





## Article

# Root Characteristics and Metal Uptake of Maize (*Zea mays* L.) under Extreme Soil Contamination

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**Abstract:** Root characteristics and metal uptake in the maize hybrid Naudi were studied in a pot trial, using soil artificially highly polluted with Zn, Cu, Co, Cd, and Pb. The addition of these metals as sulfates decreased the soil pH and increased electrical conductivity. As a result of increased bioavailability in the soil pore water, significantly higher concentrations of metals, particularly Pb, Cu, and Co, were found in the shoot tissues of maize at the 3–4 leaf stage. While the lowest increase was in Cd (0.89 mg kg<sup>-1</sup> vs. 0.33 mg kg<sup>-1</sup> for controls), Zn reached a critical threshold of 75 mg kg<sup>-1</sup> vs. 2.76 mg kg<sup>-1</sup>. Fresh and dry weights of shoots and roots, as well as root length, were markedly reduced, whereas root diameter, tip density, and the branching index increased considerably. A significant adaptation strategy by maize in the polluted soil was an increased fraction of coarse root length and a decreased fraction of finer roots. We conclude that maize is very sensitive to multiple metal pollution, suggesting its potential use as a test plant to evaluate contaminated soils. As length was the most affected root characteristic, measurement of this parameter could be a way of screening genotypes for tolerance to metal contamination and possibly salinity. There also is future scope for investigating whether K fertilization might mitigate metal phytotoxicity, in view of the negative correlations between the shoot K concentration and concentrations of the supplied metals.

**Keywords:** electrical conductivity; maize; root length and diameter; soil metal contamination; soil pore water



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## 1. Introduction

Soil pollution by heavy metals (HMs) potentially poses a significant threat to the environment and human health through multiple absorption pathways, including the ingestion of contaminated food [1,2].

HMs belong to the group of non-biodegradable, persistent, inorganic chemicals with high atomic mass (>20) and density (>5 g cm<sup>-3</sup>) that have cytotoxic, genotoxic, and mutagenic effects on humans and animals. Metals can enter the food chain through contaminated soil, water, and atmosphere [3], and their mobility across environmental compartments is also a cause of great concern. Toxicity in humans includes damage to the nervous system, liver, kidneys, heart, blood vessels, and bone tissue [4].

Soil contamination by heavy metals derives from both the soil parent material (lithogenic source) and various anthropogenic sources. Most metal(loid)s occur naturally in soil parent materials, mainly in forms that are not readily bioavailable for plant uptake. Metals occurring naturally in the soil environment as a result of pedogenic weathering processes are present at trace levels, and rarely cause toxicity [5]. The main anthropogenic sources include phosphate fertilizers, sewage sludge, and pesticides, as well as emissions from power plants, metallurgical and cement factories, and vehicles [6,7]. In the last decades,

industrial activities have also caused serious soil metal contamination worldwide through the incorrect disposal of electronic waste [2,8].

Metal uptake by plant roots is related to the availability of metals in the soil nutrient solution, which depends on several factors, mainly soil pH, but also water-holding capacity, the presence of hydrous ferric oxide, soil density, the type of charge in soil colloids, the degree of complexation with ligands, and the specific soil surface area [9,10]. High metal availability also causes the contamination of water bodies and groundwater through leaching, particularly under acidic conditions [11].

Heavy metals can persist in the environment for many decades or even indefinitely [12], with non-essential elements, such as cadmium (Cd), lead (Pb), and mercury (Hg) causing particular concern. Toxic levels of HMs were found to hamper normal plant functioning, disrupting metabolic processes by altering the permeability, potential, and enzymatic activity of cell membranes in higher plants, including maize [13,14]. Metals also negatively interact with several vital cellular biomolecules, such as nuclear proteins and DNA, leading to an excessive increase in reactive oxygen species (ROS) [3], and are able to disrupt the functionality of essential metals in biomolecules, such as pigments or enzymes [15]. These physiological changes ultimately impair shoot and root growth and productivity in many plants, depending on the contamination level [9]. Available studies on plant response to soil contamination mainly focus on shoot and root biomass damage, while root characteristics and architecture are given little attention despite their importance in plant adaptation to metal pollution. There is currently growing interest in understanding the effects of various metals on cereals, particularly maize, due to its extensive cultivation and increased soil contamination level.

In maize, metal pollution causes serious metabolic, physiological, and morphological alterations, with the extent of yield losses depending on the metal in question, the level of contamination, the combined presence of various metals, and the genetic tolerance level [16,17].

Cadmium is extremely toxic in maize, causing the most severe reductions in shoot and root biomass and grain yield compared with other metals [16]. The primary effects of Cd toxicity in this crop are stunted growth and perturbation of cell membranes [14], along with cellular damage by ROS [18]. In wheat, cadmium causes stomatal closure and a reduction in photosynthetic C assimilation and transpiration [19].

In rice, copper can bind to cell membranes or induce lipid peroxidation, which results in membrane damage and the production of free radicals in different organelles [20]. Copper toxicity is generally associated with decreased photosynthetic competence, low quantum efficiency of photosystem II (PSII), and reduced cell elongation [3].

Elevated soil zinc contamination is reported to cause a decline in initial and maximum chlorophyll fluorescence, resulting in the repression of PSII activity in many plants [21].

All these alterations at the physiological level lead to changes to the shoot and root morphological traits. There is a lack of information about maize, hence the need for further investigation—particularly at the root level and when various metals are present together, as two or more contaminants present in the soil at toxic levels may give rise to antagonistic or inhibitory effects [16].

Against this background, the aim of the present study was to artificially apply a mixture of essential (Co, Cu, Zn) and non-essential (Cd, Pb) metals at high contamination levels to assess the effects of multiple contamination on (i) metal bioavailability in the soil pore water; (ii) shoot metal accumulation in a model maize hybrid, Naudi; and (iii) plant growth, with particular reference to root characteristics as possible markers of hazardous contamination and plant tolerance.

In particular, this study revealed the effects of contamination on a series of root parameters, including length, surface area, diameter, and number of tips and branches.

## 2. Materials and Methods

### 2.1. Experimental Set-Up

The maize hybrid Naudi (FAO Class 400; Caussades Semences, Caussade, France) was grown for 41 days during May–June 2018 in tapered cylindrical pots (19 cm height × 22.5 cm top diameter, ~6 L volume). The experiment followed a randomized block design with four replicates ( $n = 4$ ) and including a metal-contaminated treatment vs. untreated controls. The soil, collected from the agricultural fields of the experimental farm of the University of Padua in Legnaro (northeast Italy), was alkaline (pH = 8.18) silty-loam, with a cation exchange capacity (CEC) of 11.4 cmol (+) kg<sup>-1</sup> and 1.71% organic matter. Before filling the pots, the soil was supplied with a ternary fertilizer at a rate corresponding to 32 kg N, 96 kg P<sub>2</sub>O<sub>5</sub>, and 96 kg of K<sub>2</sub>O per hectare at a depth of 0.3 m.

In the contaminated treatment, a mixture of metals, i.e., Co, Cd, Cu, Pb, and Zn, was added to the soil. The metals were applied as sulfates, with the exception of Pb, which was applied as acetate. After solubilization in pure water, the solution was gradually sprayed onto the soil and accurately mixed in order to ensure homogeneous contamination. The objective was to reach contamination levels about 7–9 times in excess of the maximum permitted Italian Guideline Values (IGVs) for “Green public, private and residential areas” (Italian Legislative Decree 152/2006); the maximum variations (~×9) were for the non-essential Cd and Pb (Table 1). The soil was contaminated about 6 months prior to maize cultivation, to allow for soil and metal stabilization.

**Table 1.** Total element concentrations (different units refer to soil dry weight (DW); ± standard error (SE);  $n = 4$ ), pH and electrical conductivity (EC) in soil contaminated with heavy metal (HM) vs. uncontaminated controls (Unt), and % variation vs. Unt. Letters in brackets indicate significant differences between treatments (Newman–Keuls test,  $p \leq 0.05$ ). The elements artificially added to the soil are highlighted in bold.

