forests

Article

Remediation of Pb and Cd Polluted Soils with Fulvic Acid

Aslihan Esringü [1,*], Metin Turan [2] and Asli Cangönül [3]

[1] Department of Landscape Architecture, Faculty of Architecture and Design, Atatürk University, 25100 Erzurum, Turkey
[2] Department of Genetics and Bioengineering, Yeditepe University, 34755 Istanbul, Turkey; m_turan25@hotmail.com or metin.turan@yeditepe.edu.tr
[3] Humintech Gmbh, 41517 Grevenborch, Germany; a.cangoenuel@humintech.com
* Correspondence: esringua@atauni.edu.tr; Tel.: +90-5438364836

Abstract: Heavy metal pollution is among the important environmental problems in the world. Many techniques have already been used to remove the heavy metals such as lead (Pb) and cadmium (Cd). Among them, the phytoremediation method is an environmentally friendly and green technology. This study was carried out to determine the efficiency of fulvic acid (FA) application in removing Pb and Cd from polluted soil using *Tagetes eracta* L. and *Zinnia elegans* Jacq. ornamental plants. The results indicated that, FA application, number of flower per plants, and plant fresh weight of *Tagetes eracta* plants and *Zinnia elegans* plants increased 187.5%, 104.5% and 155.5%, 57.7%, respectively with application of 7000 mg L^{-1} FA at 100 mg kg^{-1} Pb pollution condition, whereas 42.85%, 16.5%, and 44.4–36.1% with application of 7000 mg L^{-1} FA at 30 mg $kg^{\pm 1}$ Cd pollution condition, respectively. With the FA application in the *Zinnia elegans* plant, the root part has accumulated 51.53% more Pb than the shoot part. For Cd, the shoot part accumulated 35.33% more Cd than the root. The effect of FA application on superoxide dismutase (SOD), peroxidase (POD) and, catalase (CAT) of the *Tagetes eracta* were decreased as 32.7%, 33.1%, and 35.1% for Pb, 21.2%, 25.1%, and 26,1%, for Cd, and 15.1%, 22.7%, and 37.7% for Pb, and 7.55%, 18.0%, and 18.8% for Cd were in *Zinnia elegans* respectively. In conclusion, *Tagetes eracta* and *Zinnia elegans* can not be recommended for remediation of Pb and Cd polluted area, but FA can be recommended for Pb and Cd stabilization in polluted soil.

Keywords: *Zinnia elegans*; *Tagetes erecta*; lead; cadmium; phytoremediation; remediation parameters

Citation: Esringü, A.; Turan, M.; Cangönül, A. Remediation of Pb and Cd Polluted Soils with Fulvic Acid. *Forests* **2021**, *12*, 1608. https://doi.org/10.3390/f12111608

Academic Editors: Tatiana Minkina, Svetlana Sushkova and Vishnu Rajput

Received: 17 September 2021
Accepted: 12 November 2021
Published: 22 November 2021

1. Introduction

Heavy metals (HM) released to the environment as a result of the activities of industrial enterprises such as mining activities, paint, artificial fertilizer production, urban or industrial waste are important factors in the deterioration of the natural balance and the destruction of ecology. Mineral processing and mining activities in particular cause soil pollution by producing large amounts of metal-rich waste materials [1]. This heavy metal stress (HMS) caused by environmental pollutants. It limits the growth of plants and decreases the yield and quality of the product [2]. The ever-increasing concentrations of HM in the environment disrupt the ecosystem balance and increase economic loss with deterioration in human health [3]. HM, especially Cadmium (Cd) and Lead (Pb), have emerged as serious toxic environmental pollutants in recent years due to the excessive release of HM such as industrial manufacturing and agricultural input production and use [4]. High concentrations of Cd and Pb adversely affect plant growth by disrupting natural ecosystems, polluting the soil, and reducing fertility and plant nutrient uptake [5]. In addition, HMS in high concentrations in the soil can adversely affect the morphology, anatomy and physiology of the plants and cause phytotoxic, effects, leading to a decrease in the yield of the plants and their death at excessive concentrations [6,7].

HM in high concentrations in the plant growing environment affect plant production negatively by affecting the physiological processes of plants, causing imbalances in nutrient uptake, showing toxic effects and damaging biodiversity [8,9]. Pb or Cd stress decreases the intake of P, K, Ca, Cu, Fe, Mn and Zn elements in the plant tissue and ultimately causes plant death by reducing the growth of the plant [10].

Heavy metal pollution, which has become a global problem, threatens plants, wildlife and human populations [11]. Remediation of soils contaminated with cadmium and lead includes expensive technologies such as excavation and removal of soils to safe storage areas [12].

Phytoremediation is known as a sustainable green technology used for remediation of contaminated areas due to biotic and abiotic factors [13]. The success of the phytoremediation technique depends on the factors affecting the usefulness and mobility of metals in soil and water [14]. Phytoremediation is a plant-based technology that uses hyperaccumulator plants to eliminate or reduce toxic metal content in the soil and water environment. Depending on the presence of HM in the growing medium, they cause oxidative stress in plants through metabolic changes such as reduction of proline and glutathione, binding to the sulfhydryl protein group and inhibiting the activity of antioxidant enzymes by causing changes in the physiology of plants [15]. When the metal concentration in the agricultural production areas exceeds the tolerance limits of the cultivated plants, it accumulates in the plant tissues and harms the plant growth and cell metabolic activity. It also negatively affects plant growth by causing overproduction of reactive oxygen species (ROS), which reduces plant biomass and even seed germination [10]. The high percentage of HM in the growth medium reduces the chlorophyll content by affecting various physiological and biochemical processes of the plants [16].

Achieving the desired goal in the improvement of the contaminated areas with the herbal extraction method actually varies according to the concentration of the metals in the environment and their presence together or separately. Because the mobility of HM in the soil is very low, the mobility of the HM in question is increased and their uptake by plants is encouraged. However, it has been stated today that many inorganic and organic agents increase this intake, one of them being fulvic acid, and it has been suggested that the use of FAs will increase it [17]. Today, the plant growth potential of non-hyperaccumulative plants can be enhanced with chelators such as metal ions, biochar and microorganisms. The phyto-extraction ability of a plant can be enhanced by the addition of various chelators and is known as induced phyto-extraction [18]. Chelators aid metal availability for plants by controlling the aggregation of metals [19].

Plants used for phytoremediation should have high biomass, rapid growth, common fibrous root systems, and should be easy to grow, easy to harvest, high tolerance to toxic metals and easy to manipulate [16].

Lead (Pb) is not an essential nutrient for the growth of plants. On the contrary, it is a heavy metal and even low concentrations of 30–300 $\mu g\ g^{-1}$ are toxic to plants [20,21]. Combustion of leaded gasoline and pesticides are shown as man-made resources of lead toxic metal. The effects of lead toxic metals on plants are expressed as seed germination, root elongation, seedling growth and inhibition of ATP production, lipid peroxidation [22].

