, there was no significant relationship between the microorganism indexes and phytoextraction ability. This may be attributed to the insignificant correlation between microbial diversity indexes and Cu concentration.

4.2. Phytoextraction Performance of *F. chinensis* and *S. matsudanas* × *alba*

A high accumulation of metals in roots and low transport of heavy metals to the shoots were reported to be the key mechanisms in protecting plant organs involved in photosynthesis [56]. High phytoremediation ability can be found in fast-growing woody species, mainly fixed in the roots [28]. The Cu concentration of the below-ground parts of two woody species was higher than that in the above-ground tissues, even after bioaugmentation measures, especially in the tissues of *S. matsudanas* × *alba*. This is due to the low Cu mobility in the soil [57]. Most of the heavy-metals tests showed that the highest accumulation was recorded in the roots of willow, followed by the leaves and stems, which supported our current results [58].

According to the Cu concentration in the above-ground and below-ground parts, the transfer ability of *F. chinensis* was stronger than that of *S. matsudana* × *alba*. Cu is accumulated and stored in roots, and then transported to the above-ground parts, which is a way for the plant to resist heavy metal [59]. When stress occurs, roots can also make an adaptive response, by influencing the behavior of the above-ground parts through the transmission of information materials [56]. It was reported that the root activity of *F. chinensis* was significantly higher than that of *S. matsudana* under high Cu-concentration stress ($>500 \text{ mg} \cdot \text{kg}^{-1}$) [60]. Therefore, it can be concluded that *S. matsudana* × *alba* has a stronger ability to fix Cu in the roots system, and *F. chinensis* has a stronger potential to extract Cu.

In general, the phytoremediation potential in the former research showed different patterns that were dependent on the heavy metal type and tree genotype [61]. The phytoextraction performance of *F. chinensis* was promoted by the increase in Cu concentration in the plant tissue under bioaugmentation, while for *S. matsudana* × *alba*, it was the dry biomass. The phytoremediation mode of the two woody plants was different, which may be due to the difference of the translocation factor.

5. Conclusions

The phytoextraction of two woody plants was promoted by exogenous plant debris and microbial agents, due to the improvement in physical soil characteristics and copper bioavailability. Plant composts play the best role for soil improvement and copper phytoextraction in comparison with woody chips and EM agents. The best performance of

F. chinensis was in the T15 treatment (30% plant composts + 7.5% wood chips + 40 mL·m⁻²) with phytoextraction of 33.66 mg·m⁻², as well as 4.32 mg·m⁻² in the T16 treatment (30% plant composts + 15% wood chips) of *S. matsudana* × *alba*. The Cu accumulation in the above-ground parts of *F. chinensis* was attributed to the Cu concentration of plant tissues, while biomass was the primary determinant of *S. matsudana* × *alba*. Thus, according to the promoting effect of different tree species, we need to choose targeted strengthening measures to improve the efficiency of phytoremediation.

