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Dendroremediation Potential of Six *Quercus* Species to Polluted Soil in Historic Copper Mining Sites

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Abstract: Green remediation of severely contaminated soils around mining sites can be achieved using suitable woody plants such as *Quercus* species, but their phytoremediation potential has not been well evaluated yet. Six *Quercus* species, which were popular in ecological restoration and landscape application in east China, were selected and evaluated for their phytoremediation potential of metal polluted soil using a pot experiment that lasted for 150 d. The results suggested that *Quercus* species exhibited high tolerance to multi-metal contamination of Cu (9839 mg·kg^{-1}), Cd (8.5 mg·kg^{-1}), and Zn (562 mg·kg^{-1}) with a tolerance index (TI) ranging from 0.52 to 1.21. Three *Quercus* (*Q. pagoda*, *Q. acutissima*, and *Q. nuttallii*) showed relatively higher tolerance with TIs of 1.08, 1.09, and 1.21, respectively. Above-ground tissues accounted for most of the total biomass in T1 (mixture of clean and polluted soil, 50%) and T2 (100% polluted soil) treatments for most species. The Cu contents in plant tissues were in the order of root > leaf > stem, whereas Zn exhibited the order of leaf > stem > root, and Cd showed divergent mobility within the *Quercus* species. All the *Quercus* species exhibited higher capacity for Zn phytoextraction with translocation factor (TF) over 1 and Cu/Cd phytostabilization with TFs lower than 1. The analytic hierarchy process-entropy weight model indicated that *Q. virginiana* and *Q. acutissima* were two excellent species with evident phytoremediation capacity of Cu, Cd, and Zn co-contaminated soil. Taken together, *Quercus* species showed great potential for phytoremediation of soils severely polluted by Cu, Cd, and Zn around historic mining sites. Application of *Quercus* species is a green remediation option with low-maintenance cost and prospective economic benefit for phytomanagement of historic mining sites.

Keywords: copper; cadmium; zinc; *Quercus* species; phytostabilization

Citation: Cao, Y.; Yu, L.; Dang, N.; Sun, L.; Zhang, P.; Cao, J.; Chen, G. Dendroremediation Potential of Six *Quercus* Species to Polluted Soil in Historic Copper Mining Sites. *Forests* **2023**, *14*, 62. https://doi.org/10.3390/f14010062

Academic Editors: Luigi Sanita' di Toppi, Ewa Joanna Hanus-Fajerska and Martin Backor

Received: 27 October 2022
Revised: 23 December 2022
Accepted: 24 December 2022
Published: 29 December 2022

1. Introduction

Mining activities produce substantial waste (e.g., mining gangue, mine tailings, and metallurgical slag), by-products of mining and mineral processing with high levels of metal contaminations, and are generally deposited in the open air. The metal contaminants released from the by-products can be transported through wind or water, may severely pollute the surrounding soil and water environment [1,2], and raise critical threats to ecosystem safety and human health [3,4]. It is estimated that annual discharge of mine tailings in the world exceeds 10 billion tons [5]. In China alone, more than 12,000 tailings ponds exist, which have severely polluted the local land resources with approximately 2000 ha [1,6]. To minimize the deleterious impacts on the surrounding environment, numerous studies have focused on the remediation and ecological restoration of mining polluted sites with various techniques [7,8].

Phytoremediation, a promising eco-friendly technique, has attracted more attention to remediate the metal-polluted areas, which can reduce the environmental risks of contaminant transfer using plant extraction or immobilization of potentially toxic metals [4,9]. For example, plants with limited translocation capacity of metals from root to above-ground tissues are suitable for phytostabilization to reclaim vegetation of historic mining sites [10,11]. It can be considered as a cost-effective and long-term rehabilitation technology for large scales of mining area [1,12]. Generally, vegetation establishment could be beneficial for soil stabilization, carbon sequestration, and soil fertility [1,13]. However, few plant species could survive in severely contaminated mining areas due to the destroyed soil structure and low soil fertility, or the surviving plants usually have lower above-ground biomass, which restricts the recovery of mining-affected areas. Therefore, it is important to select appropriate trees with high tolerance to metal contamination and phytoremediation potential for polluted sites.

Quercus spp., a key woody species, is of relatively high tolerance to abiotic stress, which has been applied economically worldwide, and widely distributes in Asia, North America, Europe, and Africa [14,15]. Several studies have suggested that the *Quercus* species are suitable for afforestation in severely metal-contaminated sites because of their massive/deep root systems, high metal tolerance, and uptake capacities [16–19]. For example, *Quercus variabilis* is reported to have great potential for phytostabilization of antimony (Sb), with roots accumulating 1624 mg·kg^{-1} Sb [20]. In another study, *Quercus virginiana* grew well in lead/zinc tailings and exhibited the highest metal tolerance (TI = 0.97–1.04) among the selected woody species [21]. Considering the great remediation potential of *Quercus* species, it is necessary to evaluate the overall performance of these woody plants in phytomanagement of mining activities-polluted sites.

The polluted areas caused by mining activities are usually influenced by multiple metals concurrently, thereby causing multi-metal pollution cases [22]. However, some works on woody species only focus on their metal uptake or biomass production via hydroponic with single pollutants included [17,23]. The conclusions reached by these studies cannot adequately represent the real soil/filed conditions. The aims of the current study were to comprehensively evaluate the phytoremediation potential of six *Quercus* species with wide adaptation to abiotic stress. Here, six *Quercus* species one-year-old saplings were cultivated in pots with highly metal-polluted soils in copper (Cu) mining sites for 150 days. The characteristics of plant growth, physiological responses, and metal accumulation capacity were recorded in different treatments and integrated in the framework of the analytic hierarchy process method-entropy weight (AHP-EW) model for woody species evaluation [24]. The results would provide useful guidance for phytomanagement of *Quercus* species in severely metal-polluted areas caused by mining activities.

2. Materials and Methods

2.1. Soil Preparation

The polluted soil was collected from a historic Cu mining site located in Bijiashan area in Tongling city, Anhui Province, China (30°45′ N, 117°42′ E). The clean control soils (yellow soil) were collected from Hangzhou city, Zhejiang Province, China (30°06′ N, 119°96′ E). The mixture of clean and polluted soils (1:1) was set as T1 treatment. The clean and polluted soil samples were collected from the upper layer (0–20 cm) and set as treatments of control and T2 (100% polluted soil), respectively. The chemical characteristics of these three treatments were presented in Table 1. Compared with the national soil quality standard (GB15618-2018), the soil Cd content extremely exceeded the risk intervention values (3.0 mg·kg^{-1}), the soil Cu content extremely exceeded the risk screening value (100 mg·kg^{-1}), and the soil Zn content was slightly higher than the risk screening value (250 mg·kg^{-1}), for treatments T1 and T2. Before transplanting the plants, soils were air-dried and sieved through a 2 mm mesh, then mixed and weighed into pots.