The toxic effects of cadmium toxic metals on plants are expressed as chlorosis, curling of leaves and stunting, induction of lipid peroxidation, changing mineral uptake, inhibiting nitrate reductase activity in shoots [22]. Cadmium, which is among the top 20 toxic substances, is in the 7th place [23]. Cadmium is easily absorbed by plant roots and accumulates in its organs, significantly affecting physiological processes and inhibiting plant growth and development. Decreased photosynthesis is common in plants exposed to Cd ions [24]. It has been reported that the high concentration of Cd in the growth medium causes significant changes in the content of iron, manganese, phosphorus, potassium and boron elements [25]. Although the tolerance of plants to cadmium varies by species, a concentration greater than 5–10 Cd $\mu g\ g^{-1}$ in plant dry matter toxic to all living things [9].

Zinnia elegans is a plant belonging to the Asteraceae family, native to Mexico and Central America and grown as cut flower. It is an ornamental plant with high commercial value due to its long flowering, high drought tolerance and various colors [26]. *Zinnia* *elegans* is an annual flowering plant, ability to grow in toxic metals, having fast growth, high biomass, easy to cultivate and harvest and is mainly cultivated for the purpose of flower production [27]. *Tagetes erecta* (Marigold) is an ornamental plant belonging to the Asteraceae family. It is one of the easiest outdoor ornamental plants to grow in gardens and parks. Tagetes species are native to the North and South America continents. The flowers are bright yellow, brownish yellow or orange. *Tagetes erecta* (Mexican marigold) yellow multilayered blooming marigold. It is common to grow it as one of the most suitable summer ornamental plants for parks, gardens, balconies and terraces that receive sunlight for a long time. Marigold adapts to any soil, any climate. In this respect, it is easily grown all over the world [28]. The use of ornamental plants in urban areas not only cleans contaminated areas but also contributes to the beautification of the environment [29].

Heavy metal contamination, the persistence of these elements in the environment and the toxicological consequences on plants, wildlife and human populations have become an important global problem in the destruction of biodiversity, especially accelerated by the industrialization process and exponentially due to global climate change [11].

The aim of this study is to evaluated efficiency of fulvic acid on Pb and Cd uptake from Pb and Cd contaminated soil, and the phytoremediation capacity and the hyperaccumulator properties of *Zinnia elegans*, *Tagetes erecta plant.*

2. Materials and Methods

This study to evaluate efficiency of fulvic acid on Pb and Cd uptake from Pb and Cd contaminated soil, and the phytoremediation capacity was conducted using a randomized complete block design. Two pollutant (Cd and Pb), one chelating agent (Fulvic acid; FA), and five replication (Control, Cd, Cd + FA, Pb, Pb + FA) with the soilmixture (1:1:1 torf:soil:sand) prior to filling into 2 L pots). To create the heavy metal stress conditions, soil was mixed with lead (100 mg Pb kg^{-1} as $Pb(NO_3)_2$) and with cadmium (30 mg Cd kg^{-1} as $3CdSO_4H_2O$). Fulvic acid (FA) solutions were prepared by dissolving 350 g Fulvofeed® in one liter water (350,000 mg L^{-1}). The commercial product Fulvofeed® (Humintech GmbH, Grevenbroich, Germany) was used as the fulvic acid (FA) source. The content of Fulvofeed® on dry basis was 90–95% fulvic acid, 5% humic acid, 2% potassium as K_2O, 1% total organic nitrogen. The element content of Fulvofeed® (%, mass) was C—32, H—2.8, N—1.3. The ash content was 22.9 %. The element content on ash-free basis (% wt) was C—49.1, H—5.63, N—1.29, O—44.38. The pH was between 9 and 10. The particle size of insoluble constituents was less than100 µm. FA solutions were further diluted to obtain a concentration of 7000 mg L^{-1}. FA solutions were applied 20 L ha^{-1} to the soil three times a week, starting the day before planting. These concentrations are the maximum permissible limits according to U.S. Environmental Protection Agency and World Health Organization [30,31]. To simulate Cd and Pb pollutant soil, the incubation process continued as a 8 week after Cd and Pb application soil mixture. During the incubation period, the water level was kept 30% below the soil field capacity and the ambient temperature was 29 degrees. At the end of the 8 week The soil used in the experiment is classified as Ustorthents [32] and consists of 35% sand, 35% silt, 30% clay, 1.2% organic matter. The pH of the soil was 5.61. Cation exchange capacity and the electrical conductivity of the soil were measured as 15.5 cmol(+) kg^{-1} and 1.15 dS m^{-1}, respectively. The macronutrient content of the soil was as follows: total N: 1.15%, P: 15.5 mg kg^{-1}, K: 2.2 cmol(+) kg^{-1}, Ca: 11.0 cmol(+) kg^{-1}, Mg: 2.25 cmol(+) kg^{-1}. The microelements were found as Zn: 2.30 mg kg^{-1}, Cu: 1.1 mg kg^{-1}, Fe: 0.5 mg kg^{-1}, Mn: 1.8 mg kg^{-1}. Cd 0.05 mg kg^{-1}, Pb 0.02 mg kg^{-1}. After harvest soil Cd and Pb contentration was 0.4 mg kg^{-1} for Pb, and 2.1 mg kg^{-1} for *Tagetes eracta* and 0.05 mg kg^{-1} for Pb and 1.1 mg kg^{-1} for *Zinnia elegans.*

2.1. Plant Growth Parameters

Harvest took place 90 days after seedling planting. Plant height (mm), plant fresh weight (gr), plant dry weight (gr), stem diameter (mm), plant flower number, flower stem length (mm), flower diameter (mm), flower stem thickness (mm), flower fresh weight (gr), flower dry weight (gr), and chlorophyll content (SPAD) were measured. At the end of the experiment, all plants were harvested. The fresh weights of the plants were measured with an electronic balance (CX-600), incubated at 68 °C for 48 h, then measured again for dry weight. Leaf chlorophyll readings of *Zinnia elegans* L. and *Tagetes Erecta* plants were determined with a portable chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Osaka, Japan).

2.2. Plant and Soil Analyses

For mineral analysis, samples from the roots and leaves of *Zinnia elegans L.* and *Tagetes Erecta* plants were dried in an oven at 68 °C for 48 h and then ground. The Kjeldahl method with a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) was used to determine the total N. An inductively coupled plasma spectrophotometer (Optima 2100 DV, ICP/OES; Perkin-Elmer, Shelton, CT, USA) was used to determine tissue K, P, Mg, Na, Fe, Zn, B, Ca, S, Pb, Cd, Mn, B and Cl [33].

After 8 weeks of incubation with HM, soil samples from each plot were taken from a 0- to 10-cm depth to determine baseline soil properties, heavy metal pollution degree, and soil element fractions using sequential extraction.

Physical and chemical analyses were performed after soil samples were air dried, crumbled, and passed through a 2-mm sieve [34].

Soil pH was determined according to McLean [35]. The method of Rhoades (1996) [36] was used to measure electrical conductivity (EC). Kjeldahl method [37] and the sodium bicarbonate method of Olsen et al. [38] were used to determine the organic N and plant-available P contents, respectively. Soil organic matter was determined using the Smith–Weldon method [39]. The exchangeable cations were determined according to Thomas [40]. Diethylenetriaminepentaacetic acid (DTPA) extraction methods were used to determine the microelements in the soils [41].

Catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activities were determined based on the method given by Sahin et al. [42].

2.3. Remediation Parameters

The translocation factor [43], bioconcentration factor [44], bioaccumulation factor [45] phytoextraction potential [46,47], transfer index [48] and transfer factor [49] were calculated to evaluate the effectiveness of remediation applied using fulvic acid.

$$\text{Translocation factor (TLF)} = [\text{M}]\text{shoots}/[\text{M}]\text{roots}$$

$$\text{Total bioconcentration factor (BCFT)} = [\text{M}]\text{planttissue}/[\text{E}]\text{soil}$$

$$\text{Transfer factor (TF)} = [\text{M}]\text{total}/[\text{E}]\text{soil}$$

where [M] shoots is the metal concentration in the shoots (mg kg^{-1}); [M] roots is the metal concentration in the roots (mg kg^{-1}); [M] plant tissue is the metal concentration in plant tissue (root or shoot or grain) (mg kg^{-1}); [M] total is the metal concentration in plant grain and shoot and root (mg kg^{-1}); and [E] soil is the total metal concentration in the soil at harvest time (mg kg^{-1}).

2.4. Statistical Analysis

A completely randomized factorial design was applied in this study. The data were subjected to ANOVA. The statistical software IBM SPSS Statistics 20 was used for all analyses. Results were considered to be significant at the $p \leq 0.05$ level.

3. Results and Discussion

3.1. Yield and Yield Components of Plants

Our results showed that the highest number of flower perplants, plant height, stem diameter, flower diameter length, flower diameter, flower stem thickness, flower fresh weight, flower dry weight chlorophyll reading value, plant fresh weight and dry weight of *Tagetes eracta* and *Zinnia elegans* plants were obtained when FA was applied at a concentration of 7000 mg L^{-1}, whereas the lowest value were found control and follow without FA application both Pb pollution and Cd pollution treatment (Tables 1 and 2). These findings indicate that, FA application, number of flower perplants, plant height, stem diameter, flower stem thickness, flower fresh weight, flower dry weight, chlorophyll reading value, plant fresh weight and dry weight of *Tagetes eracta* plants increased 187.5%, 5.8%, 17.3%, 21.6%, 176.7%, 197.8%, 28.6%, 104.5% and 120.3% respectively with application of 7000 mg L^{-1} FA at 100 mg kg^{-1} Pb pollution condition, whereas flower perplants, flower stem thickness, flower fresh weight, flower dry weight, and plant fresh weight of *Tagetes eracta* plants increased 42.85%, 39.2%, 10.7%, 163.1% and 16.5%, with application of 7000 mg L^{-1} FA at 30 mg kg^{-1} Cd pollution condition, respectively (Table 1).

Table 1. Effect of different growing medium on growth parameters of *Tagetes eracta*.

Growth Medium	Number of Flower (Per Plant)	Plant Height (cm)	Stem Diameter (mm)	Flower Diameter Length (mm)	Flower Diameter (mm)	Flower Stem Thickness (mm)
Control	1.60 b	29.10 b	7.33 c	7.9 a	53.07 c	2.60 c
Pb + FA	4.60 a	30.80 ab	8.60 a	6.4 b	64.53 b	3.17 ab
Pb	5.20 a	31.70 a	7.97 b	7.9 a	76.38 a	3.63 a
Cd + FA	2.80 b	26.30 c	6.60 d	7.7 a	47.90 c	2.98 c
Cd	2.20 b	30.20 ab	6.48 d	8.0 a	45.13 c	2.87 bc
Growth Medium	**Flower Fresh Weight (g plant^{-1})**	**Flower Dry Weight (g plant^{-1})**	**Chlorophyll Reading Value (SPAD)**	**Plant Fresh Weight (g plant^{-1})**	**Plant Dry Weight (g plant^{-1})**	
Control	6.54 b	0.95 b	34.14 b	14.84 ab	2.51 b	
Pb + FA	18.10 a	2.83 a	43.90 a	30.38 a	5.53 a	
Pb	17.19 a	2.50 a	35.24 b	32.07 a	5.55 a	
Cd + FA	7.24 b	1.01 b	33.86 b	17.29 b	2.34 b	
Cd	5.45 b	0.74 b	31.62 b	13.40 b	2.31 b	

Notes. Different letters within the columns show significant differences at $p < 0.05$ for heavy metal and FA applications; Control (uncontaminated soil), Pb (contaminated soil with Pb), Cd (contaminated soil with Cd), Pb + FA (fulvic acid application on contaminated soil with Pb), Cd + FA (fulvic acid application on contaminated soil with Cd).

Table 2. Effect of different growing medium on growth parameters of *Zinnia elegans*.

Growth Medium	Number of Flower (Per Plant)	Plant Height (cm)	Stem Diameter (mm)	Flower Handle Length (mm)	Flower Diameter (mm)	Flower Stem Thickness (mm)	Root Length (cm)
Control	1.80 c	23.60 c	4.45 b	8.45 b	61.69 ns	3.34 ab	28.20 b
Pb + FA	4.20 a	28.70 b	7.01 a	11.51 a	59.42	3.86 a	26.60 b
Pb	4.60 a	29.10 b	5.80 b	10.01 ab	62.72	3.47 ab	32.20 ab
Cd + FA	2.60 b	61.30 a	4.65 b	9.33 ab	61.57	3.54 ab	38.40 a
Cd	2.20 b	22.10 c	4.74 b	8.64 b	58.76	3.15 b	33.80 ab
Growth Medium	**Flower Fresh Weight (g plant^{-1})**	**Flower Dry Weight (g plant^{-1})**	**Plant Fresh Weight (g plant^{-1})**	**Plant Dry Weight (g plant^{-1})**	**Root Fresh Weight (g plant^{-1})**	**Root Dry Weight (g plant^{-1})**	**Chlorophyll Reading Value (SPAD)**
Control	6.79 c	1.36 c	8.99 b	1.89 b	9.52 b	0.86 b	29.98 bc
Pb + FA	12.51 b	2.85 b	15.71 ab	3.52 a	15.02 a	1.22 a	33.10 ab
Pb	20.12 a	4.58 a	17.37 a	4.00 a	14.44 a	1.20 a	35.44 a
Cd + FA	14.16 b	1.26 c	5.92 c	1.94 b	10.06 b	0.88 b	27.68 cd
Cd	10.57 b	1.07 c	4.73 c	2.04 b	11.59 ab	1.03 ab	24.90 d

Notes. Different letters within the columns show significant differences at $p < 0.05$ for heavy metal and FA applications; Control (uncontaminated soil), Pb (contaminated soil with Pb), Cd (contaminated soil with Cd), Pb + FA (fulvic acid application on contaminated soil with Pb), Cd + FA (fulvic acid application on contaminated soil with Cd).