	Unit	IGV <sup>1</sup>	Unt	HM	% var.	
Element	Ca	g kg <sup>-1</sup>	62.9 ± 0.94 (a)	62.9 ± 1.68 (a)	−0.10	
	<b>Cd</b>	<b>mg kg<sup>-1</sup></b>	<b>2</b>	<b>0.39 ± 0.017 (b)</b>	<b>16.8 ± 0.198 (a)</b>	<b>+4197.00</b>
	<b>Co</b>	<b>mg kg<sup>-1</sup></b>	<b>20</b>	<b>10.3 ± 0.217 (b)</b>	<b>170 ± 1.82 (a)</b>	<b>+1549.00</b>
	<b>Cu</b>	<b>mg kg<sup>-1</sup></b>	<b>120</b>	<b>29.5 ± 0.28 (b)</b>	<b>799 ± 0.59 (a)</b>	<b>+2605.00</b>
	K	g kg <sup>-1</sup>		7.18 ± 0.23 (a)	7.06 ± 0.15 (a)	−1.67
	Mg	g kg <sup>-1</sup>		31.3 ± 0.44 (a)	30.6 ± 0.76 (a)	−2.00
	Ni	mg kg <sup>-1</sup>		24.4 ± 0.49 (a)	26.2 ± 0.03 (a)	+7.00
	P	mg kg <sup>-1</sup>		799 ± 11.8 (a)	703 ± 6.60 (a)	−12.00
	<b>Pb</b>	<b>mg kg<sup>-1</sup></b>	<b>100</b>	<b>19.6 ± 0.12 (b)</b>	<b>906 ± 6.59 (a)</b>	<b>+4523.00</b>
	S	g kg <sup>-1</sup>		0.24 ± 0.006 (b)	1.13 ± 0.035 (a)	+371.00
	<b>Zn</b>	<b>mg kg<sup>-1</sup></b>	<b>150</b>	<b>87.8 ± 0.56 (b)</b>	<b>1056 ± 36.90 (a)</b>	<b>+1103.00</b>
Parameter	pH		8.18 ± 0.08 (a)	7.19 ± 0.06 (b)	−12.00	
	EC	mS cm <sup>-1</sup>	0.485 ± 0.008 (b)	2.62 ± 0.017 (a)	+440.00	

<sup>1</sup> IGV: Italian Guideline Value for “Green public, private and residential areas” (Italian Legislative Decree 152/2006).

Three seeds per pot were sown on 14 May 2018, with the soil at field capacity. One week after germination, the plants were thinned to one plant per pot. The pots were kept outside under ambient conditions during the whole experiment, and the plants were watered with distilled water every 2–3 days, as necessary.

### 2.2. Soil Analysis

Electrical conductivity (EC) of the untreated controls and metal-polluted soil samples was measured with an EC meter in 1:2  $w/v$  (10 g dry weight (DW) of sample in 20 mL distilled water) soil-to-water extracts at 25 °C. Water extracts were obtained by shaking for 2 h, resting overnight, then filtering with Whatman no. 42 filters. Similarly, soil pH was evaluated with a pH-meter in 1.0:2.5  $w/v$  (10 g DW of soil in 25 mL distilled water) soil-to-water extracts after 2 h shaking and 10 min sedimentation at 25 °C.

The total metal concentrations of the soil in the untreated controls and metal-contaminated treatments were determined before sowing on 1 g DW < 2 mm sieved samples, with four replicates. After microwave acid digestion, following U.S. Environmental Protection Agency (EPA) method 3051 [22] (ETHOS 900, Milestone, Bergamo, Italy), samples were filtered through cellulose acetate (CA) filters (0.45 µm), diluted to 25 mL, and analyzed by inductively-coupled plasma optical emission spectrometry (ICP-OES) (SPECTRO CirOS Vision EOP, SPECTRO Analytical Instruments GmbH, Kleve, Germany). A certified reference material (ERM-CC141, JRC-IRMM, Geel, Belgium) was used to ensure measurement accuracy.

Soil pore water samples were collected from the soil in the pots across the whole trial using removable Rhizon samplers (Scientific Instruments, Vienna, Austria) placed at a depth of 10 cm. The samplers consisted of 5 cm-long porous cups with a pore size of 0.15 µm (tip with 2.8 mm diameter bulb) glued onto a 12 cm PVC tube, and fitted with a female luer lock suitable for creating a vacuum with syringes. The syringe was kept open with a wooden spacer until sample evacuation. The sample volumes, ranging from 1 to 10 mL, were maintained at 4 °C until filtering and analysis by ICP-OES.

### 2.3. Plant Analysis

Metal concentrations in plant tissues were determined from ~0.4 g oven-dried ground shoot samples. After adding 7 mL HNO<sub>3</sub> (65% *v/v*) and 1 mL H<sub>2</sub>O<sub>2</sub> (30% *v/v*) to the samples, they were microwave acid-digested, following EPA method 3052 [23]. Similar to the soil analysis, the samples were then diluted to 25 mL with distilled water, filtered at 0.45 µm with CA filters, and analyzed by ICP-OES.

At the end of the trial, the roots were separated from the shoot, and after soil washing and flotation were collected on a 500 µm mesh sieve, following the procedure of do Rosário et al. [24]. Root length, surface area, diameter, and number of tips and forks were measured by processing one-bit, 400 DPI (dots per inch) resolution, TIFF-format images of the roots, acquired with a flatbed scanner (Epson Expression 11000XL, Epson, Suwa, Japan) using the WinRhizo software (Regent Instruments, Ville de Québec, QC, Canada). The software allowed us to classify root length and surface area into various diameter classes.

### 2.4. Statistical Analyses

Statistical analyses were carried out with the Statgraphics Centurion XVII software (Manugistic, Rockville, MD, United States). ANOVAs and the Newman–Keuls test ( $p \leq 0.05$ ) were used to determine differences between means for growth parameters and metal accumulation in pore water and shoot tissues. Correlation analyses among element concentrations in the shoots, and between each element concentration in the shoots and in the pore water, were carried out using Pearson correlation coefficients.

## 3. Results

### 3.1. Total and Soil Pore Water Metal Concentrations

The levels of contamination with the metals artificially applied to the soil were in accordance with the target concentrations. Compared with the legal thresholds, total Zn exceeded the IGV by 7.04×, and Cu by 6.65×, whereas Co, Cd, and Pb concentrations were ~9× higher than the maximum permitted levels (Table 1). Metal contamination with sulfates led to a significant reduction in soil pH from the initial value of 8.18 to 7.19, and an increase in electrical conductivity from 0.48 to 2.62 mS cm<sup>-1</sup>.

The total concentrations of the main soil nutrients, such as K and P, were, as expected, similar in the contaminated soil and the controls, while S increased 3.7 times as a consequence of metal sulfate addition (Table 1). The total concentrations of metals in the uncontaminated controls were largely below the IGV, suggesting that the soil was suitable for maize growth.

As a consequence of artificial contamination, the concentrations of the supplied elements also greatly increased in the soil pore water compared with controls (Table 2). Lead (Pb) and Cd were below the detection limit in controls, but reached 1.9 µg L<sup>-1</sup> and

42  $\mu\text{g L}^{-1}$ , respectively, in the contaminated soil. Cu increased by 5.5 $\times$ , Zn by 82 $\times$ , and Co by as much as ~1500 $\times$  compared with controls. Thus, the solubility of Zn and especially Co increased proportionally more than their total increase. Among the macronutrients, K was also found to have increased its concentration in pore water by 6 $\times$  vs. uncontaminated controls (12.2  $\text{mg L}^{-1}$  vs. 1.72  $\text{mg L}^{-1}$ , respectively), as did Ca by 3 $\times$  (542 vs. 132  $\text{mg L}^{-1}$ , respectively) and Mg by ~2 $\times$  (88 vs. 30  $\text{mg L}^{-1}$ , respectively).

**Table 2.** Metal concentrations ( $\text{mg L}^{-1}$ ;  $\pm$  SE;  $n = 4$ ) in soil pore water extracted from pots of uncontaminated controls (Unt) vs. HM-contaminated treatment, and % variation vs. Unt. Letters in brackets indicate significant differences between treatments (Newman–Keuls test,  $p \leq 0.05$ ). Elements artificially added to the soil are highlighted in bold.

