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Phytoremediation of Cadmium-, Lead-, and Nickel-Polluted Soils by Industrial Hemp

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Abstract: The restoration of polluted soils is crucial for ecosystem recovery services. Evidently, phytoremediation is a biological and sustainable technique that includes the use of plants to remediate heavy-metal-contaminated land; the plants should be tolerant to the contamination and capable of uptake or immobilization of the heavy metals in the soil. Moreover, defining an economically efficient approach to the remediation of a contaminated area, with the possibility of further utilization of phytoremediation biomass, renders energy crops a great option for this technique. Energy crops, in fact, are known for their ability to grow with low agricultural input, and later, the biomass product can be used to produce biofuels, bioenergy, and bioproducts in a sustainable and renewable way, creating economic potential, especially when these crops are cultivated in marginal lands. The aim of this work is to test two monoecious industrial hemp varieties in different levels of Cd, Pb, and Ni in soil. Both varieties were tolerant to levels of Cd and Pb contamination that were higher than the limit for commercial and industrial use, while Ni showed a significant effect at all the tested concentrations. The variety Futura 75 performed better than Kc Dora in terms of productivity and tolerance.

Keywords: heavy metal; contaminated soil; *Cannabis sativa* L.; phytoextraction

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1. Introduction

The challenges that agriculture will face in the near future will be determined by the growth of the world's population, which is directly linked to the increase in the use of natural resources, the finite availability of agricultural land, and climate change, which is leading to higher temperatures and greater variability in precipitation, with an increase in extreme weather events [1]. Therefore, lands that are suitable for food production can be hindered by soil contamination [2]. In this context, the adoption of sustainable farming systems to restore ecosystems while sequestering atmospheric carbon will be necessary to overcome these challenges [3].

Human activities are the primary source of soil contamination with heavy metals. For example, the residues from mining, pesticides, and herbicides that are used in agricultural activities; residues from the petroleum industry or its derivates; residues from battery production; and the inappropriate discard of electronic components are some of the human actions that result in soil contamination with heavy metals [4]. Among all the contaminants that can compromise the quality of the soil, heavy metals can be hazardous for human health and the ecosystem in general, despite some of the heavy metals being used by humans and animals as micronutrients due to the process of bioaccumulation in the food chain and the impossibility of degradation [5]. The most common heavy-metal pollution that has originated from agriculture concerns Zn, As, Cd, Pb, Cu, Se, and U. Soil contamination of Cd, Pb, As, and Hg originates from mining and smelting activities, while Cd, Hg, Cr, As, Cu, Co, Ni, and Zn contamination originates from waste disposal. Another source of heavy metal contamination is atmospheric deposition in proximity to urban areas, which is relevant for As, Pb, Cu, Cd, Cr, Zn, and Hg. Overall, the most common cases of soil pollution from heavy metals concern As, Pb, Cr, Hg, Cu, Cd, and U [6]

Furthermore, excess absorption of heavy metals by humans and animals can cause serious health problems, for example, by damaging the nervous system or generate tumors [7]. Metal toxicity is due to the ability of these metals to alter biological mechanisms at the cellular and the molecular level. For example, Cr, Be, As, V, Cu, Ni, are genotoxic, i.e., they cause DNA mutation. Pb and Cd increase the incidence of tumors and cancers indirectly, decreasing the efficiency of the immune system in repairing chemical damage affecting the DNA [8,9]. A European commission report estimated a total number of 2.5 million potentially contaminated sites in Europe, and it is expected that 340,000 of these sites are contaminated and likely to require remediation, showing the significance of this problem [10]. The most frequent contaminants are heavy metals, affecting 35% of European soils [11,12].

Soil decontamination can be attained by following different paths: using chemical, physical, or biological techniques or a mix of them [13]. Phytoremediation is a biological technique for decontaminating the soil that uses plants to extract or stabilize the contaminants [14]. The plants are selected based on several criteria, such as tolerance to heavy metals, high biomass yield, deep and extensive root system, and awareness of using low agronomic inputs [15].

Many energy crops meet these requirements, and the biomass that they obtain on contaminated soils can be used as a feedstock for energy production (heat, biofuels, biogas) or in the bioproducts field (textile, paper, mats, bioplastics) with low environmental and health risks [16].

Currently, the use of land to cultivate crops for bioenergy has become an important policy objective, set out in RED II (Renewable Energy Directive, 2018 EU) [17]. Several industrial crops have been evaluated, such as giant reed [18], switchgrass [19], castor [20], safflower [15], camelina [21], flax [22], and kenaf [23]. Among all these crops, hemp appears to show a phytoremediation potential, with the possibility of reusing the biomass in several methods of conversion [24].

Various studies on industrial hemp (*Cannabis sativa* L.) have demonstrated the ability of this plant to accumulate toxic trace metals such as lead, cadmium, magnesium, copper, chromium, and cobalt and, therefore, reclaim contaminated soil while offering different end uses for its biomass. Mihoc et al. (2012), Canu et al. (2022), De Vos et al. (2023), and Shi et al. (2012) observed that hemp can offer a sustainable and economic solution for soil decontamination [25–28].

Historically, hemp has been grown for its long bast fibers and seeds, although, in modern times, it can also be grown for energy production [29]. Its high cellulose content renders hemp an attractive annual crop for second-generation bioethanol production [30].

Hemp can be grown under various agroecological conditions, varying in temperature, photoperiod, and soil water availability, by choosing planting date and variety according to the local condition [31,32]. In addition, hemp varieties can be classified according to several attributes such as geographic origin, end use (fiber or seed), ripening time, and reproductive system (dioecious or monoecious) [31].

As reported by the European Environmental Agency in the industrial pollution profiles of countries, the most abundant heavy metals from industrial waste in Italy, considering the period from 2007 to 2016, were cadmium, lead, and nickel [33,34]. For this reason, this research aims to evaluate the adaptability of two monoecious industrial hemp varieties: Futura 75, a French late-ripening cultivar which is one of the most cultivated varieties of industrial hemp in South Europe due to its excellent acclimatization to high temperatures; and KC Dora, a Hungarian variety that can achieve high biomass and seed yield in a broad spectrum of climatic conditions, including those of the Mediterranean area.

Both varieties were tested under three different levels of cadmium, lead, and nickel soil contamination in order to assess their phytoremediation potential and the effects of the pollutants on the yield of hemp in the southern Mediterranean area.

2. Materials and Methods

A two-year experiment (2020/2021) was carried out at the Department of Agriculture, Food and Environment—University of Catania (Sicily, Italy). In a block-randomized experimental design, the following factors were studied in pots with three replications in order to evaluate the tolerance of two varieties of *Cannabis sativa* L. (Futura 75 and KC Dora) in soils contaminated with three heavy metals (Ni, Cd, Pb) that were applied in the soil as nitrate (Cd (NO_3), Pb (NO_3), Ni (NO_3)). The amount of the single contaminant in the soil was decided according to the Italian law limit, which was referred to in sites for commercial and industrial use, as reported in D.Lgs 3 April 2006 n.152 (2006) [35] (Table 1).

Table 1. Heavy metal concentration at the legal limit for commercial and industrial sites and the levels of contamination applied to the experimental pots.

Contaminant	Cd	Pb	Ni
Legal limit (mg kg^{-1})	15	1000	500
Concentration I (mg kg^{-1})	60	1000	500
Concentration II (mg kg^{-1})	90	1500	1000
Concentration III (mg kg^{-1})	120	2000	1500
Concentration IV (mg kg^{-1})	150		

The non-contaminated soil was investigated as a control group.