Table 1. Characteristics of the control, T1 (mixture of clean and polluted soil, 50%), and T2 (100% polluted soil) treatment soils.

Treatments	pH	OM ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	TP ($g \cdot kg^{-1}$)	Heavy Metal Content ($mg \cdot kg^{-1}$)			Risk Screening Value ($mg \cdot kg^{-1}$)		
					Cu	Cd	Zn	Cu	Cd	Zn
control	6.9 ± 0.1	43.1 ± 2.5	1.7 ± 0.4	0.5 ± 0.05	71 ± 12	0.4 ± 0.0	99 ± 10	100	0.3	250
T1	7.2 ± 0.1	32.0 ± 1.3	1.2 ± 0.2	1.4 ± 0.05	4366 ± 201	3.8 ± 0.3	270 ± 10			
T2	7.4 ± 0.2	23.4 ± 3.9	0.9 ± 0.1	3.0 ± 0.15	9839 ± 212	8.5 ± 0.3	562 ± 14			

Data represents the means ± standard deviation (n = 3). OM, organic matter; TN, total nitrogen; TP, total phosphors; Cu, copper; Cd, cadmium; Zn, zinc.

2.2. Plant Cultivation

The *Quercus* species tested were *Quercus fabri*, *Quercus pagoda*, *Quercus phellos*, *Quercus nuttallii*, *Quercus acutissima*, and *Quercus virginiana*, which were popular in ecological restoration and landscape application in east China, with wide adaptation to abiotic stress and ornamental or timber value. The healthy potted one-year-old *Quercus* saplings were obtained from the local nursery in Hangzhou city. Saplings with similar size (average height of 65.7 cm and average base stem diameter of 5.01 mm, Table S1) were selected and removed from the original pot, their roots were washed clean and they were transplanted in polyvinylchloride (PVC) tubes (height 25.0 cm, diameter 11.0 cm) with 1.5 kg soil. Each PVC tube contained one sapling. Each treatment had six *Quercus* species, with five pots per species, and all the pots were arranged randomly in the greenhouse of the Research Institute of Subtropical Forestry, Chinese Academy of Forestry. The day/night temperature was 23–38/18–23 °C and relative humidity was 60%–65%. During the test, the saplings were irrigated with 300 mL/pot tap water once every two days. The *Quercus* saplings were cultivated for 150 d from April to September and then harvested after height and base diameter were recorded.

2.3. Photosynthetic Parameters

Before harvesting, three mature leaves of each *Quercus* sapling were selected for determination of net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) using a LiCor-6800 portable photosynthesis system (LiCor Inc., Lincoln, NE, USA). The measurements were carried out with a light intensity of 1000 μmol photon $m^{-2}s^{-1}$ from 9:00 a.m. to 14:00 p.m. The air-flow through the sample chamber was set at 500 $\mu mol \cdot s^{-1}$ and the CO_2 concentration in the sample chamber was 400 $\mu mol \cdot mol^{-1}$.

The foliar pigment of chlorophyll a + b (*Chl* a + b), chlorophyll a (*Chl* a), chlorophyll b (*Chl* b), and carotenoids (*Car*) were determined using a spectrophotometer (UV-1800, MAPADA, Shanghai, China) after being extracted with a mixed solution of ethanol and acetone (*v:v*, 2:1).

2.4. Element Determination

All harvested saplings were washed with tap water to remove the soil particles adhered to the roots and then rinsed three times with deionized water. Each sapling was divided into root, stem, and leaf, and dried in an oven for 1 h at 105 °C and 72 h at 75 °C. Then the dried samples were grounded into a fine powder using a stainless steel mall mill (Retsch MM400, Germany). The subsample (0.25 g) was digested with a mixture of concentrated nitric acid and hydrogen peroxide (4 mL, 1 mL) in a hot block system (ED36, Lab Tech, USA) [25]. The digestion was diluted to 50 mL and Cd, Cu, and Zn were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES, Perkin Elmer Optima 8000, Waltham, MA, USA). The reference material (poplar leaves, GBW 07603, National Research Center for Certified Reference Materials, Beijing, China) was applied to control the quality of plant analysis [25]. Good agreement was obtained between our method and certified values (Table S2).

Air-dried soils (0.50 g) were sieved (sieve < 0.15 mm) in preparation for soil metal measurement. The soils were digested with a mixture of 3 mL HNO_3 and 1 mL hydrochloric acid (HCl) to determine the total metal content. The available metal content of soils in each treatment (5.00 g) were extracted by a mixture of 25 mL 0.005 M diethylenetriaminepentaacetic acid (DTPA), 0.01 M $CaCl_2$, and 0.1 M triethanolamine (TEA) at room temperature (24 °C). All filtrates were analyzed for metal contents using ICP-AES.

2.5. Analytic Hierarchy Process and Entropy Weighted Method

The indicators across six *Quercus* species were divided into plant growth, photosynthesis, and metal accumulation. The judgment matrix was set up and the importance weights were provided guided by the expert decision-making method and described in Cao et al. [24] (Table S3). The data of each pattern was normalized before calculating with AHP-EW model (Figure S1).

Evaluation normalization with modification according to Cao et al. [24]:

$$y_{ij} = \frac{x_{ij} - x_{j\ min}}{x_{j\ max} - x_{j\ min}} \quad (1)$$

where y_{ij} is the jth index value of the ith unit treated without dimension standardization. x_{ij} is the value of the jth parameter for the ith *Quercus* species, $i = 1, 2, \ldots n$, $j = 1, 2, \ldots J$. $x_{j\ max}$ and $x_{j\ min}$ are the maximum and minimum values of the given index, respectively.

Characteristic proportion (Y_{ij}) calculation is as follows:

$$Y_{ij} = \frac{y_{ij}}{\sum\limits_{i=1}^{m} y_{ij}} \quad (2)$$

which satisfies $0 \leq Y_{ij} \leq 1$.