Element	Metal Concentration in Soil Pore Water ( $\text{mg L}^{-1}$ )		
	Unt	HM	% var.
Ca	132.07 $\pm$ 21.230 (b)	542.730 $\pm$ 17.59 (a)	+311
<b>Cd</b>	<b>b.d.l.</b> <sup>1</sup>	<b>0.042 <math>\pm</math> 0.003 (a)</b>	—
<b>Co</b>	<b>0.0004 <math>\pm</math> 0.00007 (b)</b>	<b>0.620 <math>\pm</math> 0.093 (a)</b>	<b>+154,900</b>
<b>Cu</b>	<b>0.02 <math>\pm</math> 0.002 (b)</b>	<b>0.131 <math>\pm</math> 0.008 (a)</b>	<b>+555</b>
K	1.72 $\pm$ 0.291 (a)	12.170 $\pm$ 1.229 (b)	+606
Mg	30.25 $\pm$ 3.732 (b)	88.360 $\pm$ 8.839 (a)	+192
Ni	0.005 $\pm$ 0.0006 (a)	0.004 $\pm$ 0.0005 (b)	−20
P	0.032 $\pm$ 0.016 (a)	0.107 $\pm$ 0.034 (a)	+234
<b>Pb</b>	<b>b.d.l.</b>	<b>0.0019 <math>\pm</math> 0.001 (a)</b>	—
S	55.03 $\pm$ 4.348 (b)	482.300 $\pm$ 21.220 (a)	+776
<b>Zn</b>	<b>0.022 <math>\pm</math> 0.011 (b)</b>	<b>1.834 <math>\pm</math> 0.447 (a)</b>	<b>+8236</b>

<sup>1</sup> b.d.l.: below detection limit by inductively-coupled plasma optical emission spectrometry (ICP-OES).

### 3.2. Shoot Metal Accumulation and Plant Biomass

As a result of soil contamination and high solubility, shoot concentrations of the applied metals increased considerably compared with controls. Zinc increased by 27 $\times$  (75.8  $\text{mg kg}^{-1}$  vs. 2.76  $\text{mg kg}^{-1}$  DW), Cu by 34 $\times$  (23.7  $\text{mg kg}^{-1}$  vs. 0.71  $\text{mg kg}^{-1}$  DW), Co by 271 $\times$  (9.75  $\text{mg kg}^{-1}$  vs. 0.032  $\text{mg kg}^{-1}$  DW), Cd by 2.7 $\times$  (0.89  $\text{mg kg}^{-1}$  vs. 0.34  $\text{mg kg}^{-1}$  DW), and Pb by 58 $\times$  (1.85  $\text{mg kg}^{-1}$  vs. 0.032  $\text{mg kg}^{-1}$  DW). The highest increases were therefore in Co and Pb, although their absolute values were low, i.e., a few  $\text{mg kg}^{-1}$  DW (Table 3).

**Table 3.** Element concentrations ( $\text{mg kg}^{-1}$  DW;  $\pm$  SE;  $n = 4$ ) in shoots of maize plants var. Naudi at the 3–4 leaf stage, after 41 days of cultivation in uncontaminated controls (Unt) vs. HM-contaminated soil, and % variation vs. Unt. Letters in brackets indicate significant differences between treatments in the same metal (Newman–Keuls test,  $p \leq 0.05$ ). Elements artificially added to the soil are highlighted in bold.

Element	Shoot Metal Concentration ( $\text{mg kg}^{-1}$ )		
	Unt	HM	% var.
Ca	9601.000 $\pm$ 836.000 (b)	19,182.000 $\pm$ 2438.000 (a)	+100
<b>Cd</b>	<b>0.337 <math>\pm</math> 0.169 (b)</b>	<b>0.897 <math>\pm</math> 0.448 (a)</b>	<b>+166</b>
<b>Co</b>	<b>0.036 <math>\pm</math> 0.018 (b)</b>	<b>9.754 <math>\pm</math> 4.877 (a)</b>	<b>+26,994</b>
<b>Cu</b>	<b>0.706 <math>\pm</math> 0.353 (b)</b>	<b>23.700 <math>\pm</math> 11.850 (a)</b>	<b>+3257</b>
K	2514.000 $\pm$ 1250.000 (a)	1600.000 $\pm$ 800.100 (b)	−36
Mg	614.500 $\pm$ 307.3 (b)	2506.000 $\pm$ 1253.000 (a)	+308
Ni	0.991 $\pm$ 0.496 (a)	0.124 $\pm$ 0.062 (b)	−87
P	295.900 $\pm$ 148.000 (a)	331.900 $\pm$ 165.900 (a)	+12
<b>Pb</b>	<b>0.032 <math>\pm</math> 0.016 (b)</b>	<b>1.851 <math>\pm</math> 0.925 (a)</b>	<b>+5684</b>
S	429.500 $\pm$ 214.700 (b)	2929.000 $\pm$ 1465.000 (a)	+582
<b>Zn</b>	<b>2.762 <math>\pm</math> 1.380 (b)</b>	<b>75.800 <math>\pm</math> 37.900 (a)</b>	<b>+2644</b>



Mineral nutrition was also assessed and found to be significantly altered following soil contamination: accumulation of K was lower (−36% vs. controls), but of Ca, Mg, and S was higher ( $p \leq 0.05$ ). More precisely, Ca doubled, Mg increased by 4.1×, and S by 6.8×. Phosphorus also slightly increased (+12% vs. controls) but the variation was not significant. Among the toxic metals, shoot Ni was found to be significantly reduced (by 87%) in polluted soil (Table 3).

Correlation analysis among element concentrations in the shoots of both contaminated and control plants together revealed positive correlations among all the contaminants applied to the soil, i.e., Cd, Co, Cu, Pb, and Zn (Table 4). Among the macronutrients, P was not correlated with any of the analyzed elements, whereas K was significantly negatively correlated with the uptake and translocation to the shoot of the applied metals; only the K–Cu correlation was not significant, but still negative.

**Table 4.** Pearson correlation coefficients among element concentrations in the shoots of maize plants var. Naudi at the 3–4 leaf stage after 41 days of cultivation. Analysis includes both uncontaminated controls and metal-contaminated treatments.

	Ca	Cd	Co	Cu	K	Mg	Ni	P	Pb	S	Zn
Ca		0.94 **	0.98 **	0.97 **	−0.74 *	0.97 **	−0.75 ns	0.15 ns	0.82 *	0.97 **	0.92 **
Cd	0.94 **		0.96 **	0.89 **	−0.80 *	0.97 **	−0.62 ns	0.02 ns	0.66 ns	0.92 **	0.86 **
Co	0.98 **	0.96 **		0.98 **	−0.71 *	0.97 **	−0.84 *	0.17 ns	0.78 *	0.97 **	0.89 **
Cu	0.97 **	0.89 **	0.98 **		−0.62 ns	0.91 **	−0.82 *	0.21 ns	0.84 **	0.97 **	0.90 **
K	−0.74 *	−0.80 *	−0.71 *	−0.62 ns		−0.83 *	0.70 ns	0.48 ns	−0.69 ns	−0.79 *	−0.78 *
Mg	0.97 **	0.97 **	0.97 **	0.91 **	−0.83 *		−0.82 *	0.05 ns	0.74 *	0.96 **	0.92 **
Ni	−0.75 ns	−0.62 ns	−0.84 *	−0.82 *	0.70 ns	−0.82 *		0.27 ns	−0.70 ns	−0.83 *	−0.83 *
P	0.15 ns	0.02 ns	0.17 ns	0.21 ns	0.48 ns	0.05 ns	0.27 ns		−0.10 ns	0.02 ns	−0.08 ns
Pb	0.82 *	0.66 ns	0.78 *	0.84 **	−0.69 ns	0.74 *	−0.70 ns	−0.10 ns		0.86 **	0.79 *
S	0.97 **	0.92 **	0.97 **	0.97 **	−0.79 *	0.96 **	−0.83 *	0.02 ns	0.86 **		0.96 **
Zn	0.92 **	0.86 **	0.89 **	0.90 **	−0.78 *	0.92 **	−0.83 *	−0.08 ns	0.79 *	0.96 **	

\* Significance at  $p \leq 0.05$ ; \*\* significance at  $p \leq 0.01$ ; ns: not significant.