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References

1. Wei, B.G.; Yang, L.S. A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. *Microchem. J.* **2010**, *94*, 99–107. [\[CrossRef\]](#)
2. Cai, M.F.; McBride, M.B.; Li, K.M. Bioaccessibility of Ba, Cu, Pb, and Zn in urban garden and orchard soils. *Environ. Pollut.* **2016**, *208*, 145–152. [\[CrossRef\]](#)
3. Yruela, I. Copper in plants: Acquisition, transport and interactions. *Funct. Plant Biol.* **2009**, *36*, 409–430. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Khan, F.I.; Husain, T.; Hejazi, R. An overview and analysis of site remediation technologies. *J. Environ. Manag.* **2004**, *71*, 95–122. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Blasco, I.N.; Duran, A.; Sirera, R.; Fernández, J.M.; Alvarez, J.I. Solidification/stabilization of toxic metals in calcium aluminate cement matrices. *J. Hazard. Mater.* **2013**, *260*, 89–103. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Pulford, I.D.; Watson, C. Phytoremediation of heavy metal-contaminated land by trees—a review. *Environ. Int.* **2004**, *29*, 529–540. [\[CrossRef\]](#)
7. Daniela, B.; Angela, C.; Alessandro, B.; Stefano, C. Different behaviours in phytoremediation capacity of two heavy metal tolerant poplar clones in relation to iron and other trace elements. *J. Environ. Manag.* **2014**, *146*, 94–99.
8. Vincent, G.; Shang, K.K.; Zhang, G.W.; Labrecque, M. Preliminary results of the tolerance to inorganic contaminants and phytoextraction potential of twelve ornamental shrub species tested on an experimental contaminated site. *Iforest-Biogeoosci. For.* **2018**, *11*, 442–448. [\[CrossRef\]](#)
9. Shang, K.K.; Zhang, G.W.; Jiang, Y. The phytoextraction ability of 54 woody species on Cu, Pb, Zn in soil. *Chin. J. Ecol.* **2019**, *38*, 3723–3730.
10. Liphadzi, M.S.; Kirkham, M.B.; Mankin, K.R.; Paulsen, G.M. EDTA-assisted heavy-metal uptake by poplar and sunflower grown at a long-term sewage-sludge farm. *Plant Soil* **2003**, *257*, 171–182. [\[CrossRef\]](#)
11. Bradford, K.R.; George, O.K.; Calvin, G.M.; John, D.N.; Cathy, A.N. Long-term effects of spring wheat–soybean cropping systems on weed populations. *Field Crops Res.* **2005**, *97*, 197–208.
12. Liao, X.Y.; Chen, T.B.; Yan, X.L.; Nie, C.J. Enhancement of heavy metal removal in phytoremediation of soils contaminated with heavy metals. *Acta Sci. Circumstantiae* **2007**, *27*, 881–893.
13. Hande, A.S.; Deshpande, A.A. Methodology for design & fabrication of portable organic waste chopping machine to obtain compost—a review. *Int. J. Innov. Res. Sci. Technol.* **2014**, *1*, 132–135.
14. Madar, R.; Singh, Y.; Meena, M.C.; Gaund, S.; Das, T.K.; Verma, R.K.; Halli, H. Crop residue and potassium management on crop and soil properties of maize and wheat in no-tillage systems. *Commun. Soil Sci. Plant Anal.* **2021**, *52*, 769–791. [\[CrossRef\]](#)
15. Covelo, E.F.; Vega, F.A.; Andrade, M.L. Competitive sorption and desorption of heavy metals by individual soil components. *J. Hazard Mater.* **2007**, *140*, 308–315. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Zheljazkov, V.D.; Warman, P.R. Phytoavailability and fractionation of Cu, manganese and zinc in soil following application of two composts to four crops. *Environ. Pollut.* **2004**, *131*, 187–195. [\[CrossRef\]](#)