The soil (Andisol, USDA) that was used was taken from the area of Mount Etna and was sampled at a depth of 30 cm.

At the start of the experiments, the soil was analyzed by collecting 1 kg of soil that had been dried in an oven (Herather, Thermo Fisher Scientific Inc., Waltham, MA, USA), at a temperature ranging from 25 to 30 °C and then sieved through a 2 mm mesh.

The sample size was measured, and electrical conductivity was measured in 1:1 soil/distilled water suspensions after 1 h by using conductivity electrodes (Hydros 21, Meter Group Inc., Pullman, WA, USA).

For the measurement of pH (H_2O), a pH meter P.H. 7 Vio (XS Instruments, Carpi, Italy) was used. Soil organic matter was determined via the Walkley–Black procedure [36].

Quantification of the total metal content (Cd, Ni and Pb) of the soil was performed by using the aqua regia digestion samples according to ISO 11466 (ISO, 1998) [37], and after filtration, the heavy metals in the soil were detected by flame atomic absorption spectrometry (AAnalyst 200 AA spectrometer, PerkinElmer, Waltham, MA, USA).

Furthermore, heavy metal bioavailability in the soil was determined according to ISO 17402 [38], by using an EDTA (Merck KGaA, Darmstadt, Germany) concentration of 0.05 M, pH 7.5 (close to soil pH) to a volumetric ratio of 1:20 in 1 g of soil, which was agitated for 24 h. Atomic absorption spectrometry was performed on the filtrate solution to quantitatively determine the available heavy metals.

Seeds were germinated in petri dishes, and each germinated seed was planted in peat pots and was transferred two weeks later to a contaminated pot (three plants per pot). Throughout the growing cycle, the seedlings were kept in well water. A nearby weather station recorded the main meteorological parameters. Over the 2 growing seasons (April–September), the range of the minimum temperatures was 6.7 °C to 19.8 °C and 5.3 °C to 21.1 °C in the 1st and 2nd years, respectively, while the range of maximum temperatures was 14.9 °C to 31.9 °C and 17.4 °C to 35.7 °C in the 1st and 2nd years, respectively.

The plants in each of the pots were harvested and fractionated into stems, leaves, and seeds. The biomass was then weighed and dried in an oven at 65 °C to a constant weight.

Roots were also collected, washed with ultrapure water to remove soil particles, freshly weighed, and oven-dried at 65 °C to obtain a stable weight.

After each sample was ground with a mill on a 1 mm sieve (IKA M20), 1 g of biomass was combusted in a muffle furnace at 550 °C for 5 h. Digestion of the biomass samples for heavy metals was performed with 10 mL of 1:1 nitric acid solution (65% nitric acid, Merck KGaA, Darmstadt, Germany).

Atomic absorption spectrometry (AAnalyst 200 AA Spectrometer, PerkinElmer, Waltham, MA, USA) was used to quantify the total heavy metals in the extract [19].

Data Analysis

The tolerance index (*TI*), bioconcentration factor (*BCF*), accumulation index (*mAI*), and translocation factor (*TF*) were calculated in order to evaluate the tolerance of the two industrial hemp varieties [19].

The tolerance index (*TI*) was calculated to assess the tolerance of the plants at the increasing levels of contaminants in the soil [14,19,39]. The *TI* was obtained by dividing the dry aboveground biomass of contaminated plants (g pot^{-1}) by the dry aboveground biomass of control plants (g pot^{-1}).

$$TI = \frac{dry\ aboveground\ biomass\ weight\ of\ contaminated\ plants,\ \text{g pot}^{-1}}{dry\ aboveground\ biomass\ weight\ of\ control\ plants,\ \text{g pot}^{-1}} \quad (1)$$

The modified accumulation index (*mAI*) was calculated to assess the ability of the plant to absorb the heavy metal from the soil [14,19]. It was obtained via the ratio between the metal accumulation in the contaminated plant (mg kg^{-1}) and the heavy metal accumulation in the control plants (mg kg^{-1}).

$$mAI = \frac{metal\ accumulation\ in\ the\ contaminated\ plants,\ \text{mg kg}^{-1}}{metal\ accumulation\ in\ the\ control\ plants,\ \text{mg kg}^{-1}} \quad (2)$$

The ability of the plant to uptake and accumulate the metal in the biomass was determined via the modified bioconcentration factor (*mBCF*). Soil bioavailable metal content, as determined by EDTA extraction, represents the level of heavy metal potentially extracted by the plant. Thus, this factor may represent the ability of the metal to be translocated in plants [14,19,40]. This was determined as the relationship between the heavy metal in the plant fraction (mg kg^{-1}) and the bioavailable metal in the soil (mg kg^{-1}).

$$mBCF = \frac{metal\ concentration\ in\ the\ plant\ fraction,\ \text{mg kg}^{-1}}{bioavailable\ metal\ concentration\ in\ the\ soil,\ \text{mg kg}^{-1}} \quad (3)$$

The translocation factor (*TF*) is expressed as the relationship between the concentration of metal in the aboveground fraction of the plant (mg kg^{-1}) and the concentration of metal in the root fraction of the plant (mg kg^{-1}) [19,41]. It was established as the concentration of metals in the aboveground plant fraction (mg kg^{-1}) divided by the concentration in the belowground plant fraction (mg kg^{-1}).

$$TF = \frac{metal\ concentration\ in\ the\ aboveground\ plant\ fraction,\ \text{mg kg}^{-1}}{metal\ concentration\ in\ the\ belowground\ plant\ fraction,\ \text{mg kg}^{-1}} \quad (4)$$

Potentially suitable for phytoextraction are plants with *mBCF* and *TF* indices greater than one (>1) [42].

Data were statistically analyzed by using R-4.2.3 software (R Core Team, 2013). The pollutants and their levels were treated as the main factors, and Tuckey's HSD test was used to isolate the means. The normality of the residual distribution was tested by using the Shapiro test. Differences in productivity and heavy metal concentrations between years were tested by using ANOVA.

Person's correlation matrix, based on the yields of the biomass fractions and the heavy metal concentrations in the fractions of the plants, was applied to interpret and visualize the multivariate data [15,43].

3. Results

3.1. Soil Characterization

The soil was characterized as sandy soil (Andisol, USDA), with neutral pH, low nitrogen, and high iron content (Table 2).

Table 2. Physical and chemical characteristics of the soil.

Physical Characteristics	
Clay (%)	3.0
Silt (%)	4.1
Sand (%)	92.9
Texture	Sandy
Conductivity (µS/cm)	34.2
Chemical Characteristics	
pH	7.4
Organic matter (%)	0.86
Fe (mg kg^{-1})	23.6
P (mg kg^{-1})	7
Mn (mg kg^{-1})	0.1
Cu (mg kg^{-1})	21.8

Soil bioavailable Cd, Ni, and Pb concentrations at the sowing time showed no differences between the pots that were used for Futura 75 and KC Dora (Table 3).

Table 3. Total and available heavy metal (mg kg^{-1}) in the soil.