On the other hand, number of flower perplants, plant height, stem diameter, flower hand length, flower diameter length, flower stem thickness, flower fresh weight, flower dry weight, plant fresh weight, plant dry weight and chlorophyll reading value of *Zinnia elegans* plants increased 155.5%, 21.6%, 57.5%, 36.2%, 15.5%, 84.2%, 109.5%, 74.7%, 86.2%, 57.7%, 41.8% and 10.1 with application of 7000 mg L^{-1} FA at 100 mg kg^{-1} Pb pollution condition respectively, on the other hand, number of flower perplants, plant height, stem diameter, flower hand length, flower diameter, flower stem thickness, root length, flower fresh weight, dry weight, root fresh weight, root dry weight and chlorophyll reading value of *Zinnia elegans* plants increased 44.4%, 159.7%, 4.4%, 10.4%, 1.0%, 5.9%, 36.1%, 108.5%, 2.6%, 5.6% and 1.0%, with application of 7000 mg L^{-1} FA at 30 mg kg^{-1} Cd pollution condition, respectively (Table 2).

3.2. Efficiency of Fulvic Acid on Uptake of Macro and Micro Nutrient Content

Lead and cadmium pollution decreased all mineral elements contents in the shoots and roots. Fulvic acid application does not affect the N, P, K, Ca, Mg, Na, Cl, S, Fe, Mn, B, Cu and Zn element contents in the shoots and roots of *Tagates eracta* and *Zinnia elegans* plants (Tables 3–6).

Table 3. The root part macro and micro element analysis results of *Tagetes eracta*.

Growth Medium	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	Cl	S
Control	1.12 a	1.33 a	0.61 a	0.36 a	0.96 a	68.10 a	0.65 ns	0.06 a
Cd	1.11 a	1.15 b	0.62 a	0.37 a	0.82 b	61.82 a	0.48	0.05 bc
Cd + FA	0.95 b	1.13 b	0.52 b	0.34 ab	0.06 c	54.41 bc	0.35	0.04 c
Pb	1.04 a	1.21 ab	0.55 b	0.32 bc	0.81 bc	60.35 ab	0.55	0.05 ab
Pb + FA	0.95 b	1.11 b	0.54 b	0.31 c	0.06 c	49.32 c	0.40	0.04 c

Growth Medium	Pb	Cd	Fe (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
Control	0.43 c	0.24 c	394.27 a	11.59 ab	3.085 a	1.12 a	4.33 a
Cd	0.42 c	4.54 b	332.87 b	12.03 a	2.16 c	0.80 b	3.50 b
Cd + FA	0.45 c	16.66 a	302.88 b	9.29 c	1.77 c	0.78 b	3.30 b
Pb	6.61 b	0.26 c	377.65 a	10.84 bc	2.62 b	0.90 bc	3.25 b
Pb + FA	18.27 a	0.25 c	322.45 b	10.29 bc	1.85 c	0.95 b	3.15 b

Table 4. The shoot part macro and micro element analysis results of *Tagetes eracta*.

Growth Medium	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	Cl	S
Control	1.56 a	0.14 a	1.01 a	0.55 a	0.15 a	87.75 a	0.65 ns	0.09 a
Cd	1.45 ab	0.12 b	0.89 ab	0.53 a	0.11 bc	80.02 ab	0.46	0.08 a
Cd + FA	1.41 b	0.12 b	0.85 bc	0.50 a	0.13 ab	81.47 ab	0.77	0.08 ab
Pb	1.28 c	0.12 b	0.82 bc	0.44 b	0.12 ab	70.42 bc	0.55	0.07 ab
Pb + FA	1.24 c	0.10 c	0.71 c	0.42 b	0.09 c	63.28 c	0.39	0.06 b

Growth Medium	Pb	Cd	Fe (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})
Control	0.38 c	0.19 c	39.16 a	13.02 a	3.70 a
Cd	0.35 c	3.6 b	35.08 ab	11.98 ab	2.71 ab
Cd + FA	0.37 c	14.01 a	34.97 ab	11.15 ab	3.90 a
Pb	5.54 b	0.21 c	33.05 b	10.44 ab	3.17 ab
Pb + FA	15.43 a	0.22 c	29.47 b	9.49 b	2.32 b

Table 5. The root part macro and micro element analysis results of *Zinnia elegans*.

Growth Medium	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	Cl	S
Control	0.86 ab	0.99 b	0.46 a	0.31 a	051 cd	46.16 ab	0.27 ns	0.038 b
Cd	0.92 a	1.12 a	0.48 a	0.28 ab	0.07 a	52.31 a	0.50	0.046 a
Cd + FA	0.81 c	1.02 b	0.47 a	0.27 b	0.59 bc	42.58 bc	0.36	0.036 bc
Pb	0.74 c	0.90 c	0.39 b	0.27 b	0.05 d	39.54 c	0.25	0.033 c
Pb + FA	0.80 c	0.95 bc	0.44 a	0.26 b	0.69 ab	51.67 a	0.47	0.043 a
Growth Medium	**Pb**	**Cd**	**Fe (mg kg^{-1})**	**Mn (mg kg^{-1})**	**B (mg kg^{-1})**	**Cu (mg kg^{-1})**	**Zn (mg kg^{-1})**	
Control	0.38 c	0.24 c	303.84 b	8.43 ab	1.35 a	0.92 a	2.13 a	
Cd	0.36 c	4.11 b	348.53 a	9.47 a	2.39 a	0.55 d	1.22 b	
Cd + FA	0.40 c	13.95 a	296.29 bc	8.84 a	1.67 b	0.54 d	1.15 b	
Pb	8.54 b	0.27 c	276.49 c	7.22 b	1.23 b	0.60 c	1.20 b	
Pb + FA	22.96 a	0.25 c	290.10 bc	8.30 b	2.22 a	0.75 b	1.30 a	

Table 6. The shoot part macro and micro element analysis results of *Zinnia elegans*.

Growth Medium	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	Cl	S
Control	1.13 a	0.11 a	0.75 a	0.44 a	0.11 a	72.90 a	0.67 a	0.07 ns
Cd	1.01 ab	0.10 a	0.75 a	0.39 ab	0.10 ab	63.6 ab	0.45 ab	0.07
Cd + FA	1.04 ab	0.88 b	0.59 b	0.34 c	0.07 b	56.9 b	0.32 b	0.06
Pb	0.96 b	0.10 a	0.67 ab	0.38 bc	0.09 ab	67.98 ab	0.62 ab	0.07
Pb + FA	0.78 c	0.99 b	0.74 a	0.39 ab	0.09 ab	60.10 ab	0.45 ab	0.06
Growth Medium	**Pb**	**Cd**	**Fe (mg kg^{-1})**	**Mn (mg kg^{-1})**	**B (mg kg^{-1})**	**Cu (mg kg^{-1})**	**Zn (mg kg^{-1})**	
Control	0.33 c	0.17 c	30.98 a	9.39 ns	3.58 a	1.10 a	3.56 a	
Cd	0.33 c	3.05 b	29.42 a	8.86	2.77 ab	0.75 b	2.34 b	
Cd + FA	0.32 c	11.43 a	23.91 b	7.34	2.00 b	0,72 b	2.30 b	
Pb	7.1 b	0.18 c	28.06 ab	8.54	3.08 ab	0.80 bc	2.22 b	
Pb + FA	15.43 a	0.19 c	27.75 ab	8.99	2.57 ab	0.85 b	2.34 b	

Notes. Different letters within the columns show significant differences at $p < 0.05$ for heavy metal and FA applications; improvement factor (%); PP, plant extraction potential (kg ha^{-1}); BCFT, bioconcentration factor (stem and root) relative to total soil element (B, Pb or Cd); TF, transfer factor; TLF, translocation factor.

