

Phytomanagement of Chromium-Contaminated Soils Using *Cannabis sativa* (L.)

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Received: 8 July 2020; Accepted: 13 August 2020; Published: 19 August 2020

Abstract: This study aimed to assess whether hemp (*Cannabis sativa* L.) behaves as a Cr(III)-tolerant or a hyperaccumulator species and whether it could be a suitable species for the phytomanagement of Cr(III)-contaminated soils. The experiment was conducted in pots under greenhouse conditions comparing two fiber hemp (Fibranova and Carmagnola) and two seed hemp (Futura 75 and Fedora 17) varieties under four different soil Cr levels (24.3, 40.1, 55.8, and 87.4 mg kg⁻¹) supplied with tannery sludge. The Cr level did not significantly influence hemp biomass production or Cr accumulation in the aboveground biomass. Focusing on marketable fractions, Cr uptake was 0.03 ± 0.04 mg plant⁻¹ in the stems of fiber varieties and 0.60 ± 0.17 mg plant⁻¹ in the seeds of seed varieties. The only significant accumulation of Cr content was indeed observed in the root system, where it reached 0.63 mg plant⁻¹ for the fiber varieties and 1.76 mg plant⁻¹ for the seed varieties in the fertilization with 200% N by tannery sludge (T200) treatment. The Cr translocation factor (aboveground-to-belowground biomass) decreased from 2.17 to 0.37, increasing the Cr level applied from 24.3 to 87.4 µg g⁻¹. The maximum Cr concentrations in aboveground biomass fractions (average value of 40.4 mg kg⁻¹) were found in the seeds, regardless of treatment. The low Cr content in the aboveground biomass suggests that hemp can be considered an excluder species, valid as a candidate for Cr-contaminated soils' phytomanagement.

Keywords: chromium; polluted soil; hemp; soil phytomanagement

1. Introduction

Hemp (*Cannabis sativa* L.) comprises several varieties of plants that are intended for agricultural and industrial purposes. Hemp is one of the most ancient cultivated crops and its use is estimated to date back 10,000 years [1]. The fact that it is a multipurpose crop that can provide fiber, cellulose, seeds, and seed oil, as well as valuable metabolites such as its cannabinoids, has led to considerable interest in its research and cultivation [2]. Hemp fibers have excellent mechanical properties [3] and can be used in the textile industry, building and insulation industries, paper production, and development of new materials such as biocomposites. Hemp seeds have been used to treat various disorders for thousands of years [4], but their potential as functional foods with numerous health benefits for humans and animals has only been stated in recent years. Indeed, the seed oil characteristics such as the omega-6/omega-3 ratio, which is normally between 2:1 and 3:1 [5], make it optimal for human health. In addition, hemp seed oil can be used in cosmetics, industrial coatings, varnishes, and paints. Depending on the product desired (fiber or seed), the hemp variety should be carefully chosen.

Due to its biological characteristics such as rapid growth, high biomass production, well-developed root system, adaptability, and low susceptibility to diseases and pests [6,7], hemp has been seen as a valid candidate in phytoremediation. The latter consists of an array of techniques that use plants and their associated microorganisms to reduce, remove, degrade, or immobilize organic and inorganic contaminants in order to obtain in situ environmental restoration and reclamation of contaminated soils. Phytoremediation, through vegetation cover, may also have the ability to reduce windblown soil and the re-entrainment of particulate pollutants that would impact human health or other receptors [8]. It is considered to be a relatively inexpensive, environmentally friendly technology, as well as an effective alternative to other destructive physical or chemical processes used for the decontamination of soils. In the last three decades, several studies have reported the potential of hemp for the phytoremediation of soils contaminated by heavy metals such as Ni, Pb, Zn, Cu, and Cd [9–11]. A few studies [12,13] attempted to assess this potential in chromium (Cr)-contaminated soils, demonstrating its capability to tolerate Cr and store it in the roots. Although this field of study has grown substantially over the past decade, phytoremediation still presents several limitations concerning its efficacy and commercial implementation [14]. The difficulties lie mainly in the low efficiency of phytoextraction for most soil contaminants, as well as the resulting time constraints [15]. In addition, the Cr excess can affect the uptake of several nutrients in plants, as already reported in several studies [16–19].