		Total	Available
		H.M. in soil (mg kg^{-1})	H.M. in soil (mg kg^{-1})
Cd	Control	1.7 ± 0.1	1.1 ± 0.1
	60	59.0 ± 2.3	36.0 ± 2.1
	90	88.2 ± 1.4	55.3 ± 2.0
	120	119.4 ± 1.4	80.2 ± 2.3
	150	150.5 ± 2.6	112.9 ± 1.5
Pb	Control	39.6 ± 0.0	19.3 ± 0.0
	1000	1075.5 ± 46.9	570.9 ± 7.1
	1500	1546.6 ± 11.9	1116.2 ± 57.9
	2000	1808.1 ± 32.3	1465.9 ± 53.7
Ni	Control	40.3 ± 5.7	8.7 ± 1.9
	500	508.2 ± 43.1	331.0 ± 14.4
	1000	1047.3 ± 44.5	753.6 ± 29.5
	1500	1491.5 ± 18.7	1153.9 ± 16.1

In Cd-contaminated soil, the bioavailability ranged from 60.2% at the lowest level of Cd-contamination (Cd_{60}) to 75.0% at the highest level of contamination. The bioavailability of Ni in soil underwent a considerable increase from a low to a high level of contamination, ranging from 21.7% to 77.4%. In Pb-contaminated soil, the bioavailability ranged from 48.7% to 81.1%.

3.2. Morphological Measurement

The two studied hemp varieties differed in morphology but showed similar behavior in response to the heavy metal contamination (Table 4). All the plants of both Futura 75 and KC Dora varieties that were sown in uncontaminated soil survived until harvesting, while the plant survival rate decreased at high levels of contamination, particularly at Cd_{150} and Ni_{1500}, with the rate of survival approaching 50%. In uncontaminated soil, Futura

75 grew taller than KC Dora. Cd contamination did not reduce plant height and basal diameter, except for the highest concentration (Cd_{150}) in Futura 75 and at concentrations higher than 120 mg/kg in KC Dora. Ni-contamination induced the largest plant height and basal diameter reduction in both varieties. Both varieties were little affected by the lowest level of Pb contamination (Pb_{1000}), but a significant reduction in plant height and basal diameter was observed at the two higher concentrations (Pb_{1500}, Pb_{2000}).

Table 4. Plant survival per pot, height of the plant, and basal diameter. Multiple comparisons between means were performed within the different morphological measurements. Different letters indicate significant differences between the means (according to HSD at $p \leq 0.05$).

Variety	Cont	Conc.	Plant Survival (%)	Average Height (cm)	Avarage Diameter (mm)
Futura 75	Control		100 a	81.9 ± 9.6 a	4.8 ± 1.0 a
	Cd	60	100 a	88.3 ± 7.3 a	4.6 ± 0.2 a
	Cd	90	93 ab	75.4 ± 7.7 a	4.6 ± 0.3 a
	Cd	120	73 ab	80.1 ± 10.3 a	4.5 ± 0.8 a
	Cd	150	57 b	72.3 ± 7.3 a	4.3 ± 0.4 a
	Ni	500	93 ab	66.3 ± 4.1 a	3.6 ± 0.3 a
	Ni	1000	87 ab	63.0 ± 2.3 a	3.7 ± 0.3 a
	Ni	1500	53 b	64.9 ± 18.6 a	3.7 ± 0.8 a
	Pb	1000	87 ab	78.3 ± 16.4 a	4.8 ± 1.5 a
	Pb	1500	80 ab	60.4 ± 3.4 a	3.7 ± 0.6 a
	Pb	2000	73 ab	63.2 ± 9.5 a	3.5 ± 0.6 a
KC Dora	Control		100 a	77.9 ± 10.2 a	4.2 ± 1.9 a
	Cd	60	93 a	76.7 ± 5.1 a	4.9 ± 0.5 a
	Cd	90	93 a	77.3 ± 14.9 a	4.5 ± 1.1 a
	Cd	120	73 ab	65.3 ± 2.3 a	4.1 ± 0.8 a
	Cd	150	6 ab	55.3 ± 16.2 a	3.6 ± 1.3 a
	Ni	500	87 a	71.0 ± 8.4 a	4.4 ± 0.9 a
	Ni	1000	87 a	62.1 ± 12.7 a	4.0 ± 0.5 a
	Ni	1500	47 b	47.4 ± 11.4 a	3.0 ± 0.6 a
	Pb	1000	80 ab	76.5 ± 8.9 a	4.8 ± 0.9 a
	Pb	1500	87 a	75.4 ± 11.4 a	4.9 ± 0.2 a
	Pb	2000	73 ab	69.9 ± 4.6 a	3.8 ± 2.5 a

3.3. Plant Biomass Production

Biomass production can be observed in Figure 1. The two hemp varieties did not differ in biomass productivity on uncontaminated soil. However, in heavy-metal-contaminated soil, Futura 75 showed greater tolerance than KC Dora, in particular at Cd_{150}, Ni_{1500}, and Pb_{2000}, for which the biomass yield reduction in comparison with the uncontaminated control was 32%, 38%, and 38%, respectively, for Futura 75 and 47%, 71%, and 44%, respectively, for KC Dora. Both industrial hemp varieties recorded the greatest reduction in biomass yield in Ni-contaminated soil.

Regarding the biomass production, a significant difference was observed in both varieties for the dry weight of stems and leaves, whereas a not significant difference was observed in the dry weight of the roots and seeds (Table 5).