Entropy value (e_j) is determined as follows:

$$e_j = -\frac{1}{\ln m} \sum_{i=1}^{m} Y_{ij} \cdot \ln Y_{ij} \quad (3)$$

where if $Y_{ij} = 0$ or $Y_{ij} = 1$, $Y_{ij} \ln(Y_{ij}) = 0$.

Entropy weight (W_d) calculation is as follows:

$$W_j = \frac{1 - e_j}{\sum\limits_{j=1}^{n} (1 - e_j)} \quad (4)$$

The priority weight (λ_i) of remediation indices of *Quercus* species is as follows:

$$\lambda_i = \sum_{j=1}^{J} \left(W_j \cdot x_{ij} / \sum_{i=1}^{n} x_{ij} \right) \quad (5)$$

Finally, a comprehensive weight combining the AHP and EW methods were obtained.

$$\mathrm{F} = \sum (\lambda_i \times \sum W_j y_{ij}) \quad (6)$$

2.6. Calculation and Statistical Analysis

To evaluate the tolerance of the six *Quercus* species to metal pollution, tolerance index (TI) based on biomass (dry weight) was calculated as follows according to Metwally et al. [26]:

$$TI = \frac{B_{treatment}}{B_{control}} \quad (7)$$

where $B_{treatment}$ (g per sapling) is the plant biomass in T1 and T2 treatments, and $B_{control}$ (g per sapling) is plant biomass in control. Higher values indicate *Quercus* saplings with a stronger tolerant capacity.

The bioconcentration factor (BCF) can evaluate plant capacity in metal accumulation from soil, which was determined by the ratio of metal concentration in plant tissues to that in the soil [27]. Here, BCF at the end of the experiment was determined as:

$$BCF = \frac{C_{harvested\ tissues}}{C_{soil}} \quad (8)$$

where $C_{harvested\ tissues}$ is the total metal contents in harvested tissues (root, stem, or leaf) and C_{soil} is the soil metal concentration.

The translocation factor (TF) can indicate the plant capacity for translocating metal from roots to above-ground tissues, determined as the ratio of metal contents in shoots to roots [28].

$$TF = \frac{C_{above\text{-}ground\ tissues}}{C_{roots}} \quad (9)$$

where $C_{above\text{-}ground\ tissues}$ and C_{roots} are total metal contents in above-ground tissues (leaf and stem) and roots, respectively.

The homogeneity of variance and normality were tested for all data. A two-way analysis of variance (ANOVA) for treatments and species were performed, followed by Tukey's post-hoc test ($p < 0.05$) using the DPS 13.01 (Zhejiang University, Hangzhou, China).

3. Results

3.1. Plant Growth and Biomass Production

After 150 days exposure, all *Quercus* species has a survival of 100% and performed without any visible symptoms caused by metal stress. Meanwhile, the tested *Quercus* species varied greatly in growth performance in the T2 treatment (Tables 2 and S4). Significant differences in plant height or stem diameter were observed among the six *Quercus* species (Table S4) despite the similar initial size before treatment, while no apparent difference in these two parameters were found between T1/T2 treatments and control ($p > 0.05$). The total biomass of *Quercus* species (except for *Q. nuttallii*) in T1 treatment were markedly decreased by 5.1%–44.0% compared with control treatment (Table 2), while the above-ground biomass of *Q. nuttallii* dramatically increased by 29.4% and 13.2%, respectively, in T1 and T2 treatments compared with control treatment ($p < 0.05$). Interestingly, although the biomass (root or above-ground tissues) of *Q. phellos* was markedly reduced by T1 and T2 treatments, this species still displayed the highest biomass production among the tested *Quercus* species (except for root biomass in T1 treatment).

Tolerance index was calculated according to the biomass of *Quercus* species grown in T1 and T2 treatments compared to that in control, which differed markedly among the six species ($p < 0.05$). Three *Quercus* species (*Q. pagoda*, *Q. acutissima*, and *Q. nuttallii*) exhibited relatively higher TIs (1.08–1.21). Among them, *Q. nuttallii* showed the greatest TI values with 1.29 and 1.21 in T1 and T2 treatments, respectively.

Table 2. Biomass (g, DW) and tolerance index (TI) of six *Quercus* species cultivated in control, T1 (mixture of clean and polluted soil, 50%) and T2 (100% polluted soil) treatments for 150 d.

Species	Treatments	Above-Ground Biomass	Root Biomass	Total Biomass	TI
	Control	28.3 ± 1.7	28.3 ± 1.6	56.6 ± 0.2	–
Q. fabri	T1	14.2 ± 2.7	17.4 ± 1.8	31.7 ± 1.2	0.56 ± 0.02
	T2	14.4 ± 1.0	14.9 ± 1.4	29.3 ± 0.6	0.52 ± 0.01
	Control	18.9 ± 1.8	14.8 ± 2.6	33.7 ± 3.0	–
Q. pagoda	T1	12.6 ± 1.7	8.7 ± 2.9	21.3 ± 4.7	0.64 ± 0.16
	T2	18.0 ± 1.7	18.2 ± 4.1	36.2 ± 5.8	1.09 ± 0.27
	Control	52.3 ± 2.7	41.8 ± 1.2	95.6 ± 1.7	–
Q. phellos	T1	38.3 ± 7.3	20.4 ± 2.1	58.7 ± 5.3	0.61 ± 0.07
	T2	37.4 ± 5.6	31.3 ± 0.9	68.7 ± 6.3	0.72 ± 0.05
	Control	23.5 ± 4.2	12.1 ± 3.5	35.6 ± 6.9	–
Q. nuttallii	T1	30.4 ± 7.0	13.9 ± 1.7	44.3 ± 8.4	1.29 ± 0.42
	T2	26.6 ± 3.3	15.6 ± 1.7	42.2 ± 5.0	1.21 ± 0.23
	Control	26.1 ± 5.3	28.5 ± 6.2	54.5 ± 0.9	–
Q. acutissima	T1	23.4 ± 5.2	28.4 ± 1.1	51.8 ± 4.2	0.95 ± 0.09
	T2	28.5 ± 7.6	30.2 ± 5.1	58.8 ± 2.7	1.08 ± 0.04
	Control	27.3 ± 1.8	19.9 ± 1.8	47.4 ± 3.1	–
Q. virginiana	T1	25.7 ± 4.0	16.0 ± 2.8	41.7 ± 4.5	0.88 ± 0.04
	T2	24.5 ± 1.0	21.7 ± 4.9	46.2 ± 4.8	0.98 ± 0.11
	T	**	****	****	ns
Significances	S	****	****	****	*
	T×S	**	****	****	ns

Data represent the mean of three replicates ± SD (n = 3). *p* values of the two-way ANOVAs of soil treatments (T), oak species (S), and their interactions (T × S) were also shown. ns, not significant; *, $p \leq 0.05$; **, $p \leq 0.01$; ****, $p \leq 0.0001$.