3.3. Efficiency of Fulvic Acid on Uptake of Pb and Cd in Shoots and Roots Part of Plants

The application of FA significantly affected Pb, and Cd uptakes of the plants under Pb and Cd-contaminated soils. Pb and Cd concentrations in plant shoots and roots increased with the fulvic acid application. The effect of FA application on the uptake of shoot and root parts of the *Tagetes eracta* plant in Pb and Cd uptake amounts were determined as 178.52% and 176.40% for Pb, 290% and 266.96% for Cd, respectively, compared to the control. The effect of chelation application on the intake of shoot and root parts of *Zinnia elegans* plant in Pb and Cd uptake amounts were determined as 117.32% and 168.85% for Pb, 274.75% and 239.42% for Cd, respectively, compared to the control. With the FA application in the *Tagetes eracta* plant, the shoot part accumulated 6.75% more Pb and 23.53% Cd compared to the root part. With the FA application in the *Zinnia elegans* plant, the root part has accumulated 51.53% more Pb than the shoot part. For Cd, the shoot part accumulated 35.33% more Cd than the root. It was determined that FA application increased the Pb and Cd uptake in shoots and leaves at a much higher rate compared to the control. Thus, the highest Pb, and Cd uptakes were observed from plant shoots followed by roots (Tables 3–6).

3.4. Effects of Fulvic Acid Applied under Heavy Metal Stress on Enzymatic Activity

It is known that the enzyme activities of plants grown in soils contaminated with various heavy metals are affected by stress [50,51]. In this study, the application of FA significantly affected enzyme activity of *Tagetes eracta and Zinnia elegans* at Pb, and Cd pollution. The effect of FA application on Superoxide dismutase (SOD), peroxidase (POD) and, Catalase (CAT) of the *Tagetes eracta* were decreased as 32.7%, 33.1%, and 35.1% for Pb, 21.2%,25.1%, and 26,1%, for Cd, and *Zinnia elegans* SOD, POD and CAT activity were 15.1%, 22.7%, and 37.7% for Pb, and 7.55%,18.0%, and 18.8% for Cd, respectively (Table 7). Obtained results showed that, the activities of SOD, CAT and POD were reduced after treatment with FA. This result indicates that FA may serve directly as an antioxidant to eliminate excess ROS, or as a signal molecule to promote antioxidant production. In consistent with our results, Yildirim et al. [52] reported that FA treatment in garden cress under cadmium stress reduced SOD and CAT activity.

Table 7. Antioxidant enzyme activity of *Tagetes eracta* and *Zinnia elegans*.

	Tagetes eracta			***Zinnia elegans***		
Growth Medium	SOD	POD	CAD	SOD	POD	CAT
			EU gr leaf^{-1}			
Control	1980 c	18,780 c	555 c	1720 c	20,438 c	678 c
Pb+FA	1345 e	12,550 e	360 e	1460 e	15,780 e	415 e
Pb	2546 b	2908 b	780 b	2834 b	33,652 b	934 b
Cd+FA	1560 d	13,869 d	410 d	1590 d	16,750 d	550 d
Cd	3652 a	31,687 a	934 a	3005 a	34,970 a	1040 a

Note: EU: Enzyme unite, SOD: Superoxide dismutase, POD: peroxidase, CAT: Catalase. Different letters within the columns show significant differences at $p < 0.05$ for heavy metal and FA applications; Control (uncontaminated soil), Pb (contaminated soil with Pb), Cd (contaminated soil with Cd), Pb + FA (fulvic acid application on contaminated soil with Pb), Cd + FA (fulvic acid application on contaminated soil with Cd.

3.5. Effects FA on Pb and Cd Remediation Factors of Tagetes eracta and Zinnia Elegans Grown in Contaminated Soil

Translocation Factor (TLF): It is a ratio that indicates the ability of a plant to transport metals from its roots to the harvestable part of the plant. In other words, it is used to measure the amount of heavy metal transferred from one organ to another. The heavy metal concentration in the stem of the plant is calculated by proportioning to the root. TLF factor in plants is usually less than 1 FA application doesn't affected TLF (Figure 1). Hyperaccumulator plants are plants that can accumulate HM such as Pb, Cd, Hg, Se, in their body when compared with cultivated plants [53,54]. Translocation factors are used to evaluate the hyperaccumulatory properties of plants, revealing the rates of leaf and fruit transport from the root zone to the stem, from the stem to the stem. As the ideal value used for phytoextraction, BCF > 100 and TLF > 1 are expected to have accumulator plant characteristics [55]. Plants with bioconcentration and translocation factors >1 can be used as bioaccumulators, and those with bioconcentration values >2 are considered high accumulators. can be used as a phytostabilizer if bioconcentration factors >1 and translocation factors <1, and as phytoextractor if bioconcentration factors <1 and translocation factors >1 [56]. Data presented in Figure 1 shows the values of TLF factors of Pb and Cd. *Tagetes eracta* and *Zinnia Elegans* show low TLF to Pb and Cd and therefore, are not suitable in phytoremediation studies and It cannot be considered as a hyperaccumulator plant.

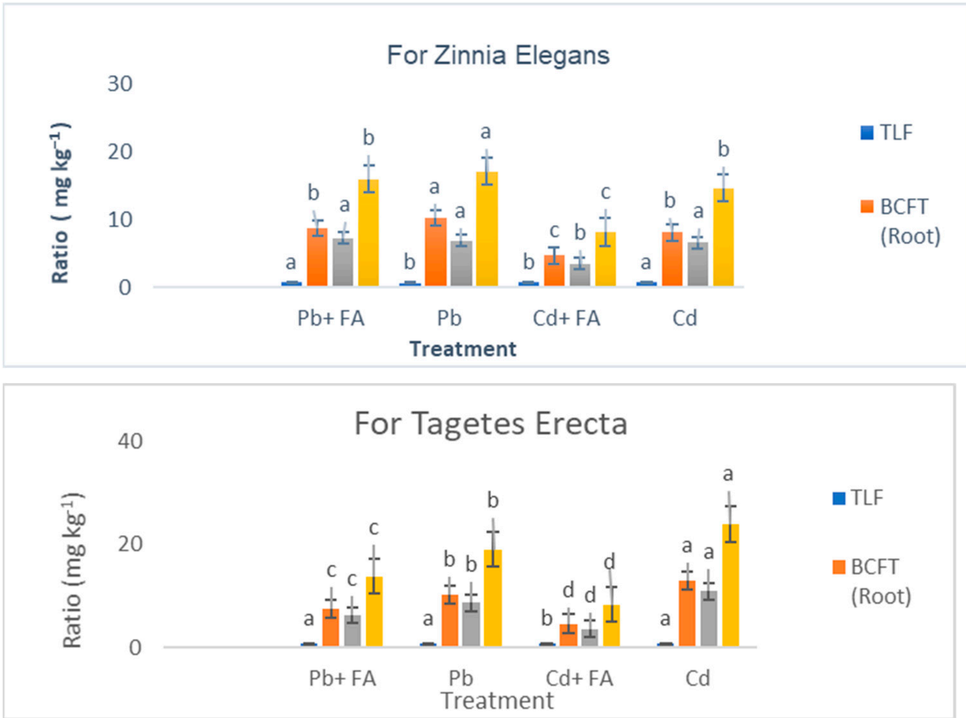

Figure 1. Effects of FA application on remediation component of *Tagetes eracta* and *Zinnia Elegans* under growth with different Pb and Cd amendments. Notes. Different letters within the columns show significant differences at *p* 0.05 for heavy metal and FA applications; Remediation Factors, Bio-concentration factor (BCFT) (stem and root) relative to total soil element (B, Pb or Cd); Translocation factor (TLF).