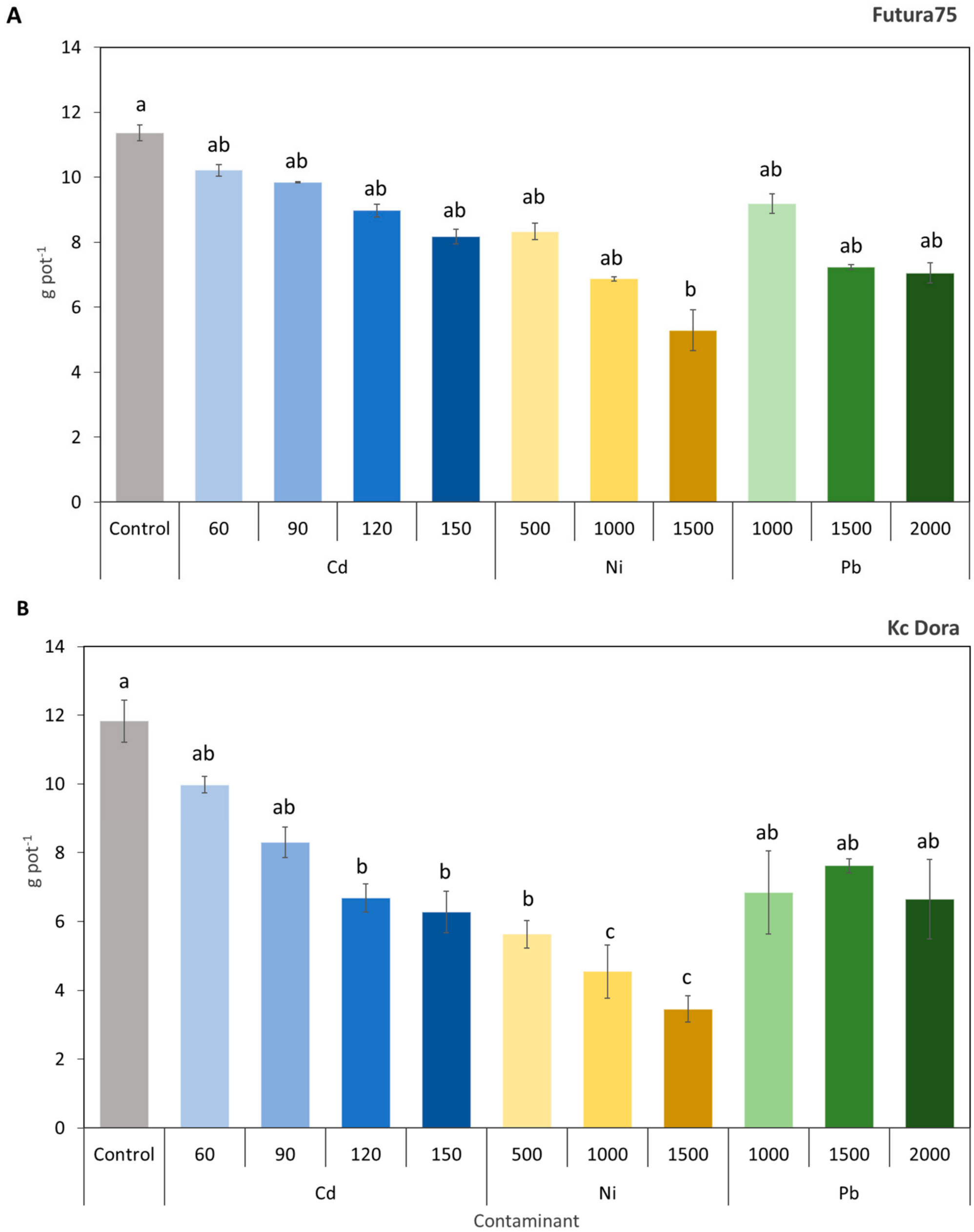

Figure 1. Aboveground biomass of Futura 75 (**A**) and KC Dora (**B**). Different letters indicate significant differences between the means (according to HSD at $p \leq 0.05$).

The biomass of the stems was significantly reduced at the concentration of Ni_{1500} and Pb_{2000} for Futura 75 and Ni_{1000} and Ni_{1500} for KC Dora. The production of leaves in the two varieties was affected by the concentration of the heavy metals: a significant reduction was observed in Cd_{120} for Futura 75 and in Ni_{1500} for KC Dora. Seed yield ranged between 0.4 and 1.5 g pot^{-1} for Futura 75, while in KC Dora, seed yield ranged between 0.3 and 1.2 g pot^{-1}. In both varieties, the highest productivity of seeds was recorded in the untreated pots.

Table 5. Average of the weight of the different compounds of the biomass (roots, stems, leaves, and seeds) in relation to different contaminants and concentrations. The multiple comparisons were performed within the fractions of the plants. Different letters indicate significant differences between the means (according to HSD at $p \leq 0.05$).

Variety	Cont.	Conc.	Average Roots Biomass (g)	Average Stems Biomass (g)	Average Leaves Biomass (g)	Average Seeds Biomass (g)
Futura 75	Control		1.6 a	6.4 ab	3.5 a	1.5 a
	Cd	60	3.2 a	6.9 a	2.7 ab	0.6 a
		90	1.4 a	5.2 ab	3.8 ab	0.9 a
		120	1.2 a	5.3 ab	3.3 b	0.4 a
		150	1.3 a	4.3 ab	3.1 ab	0.7 a
	Ni	500	1.1 a	4.0 ab	3.8 ab	0.6 a
		1000	1.2 a	4.1 ab	1.9 ab	0.9 a
		1500	0.9 a	3.0 b	1.6 ab	0.7 a
	Pb	1000	1.2 a	5.7 ab	2.7 ab	0.7 a
		1500	1.6 a	3.9 ab	2.5 ab	0.8 a
		2000	1.0 a	3.4 b	2.8 ab	0.8 a
KC Dora	Control		2.0 a	6.6 a	4.0 a	1.2 a
	Cd	60	1.6 a	5.5 ab	3.3 ab	1.3 a
		90	1.6 a	5.2 ab	2.3 ab	0.8 a
		120	0.9 a	4.2 ab	1.7 ab	0.8 a
		150	1.1 a	3.9 ab	2.1 ab	0.3 a
	Ni	500	0.9 a	2.8 ab	2.2 ab	0.6 a
		1000	0.7 a	2.5 b	1.5 ab	0.6 a
		1500	0.6 a	2.0 b	1.2 b	0.3 a
	Pb	1000	1.8 a	4.2 ab	2.0 ab	0.7 a
		1500	1.6 a	4.3 ab	2.3 ab	1.0 a
		2000	1.6 a	3.8 ab	2.0 ab	0.9 a

3.4. The Concentration of Heavy Metals in the Different Parts of the Plants

At low levels of cadmium contamination, the highest Cd concentration among plant organs in Futura 75 was observed in the leaves. At high levels of contamination, above Cd_{120}, the plants decreased the translocation of the heavy metal from the roots toward the aboveground organs, leading to a higher concentration of cadmium in the roots. KC Dora showed a larger translocation tendency for cadmium than Futura 75, which led to similar concentrations in roots and leaves at all levels of soil contamination. Cadmium concentration in the aboveground organs did not increase linearly with the concentration in the soil, suggesting the existence of a limitation factor for the translocation. Cadmium concentration in the seeds was lower than 3 $\mu g\ g^{-1}$ at any level of soil contamination.

Futura 75 showed a higher nickel uptake and translocation than KC Dora: nickel concentration in the plant tissues was higher in Futura 75 than in KC Dora in roots, leaves, stems, and seeds. A significant difference was observed in all the concentrations. Regarding the aboveground biomass, the highest concentration was observed in the leaves of Futura 75, with a concentration of 26%, 57%, and 87% for Ni_{500}, Ni_{1000}, and Ni_{1500}, respectively. In comparison, the concentration of Ni in the leaves increased in KC Dora, with a percentage of 16%, 30%, and 31% in Ni_{500}, Ni_{1000}, and Ni_{1500}.

Lead translocation potential from the roots to the aboveground organs was low for both Futura 75 and KC Dora. Both varieties showed higher lead concentration in the roots, reaching over 100 $\mu g\ g^{-1}$ at Pb_{2000}. Lead concentration was lower in the aboveground organs, staying below 40 $\mu g\ g^{-1}$ in the stem and the leaves and below 20 $\mu g\ g^{-1}$ in the seeds at the highest level of lead soil contamination for both varieties. The concentration of the contaminants can be observed for cadmium in Figure 2, for nickel in Figure 3, and for lead in Figure 4.