3.2. Photosynthesis and Foliar Pigments

The P_n and T_r of all *Quercus* species declined markedly by 9.3%–55.0% and 9.1%–65.7% in T2 treatment, respectively, while those of *Q. fabri* and *Q. virginiana* in T1 treatment were significantly elevated compared to their respective control treatment (Figure 1). Similar results were also observed in g_s and C_i under T2 treatment. Contrarily, significant enhancements in T_r and g_s were observed in *Q. fabri*, *Q. nuttallii*, and *Q. virginiana* under T1 treatment compared to control treatment.

Q. fabri had the highest content (4.46 and 4.13 mg·g^{-1}) of *Chl* a + b in T1 and T2 treatments, respectively (Table 3). In T2 treatment, the content of photosynthetic pigments in *Q. pagoda* and *Q. phellos* markedly decreased by 20.0%–23.0% and 28.8%–33.3%, respectively, compared to control treatment. Although *Q. phellos* exhibited a decreased trend in foliar pigments (including *Chl* a, *Chl* b, *Chl* a + b, *Car*) in T1 and T2 treatments compared with control treatment, it still possessed a relative higher amount of these foliar pigments. Additionally, the contents of *Chl* a, *Chl* b, and *Chl* a + b in the leaves of four species (*Q. fabri*, *Q. nuttallii*, *Q. acutissima*, *Q. virginiana*) were all increased by T1 and T2 treatments, and higher level of multi-metals inhibited the increase in chlorophyll components. Leaf carotenoids was increased by 4.3%–37.0% in *Q. fabri*, *Q. nuttallii*, *Q. acutissima*, and *Q. virginiana*.

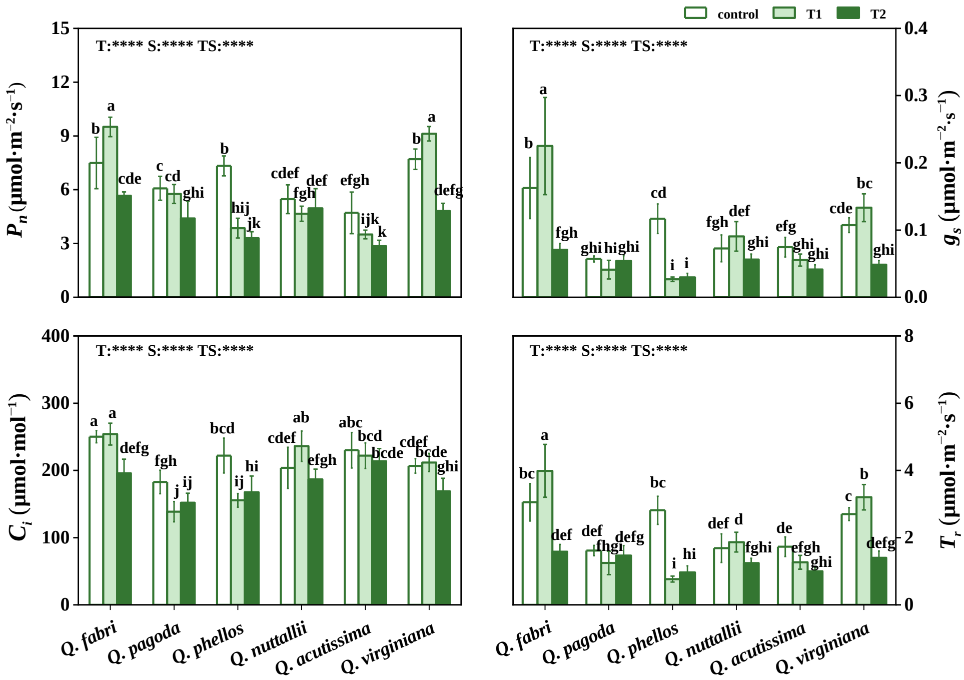

Figure 1. Photosynthetic rate (P_n, μmol.m^{-2}s^{-1}), stomatal conductance (*gs*, mol H_2O m^{-2}s^{-1}), intercellular CO_2 concentration (*Ci*, μmol CO_2 mol air^{-1}), and transpiration rate (*Tr*, mmol H_2O m^{-2}s^{-1}) in fresh leaves of six *Quercus* species cultivated in control, T1 (mixture of clean and polluted soil, 50%), and T2 (100% polluted soil) treatments that lasted for 150 d. Data represent the mean of three replicates ± SD (n = 3). *p* values of the two-way ANOVAs of soil treatments (T), *Quercus* species (S), and their interactions (T × S) were also shown. ns, not significant; **** $p \leq 0.0001$. The different letters showed the significance at level of 0.05.

Table 3. Photosynthetic pigments (mg·g^{-1} fresh weight) in leaves of six *Quercus* species cultivated in control, T1 (mixture of clean and polluted soil, 50%) and T2 (100% polluted soil) treatments for 150 d.