17. Walker, D.J.; Clemente, R.; Roig, A.; Bernal, M.P. The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. *Environ. Pollut.* **2003**, *122*, 303–312. [\[CrossRef\]](#)
18. Li, T.Q.; Yang, X.E. Transfer of Arsenic from Agricultural Soil to Food Chain. *Guangdong Trace Elem. Sci.* **2004**, *11*, 1–10.
19. Labrecque, M.; Hu, Y.H.; Vincent, G.; Shang, K.K. The use of willow microcuttings for phytoremediation in a copper, zinc and lead contaminated field trial in Shanghai, China. *Int. J. Phytoremed.* **2020**, *22*, 1331–1337. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Kavita, B.; Shukla, S.; Kumar, G.N.; Archana, G. Amelioration of phytotoxic effects of Cd on mung bean seedlings by gluconic acid secreting rhizobacterium *Enterobacter asburiae* PSI3 and implication of role of organic acid. *World J. Microbiol. Biotechnol.* **2008**, *24*, 2965–2972. [\[CrossRef\]](#)
21. Venkatesh, N.M.; Vedaraman, N. Remediation of soil contaminated with copper using Rhamnolipids produced from *Pseudomonas aeruginosa* MTCC 2297 using waste frying rice bran oil. *Ann. Microbiol.* **2012**, *62*, 85–91. [\[CrossRef\]](#)
22. Kim, C.; Zhou, Q.; Deng, B.; Thornton, E.C.; Xu, H. Chromium(VI) reduction by hydrogen sulfide in aqueous media: Stoichiometry and kinetics. *Environ. Sci. Technol.* **2011**, *35*, 2219–2225. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Liu, R.W. Effects of EM and organic fertilizer on the bio-availability of heavy metal. *Hubei Agric. Sci.* **2009**, *48*, 1352–1354.
24. Cui, S.; Wang, G.; Zheng, F.F.; Wang, L.H. Effects of tea polyphenols and EM on fecal pollutant emission of growing minks. *Chin. J. Anim. Sci.* **2018**, *54*, 108–112.
25. Liu, S.H.; Liu, S.Q.; Zhang, Z.K.; Wei, H.; Zhang, Y.; Ma, L.; Yang, X.J.; Dou, J. Impact of effective microorganisms on microbial communities and enzyme activities in rhizosphere soil of continuously cropped garlic. *Plant Nutr. Fertil. Sci.* **2011**, *17*, 718–723.
26. Yu, Z.Q.; Xu, Y.Q.; Li, F.L.; Liu, D.; He, L.B.; Wang, M.J.; Wang, X.; Dong, S.J.; Hu, B.Z. Effects of composting mulch and organic fertilizer fermented by EM on cucumber quality. *Crops* **2015**, *3*, 104–110.
27. Allison, S.D.; Czimczik, C.I.; Treseder, K.K. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. *Glob. Change Biol.* **2008**, *14*, 1156–1168. [\[CrossRef\]](#)
28. Shang, K.K.; Hu, Y.H.; Vincent, G.; Labrecque, M. Biomass and phytoextraction potential of three ornamental shrub species tested over three years on a large-scale experimental site in Shanghai, China. *Int. J. Phytoremed.* **2020**, *22*, 10–19. [\[CrossRef\]](#)
29. GB/T 36197; Soil Quality—Guidance on Sampling Techniques. Standardization Administration of China: Beijing, China, 2018.
30. NY/T 1121.4; Soil Testing Part 4: Method for Determination of Soil Bulk Density. Ministry of Agriculture of China: Beijing, China, 2006.
31. LY/T 1237; Determination of Organic Matter in Forest Soil and Calculation Carbon-Nitrogen Ratio. National Forestry and Grassland Administration of China: Beijing, China, 1999.
32. LY/T 1228; Determination of Hydrolyzable Nitrogen in Forest Soil. National Forestry and Grassland Administration of China: Beijing, China, 1999.
33. LY/T 1243; Determination of Cation Exchange in Forest Soil. National Forestry and Grassland Administration of China: Beijing, China, 1999.
34. LY/T 1239; Determination of pH Value in Forest Soil. National Forestry and Grassland Administration of China: Beijing, China, 1999.
35. HJ 802; Soil Quality—Determination of Conductivity—Electrode Method. Ministry of Ecology and Environmental Protection of China: Beijing, China, 2016.
36. Seguin, V.; Gagnon, C.; Courchesne, F. Changes in water extractable metals, pH and organic carbon concentrations at the soil-root interface of forested soils. *Plant Soil* **2004**, *260*, 1–17. [\[CrossRef\]](#)
37. Beckers, B.; De Beeck, M.O.; Weyens, N.; Van Acker, R.; Van Montagu, M.; Boerjan, W.; Vangronsveld, J. Lignin engineering in field-grown poplar trees affects the endosphere bacterial microbiome. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 2312–2317. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Xu, N.; Tan, G.C.; Wang, H.Y.; Gai, X.P. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *Eur. J. Soil Biol.* **2016**, *74*, 1–8. [\[CrossRef\]](#)
39. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Pena, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [\[CrossRef\]](#)
40. Kou, S.M.; Vincent, G.; Gonzalez, E.; Pitre, F.E.; Labrecque, M.; Brereton, N.J.B. The response of a 16S ribosomal RNA gene fragment amplified community to lead, zinc, and copper pollution in a Shanghai field trial. *Front. Microbiol.* **2018**, *9*, 366–388. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Ali, H.; Khan, E.; Sajad, M.A. Phytoremediation of heavy metals—Concepts and applications. *Chemosphere* **2013**, *91*, 869–881. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Wang, X.; Jia, Z.X.; Liu, L.J.; Li, J.Q.; Zhang, C.C.; Wang, S.F. Optimization of molding process parameters for sheet molding compound composites based on range analysis. *China Plast.* **2022**, *36*, 77–83.
43. Schmid, C.J.; Murphy, J.A.; Murphy, S. Effect of tillage and compost amendment on turfgrass establishment on a compacted sandy loam. *J. Soil Water Conserv.* **2017**, *72*, 55–64. [\[CrossRef\]](#)
44. Logsdon, S.D.; Sauer, P.A.; Shipitalo, M.J. Compost improves urban soil and water quality. *J. Water Resour. Protect.* **2017**, *9*, 345–357. [\[CrossRef\]](#)