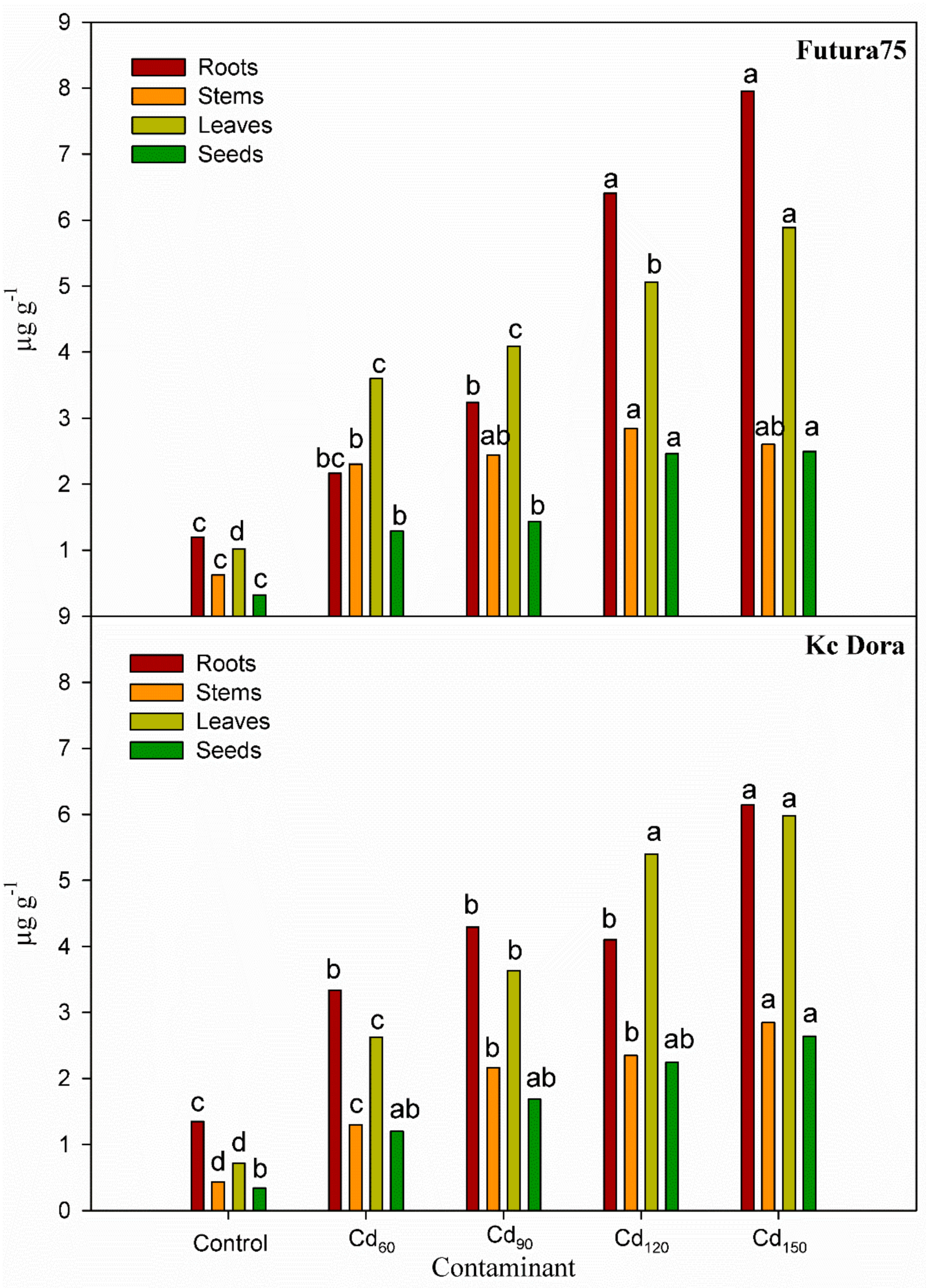

Figure 2. Concentration of cadmium (µg g^{-1}) in roots, stems, leaves, and seeds in Futura 75 and KC Dora. The comparisons were performed within the fractions of the plants. Different letters indicate significant differences between the means (according to HSD at $p \leq 0.05$).

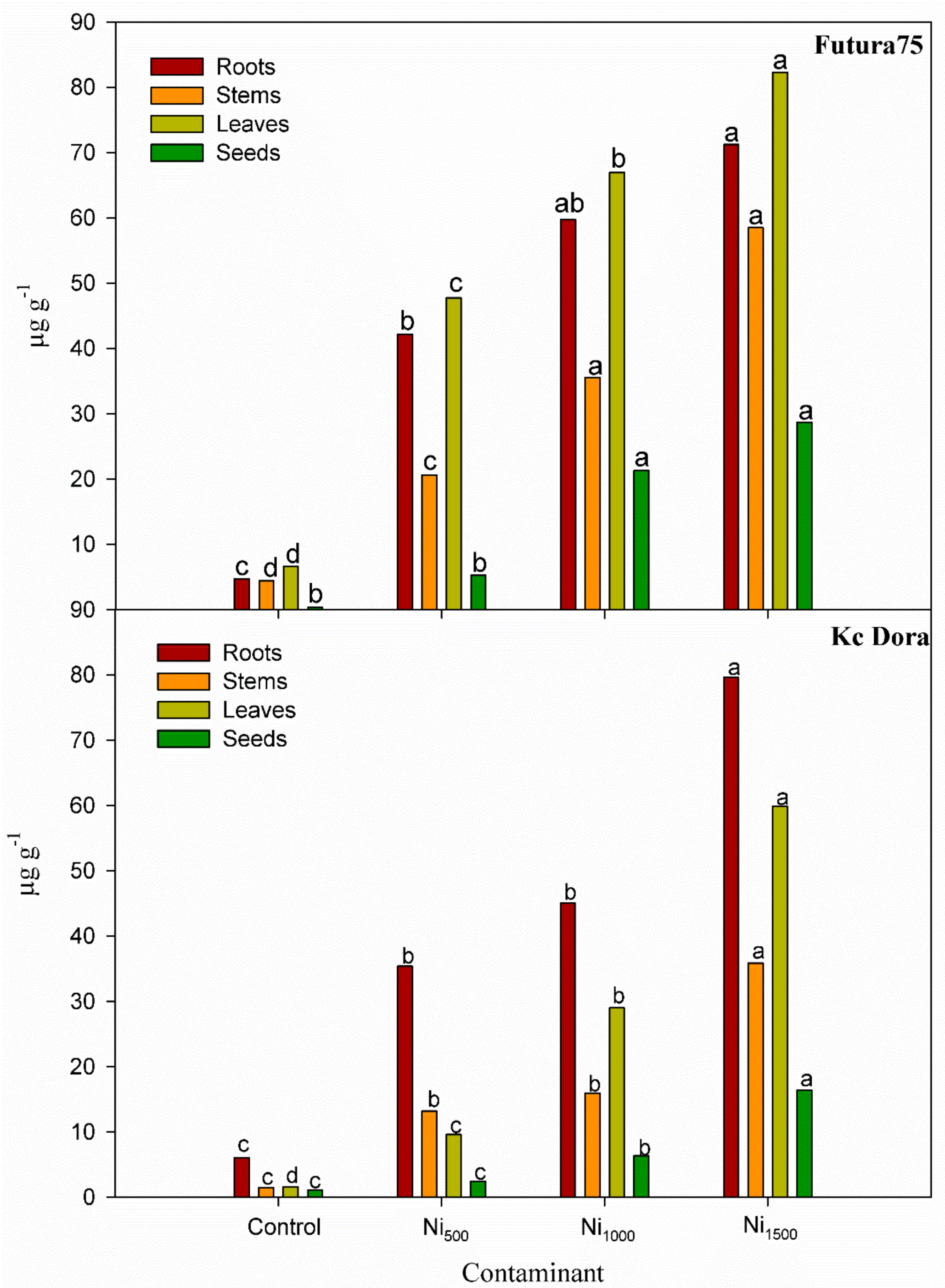

Figure 3. Concentration of nickel (μg g^{-1}) in roots, stems, leaves, and seeds in Futura 75 and KC Dora. The comparisons were performed within the plant fractions. Different letters indicate significant differences between the means (according to HSD at $p \leq 0.05$).