Quercus Species	Treatments	*Chl* a	*Chl* b	*Chl* (a + b)	*Car*
Q. fabri	Control	2.68 ± 0.15	0.87 ± 0.05	3.55 ± 0.20	0.49 ± 0.03
	T1	3.41 ± 0.15	1.05 ± 0.04	4.46 ± 0.19	0.57 ± 0.02
	T2	3.14 ± 0.27	0.99 ± 0.09	4.13 ± 0.36	0.53 ± 0.04
Q. pagoda	Control	2.24 ± 0.11	0.74 ± 0.05	2.98 ± 0.16	0.40 ± 0.01
	T1	2.26 ± 0.03	0.72 ± 0.01	2.98 ± 0.04	0.42 ± 0.00
	T2	1.74 ± 0.09	0.57 ± 0.01	2.30 ± 0.08	0.32 ± 0.03
Q. phellos	Control	2.83 ± 0.22	0.87 ± 0.09	3.69 ± 0.30	0.52 ± 0.03
	T1	2.54 ± 0.17	0.80 ± 0.06	3.34 ± 0.22	0.49 ± 0.03
	T2	1.95 ± 0.05	0.58 ± 0.04	2.54 ± 0.09	0.37 ± 0.01
Q. nuttallii	Control	1.34 ± 0.10	0.42 ± 0.04	1.76 ± 0.13	0.26 ± 0.02
	T1	1.79 ± 0.17	0.61 ± 0.06	2.40 ± 0.22	0.35 ± 0.03
	T2	1.61 ± 0.07	0.52 ± 0.03	2.12 ± 0.10	0.31 ± 0.02
Q. acutissima	Control	1.32 ± 0.03	0.41 ± 0.03	1.73 ± 0.05	0.27 ± 0.01
	T1	1.97 ± 0.09	0.61 ± 0.03	2.58 ± 0.12	0.37 ± 0.02
	T2	1.64 ± 0.13	0.53 ± 0.01	2.17 ± 0.13	0.33 ± 0.03
Q. virginiana	Control	2.38 ± 0.14	0.81 ± 0.05	3.19 ± 0.19	0.47 ± 0.03
	T1	2.74 ± 0.12	0.87 ± 0.04	3.61 ± 0.16	0.50 ± 0.02
	T2	2.59 ± 0.13	0.84 ± 0.07	3.43 ± 0.19	0.49 ± 0.01
Significances	T	****	****	****	****
	S	****	****	****	****
	T×S	****	****	****	****

Chl a, chlorophyll a; *Chl* b, chlorophyll b; *Chl* (a + b), chlorophyll a and b; *Car*, carotenoid. Data represent the mean of three replicates ± SD (n = 3). *p* values of the two-way ANOVAs of soil treatments (T), oak species (S), and their interactions (T × S) were also shown. ns, not significant; ****, $p \leq 0.0001$.

3.3. Accumulation of Nutrient Elements in Plants

Generally, leaves accumulated higher concentrations of macro-elements (Ca, Mg, and K) than those in roots in all the treatments (Figure 2). Increases in leaf Ca content were evident in *Q. fabri*, *Q. phellos*, and *Q. nuttallii*, which increased by 14.9%–46.2% in T1 and T2 treatments compared with control treatment. The root Ca contents in most *Quercus* species were also increased (3.6%–71.4%) in mine contaminated soils (T1 and T2) compared to control soil (Figure 2). It was noteworthy that Fe contents in the root of *Quercus* species (except for *Q. nuttallii*) dramatically increased by approximately 78%–252% in T1 treatments compared with control.

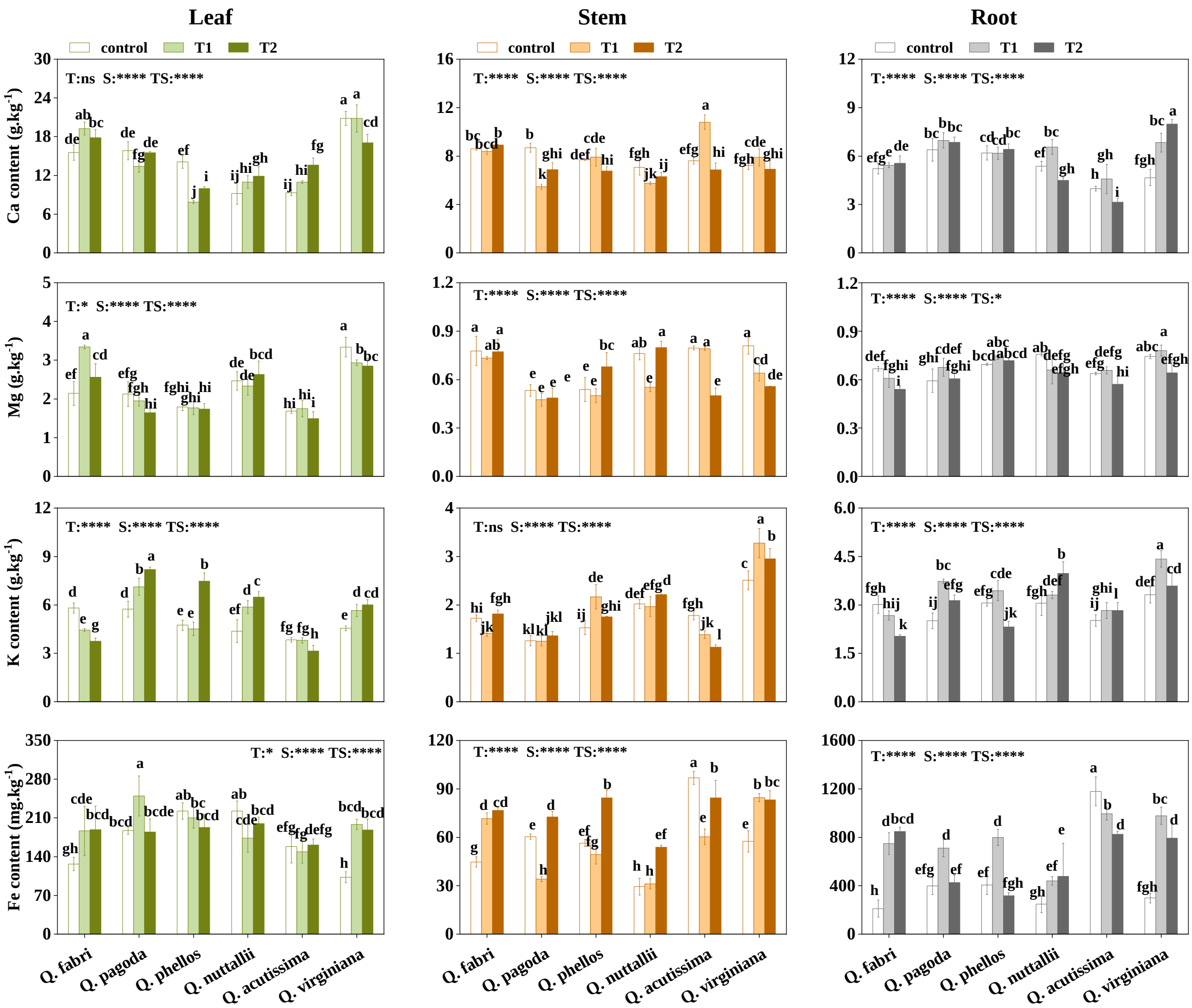

Figure 2. Nutrient elements (Ca, Mg, K, and Fe) of six *Quercus* species cultivated in control, T1 (mixture of clean and polluted soil, 50%), and T2 (100% polluted soil) treatments for 150 d. Data represent the mean of three replicates ± SD (n = 3). p values of the two-way ANOVAs of soil treatments (T), *Quercus* species (S), and their interactions (T × S) were also shown. ns, not significant; *, $p \leq 0.05$; **** $p \leq 0.0001$. The different letters showed the significance at level of 0.05.