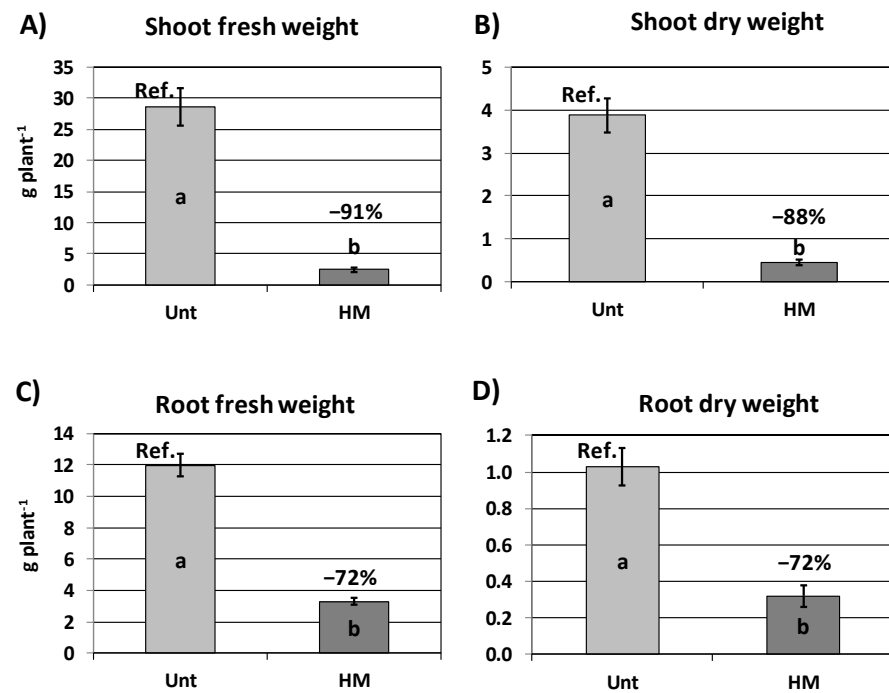
Correlation analyses among element concentrations in the shoots in each treatment dataset separately (contaminated soil and controls) revealed only a few significant coefficients (Table S1), likely due to the more limited range of variation and number of replicates. In controls, K was negatively correlated with the applied metals, although significantly only with Cd ( $r = -0.92$ ;  $p < 0.05$ ). In contrast, K was positively but not significantly correlated with Cd, Co, Cu, and Zn in the contaminated soil.

The effect of the concentration of each element in pore water on its accumulation in the shoots was evaluated considering the whole data set, and we found generally positive correlations, although it was not the rule. Potassium showed a negative correlation, as did Zn (Table S2), which we assume to be due to the vertical drift of elements in pore water towards the deeper soil layers with poor root colonization.

Regarding growth analysis, both the shoot and root fresh weights were markedly reduced by contamination, the impairments being greater in the shoot than in the root system (−91% and −72%, respectively;  $p \leq 0.05$ ) (Figure 1). Similar reductions in the roots were recorded in terms of dry biomass (−72%;  $p \leq 0.05$ ), while above ground there was less damage in terms of dry biomass than of fresh biomass (−88% vs. −91%; both  $p \leq 0.05$ ), suggesting altered water status/turgor in metal-stressed plants.

### 3.3. Root Characteristics

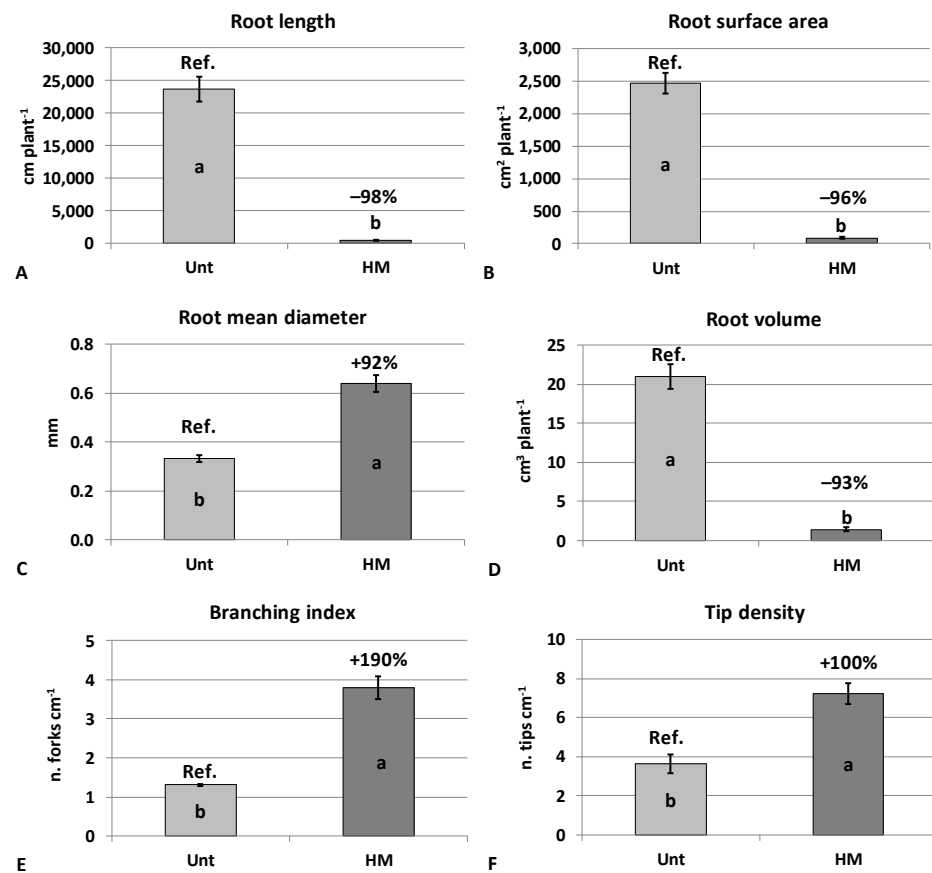
The reductions due to soil contamination in the other root parameters, i.e., length, surface area, and volume, were even greater than in root biomass: −98%, −96%, and −93%, respectively (Figure 2). On average, the variations ranged from 250 m plant<sup>−1</sup> to 4.5 m plant<sup>−1</sup> of root length, and from 2500 cm<sup>2</sup> plant<sup>−1</sup> to 89 cm<sup>2</sup> plant<sup>−1</sup> of root surface area.



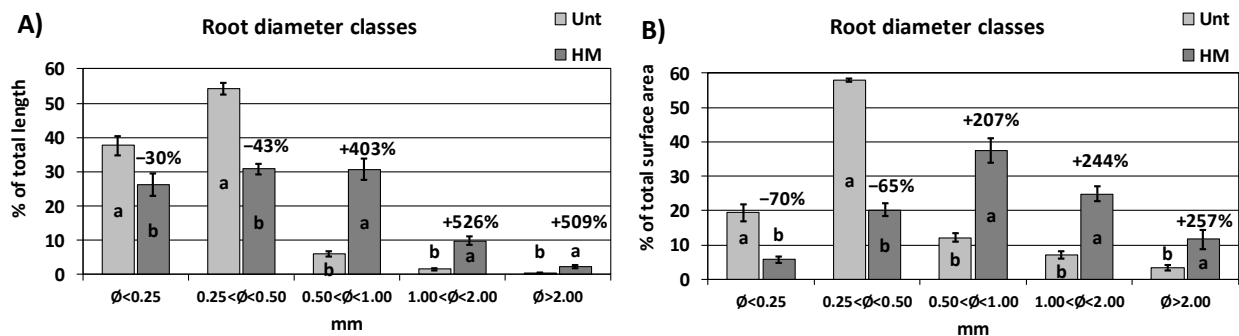
**Figure 1.** Shoot fresh (A) and dry (B) weight, and root fresh (C) and dry (D) weight ( $\text{g plant}^{-1}$ ;  $\pm$  SE;  $n = 4$ ) of maize plants var. Naudi at the 3–4 leaf stage, after 41 days of cultivation in uncontaminated controls (Unt) vs. HM-contaminated soil. Numbers above the histograms refer to the % variations vs. Unt. Letters indicate significant differences between treatments in the same parameter (Newman–Keuls test,  $p \leq 0.05$ ).