Bioconcentration (BCF) is used to determine the ratios of a metal removed by the plant, and the evaluation using the value can be evaluated separately according to the total and exchangeable amounts of HM in the soil. Root and stem BCF factors for Pb and Cd HM of *Tagetes eracta* and Zinnia plants are shown in Figure 1. Based on this table, while the BCF root values of *Tagetes eracta* and Zinnia plants for Pb and Pb + FA application were found as 7.51, 10.32, 8.71 and 10.20, respectively, while for Cd and Cd + FA applications, they were listed as 4.63, 13.02, 4.67 and 8.07. As a result of the calculated values, it has been revealed that the FA application is more effective in Cd metal deposition than Pb metal deposition. While the BCF shoot values of *Tagetes eracta* and Zinnia plants for Pb and Pb + FA application were found as 6.30, 8.71, 7.24 and 6.86, respectively, they were listed as 3.67, 10.94, 3.47 and 6.57 for Cd and Cd + FA applications. As a result of the calculated values, it has been revealed that the FA application is more effective in Cd metal deposition than Pb metal deposition.

Transfer Factor (TF). The ability of a species to transfer metal from the soil to the upper parts of the plant is estimated using TF. The TF of a metal is calculated as the ratio of the total metal concentration of the root and stem parts of the plant at harvest time to the total metal concentration in the soil at the time of harvest. FA application affected the transition and TF of Pb and Cd from soil to plants. With the FA application, the TF values of the plant decreased.

Heavy metal transfer factors of *Tagetes eracta* and *Zinnia elegans* can be seen in Figure 1. According to this figure, the lowest TF in Pb + FA and Cd + FA applications in *Tagetes eracta* plants are 8.3 and 13.80 respectively. It was calculated as 15.95 and 8.13 for Pb + FA and Cd + FA applications in *Zinnia elegans* plant, respectively. In this case, FA application causes lead and cadmium to be stabilized in the root zone of the plant, reducing its ability to transport from soil to the upper part of the plant in *Tagetes eracta* and *Zinnia elegans* plants (Figure 1). Similar result was obtained from Yıldırım et al. [52] in garden cress plants.

4. Conclusions

It was carried out to determine whether landscape plants such as *Tagetes eracta* and *Zinnia elegans* are accumulator plants in a cadmium and lead polluted area. FA application was made to increase the efficiency of HM in the soil and to increase their uptake by the plant. The results obtained showed that *Tagetes eracta* and *Zinnia elegans* planted the ability to accumulate Pb and Cd in their tissues (shoots and roots), but it was determined that it did not have hyperaccumulatory properties. Cd accumulation in plant shoots was higher than accumulation from Pb, but Pb accumulation in plant root was higher than Cd. BCFT root value for Cd and Pb in *Tagetes eracta* was higher than BCFT shoot value, in *Zinnia elegans* BCFT shoot concentration for Cd and Pb was lower than BCFT root concentration. With the FA application, the TF values of the plant decreased and causes lead and cadmium to be stabilized in the root zone of the plant, reducing its ability to transport from soil to the upper part of the plant in *Tagetes eracta* and *Zinnia elegans* plants. It is a result of the fact that the application of fulvic acid caused the Pb and Cd HM in the soil to stabilize instead of increasing the uptake ability of the plant, and the TLF factor was less than 1.

In many studies conducted to date, there are many results that increase or decrease the mobility of HM such as humic and fulvic acids. Many studies showing that HM in the soil are easily taken up by the plant or reduced their uptake have also shown that it affects plants by increasing or decreasing the accumulation of Pb and Cd in total or exchangeable form. While there are results in the direction that these types of chelating agents increase the mobility of Pb and Cd in the soil, contrary to these results, it has been determined that fulvic acid applications serve to reduce the uptake of the plant by immobilizing, that is, to stabilize the soil, and to eliminate the uptake of these HM by the plant. Contrary to the views that humic acid and fulvic acid act in the same direction, that is, increase the mobility of HM in the soil, it has been revealed that fulvic acid is an important stabilizer in remediation technology with its high negative charges and high caton adsorption capacity. In many previous studies, inhibitory effects of humic substances on HM in acidic soils have been reported.

On the contrary, the humic acid content of humic substance, which is very high relative to the fulvic acid content, increases the heavy metal availability. Therefore, it can be said that humic acids are effective for heavy metal bioremediation because humic acids can interact with metals to form metal-humic complexes. On the other hand, it has been shown that fulvic acids inhibit metal availability and can be used to reduce metal accumulation in polluted acidic soils. Many studies have shown that humic acid applications, like ethylenediamine tetra acetic acid applications, increase the mobility of HM in the soil and promote phytoremediation, especially in alkaline soils with high pH. In conclusion, the application of FA may be suggested as an effective treatment for reducing the Cd and Pb uptake of the plants by stabilizing Cd and Pb in soil and preventing translocation of Cd and Pb from the roots of plant to its shoot and leaves. *Tagetes eracta* and *Zinnia elegans* can not be recommended for remediaion of Pb and Cd polluted area, but FA can be recommended for Pb and Cd stabilization in polluted soil.

Author Contributions: M.T. designed the study. A.E. performed the experiments. M.T. and A.C. made the chemical analysis. M.T. revised the English composition. A.E., M.T. and A.C. analyzed the experiment data. All authors discussed the results and commented on the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Humintech Gmbh, 41517 Grevenborch, Germany.

Informed Consent Statement: Informed Consent Statement accepted.

Data Availability Statement: Journal can be use our data.