As a result, an alternative approach named “phytomanagement” was developed. It concerns the use of contaminated soils to produce marketable biomass, increasing the sustainability and profitability of polluted areas without causing any substantial health risks for humans or animals [20]. In phytomanagement, the aim is not finding hyperaccumulator species but rather species adapted to the contaminated sites that produce valuable biomass for the production of bioenergy [21], fiber [22], timber, pulp, fodder, and so forth [23]. Hemp could be a valid species for phytomanagement as, in addition to its favorable agronomic features, it can also offer valuable biomass production of marketable quality [15]. However, the hemp potential for the phytomanagement of soil specifically contaminated by Cr(III) (Cr hereafter) has not yet been entirely explored.

Within this framework, the scope of our study was to assess whether hemp behaves as a tolerant or hyperaccumulator species in the presence of Cr, whether its behavior is different in cultivars for fiber or seed, and whether it could be a suitable species for the phytomanagement of Cr-contaminated soils instead of classic phytoremediation.

2. Materials and Methods

2.1. Experimental Layout

The experiment took place in a greenhouse at the “L. Toniolo” Experimental Farm of the University of Padova (45°20' N, 11°57' E, northeast Italy) from April to September 2019. Adopting a completely randomized design, the performance of 4 varieties of hemp was evaluated under 4 different Cr application levels, with 5 replicates for a total of 80 pots. Pots were located in a greenhouse to create the best conditions for Cr uptake, avoiding its leaching and maximizing plants' evapotranspiration. Of the 4 hemp varieties, 2 were fiber hemp (Fibranova and Carmagnola) and 2 were seed hemp (Futura 75 and Fedora 17). Four Cr application levels were tested using tannery sludge (Table 1) to integrate or cover mineral N hemp requirements (150 kg N ha⁻¹) [24]: (i) Fertilization with 50% N by tannery sludge and 50% N by mineral (NH₄NO₃) fertilizer (T50), (ii) fertilization with 100% N by tannery sludge (T100), (iii) fertilization with 200% N by tannery sludge (T200), and (iv) with 100% N only by mineral fertilization (Tmin). The T200 treatment was studied in order to increase the level of Cr application without changing the typical nutrients:Cr ratio of sludge.

Table 1. Tannery sludge chemical–physical characteristics.

Parameter	Value
Dry matter	89.5%
Chromium(III) *	27.5 g kg ^{−1}
Total nitrogen *	55 g kg ^{−1}
Total phosphorus *	10.0 g kg ^{−1}
Potassium *	15 g kg ^{−1}

* Values refer to sludge fresh weight.

The P and K contents in the tannery sludge were taken into consideration to calculate the amount of mineral P and K added to reach hemp requirements (150 kg P₂O₅ ha^{−1} and 100 kg K₂O ha^{−1}) [24]. Mineral P and K were added as triple superphosphate and potassium sulfate, respectively. On the basis of tannery sludge composition, the Cr added in the Tmin, T50, T100, and T200 treatments was 0, 37.5, 75, and 150 kg ha^{−1}, respectively. On the pot base, the Cr added in the T50, T100, and T200 treatments was 0.268, 0.532, and 1.073 g pot^{−1}, respectively, which corresponded to an increase of 65%, 129.6%, and 259.7%, respectively, compared to the soil Cr content in Tmin.

The trial was conducted in PVC pots with a volume of 18 L and a diameter of 30 cm. Each pot was filled with 17 kg of fluvi-calcaric cambisol according to the FAO-UNESCO classification (sand 33%, silt 47%, clay 20%). The soil used to fill pots had an average Cr content of 24.3 ± 0.4 mg kg^{−1}.

2.2. Plants Management

The hemp was sown directly in each pot on 10th April 2019 using 4 seeds pot^{−1}. After 2 weeks, only one plant per plot was left. Mineral and/or tannery sludge (TS) fertilization was performed a few hours before sowing. In the first two months of the growing cycle, irrigation was manually managed to supply twice a week a water quantity necessary to reach field capacity. This quantity was calculated after measurement of the volumetric water content in each pot using a Field Scout TDR 100 (Spectrum Technologies Inc., Aurora, IL, USA). Due to the high evapotranspiration caused by the high temperatures recorded, two months later, an automatized drip irrigation system was installed to obtain a daily water supply of 1 L pot^{−1}. During the hemp growing cycle, weeds were removed manually weekly and left on the soil surface. In addition, the height and number of leaves on each plant were measured with the same frequency. Pest management was conducted with a single treatment using insecticide and acaricide on 5th July 2019 to address some signs of pests such as aphids (Aphidoidea), brown marmorated stink bug (*Halyomorpha halys*), and, to a lesser extent, some mealybugs (Pseudococcidae) detected on top of and under the leaf surfaces of some plants between the end of June and first days of July.