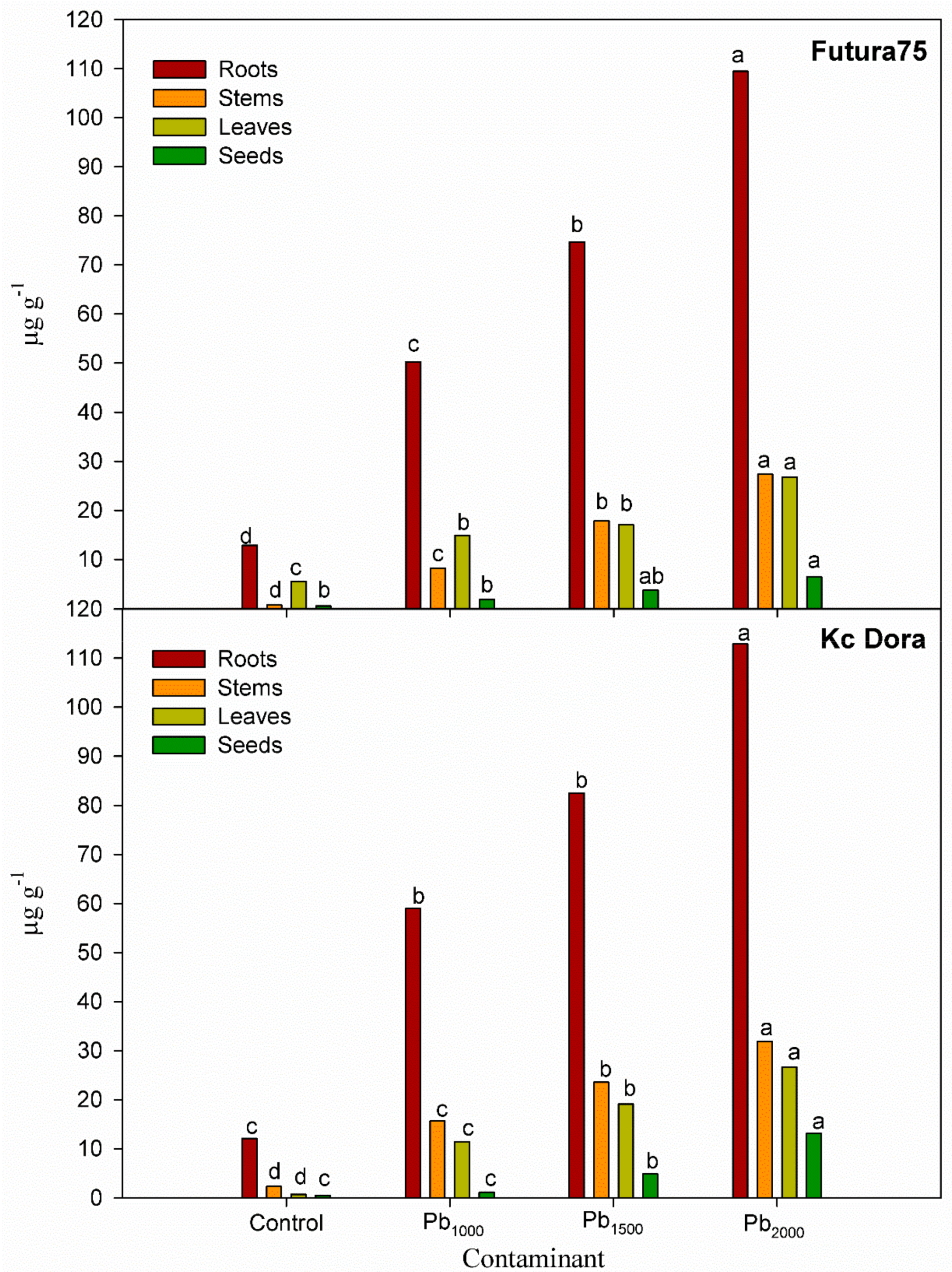

Figure 4. Concentration of lead (μg g^{-1}) in roots, stems, leaves, and seeds in Futura 75 and KC Dora. The comparisons were performed within the fractions of the plant. Different letters indicate significant differences between the means (according to HSD at $p \leq 0.05$).

3.5. Evaluating the Tolerance and the Potential Phytoextraction by Phytoremediation Index and Factors

The several indices and factors can be calculated to evaluate the adaptability to soil contamination (*TI*) and the phytoextraction potential (*mAI*, aboveground and belowground *mBCF* and *TF*) (Table 6). The TI shows the adaptability of the two industrial hemp varieties for growing in soils that were contaminated with progressive levels of cadmium, nickel, and lead.

Table 6. Phytoremediation indices and factors of phytoremediation extraction of Futura 75 and Kc Dora.

Varieties	H.M.—Conc		*TI*	*mAI*	*mBCF* Aboveground	*TF*	*mBCF* Belowground
Futura 75	Cd	60	0.90	3.66	0.28	3.32	0.09
		90	0.87	4.06	0.20	2.47	0.08
		120	0.79	5.26	0.16	1.62	0.10
		150	0.72	5.58	0.13	1.38	0.09
	Ni	500	0.73	6.55	0.34	1.76	0.20
		1000	0.60	10.90	0.26	2.07	0.13
		1500	0.46	14.92	0.16	2.38	0.07
	Pb	1000	0.81	3.68	0.05	0.50	0.10
		1500	0.64	5.71	0.03	0.52	0.07
		2000	0.62	8.94	0.05	0.55	0.08
KC Dora	Cd	60	0.84	3.45	0.14	1.54	0.09
		90	0.63	5.03	0.14	1.74	0.08
		120	0.57	6.72	0.15	2.44	0.06
		150	0.53	8.40	0.15	2.04	0.07
	Ni	500	0.62	6.02	0.12	0.71	0.17
		1000	0.51	12.27	0.11	1.14	0.10
		1500	0.35	26.79	0.15	1.41	0.11
	Pb	1000	0.75	8.12	0.04	0.49	0.09
		1500	0.64	13.39	0.04	0.58	0.07
		2000	0.56	20.19	0.06	0.60	0.09

The tolerance index decreased for the increasing level of soil contamination for both hemp varieties and all the heavy metals that were tested. The lowest TI score was observed at Ni_{1500} (0.46 and 0.35 for Futura 75 and KC Dora, respectively). Futura 75 showed higher TI than KC Dora for all the heavy metals at all the levels of contamination.

The mAI, which assesses the amount of the heavy metal uptake, increased for the increasing level of soil contamination for Futura 75 and KC Dora, indicating that the plants can phytoextract a higher amount of heavy metals from soil with high heavy metal concentrations. The highest mAI score was observed in KC Dora at Ni_{1500} e Pb_{2000}. KC Dora showed higher values of mAI than Futura 75. The comparison of aboveground and belowground mBCF gives insight into the heavy metal partitioning between plant organs. Both factors tend to decrease at high contamination levels. Under cadmium and nickel contamination, Futura 75 showed a higher aboveground mBCF than KC Dora, suggesting a better suitability for the uptake and removal of the heavy metal from the soil.

Under lead and nickel contamination, both Futura 75 and KC Dora had increasing TF scores for increasing soil concentrations. Under cadmium contamination, only KC Dora had increasing TF scores for the increasing soil Cd concentration, while the TF of Futura 75 decreased.

3.6. Correlation of the Main Factor between the Two Varieties of Industrial Hemp

A multivariate analysis was carried out to assess the effect of metal contaminants at different concentrations on variables for cadmium, nickel, and lead (Figures 5–7).