Acknowledgments: We are very grateful to the Humintech GmbH for generous support.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Monterroso, C.; Rodrıguez, A.F.; Chaves, R.; Diez, J.; Becerra Castro, C.; Kidd, P.S.; Macıas, F. Heavy metal distribution in mine-soils and plants growing in a Pb/Zn-mining area in NW Spain. *Appl. Geochem.* **2014**, *44*, 3–11. [CrossRef]
2. Shanker, A.K.; Cervantes, C.; Loza-Tavera, H.; Avudainayagam, S. Chromium toxicity in plants. *Environ. Int.* **2005**, *31*, 739–753. [CrossRef]
3. Lal, S.; Ratna, S.; Saida, O.B.; Kumar, R. Biosurfactant and exopolysaccharide-assisted rhizobacterial technique for the remediation of heavy metal contaminated soil: An advancement in metal phytoremediation technology. *Environ. Technol. Innov.* **2018**, *10*, 243–263. [CrossRef]
4. Chen, Y.; Chao, Y.; Li, Y.; Lin, Q.; Bai, J.; Tang, L.; Wang, S.; Ying, R.; Qiu, R. Survival strategies of the plant-associated bacterium Enterobacter sp. strain EG16 under cadmium stress. *Appl. Environ. Microbiol. Open Access* **2016**, *82*, 1734–1744. [CrossRef] [PubMed]
5. Shaheen, N.; Irfan, N.M.; Khan, I.N.; Islam, S.; Islam, M.S.; Ahmed, K. Presence of HM in fruits and vegetables: Health risk implications in Bangladesh. *Chemosphere* **2016**, *152*, 431–438. [CrossRef]
6. Daud, M.K.; Quiling, H.; Lei, M.; Ali, B.; Zhu, S.J. Ultrastructural, metabolic and proteomic changes inleaves of upland cotton in response to cadmiumstress. *Chemosphere* **2015**, *120*, 309–320. [CrossRef]
7. Fahr, M.; Laplaze, L.; El Mzibri, M.; Doumas, P.; Ben-daou, N.; Hocher, V.; Bogusz, D.; Smouni, A. Assessment of lead tolerance and accumulation inmetallicolous and non-metallicolous populations ofHirschfeldia incana. *Environ. Exp. Bot.* **2015**, *109*, 186–192. [CrossRef]
8. Verbruggen, N.; Juraniec, M.; Baliardini, C.; Meyer, C.L. Tolerance to cadmium in plants: The specialcase of hyperaccumulators. *Biometals* **2013**, *26*, 633–638. [CrossRef]
9. Wiszniewska, A.; Hanus-Fajerska, E.; Muszynska, E.; Smolen, S. Comparative assessment of response to cadmium in heavy metal-tolerant shrubscultured in vitro. *Water Air Soil Pollut.* **2017**, *228*, 304. [CrossRef]
10. Muszynska, E.; Hanus-Fajerska, E.; Ciarkowska, K. Studies on lead and cadmium toxicity in Dianthus carthusianorum calamine ecotype cultivated in vitro. *Plant Biol. Vol.* **2018**, *20*, 474–482. [CrossRef]
11. Lei, K.; Giubilato, E.; Critto, A.; Pan, H.; Lin, C. Contamination and human health risk of lead in soils around lead/zinc smelting areas in China. *Environ. Sci. Pollut. Res.* **2016**, *23*, 13128–13136. [CrossRef]
12. Hölzle, I. Dry screening—Assessing, the effectiveness of contaminant reduction in recovered landfill soils Author links open overlay panel. *J. Clean. Prod.* **2018**, *172*, 1998–2008. [CrossRef]
13. Burger, A.; Alkorta, I.; Epelde, L.; Garbisu, C. From phytoremediation of soil contaminants to phytomanagement of ecosystem services in metal contaminated sites. *Int. J. Phytoremediatıon* **2018**, *20*, 384–397. [CrossRef]
14. Ali, H.; Khan, E.; Sajad, M.A. Phytoremediation of heavy metals-concepts and applications. *Chemosphere* **2013**, *91*, 869–881. [CrossRef]
15. Valko, M.; Moris, H.; Cronin, M.T.D. Metals, toxicity and oxidative stress. *Curr. Med. Chem.* **2005**, *12*, 1161–1208. [CrossRef] [PubMed]
16. Patra, D.K.; Pradhan, C.; Patra, H.K. Chromium bioaccumulation, oxidative stress metabolism and oil content in lemon grass Cymbopogon flexuosus (Nees ex Steud.) W. Watson grown in chromium rich over burden soil of Sukinda chromite mine, India. *Chemosphere* **2019**, *218*, 1082–1088. [CrossRef]
17. Sinhal, V.K.; Srivastava, A.; Singh, V.P. EDTA and citric acid mediated phytoextraction of Zn, Cu, Pb and Cd through marigold (*Tagetes erecta*). *J. Environ. Biol.* **2010**, *31*, 255–259. [PubMed]
18. Salt, D.E.; Smith, R.D.; Raskin, I. Phytoremediation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1998**, *49*, 643–668. [CrossRef] [PubMed]
19. Salt, D.E.; Rauser, W.E. Mg-ATP dependent transport of phytochelatins across tonoplast of oat roots. *Plant Physiol.* **1995**, *107*, 1293–1301. [CrossRef]
20. Komlos, J.; Traver, R.G. Long-term orthophosphate removal in a field-scale storm-water bioinfiltration rain garden. *J. Environ. Eng.* **2012**, *138*, 991–998. [CrossRef]
21. Mullane, J.M.; Flury, M.; Iqbal, H.; Freeze, P.M.; Hinman, C.; Cogger, C.G.; Shi, Z. Intermittent rainstorms cause pulses of nitrogen, phosphorus, and copper in leachate from compost in bioretention systems. *Sci. Total Environ.* **2015**, *537*, 294–303. [CrossRef] [PubMed]
22. Patra, D.K.; Pradhan, C.; Patra, H.K. Toxic metal decontamination by phytoremediation approach: Concept, challenges, opportunities and future perspectives. *Environ. Technol. Innov.* **2020**, *18*, 100672. [CrossRef]
23. Birke, M.; Reimann, C.; Rauch, U.; Landenberger, A.; Demetriades, A.; Jähne-Klingberg, F.; Oorts, K.; Gosar, M.; Dinelli, E.; Halamić, J. GEMAS: Cadmium distribution and its sources in agricultural and grazing land soil of Europe—Original data versus clr-transformed data. *J. Geochem. Explor.* **2017**, *173*, 13–30. [CrossRef]
24. Gill, S.S.; Khan, N.; Tuteja, N. Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (*Lepidium sativum* L.). *Plant Sci.* **2012**, *182*, 112–120. [CrossRef]
25. Dias, M.C.; Monteiro, C.; Moutinho-Pereira, J.; Correia, C.; Goncalves, B.; Santos, C. Cadmium toxicity affects photosynthesis and plant growth at different levels. *Acta Physiol. Plant.* **2013**, *35*, 1281–1289. [CrossRef]