3.4. Bioconcentration and Translocation of HMs in Plants

The tested *Quercus* species showed variations in Cu, Cd, and Zn accumulation in root, stem and leaf (Figure 3). Copper and Cd contents in roots were also much higher than that of leaves and stems of all selected *Quercus* species (Figure 3). The total Cu contents in *Quercus* species ranged from 4.1 to 593.9 mg·kg^{-1}, and the highest Cu content was recorded in *Q. acutissima* root (Figure 3). The stem Cd and leaf Cd were markedly higher in T2 treatment than T1 treatment, except for *Q. fabri* and *Q. acutissima*. In T1 and T2 treatments, the Zn contents exhibited wide ranges among different *Quercus* species, which accumulated 26.2–96.1, 10.2–60.4, and 7.9–25.6 mg·kg^{-1} in leaves, stems, and roots, respectively (Figure 3).

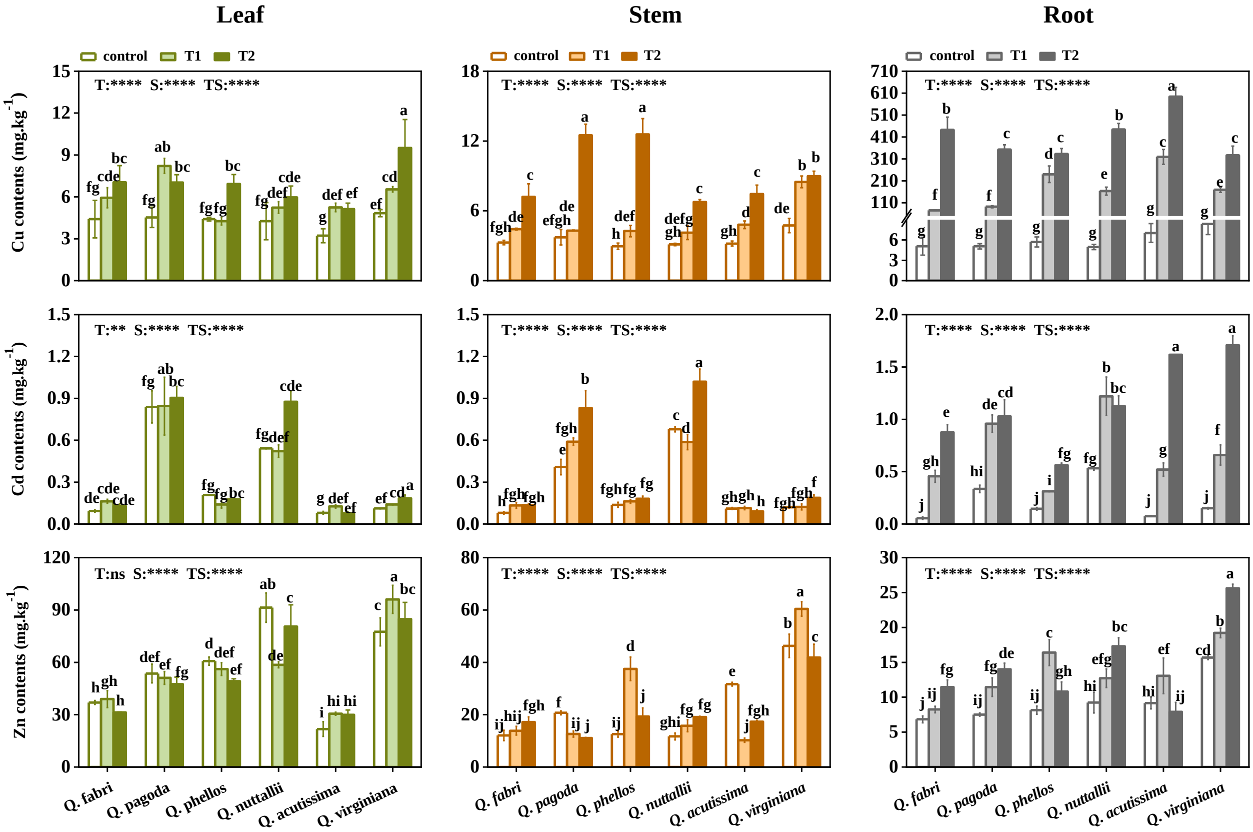

Figure 3. Metal accumulation of six *Quercus* species cultivated in control, T1 (mixture of clean and polluted soil, 50%), and T2 (100% polluted soil) treatments for 150 d. Data represent the mean of three replicates ± SD (*n* = 3). p values of the two-way ANOVAs of soil treatments (T), Quercus species (S), and their interactions (T × S) were also shown. ns, not significant; **, $p \leq 0.05$; ****, $p \leq 0.0001$. The different letters showed the significance at level of 0.05.

Meanwhile, the TF and BCF values of Cu in the tested *Quercus* species were far lower than 1 in T1 and T2 treatments (Figure 4). For Cd, the roots of *Quercus* species had relatively higher bioaccumulation capacity with larger BCF values than above-ground tissues. Contrary to Cu (TF < 0.1), the six *Quercus* species exhibited higher capacity for Zn phytoextraction with TF > 1 and markedly higher BCF values of above-ground tissues.