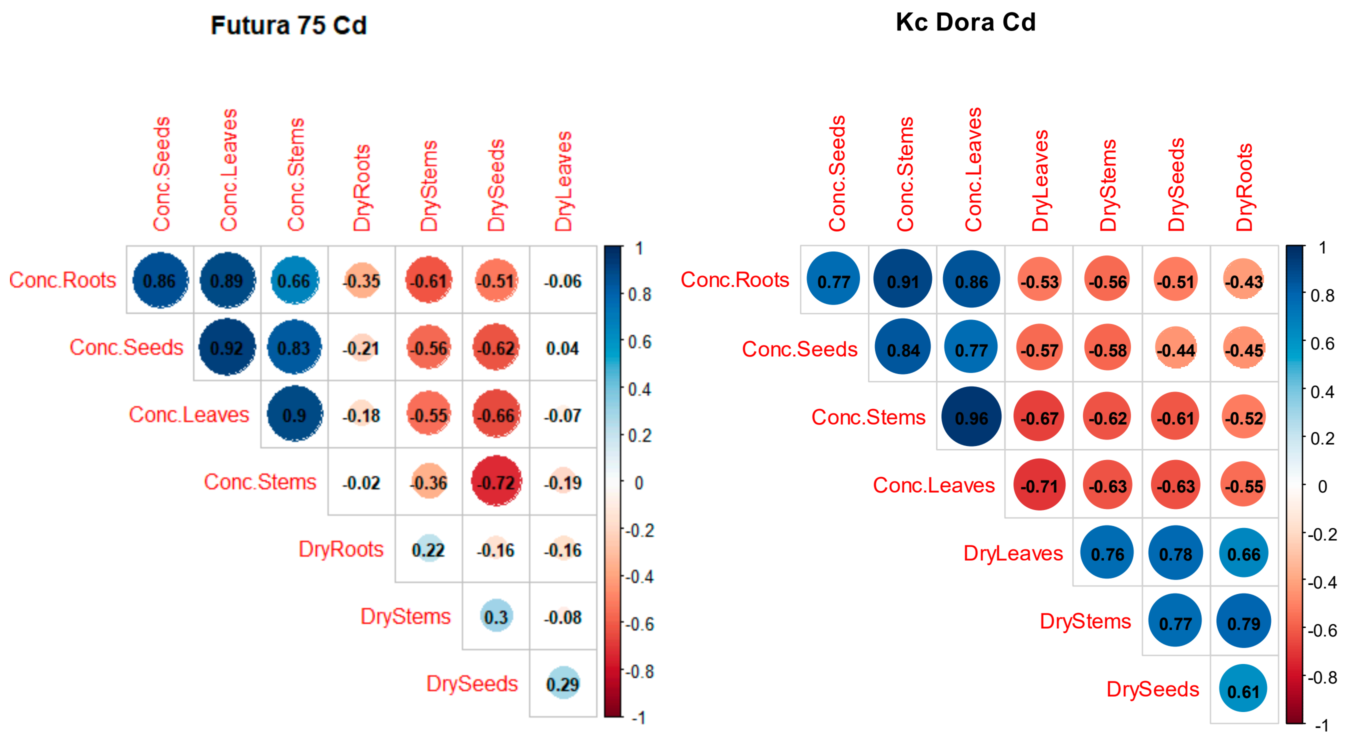

Figure 5. Correlation matrix of Cd of Futura75 and KC Dora, using as the variable the biomass yield of roots, stems, leaves, and seeds, and the concentration of the heavy metal measured in each part of the plant. The numbers within the circles represent the Pearson correlation coefficient.

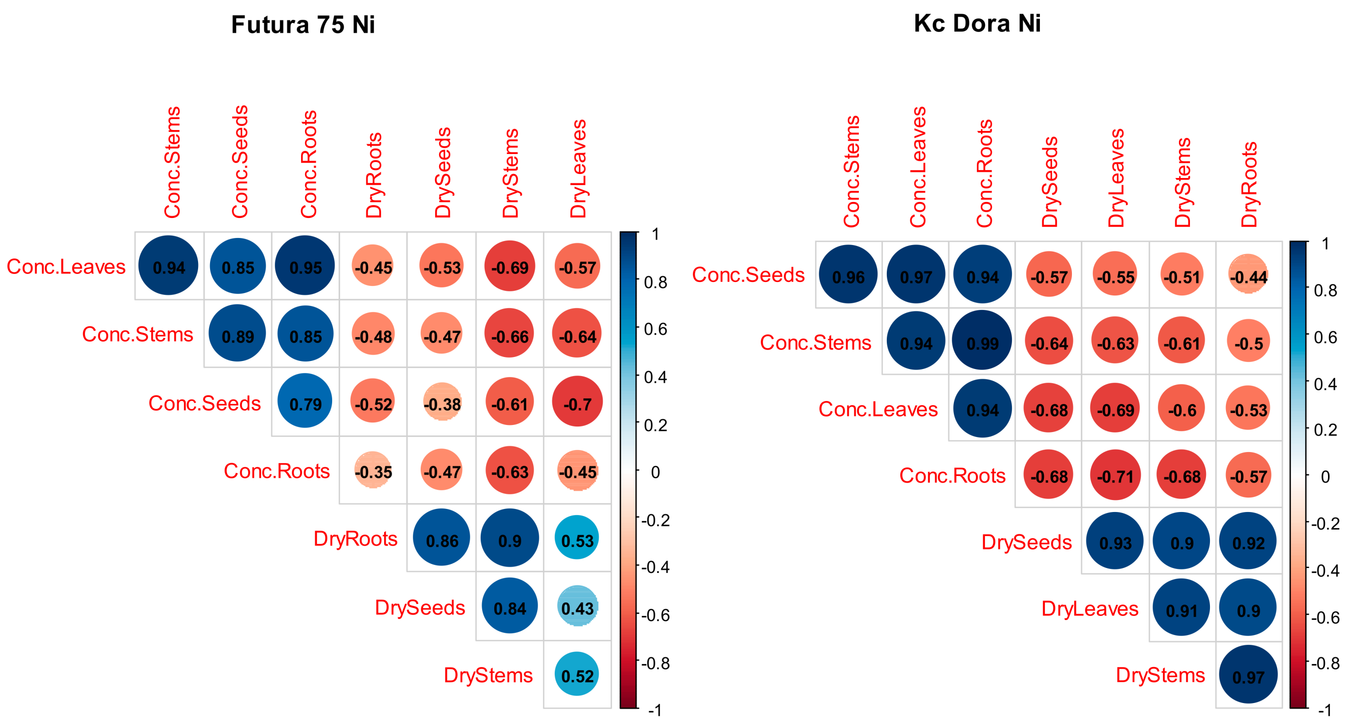

Figure 6. Correlation matrix of Ni of Futura 75 and KC Dora, using as the variable the biomass yield of roots, stems, leaves, and seeds, and the concentration of the heavy metal measured in each part of the plant. The numbers within the circles represent the Pearson correlation coefficient.