26. Gomaaa, A.A.R.; Samya, M.N.; Abdelmohsena, U.R.; Krischkec, M.; Muellerc, M.J.; Wanasa, A.S.; Desoukeya, S.Y.; Kamela, M.S. Metabolomic profiling and anti-infective potential of Zinnia elegans and Gazania rigens (Family Asteraceae). *Nat. Prod. Res.* **2020**, *34*, 2612–2615. [CrossRef] [PubMed]
27. Ehsan, N.; Nawaz, R.; Ahmad, S.; Arshad, M.; Umaır, M.; Sarmad, M. Remediation of Heavy Metal-Contaminated Soil by Ornamental Plant Zinnia (*Zinnia elegance* L.). *Asian J. Chem.* **2016**, *28*, 1338–1342. [CrossRef]
28. Padalia, H.; Moteriya, P.; Chanda, S. Green synthesis of silver nanoparticlesfrom marigold flower and its synergisticantimicrobial potential. *Arab. J. Chem.* **2015**, *8*, 732–741. [CrossRef]
29. Prapagdee, B.; Wankumpha, J. Phytoremediation of cadmium polluted soil by Chlorophytum laxum combined with chitosanimmobilized cadmium-resistant bacteria. *Environ. Sci. Pollut. Res.* **2017**, *24*, 19249–19258. [CrossRef]
30. US EPA. *Standard Methods for the Examination of Water and Wastewater, Environmental Protection Agency (EPA)*; American Publish Health Association: Washington, DC, USA, 1993.
31. WHO. *Guidelines for Drinking-Water Quality. First Addendum to Third Edition. Volume 1, Recommendations*; WHO: Geneva, Switzerland, 2006; p. 595.
32. Soil Survey Staff. Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys. In *Agriculture Handbook*; Natural Resources Conservation Service USDA: Washington, DC, USA, 1999; p. 436.
33. Mertens, D. AOAC Official Method 922.02: Plant Preparation of Laboratory Sample. In *Official Methods of Analysis*, 18th ed.; AOAC- İnternational Suite: Gaitherburg, MD, USA, 2005; Chapter 3; pp. 1–2.
34. AOAC (Association of Official Analytical Chemists). *Official Methods of Analysis*, 18th ed.; AOAC: Arlington, VA, USA, 2005.
35. McLean, E.O. Soil pH and Lime Requirement. In *Methods of Soil Analysis, Part II: Chemical and Microbiological Properties*, 2nd ed.; Page, A.L., Miller, R.H., Keeney, E.D., Eds.; ASA: Madison, WI, USA, 1982; pp. 199–224.
36. Rhoades, J.D. Salinity: Electrical Conductivity and Total Dissolved Solids. In *Methods of Soil Analysis, Part III: Chemical Methods*, 2nd ed.; Sparks, D.L., Ed.; ASA: Madison, WI, USA, 1996; pp. 417–436.
37. Bremner, J.M. Nitrogen—Total. In *Methods of Soil Analysis, Part III: Chemical Methods*, 2nd ed.; Sparks, D.L., Ed.; ASA: Madison, WI, USA, 1996; pp. 1085–1122.
38. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate (USDA Circular 939)*; U.S. Government Printing Office: Washington, DC, USA, 1954.
39. Nelson, D.W.; Sommers, L.E. Organic Matter. In *Methods of Soil Analysis, Part II: Chemical and Microbiological Properties*, 2nd ed.; Page, A.L., Miller, R.H., Keeney, E.D., Eds.; ASA: Madison, WI, USA, 1982; pp. 574–579.
40. Thomas, G.W. *Exchangable Cations: Chemical and Microbiological Properties*; ASA and SSSA: Madison, WI, USA, 1982.
41. Lindsay, W.L.; Norvell, W.A. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.* **1978**, *42*, 421–428. [CrossRef]
42. Sahin, U.; Ekinci, M.; Örs, S.; Turan, M.; Yıldız, S.; Yıldırım, E. Effects of individual and combined effects of salinity and drought on physiological, nutritional and biochemical properties of cabbage (*Brassica oleracea* var. capitata). *Sci. Hortic.* **2018**, *240*, 196–204. [CrossRef]
43. Zayed, A.; Gowthaman, S.; Terry, N. Phytoaccumulation of trace elements by wetland plants: I. Duckweed. *J. Environ. Qual.* **1998**, *27*, 715–721. [CrossRef]
44. Marques, A.; Rangel, A.; Castro, P.M.L. Remediation of heavy-metal-contaminated soils: Phytoremediation as a potentially promising cleanup technology. *Crit. Rev. Environ. Sci. Technol.* **2009**, *39*, 622–654. [CrossRef]
45. Vysloužilová, M.; Tlustoš, P.; Száková, J. Cadmium and zinc phytoextraction potential of seven clones of *Salix* spp. planted on heavy metal–contaminated soils. *Plant Soil Environ.* **2003**, *49*, 542–547. [CrossRef]
46. Kos, B.; Grcman, B.; Lestan, D. Phytoextraction of lead, zinc, and cadmium from soil by selected plants. *Plant Soil Environ.* **2003**, *49*, 548–553. [CrossRef]
47. Kos, B.; Leštan, D. Induced phytoextraction/soil washing of lead using biodegradable chelate and permeable barriers. *Environ. Sci. Technol.* **2003**, *37*, 624–629. [CrossRef]
48. Paiva, H.N.; Carvalho, J.G.; Siqueira, J.O. Translocation index of nutrients in cedro (cedrela fissilis vell.) and ipê-roxo (Tabebuia impetiginosa (mart.) standl.) seedlings submitted to increasing levels of cadmium, nickel and lead. *Rev. Árvore* **2002**, *26*, 467–473. [CrossRef]
49. Lubben, S.; Sauerbeck, D. The uptake and distribution of HM by spring wheat. *Water Air Soil Pollut.* **1991**, *57*, 239–247. [CrossRef]
50. Han, Y.; Chen, G.; Chen, Y.; Shen, Z. Cadmium toxicity and alleviating effects of exogenous salicylic acid in Iris hexagona. *Bull. Environ. Contam. Toxicol.* **2015**, *95*, 796–802. [CrossRef]
51. Goswami, S.; Das, S. Copper phytoremediation potential of *Calandula officinalis* L. and the role of antioxidant enzymes in metal tolerance. *Ecotox. Environ. Safe* **2016**, *126*, 211–218. [CrossRef]
52. Yıldırım, E.; Ekinci, M.; Turan, M.; Ağar, G.; Dursun, A.; Kul, R.; Alim, R.; Argın, S. Humic + Fulvic acid mitigated Cd adverse effects on plant growth, physiology and biochemical properties of garden cress. *Sci. Rep.* **2021**, *11*, 1–8. [CrossRef]
53. Baker, A.J.M.; Brooks, R.R. Terrestrial higher plants which hyperaccumulate metallic elements-a review of their distribution, ecology and phytocheistry. *Biorecovery* **1989**, *1*, 81–126.
54. Turan, M.; Esringü, A. Phytoremediation based on canola (*Brassica napus* L.) and Indian mustard (*Brassica juncea* L.) planted on spiked soil by aliquot amount Cd, Cu, Pb, and Zn. *Plant Soil Environ.* **2007**, *53*, 7–15. [CrossRef]

55. Liu, J.; Xin, X.; Zhou, Q. Phytoremediation of contaminated soils using ornamental plants. *Environ. Rev.* **2018**, *26*, 1. [CrossRef]
56. Takarina, N.D. Bioconcentration Factor (BCF) and Translocation Factor (TF)of Bioconcentration Factor (BCF) and Translocation Factor (TF)of HM in Mangrove Trees of Blanakan Fish Farm HM in Mangrove Trees of Blanakan Fish Farm. *Makara J. Sci.* **2017**, *21*, 77–81. [CrossRef]