In contrast, the mean root diameter, root tip density, and branching index increased significantly in polluted soil—by 92%, 100%, and 190%, respectively (Figure 2). Root diameter doubled from 0.33 to 0.66 mm, as did tip density from 3.6 to 7.2 apices  $\text{cm}^{-1}$  of root length, although the root architecture changed mainly as a consequence of the increase in the branching index from 1.3 to 3.8 forks  $\text{cm}^{-1}$  of root length.

Regarding the distribution of the total root length and surface area across root diameter intervals, contamination caused a moderately significant reduction in the fraction (%) of finer roots (Figure 3). There was a 43% reduction in root length in the 0.25–0.50 mm diameter interval, 30% in the <0.25 mm diameter interval, and an even greater reduction in root surface area (−65% and −70% for the two diameter classes, respectively). At the same time, there was a marked increase in the root length and surface area fractions of the thicker roots in contaminated soil. The fraction of roots falling into the 1–2 mm diameter interval showed the greatest increases: by 526% in length and 244% in surface area (Figure 3).



**Figure 2.** Main root parameters, i.e., length (cm plant<sup>-1</sup>) (A), surface area (cm<sup>2</sup> plant<sup>-1</sup>) (B), mean diameter (mm) (C), volume (cm<sup>3</sup> plant<sup>-1</sup>) (D), branching index (number of forks cm<sup>-1</sup>) (E), and tip density (number of tips cm<sup>-1</sup>) (F) of the maize hybrid Naudi, grown for 41 days in uncontaminated (Unt) vs. metal contaminated (HM) soil. Numbers above the histograms refer to the % variations vs. Unt. Letters indicate significant differences between treatments (Newman–Keuls test,  $p \leq 0.05$ ).



**Figure 3.** Percentages of root length (A) and surface area (B), according to different diameter ( $\Phi$ ) classes, i.e.,  $<0.25$ ,  $0.25\text{--}0.50$ ,  $0.5\text{--}1.0$ ,  $1\text{--}2$ ,  $>2$  mm ( $\pm$  SE;  $n = 4$ ), of the maize hybrid Naudi grown for 41 days in uncontaminated (Unt) vs. metal contaminated (HM) soil. Numbers above the histograms refer to the % variations vs. Unt. Letters indicate significant differences between treatments in the same diameter interval (Newman–Keuls test,  $p \leq 0.05$ ).

#### 4. Discussion

Soil is a non-renewable resource that plays an essential role in food production and safety, affecting the yield and mineral composition/quality of cultivated plants [25]. Soil quality may therefore also be characterized by its potential impact on human health through food consumption. The presence of hazardous levels of toxic metals is one of the main issues in soil quality, due to their long-term persistence and the related effects on soil



characteristics [26,27]. In this study, artificial addition of a mixture of various metals to soil was aimed at mimicking the concomitant effects of different contaminants, a type of investigation only seldom reported in the literature. The addition of high levels of cadmium (Cd), cobalt (Co), copper (Cu), lead (Pb), and zinc (Zn) as sulfates resulted in a marked decrease in soil pH, which went from alkaline to almost neutral values (Table 1). The literature documents a decrease in pH, particularly down to acidic conditions, as enhancing the concentrations of  $Zn^{2+}$  and other metal cations in the soil solution, potentially raising their bio-availability to very toxic concentrations [28]. A high metal load together with a reduction of soil pH can significantly promote the desorption of various elements from soil particles, thus causing roots to compete for uptake of the free ions [29]. This may explain why we found an alteration in mineral nutrition (decrease of shoot K and increase of Ca, Mg, S, and P) as well as increased shoot metal concentrations (Table 3). A decrease in soil pH is sustained by the release of free  $H^+$  protons into the soil solution from clay colloids, and by changes in root exudates or in root proton extrusion [30]. An alarming increase in electrical conductivity (EC) can also be expected following metal contamination [31], although in our case we can exclude that the resulting level of salinity (i.e.,  $2.62 \text{ mS cm}^{-1}$ ) had directly constrained maize growth.

The high soil contamination artificially produced in this trial significantly increased the availability of metals, as evidenced by their greater concentrations in soil pore water (Table 2). This was most evident for the more mobile elements like Zn, Co, and Cu, in addition to S, followed by the less mobile Pb and Cd, and reflected the increased metal accumulation in shoot tissue. This is in agreement with observations made by Cui et al. [32], who reported increased concentrations of Zn, Pb, and Cd in maize with increasing rates of metal loading, and higher accumulations in shoots and roots with application of S due to its acidifying capacity.

In our study, total soil metal concentrations exhibited the following hierarchy:  $Zn > Pb > Cu > Co > Cd$ ; however, in soil pore water this changed to  $Zn > Co > Cu > Cd > Pb$ , highlighting the different levels of mobility of the metals at neutral pH. However, differential plant uptake is also expected according to the metabolic role of the metal and the species/variety of plant, the hierarchy found here being  $Zn > Cu > Co > Pb > Cd$  (Table 3). Numerous studies have shown the essential role of zinc, copper, and cobalt as micronutrients, although when present in excess they can cause phytotoxicity [25,33–36]. We found that Zn and Cu had the highest levels (absolute values) of accumulation in maize shoot tissues in both contaminated and control soils, although Cu is generally reported to have a high affinity with soil organic matter and high root retention [37]. The severe metal contamination we produced was well above the threshold of  $50 \text{ mg kg}^{-1}$  for Zn and Cu cited in the literature for phytotoxicity [9,34]. This explains the marked shoot and root growth impairments (Figures 1 and 2), with the aggravating condition of multiple contamination.

The shoot Zn concentrations we measured at the 3–4 leaf stage of maize grown in contaminated soil were much higher than the controls ( $76 \text{ vs. } 2.8 \text{ mg kg}^{-1} \text{ DW}$ ) and above the average concentrations of  $66 \text{ mg kg}^{-1} \text{ DW}$  usually found in normal plants [35]. However, in contaminated sites, Zn concentrations can be as high as  $120 \text{ mg kg}^{-1}$  in mature plants and  $40 \text{ mg kg}^{-1}$  in kernels [38]. High Zn levels can cause shoot necrosis [39] as a result of alterations to membrane integrity and permeability [40], changes in mitotic activity [41], and suppression of cell expansion [42,43]. Zinc and Cu, however, remain essential in many metabolic and physiological processes, such as photosynthesis, which explains their high absorption rates [3].

There were also greater accumulations of non-essential Cd and Pb in shoot biomass in contaminated soil than in controls, reaching  $\sim 0.9 \text{ mg kg}^{-1}$  and  $\sim 1.9 \text{ mg kg}^{-1} \text{ DW}$ , respectively, but they did not exceed the EU threshold of  $0.2 \text{ mg kg}^{-1} \text{ FW}$  ( $\sim 2 \text{ mg kg}^{-1} \text{ DW}$ ) for fodder use, and are generally negligible in maize kernels [38]. Pb and Cd are recognized as being very phytotoxic but fortunately low-mobility pollutants, particularly under neutral pH conditions, as in our trial, which explains their low levels of accumulation. In

polluted sites, maximum Cd concentrations of  $5 \text{ mg kg}^{-1}$  DW in young maize plants and  $0.4 \text{ mg kg}^{-1}$  at maturity were reported by Vamerli et al. [38], and a Pb concentration of  $13.72 \text{ mg kg}^{-1}$  in mature stalks was reported by Ibrahim et al. [44].

According to Ghani [16], Cd is one of the most toxic metals in maize, causing severe reductions in shoot, root, and grain development, followed in order by Co, Hg, and Mn [45,46]. Even at low levels, cadmium can inhibit plant growth, due to oxidative cell stress [47], reduced water uptake [48], and altered seed germination [35,49,50], although some studies have indicated non-detectable phytotoxic symptoms in various crops, including maize [51–53].

For these reasons, it is quite difficult to predict the extent of phytotoxicity from a mixture of metals. A partially additive toxic effect can at least be hypothesized in view of the close positive correlations among the accumulations of all the metal contaminants in the shoot (Table 4), although Ghani [16] suggests that when two metals are added in combination, the effect derives mainly from the most toxic one. There is also much debate on the use of total or bioavailable metal abundances to define phytotoxicity thresholds, as their true roles in plant metal accumulation are still uncertain due to other soil features, such as pH, organic matter content, oxidation, etc., which affect metal mobility [54].