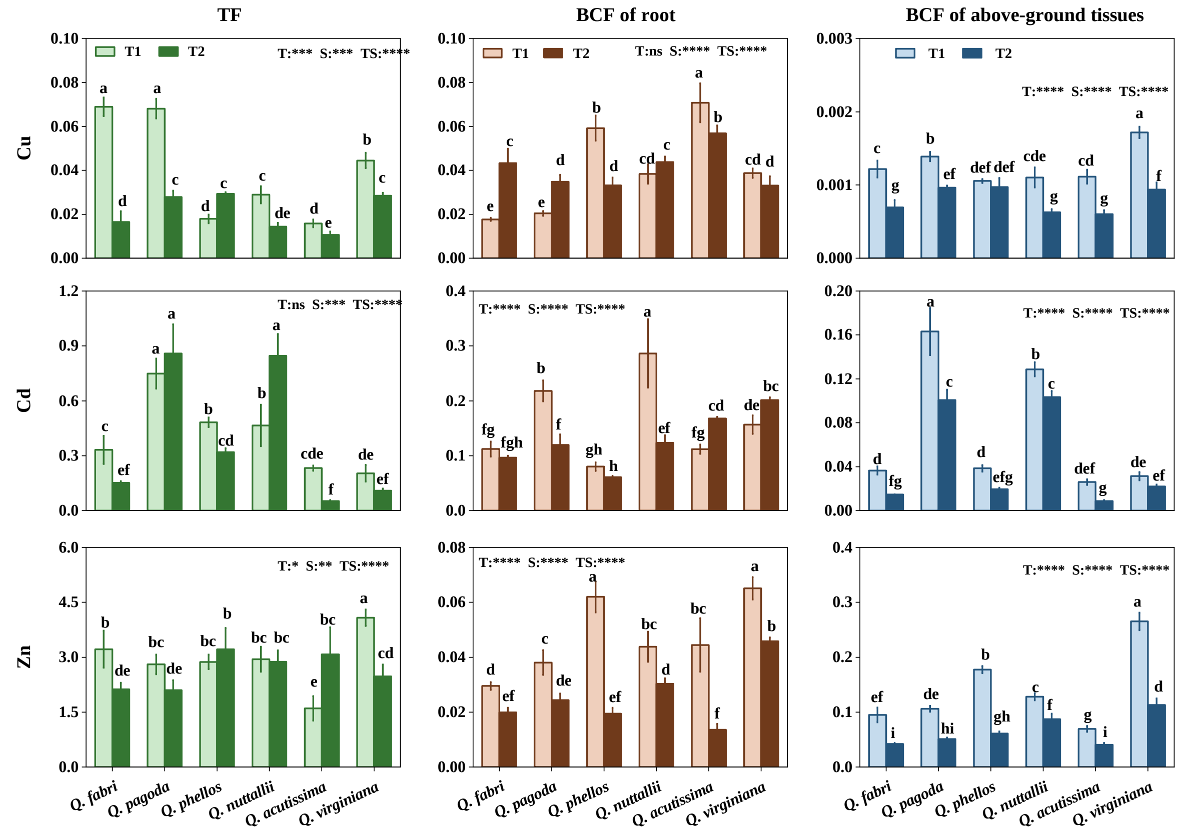

Figure 4. Translocation factor (TF) and bioaccumulation factor (BCF) of six *Quercus* species cultivated in control, T1 (mixture of clean and polluted soil, 50%), and T2 (100% polluted soil) treatments for 150 d. Data represent the mean of three replicates ± SD (n = 3). p values of the two-way ANOVAs of soil treatments (T), *Quercus* species (S), and their interactions (T × S) were also shown. ns, not significant; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$. The different letters showed the significance at level of 0.05.

4. Discussion

4.1. Quercus spp. Tolerance to HMs

Exposure to high metal levels could inhibit plant growth and induce a decrease in biomass production [29,30]. A decrease in total biomass of tested *Quercus* species (except for *Q. nuttallii*) in the T1 treatment was observed (Table 2). The reduction in biomass might result from the direct or indirect inhibition of plant growth caused by metal toxicity or alterations of physiological processes [30]. It is suggested that heavy metal stress can cause photosynthetic inhibition, water relationship disturbance, and decreased nutrient transfer ability of plants [31–34]. Decreases in *Pn* and *Gs* of the *Quercus* species tested in the T2 treatment and partly in the T1 treatment were observed, which is in line with the change in total biomass. Photosynthesis is the most important process of CO_2 assimilation in plants to maintain growth and biomass production [35,36]. Stomata are important channels for gas exchange between plants and the outside world, affecting photosynthesis and transpiration [37]. Generally, the inhibition of photosynthesis results from the stomatal closure following a decrease in CO_2 availability [38,39]. It was proved that heavy metal stress can reduce stomatal conductance, which affects transpiration and photosynthesis [40]. In addition, it will also further affect the rate of nutrient transfer from the underground part of the plant to the above-ground part [41].

The increased root biomass of *Quercus* species (except *Q. fabri*) in T1 and T2 treatments may be partly related to the fact that the root system can better absorb nutrients by changing its own growth configuration in a stressful environment [34].The dramatic increase of above-ground biomass in *Q. nuttallii* grown in T1 and T2 treatments suggests it has high tolerance to heavy metals and great phytoremediation potential according to the criterion of high biomass for plant screening [42]. The highest biomass production of *Q. phellos* among the *Quercus* species tested in T1 and T2 treatments also suggests its high phytoremediation potential.

The TI values are often used to evaluate the tolerance capacity of plants in response to metal stress [43]. A woody plant with a TI value above 0.6 is considered a tolerant plant according to the criterion proposed by Lux et al. [44]. In the current study, only *Q. fabri* had a TI below 0.6, while the other five species all had a TI greater than 0.6, which confirmed their high tolerance and reflects their great potential in phytoremediation for multiple high metals (Cu, Cd, and Zn). Moreover, the TI values of the *Quercus* species selected were generally higher in the T2 treatment than in the T1 treatment, indicating that they were less sensitive to heavily metal contamination. *Q. nuttallii* showed the highest tolerance to Cu, Cd, and Zn co-contamination among the *Quercus* species tested with the largest TIs of 1.29 and 1.21 in T1 and T2 treatments, respectively, which is also confirmed by a three-year field trial [19].

4.2. Elements Accumulation and Distribution in Plants

Excessive metal contents in soils, particularly in mine-contaminated sites, could cause phytotoxicity and further alter the uptake and utilization of the mineral elements (e.g., Fe, Ca, and Mg) [24].The higher accumulation of macro-elements (Ca, Mg, and K) in leaves than in roots (Figure 2) could partly be explained by the high mobility of these nutrient elements in plant tissues [45].On the other hand, the elements related to photosynthesis, encompassing Mg and K, were highly demanded to be translocated, and accumulated in leaves to maintain normal growth under the stressful environment [46]. The dramatic increase of root Fe was ascribed to tight binding within root cells, contributing to greater accumulation in the below-ground tissues [45]. The elements required for high concentrations are considered less sensitive and less variable to environmental variations during plant growth and development [47]. Additionally, elemental composition for homoeostatic regulation is a vital physiological mechanism to maintain the normal growth of plants suffering from environmental stresses [48].