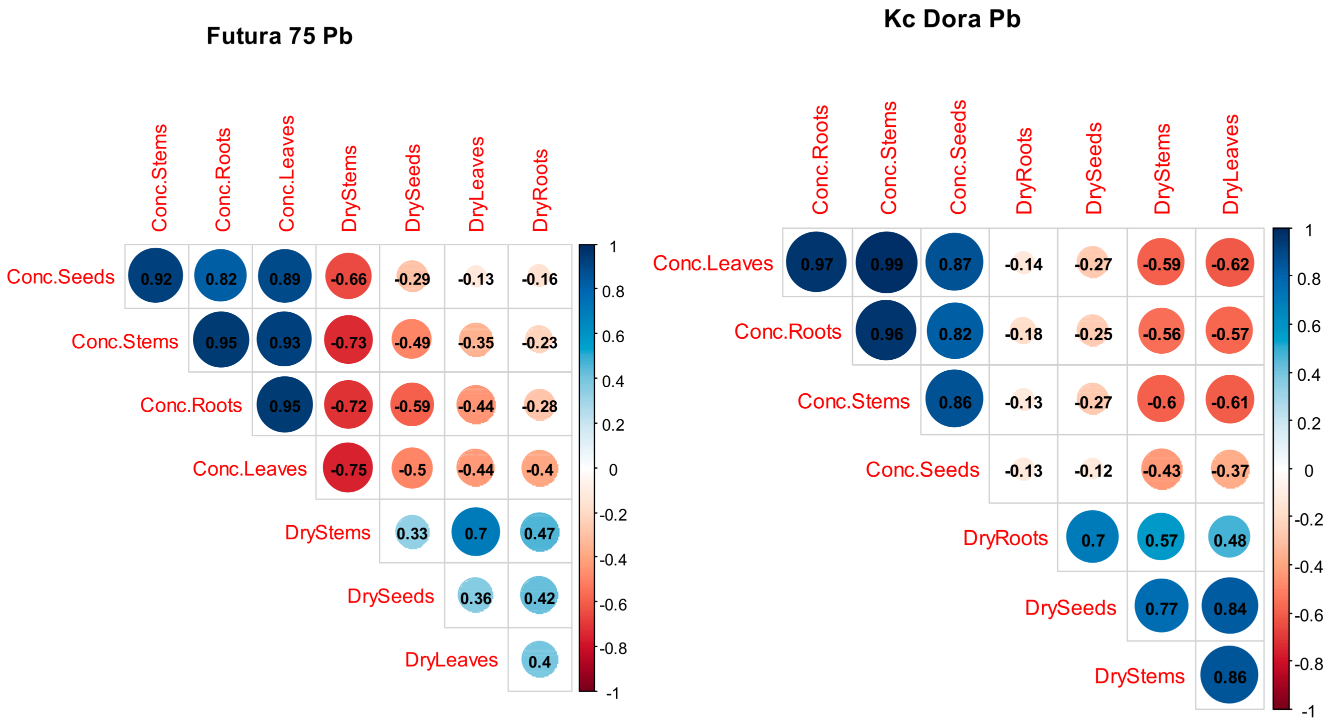

Figure 7. Correlation matrix of Pb of Futura 75 and KC Dora, using as the variable the biomass yield of roots, stems, leaves, and seeds, and the concentration of the heavy metal measured in each part of the plant. The numbers within the circles represent the Pearson correlation coefficient.

Specifically, the components of biomass yield (stems, leaves, and roots) were correlated with each other and negatively correlated with the contaminant concentration in the plant fractions (stems, leaves, seeds, and roots yield).

In fact, in cadmium-contaminated soil, in Futura 75, only the biomass of the stems and the seeds was strongly negatively affected by the concentration of cadmium in the different parts of the plant. In contrast, in KC Dora, all the biomass of the plants was strongly negatively correlated with the concentration in the various parts of the plants. Similar behavior was obtained in nickel-contaminated soil in the correlation matrix of Futura 75 and KC Dora. However, in lead-contaminated soil, the biomass of stems and leaves in KC Dora was strongly negatively correlated with the concentration of Pb in the different parts of the plant. In Futura 75, the stem biomass was strongly negatively correlated with the concentration of the heavy metal in the plant.

4. Discussion

Industrial hemp can be grown in most of the world for its high environmental adaptability [31]. The selection of the best-suited genotype for a specific environment, climatic condition, and agronomic management is crucial for crop success [32,44]. Various studies carried out on *C. sativa* have shown its potential as an accumulator for different toxic traces of metals such as lead, cadmium, magnesium, copper, chromium, and cobalt, which pose a great risk to the ecological system [24,30], making it possible to reclaim contaminated soil while it yields fiber and/or seeds [29].

All over the world, for the problem of soil contamination, hemp can provide a solution that is both economical and sustainable [22,25].

In this study, the productivity of stems in both varieties of hemp was affected by the increasing level of heavy metal, while no significant difference was observed in the seed production. However, low levels of contamination were not detrimental to the overall aboveground biomass; morphologic parameters were not affected by the heavy metal in the soil.

A similar result was observed by De Vos et al. (2023) [27], Pietrini et al. (2019) [45], and Guidi Nissim et al. (2018) [46], who reported no differences in stem height and stem diameter between the control and plants that were cultivated in a low level of soil contamination.

The present study found that Futura 75, a late ripening variety, was more tolerant than KC Dora, an early ripening variety, to high concentrations of cadmium, lead, and nickel [26,31,47].

Cadmium is considered to be one of the most phytotoxic heavy metals [27]. Linger et al. (2002) [36] showed that the photosynthetic pathway in hemp was affected by cadmium indirectly, with the uptake of water and ions by the plant, and directly in the chloroplast apparatus after entering the leaf cells. Cd concentrations up to 72 mg kg^{-1} (soil) had no negative effect on the germination of hemp. Shi et al. (2012) [28] compared 18 hemp accessions cultivated on cadmium-contaminated soils for biodiesel production. It was found that below 25 mg of cadmium per kg of dry soil, most varieties of hemp could grow quite well. Under this condition, the tolerance factor observed in hemp was high (68.6–92.3%), and the ability to store cadmium in the aerial fraction of biomass was suitable for phytoextraction, indicating that the production of this crop can be an alternative to valorize and remediate cadmium-contaminated soils.

Hemp productivity was less affected by lead contamination when compared with the highest concentration of cadmium or nickel. The translocation of lead from roots to the aerial biomass was low; therefore, the highest concentration was observed in the roots. A similar result was observed by Ahmad et al. (2016) [48] and Angelova et al. (2004) [49], who reported Pb concentrations in hemp plants in the following order: roots > stems > leaves > seeds; and by Pietrini et al. (2019) [45], who reported that hemp tends to accumulate lead mainly in the roots, with minimal translocation to the aboveground biomass, which explains the relatively low BCF for Pb that was observed in the present study.

Nickel soil contamination induced the highest reduction in biomass production among the heavy metals that were tested. Ferrarini et al. (2021) [50] reported that hemp had a reduced yield in soil that was contaminated by nickel (>500 mg kg^{-1}). Zhao et al. (2022) [22] reported a reduction in germination and biomass production even at low nickel concentrations (110 and 220 mg kg^{-1}), and both higher concentration in plant organs and higher translocation factor (*TF*) than the value observed for lead.

For cadmium and nickel, with the exception of Ni_{500}, the translocation factor was higher than 1, indicating the high suitability of hemp for the phytoextraction processes, thanks to the accumulation of the heavy metals in the aerial part of the plant.

Although the soil analysis indicated that the bio-availability of cadmium was low, the actual availability of cadmium can increase over time due to the low tendency of this metal to form complexes, while the bio-availability of lead and nickel have a higher complex rate, which reduces the bio-availability.

However, the high tolerance of hemp toward certain heavy metals in the soil renders this plant a suitable alternative for contaminated soil valorization and remediation [27].

5. Conclusions

This research highlighted the different phytoextraction capabilities among the two industrial hemp varieties and demonstrated the capability of industrial hemp to translocate metals from the soils to the aerial parts of the plants, suggesting a good potential for the phytoextraction process. Hemp showed the ability to complete its life cycle until seed ripening in heavily contaminated soils.

The two varieties were tolerant to levels of Cd and Pb contamination above the limit for commercial and industrial use, while Ni showed a significant effect at all the concentrations tested. Futura 75 performed better than Kc Dora in terms of productivity and tolerance.

The low heavy metal concentration in hemp seeds enables the utilization of this plant as a source of oil for bioenergy conversion purposes, avoiding the concerns about contaminant dispersion. The remaining biomass such as stems and leaves can be further valorized through conversion into bioenergy, raising the interest of industrial hemp. Future investigation on the bioconversion processes and on the economic viability of the entire supply chain would be useful to assess the suitability of the entire phytoremediation process.

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