In our trial, a marked nutrient imbalance was also observed alongside plant metal accumulation (Table 3). Compared to normal (controls) shoot concentrations, there was a significant decrease in K, and an increase in Ca, Mg, S, and (slightly) P, probably due to antagonistic/synergistic effects with metals [36,55]. Similar results on K were found in a pluri-contaminated site in Italian ryegrass, but not in fodder radish or sunflower, while alfalfa exhibited increased shoot K concentrations [38]. In contrast, Sarwar et al. [56] observed decreased uptake of essential nutrients, such as K, Mg, Ca, Mn, and Fe in maize under Zn–Pb–Cd contamination, suggesting a species-specific response and a strong influence of soil characteristics. However, in the contaminated treatment of our study, K concentration in shoots of maize was unexpectedly positively (although not significantly) correlated with that of metals (Table S1), likely due to the small within-group variation. As the correlations became negative and statistically significant, considering the whole data set (controls included) (Table 4), it is suggested to investigate different levels of soil contamination, in order to confirm robustly the negative impact of metals on K uptake, which may have negative physiological and morphological implications. Some negative correlations, like K–Mg and Zn–Cd, are well known in many plants [57], but our results suggest the need to explore the possibility of limiting metal uptake and phytotoxicity through adequate K fertilization. This should be experimentally verified, and root metal retention/accumulation as a detoxification strategy assessed [58].

The present trial revealed marked shoot phytotoxicity, in agreement with many studies dealing with toxic metals either individually or in combination in a variety of crops [48,59–61]. We also observed a greater reduction in shoot fresh weight than dry weight, as did Bashmakov et al. [62], possibly due to increased salinity (EC) and altered water uptake. However, root impairment was greater than shoot impairment, as Ghani [16] also found, and the most sensitive parameter was root length (Figures 1 and 2). While roots may mitigate metal toxicity by releasing chelating exudates [63,64], more effective plant defense strategies seem to be reducing the root length/surface and increasing the root diameter, which would explain the increased fraction of coarse roots and the more branched root systems we found in this trial (Figure 3). Thicker roots reduce radial Zn transport to vascular bundles [65,66], while shorter roots mean less soil exploration and hence less direct exposure to toxic elements [67], although this may increase metal drift and leaching [68]. Similar results have been observed in maize at increasing Cd concentrations, with reductions in the length of the primary root and the lateral roots [66,69], but this does not seem to be a general response in plants. For example, Bochicchio et al. [70] found no variations in *Arabidopsis thaliana* root length under Cd–Zn–Cu in hydroponics, and even increased root length and branching under Zn alone.

Metal exclusion and root cell wall and vacuole compartmentalization are general tolerance mechanisms in non-hyperaccumulator plants that allow pollutants to be separated from the main cellular physiological process [3]. Agronomic practices may also help in reducing phytotoxicity, for example through soil capping with a thin layer of uncontaminated soil [71] or adding silicon [66].

The maize variety Naudi tested here was very sensitive to high multiple-metal contamination, and was found to be both an excluder of Cd and Pb and an accumulator of Zn, in agreement with Lu et al.'s findings [36]. However, given the intra-specific variability in metal accumulation in maize [14], there is probably scope for screening tolerant genotypes.

## 5. Conclusions

Heavy metals currently represent one of the major constraints to crop growth and productivity worldwide. We found maize at the early stages to be highly sensitive to extreme multiple metal contamination, as evidenced by marked shoot and root growth impairments, although it was mainly the shoot concentrations of Zn that were hazardous. The morphological parameters studied here suggest that maize could be used as a test plant to assess the potential phytotoxicity of contaminated soils. Detecting shoot biomass impairment would be a simple method for exposing metal phytotoxicity, although root length may be more sensitive, as it was the most affected growth parameter. Measuring the root length could be a way of screening genotypes for tolerance to metal contamination and possibly salinity. In addition, there is probably an interesting scope for future investigating into whether K fertilization might mitigate metal phytotoxicity in this crop.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2073-4395/11/1/178/s1>: Table S1: Pearson correlation coefficients ( $r$ ) among element concentrations in the shoots of maize plants var. Naudi at the 3–4 leaf stage after 41 days of cultivation in uncontaminated controls (Unt) and HM-contaminated soil. Table S2: Pearson correlation coefficients ( $r$ ) between concentrations of each element in pore water and in shoots of maize plants var. Naudi at the 3–4 leaf stage after 41 days of cultivation in uncontaminated controls (Unt) and HM-contaminated soil.

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## References

1. Garty, J.; Garty-Spitz, R.L. Lichens and particulate matter: Inter-relations and biomonitoring with Lichens. In *Recent Advances in Lichenology*; Upreti, D.K., Divakar, P.K., Shukla, V., Bajpai, R., Eds.; Springer: New Delhi, India, 2015; pp. 47–85.
2. Ouabo, R.E.; Ogundiran, M.B.; Sangodoyin, A.Y.; Babalola, B.A. Ecological risk and human health implications of heavy metals contamination of surface soil in e-waste recycling sites in Douala, Cameroun. *J. Health Pollut.* **2019**, *9*, 190310. [[CrossRef](#)] [[PubMed](#)]
3. Emamverdian, A.; Ding, Y.; Mokhberdorani, F.; Xie, Y. Heavy metal stress and some mechanisms of plant defense response. *Sci. World J.* **2015**, *2015*, 756120. [[CrossRef](#)] [[PubMed](#)]