The ideal plant species for phytoremediation purposes should be capable of accumulating high levels of metals and producing a large biomass [49,50]. However, high contents of potentially toxic metals may cause phytotoxicity, e.g., 30–300 $mg \cdot kg^{-1}$ Cu in dry weight in plants could cause phytotoxicity [51]. A large amount of Cu accumulated in the roots and exceeded the phytotoxicity value (Figure 3), which implied that roots could act as a barrier for metal translocation to above-ground tissues and contribute to the metal tolerance of these woody plants.

Contrary to Cu and Cd (TF < 1), the six *Quercus* species exhibited higher capacity for Zn phytoextraction with TF > 1 and markedly higher BCF values of above-ground tissues. The current study indicated that Cu and Zn exhibited divergent mobility within the *Quercus* species, although Cu and Zn are both essential elements for plants. Here, we observed that the ratio of Zn:Cu in leaves was approximately 5.1–14.7 in T1 and T2 treatments, implying that *Quercus* species might preferentially uptake more Zn than Cu. Jeyakumar et al. [52] found that *Populus deltoides* × *yunnanensis* accumulated remarkably more Zn in leaves than Cu, and the highest BCF of Zn was 11.5 times higher than that of Cu. These results could clearly explain the significant difference in Zn amounts based on plant uptake compared to Cu accumulation.

4.3. Phytoremediation Potential of Quercus Species

Phytoremediation potential is closely linked to metal accumulation and plant biomass [53]. It is generally classified as phytostabilization and phytoextraction, and the vital criterion is the allocation of metal levels between below-ground parts and above-ground parts of the plant [42,49]. Specifically, phytostabilization of woody plants is more suitable and usually considered first in practice for phytoremediation of severely metal-contaminated sites/mine tailings, which aims to decrease the ecological risk and utilization safety of land [24].The current findings confirmed that the six *Quercus* species are good candidates for Cu and Cd phytostabilization and could decrease the potential risks of metal mobilization in the plant-soil systems, while showing potential for Zn phytoextraction, which could effectively transport Zn to above-ground tissues.

In addition to metal accumulation, plant physiological or phenotypic patterns (e.g., plant biomass, photosynthetic rate, chlorophyll) of different plant species are also associated with metal tolerance and biomass production [25]. Therefore, the AHP-EW model with little modification was used in this study to deal with the subjective and objective patterns simultaneously as described in our earlier study [24]. Here, the obtained consistency ration of judgment matrix was lower than 0.1, indicating that the consistency ration of each judgment matrix and all the judgments are reasonable [54]. The weighed order of importance was displayed in the order of accumulation capacity > plant growth > photosynthesis. The weights of the objective data were calculated with the EW method and subsequently the AHP and EW methods were combined to obtain a comprehensive weight (Table 4). The results implied that *Q. virginiana*, *Q. nuttallii*, and *Q. phellos* in T1 treatments, and *Q. virginiana*, *Q. acutissima*, and *Q. phello* in T2 treatments show the best performance in phytoremediation potentials for multi-metal contamination. The results are also consistent with the observation that they have significantly higher biomass production or metal accumulation capacity than other *Quercus* species. *Q. virginiana* was also suggested to be highly tolerant to heavy metals and maintained normal growth in Pb/Zn tailings [21]. High biomass was observed in *Q. acutissima* with 51.8 g and 58.8 g (dry weight) in T1 and T2 treatments, respectively. Moreover, *Q. virginiana* exhibited the highest BCF values of Zn and Cu, and *Q. acutissima* accumulated the highest Cd and Zn in above-ground tissues. Thus, we could conclude that these *Quercus* species have a strong capacity for revegetation and remediation of Cu, Cd, and Zn co-contamination.

Table 4. Importance weights of different criteria in T1 (mixture of clean and polluted soil, 50%) and T2 (100% polluted soil) treatments.

Quercus Species	Plant Growth		Photosynthesis		Metal Accumulation		Importance Weight	
	T1	T2	T1	T2	T1	T2	T1	T2
Q. fabri	0.268	0.037	1.00	1.00	0.049	0.138	0.383	0.355
Q. pagoda	0.000	0.144	0.27	0.40	0.293	0.233	0.204	0.256
Q. phellos	0.870	0.913	0.16	0.14	0.342	0.228	0.441	0.398
Q. nuttallii	0.715	0.430	0.18	0.30	0.447	0.390	0.449	0.375
Q. acutissima	0.629	0.705	0.08	0.03	0.305	0.482	0.333	0.416
Q. virginiana	0.611	0.507	0.77	0.68	0.582	0.555	0.645	0.576

5. Conclusions

All tested *Quercus* species survived without evident toxicity symptoms under Cu tailing contaminated treatments. The TIs of these *Quercus* species were generally greater than 0.6, implying that the *Quercus* possess high tolerance capacity for Cu, Cd, and Zn pollution in Cu tailing-contaminated soils. The contents of Cu and Cd were generally higher in roots than in leaves and stems of all *Quercus* species selected, whereas Zn preferentially accumulated more in above-ground tissues. The comprehensive evaluation of the AHP-EW model suggested that *Q. virginiana* and *Q. acutissima* are two key woody species for Cu, Cd, and Zn co-polluted sites.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14010062/s1, Table S1: Plant height (cm), base stem diameter (mm) of six Quercus species planted in control, T1, and T2 before cultivation. Table S2: The recovery data for elemental analysis using inductively coupled plasma atomic emission spectrometry (ICP-AES). Table S3: AHP method for three criteria regardless of T1 and T2 treatments. Table S4: Plant height (cm), stem diameter (mm), leaf biomass, and stem biomass (g, DW) of six Quercus species cultivated in control, T1, and T2 for 150 d. Figure S1: The framework of AHP-EW method for calculating importance weights of phytoremediation potential. Figure S2: Effects of different heavy metal-contaminated soils on malonaldehyde (MDA) content in leaves of six Quercus species.

Author Contributions: Conceptualization, G.C. and J.C.; methodology, Y.C. and L.Y.; formal analysis, Y.C. and L.Y.; investigation, L.Y.; resources, G.C., L.S. and P.Z.; data curation, L.Y.; writing—original draft preparation, Y.C.; writing—review and editing, N.D. and G.C.; supervision, G.C. and J.C.; funding acquisition, G.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Fundament Research Funds of Chinese Academy of Forestry (CAFYBB2019SZ001), and Forestry science and technology innovation project of Anhui Province (AHLYCX-2021-11).

Conflicts of Interest: The authors declare no conflict of interest.

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