45. Zhang, X.; Zheng, C.Y.; Li, S.M.; Xie, F.J.; Deng, A.X.; Zhang, J.; Song, Z.W.; Zhang, W.J. Long-term combined application of chemical and organic fertilizers decrease net greenhouse gas emission in wheat-soybean system in Huang-Huai-Hai region. *J. Plant Nutr. Fertil.* **2020**, *26*, 2204–2215.
46. Xue, Q.; Zhou, J.F. Effect of organic fertilizer application on soil microbial diversity in winter wheat fields. *Mod. Chem. Res.* **2020**, *22*, 127–128.
47. Zhou, N.Q.; Zhao, S.; Shen, X.P. Nitrogen cycle in the hyporheic zone of natural wetlands. *Chin. Sci. Bull.* **2014**, *59*, 2945–2956. [[CrossRef](#)]
48. Song, L.L.; Tie, M.; Zhang, C.H.; Hui, X.J.; Jing, K.; Chen, Z.L.; Zhang, Y. Effects of applying sewage sludge on chemical form distribution and bioavailability of heavy metals in soil. *Chin. J. Appl. Ecol.* **2012**, *23*, 2701–2707.
49. Pernezny, K.; Nagata, R.; Havranek, N.; Sanchez, J. Comparison of two culture media for determination of the copper resistance of *Xanthomonas* strains and their usefulness for prediction of control with copper bactericides. *Crop Prot.* **2007**, *27*, 256–262. [[CrossRef](#)]
50. Wang, C.L.; Zheng, W.J.; Wang, H.J.; Zeng, X.D.; Ji, S.L. The relationship between heavy metals enrichment characteristics and soil acidification for environmental media in Yantai of Shandong province. *Rock Miner. Anal.* **2012**, *31*, 361–369.
51. Lv, J.; Wen, T.C.; Guo, K.L.; Kang, J.C. Effects of distiller's grains bio-organic fertilizer and microbial agent on microbial quantities and sorghum yield. *Res. Agric. Mod.* **2013**, *34*, 502–506.
52. Ma, Y.; Rajkumar, M.; Vicente, J.A.F.; Freitas, H. Inoculation of Ni-resistant plant growth promoting bacterium *Psychrobacter* sp. strain SRS8 for the improvement of nickel phytoextraction by energy crops. *Int. J. Phytoremed.* **2011**, *13*, 126–139. [[CrossRef](#)] [[PubMed](#)]
53. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010**, *48*, 909–930. [[CrossRef](#)] [[PubMed](#)]
54. Babu, A.G.; Shea, P.J.; Oh, B.T. *Trichoderma* sp. PDR1-7 promotes *Pinus sylvestris* reforestation of lead-contaminated mine tailing sites. *Sci. Total Environ.* **2014**, *476–477*, 561–567. [[CrossRef](#)]
55. Garg, N.; Aggarwal, N. Effect of mycorrhizal inoculations on heavy metal uptake and stress alleviation of *Cajanus cajan* (L.) Millsp. genotypes grown in cadmium and lead contaminated soils. *Plant Growth Regul.* **2012**, *66*, 9–26. [[CrossRef](#)]
56. Landberg, T.; Greger, M. Differences in uptake and tolerance to heavy metals in *Salix* from unpolluted and polluted areas. *Appl. Geochem.* **1996**, *11*, 175–180. [[CrossRef](#)]
57. Massenet, A.; Bonet, A.; Laur, J.; Labrecque, M. Co-planting *Brassica napus* and *Salix nigra* as a phytomanagement alternative for Cu contaminated soil. *Chemosphere* **2021**, *279*, 130517. [[CrossRef](#)]
58. Borghi, M.; Tognetti, R.; Monteforti, G.; Sebastiani, L. Responses of two poplar species (*Populus alba* and *Populus × canadensis*) to high copper concentrations. *Environ. Exp. Bot.* **2008**, *62*, 290–299. [[CrossRef](#)]
59. Tian, S.K.; Li, T.X.; Yang, X.E.; Lu, L.L.; Peng, H.Y. Mechanisms of copper uptake, transportation and detoxification in plants. *Chin. J. Soil Sci.* **2006**, *37*, 387–394.
60. Li, Y.J. Physiological Response and Tolerance Evaluation on Six Urban Greening Seedlings under Soil Cu and Pb Contamination. Ph.D. Thesis, Beijing Forestry University, Beijing, China, 2010.
61. Pilipović, A.; Zalesny, R.S.; Rončević, S.; Nikolić, N.; Orlović, S.; Beljin, J.; Katanić, M. Growth, physiology, and phytoextraction potential of poplar and willow established in soils amended with heavy-metal contaminated, dredged river sediments. *J. Environ. Manag.* **2019**, *239*, 352–365. [[CrossRef](#)]