4. Sharifi Rad, J.; Sharifi Rad, M.; Teixeira da Silva, J.A. Effects of exogenous silicon on cadmium accumulation and biological responses of *Nigella sativa* L. (Black Cumin). *Commun. Soil Sci. Plant Anal.* **2014**, *45*, 1918–1933. [[CrossRef](#)]
5. Nejad, Z.D.; Jung, M.C.; Kim, K.H. Remediation of soils contaminated with heavy metals with an emphasis on immobilization technology. *Environ. Geochem. Health* **2018**, *40*, 927–953. [[CrossRef](#)] [[PubMed](#)]
6. Bolan, N.S.; Adriano, D.C.; Naidu, R. Role of phosphorus in (im) mobilization and bioavailability of heavy metals in the soil-plant system. In *Reviews of Environmental Contamination and Toxicology*; Springer: New York, NY, USA, 2003; pp. 1–44.
7. Kumpiene, J.; Lagerkvist, A.; Maurice, C. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments—A review. *Waste Manag.* **2008**, *28*, 215–225. [[CrossRef](#)] [[PubMed](#)]
8. Shi, Y.; Huang, Z.; Liu, X.; Imran, S.; Peng, L.; Dai, R.; Deng, Y. Environmental materials for remediation of soils contaminated with lead and cadmium using maize (*Zea mays* L.) growth as a bioindicator. *Environ. Sci. Pollut. Res.* **2016**, *23*, 6168–6178. [[CrossRef](#)]
9. Chibuike, G.U.; Obiora, S.C. Heavy metal polluted soils. Effect on plants and bioremediation methods. *Appl. Environ. Soil Sci.* **2014**, *2014*, 752708. [[CrossRef](#)]
10. Balkhair, K.S.; Ashraf, M.A. Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. *Saudi J. Biol. Sci.* **2016**, *23*, S32–S44. [[CrossRef](#)]
11. Tran, T.A.; Popova, L.P. Functions and toxicity of cadmium in plants: Recent advances and future prospects. *Turk. J. Bot.* **2013**, *37*, 1–13.
12. Bortey-Sam, N.; Nakayama, S.M.M.; Ikenaka, Y.; Akoto, O.; Yohannes, Y.B.; Baidoo, E.; Mizukawa, H.; Ishizuka, M. Human health risks from metals and change metalloids via consumption of food animals near Gold Mines in Tarkwa, Ghana: Estimation of the daily intakes and target hazard quotients (THQs). *Ecotoxicol. Environ. Saf.* **2015**, *111*, 160–167. [[CrossRef](#)]
13. Farid, M.; Shakoor, M.B.; Ehsan, S.; Ali, S.; Zubair, M.; Hani, M.S. Morphological, physiological and biochemical responses of different plant species to Cd stress. *Int. J. Chem. Biochem. Sci.* **2013**, *3*, 53–60.
14. Anjum, S.A.; Tanveer, M.; Hussain, S.; Ullah, E.; Wang, L.; Khan, I.; Shahzad, B. Morpho-physiological growth and yield responses of two contrasting maize cultivars to cadmium exposure. *Clean Soil Air Water* **2016**, *44*, 29–36. [[CrossRef](#)]
15. Ali, H.; Khan, E.; Sajad, M.A. Phytoremediation of heavy metals—Concepts and applications. *Chemosphere* **2013**, *91*, 869–881. [[CrossRef](#)] [[PubMed](#)]
16. Ghani, A. Toxic effects of heavy metals on plant growth and metal accumulation in maize (*Zea mays* L.). *Iran J. Toxicol.* **2010**, *4*, 325–334.
17. Rizvi, A.; Khan, M.S. Heavy metal induced oxidative damage and root morphology alterations of maize (*Zea mays* L.) plants and stress mitigation by metal tolerant nitrogen fixing *Azotobacter chroococcum*. *Ecotoxicol. Environ. Saf.* **2018**, *157*, 9–20. [[CrossRef](#)]
18. Lagriffoul, A.; Mocquot, B.; Mench, M.; Vangronsveld, J. Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.). *Plant Soil* **1998**, *200*, 241–250. [[CrossRef](#)]
19. Ci, D.; Jiang, D.; Dai, T.; Jing, Q.; Cao, W. Effects of cadmium on plant growth and physiological traits in contrast wheat recombinant inbred lines differing in cadmium tolerance. *Chemosphere* **2009**, *77*, 1620–1625. [[CrossRef](#)]
20. Chen, L.M.; Lin, C.C.; Kao, C.H. Copper toxicity in rice seedlings: Changes in antioxidative enzyme activities, H<sub>2</sub>O<sub>2</sub> level, and cell wall peroxidase activity in roots. *Bot. Bull. Acad. Sin.* **2000**, *41*, 99–103.
21. Tsonev, T.; Lidon, F.J.C. Zinc in plants—An overview. *Emir. J. Food Agric.* **2012**, *24*, 322–333.
22. USEPA 1995a. EPA method 3051, microwave assisted acid digestion of sediments; sludges; soils; and oils. In *Test Methods for Evaluating Solid Waste*, 3rd ed.; US Environmental Protection Agency: Washington, DC, USA, 1995.
23. USEPA 1995b. EPA Method 3052, microwave assisted acid digestion of siliceous and organically based matrices. In *Test Methods for Evaluating Solid Waste*, 3rd ed.; US Environmental Protection Agency: Washington, DC, USA, 1995.
24. do Rosário, G.; Oliveira, M.; Van Noordwijk, M.; Gaze, S.R.; Brouwer, G.; Bona, S.; Mosca, G.; Hairiah, K. Auger sampling, ingrowth cores and pinboard methods. In *Root Methods*; Springer: Berlin/Heidelberg, Germany, 2000; pp. 175–210.
25. Chahal, V.; Chand, P.; Nagpal, A.; Kaur, K.J.; Pakad, Y.B. Evaluation of heavy metals contamination and its genotoxicity in agricultural soil of Amritsar, Punjab, India. *Int. J. Res. Chem. Environ.* **2014**, *4*, 20–28.
26. Lone, M.I.; He, Z.; Stoffella, P.J.; Yang, X. Phytoremediation of heavy-metal-polluted soils and water: Progresses and perspectives. *J. Zhejiang Univ. Sci. B* **2008**, *9*, 210–220. [[CrossRef](#)] [[PubMed](#)]
27. Tóth, G.; Hermann, T.; Da Silva, M.R.; Montanarella, L. Heavy metals in agricultural soils of the European Union with implications for food safety. *Environ. Int.* **2016**, *88*, 299–309. [[CrossRef](#)] [[PubMed](#)]
28. Akhionbare, S.M.O.; Ebe, T.E.; Akhionbare, W.N.; Ac-Chukwuocha, N. Heavy metal uptake by corn (*Zea mays*) grown on contaminated soil. *Res. J. Agric. Biol. Sci.* **2010**, *6*, 993–997.
29. Rietra, R.P.J.J.; Heinen, M.; Dimkpa, C.O.; Bindraban, P.S. Synergism on yield and fertilizer use efficiency. *Commun. Soil Sci. Plant* **2017**, *48*, 1895–1920. [[CrossRef](#)]
30. Lombi, E.; Gerzabek, M.H.; Horak, O. Mobility of heavy metals in soil and their uptake by sunflowers grown at different contamination levels. *Agronomie* **1998**, *18*, 361–371. [[CrossRef](#)]
31. Ludwig, F.; Fernandes, D.M.; Mota, P.R.D.; Villas Boas, R.L. Electrical conductivity and pH of the substrate solution in gerbera cultivars under fertigation. *Hortic. Bras.* **2013**, *31*, 356–360. [[CrossRef](#)]
32. Cui, Y.; Dong, Y.; Li, H.; Wang, Q. Effect of elemental sulphur on solubility of soil heavy metals and their uptake by maize. *Environ. Int.* **2004**, *30*, 323–328. [[CrossRef](#)]



33. McIntyre, T. Phytoremediation of heavy metals from soils. *Adv. Biochem. Eng. Biotechnol.* **2003**, *78*, 97–123.
34. Mojiri, A.; Jalalian, A. Relationship between growth of *Nitraria schoberi* and some soil properties. *J. Anim. Plant Sci.* **2011**, *21*, 246–250.
35. Yaqvob, M.; Golale, A.; Masoud, S.; Hamid, R.G. Influence of different concentration of heavy metals on the seed germination and growth of tomato. *Afr. J. Environ. Sci. Technol.* **2011**, *5*, 420–426.
36. Lu, Y.; Yao, H.; Shan, D.; Jiang, Y.; Zhang, S.; Yang, J. Heavy metal residues in soil and accumulation in maize at long-term wastewater irrigation area in Tongliao, China. *J. Chem.* **2015**, *2*, 1–9. [[CrossRef](#)]
37. Vamerali, T.; Bandiera, M.; Coletto, L.; Zanetti, F.; Dickinson, M.N.; Mosca, G. Phytoremediation trials on metal- and arsenic-contaminated pyrite wastes (Torviscosa, Italy). *Environ. Pollut.* **2009**, *157*, 884–891. [[CrossRef](#)]
38. Vamerali, T.; Bandiera, M.; Lucchini, P.; Dickinson, N.M.; Mosca, G. Long-term phytomanagement of metal-contaminated land with field crops: Integrated remediation and biofortification. *Eur. J. Agron.* **2014**, *53*, 56–66. [[CrossRef](#)]
39. Mahmood, S.; Hussain, A.; Saeed, Z.; Athar, M. Germination and seedling growth of corn (*Zea mays* L.) under varying levels of copper and zinc. *Int. J. Environ. Sci. Technol.* **2005**, *2*, 269–274. [[CrossRef](#)]
40. Stoyanova, Z.; Doncheva, S. The effect of zinc supply and succinate treatment on plant growth and mineral uptake in pea plants. *Braz. J. Plant Physiol.* **2002**, *14*, 1–9. [[CrossRef](#)]
41. Rout, G.R.; Das, P. Effect of metal toxicity on plant growth and metabolism: I. Zinc. In *Sustainable Agriculture*; Springer: Dordrecht, The Netherlands, 2009; pp. 873–884.
42. Chang, H.B.; Lin, C.W.; Huang, H.J. Zinc-induced cell death in rice (*Oryza sativa* L.) roots. *Plant Growth Regul.* **2005**, *46*, 261–266. [[CrossRef](#)]
43. Fukao, Y.; Ferjani, A.; Tomioka, R.; Nagasaki, N.; Kurata, R.; Nishimori, Y.; Maeshima, M. iTRAQ analysis reveals mechanisms of growth defects due to excess zinc in Arabidopsis. *Plant Physiol.* **2011**, *155*, 1893–1907. [[CrossRef](#)]
44. Ibrahim, K.N.; Yet, Z.R.; Som, A.M.; Razali, N.; Rahaizah, N.A.M.; Othman, E.N.; Burok, N.A.; Mohd, Y.; Othman, R.; Fazli, T.; et al. Heavy metal concentration (Pb, Cu, Fe, Zn, Ni) in plant parts of *Zea Mays* L. cultivated in agricultural area near Alor Gajah, Melaka. *Am. J. Environ. Eng.* **2015**, *5*, 8–12.
45. Hough, R.L.; Hough, R.L.; Young, S.D.; Crout, N.M.J. Modelling of Cd, Cu, Ni, Pb and Zn uptake, by winter wheat and forage maize, from a sewage disposal farm. *Soil Use Manag.* **2003**, *19*, 19–27. [[CrossRef](#)]
46. He, S.Y.; Yang, X.E.; He, Z.; Baligar, V.C. Morphological and physiological responses of plants to cadmium toxicity: A review. *Pedosphere* **2017**, *27*, 421–438. [[CrossRef](#)]
47. Sandalio, L.M.; Dalurzo, H.C.; Gómez, M.; Romero-Puertas, M.C.; Del Río, L.A. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.* **2001**, *52*, 2115–2126. [[CrossRef](#)] [[PubMed](#)]
48. Khan, S.; Naz, A.; Asim, M.; Ahmad, S.S.; Youssaf, S.; Muhammad, S. Toxicity and bioaccumulation of heavy metals in spinach seedlings grown on freshly contaminated soil. *Pak. J. Bot.* **2013**, *45*, 501–508.
49. Peralta-Videa, J.R.; Gardea-Torresdey, J.L.; Gomez, E.; Tiemann, K.J.; Parsons, J.G.; Carrillo, G. Effect of mixed cadmium, copper, nickel and zinc at different pHs upon alfalfa growth and heavy metal uptake. *Environ. Pollut.* **2002**, *119*, 291–301. [[CrossRef](#)]
50. Yuan, M.; He, H.; Xiao, L.; Zhong, T.; Liu, H.; Li, S.; Deng, P.; Ye, Z.; Jing, Y. Enhancement of Cd phytoextraction by two *Amaranthus* species with endophytic *Rhizobium* sp. JN27. *Chemosphere* **2014**, *103*, 99–104. [[CrossRef](#)]
51. Alkorta, I.; Hernández-Allica, J.; Becerril, J.M.; Amezaga, I.; Albizu, I.; Garbisu, C. Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. *Rev. Environ. Sci. Biotechnol.* **2004**, *3*, 71–90. [[CrossRef](#)]
52. Vogel-Mikus, K.; Pongrac, P.; Kump, P.; Necemer, M.; Regvar, M. Colonisation of a Zn, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. *Environ. Pollut.* **2006**, *139*, 362–371. [[CrossRef](#)]
53. Liu, J.; Qian, M.; Cai, G.; Yang, J.; Zhu, Q. Uptake and translocation of Cd in different rice cultivars and the relation with Cd accumulation in rice grain. *J. Hazard. Mater.* **2006**, *143*, 443–447. [[CrossRef](#)]
54. Bandiera, M.; Dal Cortivo, C.; Barion, G.; Mosca, G.; Vamerali, T. Phytoremediation opportunities with alimurgic species in metal-contaminated environments. *Sustainability* **2016**, *8*, 357. [[CrossRef](#)]
55. MacFarlane, G.R.; Burchett, M.D. Toxicity, growth and accumulation relationships of copper, lead and zinc in the grey mangrove *Avicennia marina* (Forsk.) Vierh. *Mar. Environ. Res.* **2002**, *54*, 65–84. [[CrossRef](#)]
56. Sarwar, N.; Saifullah Malhi, S.S.; Zia, M.H.; Naeem, A.; Bibia, S.; Farida, G. Role of mineral nutrition in minimizing cadmium accumulation by plants. *J. Sci. Food Agric.* **2010**, *90*, 925–937. [[CrossRef](#)]
57. Nan, Z.; Li, J.; Zhang, J.; Cheng, G. Cadmium and zinc interactions and their transfer in soil-crop system under actual field conditions. *Sci. Total Environ.* **2002**, *285*, 187–195. [[CrossRef](#)]
58. Wang, F.Y.; Lin, X.G.; Yin, R. Effect of arbuscular mycorrhizal fungal inoculation on heavy metal accumulation of maize grown in a naturally contaminated soil. *Int. J. Phytoremediat.* **2007**, *9*, 345–353. [[CrossRef](#)] [[PubMed](#)]
59. Devi, R.; Munjral, N.; Gupta, A.K.; Kaur, N. Cadmium induced changes in carbohydrate status and enzymes of carbohydrate metabolism, glycolysis and pentose phosphate pathway in pea. *Environ. Exp. Bot.* **2007**, *61*, 167–174. [[CrossRef](#)]
60. Auda, A.M.; Ali, E.S. Cadmium and Zinc toxicity effects on growth and Mineral nutrients of carrot (*Daucus carota*). *Pak. J. Bot.* **2010**, *42*, 341–351.

61. Naz, A.; Khan, S.; Qasim, M.; Khalid, S.; Muhammad, S.; Tariq, M. Metals toxicity and its bioaccumulation in purslane seedlings grown in controlled environment. *Nat. Sci.* **2013**, *5*, 573–579. [[CrossRef](#)]
62. Bashmakov, D.I.; Lukatkin, A.S.; Revin, V.V.; Duchovskis, P.; Brazaitytė, A.; Baranauskis, K. Growth of maize seedlings affected by different concentrations of heavy metals. *Ekologija* **2005**, *3*, 22–27.
63. Fahr, M.; Laplaze, L.; Bendaou, N.; Hocher, V.; El Mzibri, M.; Didier Bogusz, D.; Smouni, A. Effect of lead on root growth. *Front. Plant Sci.* **2013**, *4*, 1–7. [[CrossRef](#)]
64. Seshadri, B.; Bolan, N.S.; Naidu, R. Review: Rhizosphere-induced heavy metal (loid) transformation in relation to bioavailability and remediation. *J. Soil Sci. Plant Nutr.* **2015**, *15*, 524–548.
65. Redjala, T.; Zelko, I.; Sterckeman, T.; Legué, V.; Lux, A. Relationship between root structure and root cadmium uptake in maize. *Environ. Exp. Bot.* **2011**, *71*, 241–248. [[CrossRef](#)]
66. Vaculík, M.; Landberg, T.; Greger, M.; Luxová, M.; Stoláriková, M.; Lux, A. Silicon modifies root anatomy, and uptake and subcellular distribution of cadmium in young maize plants. *Ann. Bot.* **2012**, *110*, 433–443. [[CrossRef](#)]
67. Mahmood, T.; Islam, K.R.; Muhammad, S. Toxic effects of heavy metals on early growth and tolerance of cereal crops. *Pak. J. Bot.* **2007**, *39*, 451–462.
68. Bandiera, M.; Mosca, G.; Vamerali, T. Phytotoxicity and metal leaching in EDDS-assisted phytoextraction from pyrite wastes with Ethiopian mustard and fodder radish. *Plant Biosyst.* **2010**, *144*, 490–498. [[CrossRef](#)]
69. Lux, A.; Martinka, M.; Vaculík, M.; White, P.J. Root responses to cadmium in the rhizosphere: A review. *J. Exp. Bot.* **2011**, *62*, 21–37. [[CrossRef](#)] [[PubMed](#)]
70. Bochicchio, R.; Sofò, A.; Terzano, R.; Terzano, R.; Gattullo, C.E.; Amato, M.; Scopa, A. Root architecture and morphometric analysis of *Arabidopsis thaliana* grown in Cd/Cu/Zn-gradient agar dishes: A new screening technique for studying plant response to metals. *Plant Physiol. Biochem.* **2015**, *91*, 20–27. [[CrossRef](#)] [[PubMed](#)]
71. Vamerali, T.; Bandiera, M.; Mosca, G. In situ phytoremediation of arsenic- and metal-polluted pyrite waste with field crops: Effects of soil management. *Chemosphere* **2011**, *83*, 1241–1248. [[CrossRef](#)]