2.3. Plant Harvest

Plant harvesting was performed on two different dates: 18th July 2019 for fiber varieties and 2nd September 2019 for seed varieties. Fiber varieties' biomass was divided into leaves, stems, and roots, whereas, for the seed varieties, it was divided into seeds, straw, and roots. The plant root system was extracted from each pot, washing off the soil with water. The dry matter content in each biomass fraction was determined by drying at 65 °C in a thermoventilated oven until it reached a constant weight.

2.4. Biomass Analysis and Elements Mass Balance

Dried biomass was then ground to 2 mm and chemically analyzed to determine its Cr, K, P, and N content. Cr, K, and P were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES, Arcos EOP, Spectro A. I. GmbH, Kleve, Germany), whereas the N content was determined using the Kjeldahl method (Kjeltec 2300 Auto Analyser; Foss-Tecator, Hillerød, Denmark).

The Cr, N, P, and K quantity uptakes in the different plant fractions (g plant^{−1}) were determined as the product of their dry biomass yields and nutrient concentrations.

2.5. Statistical Analysis

Statistical analyses were performed using Statgraphics Centurion XVIII, version 18.1.12, software (StatPoint Inc., Warrenton, VA, USA). Data were normally distributed (Shapiro–Wilk test; $p < 0.01$) and the homogeneity of variance was tested (Levene test; $p < 0.01$). Data were analyzed using two-way ANOVA to assess the effect of hemp varieties and Cr application level, as well as their interactions, on plant height; biomass fractions (i.e., roots, stems, leaves); Cr, N, P, and K concentrations in the hemp biomass; and its fractions. The two-way ANOVA was done separately within the single hemp type. When the ANOVA was significant ($p < 0.01$), the means were differentiated by Tukey's HSD (Honestly Significant Difference) test ($p < 0.01$).

A comparison of regression lines was performed using soil Cr concentration as the independent variable, root Cr concentration as the dependent variable, and hemp type as the classification factor. All variables used resulted in a normal distribution (Shapiro–Wilk test; $p < 0.01$), and 0.254782 was the average value of the residuals. The Durbin–Watson (DW) statistic was used to determine if there were any significant correlations based on the order in which they occurred in the data file. As the p -value was greater than 0.01, there was no indication of serial autocorrelation in the residuals at the 99.0% confidence level.

3. Results

3.1. Morpho-Biometric Characteristics

Plant height was not significantly influenced by Cr treatments. For both fiber and seed varieties, a significantly different height was measured at harvest time between studied genotypes, with the Carmagnola and Futura 75 being the tallest (Figure 1A,B). No Cr \times genotype interaction was found.

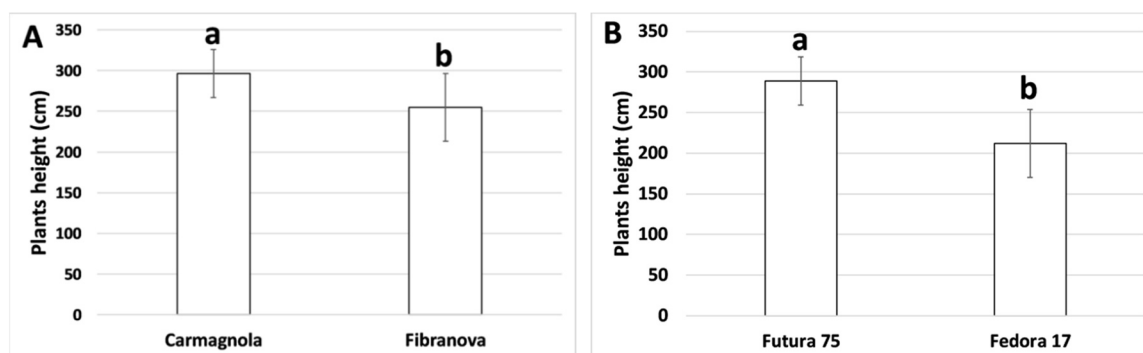


Figure 1. Plant heights of fiber (A) and seed (B) varieties at harvest time. Different letters indicate significant differences between the two varieties within each type at $p < 0.01$ (Tukey's HSD test).

Considering the whole plant biomass of the two fiber varieties, Carmagnola had a significantly ($p < 0.01$) higher biomass (+15.5%) than Fibranova (138.5 g plant⁻¹). Analyzing the single plant biomass fractions, the difference was due to a higher stem (+26.8%) biomass because no difference was observed on either the leaves or roots (Figure 2A). The chromium level and interaction with genotypes were not statistically significant for the whole biomass or the single fractions.

Taking into account the two seed varieties, Futura 75 had a significantly ($p < 0.01$) higher total biomass (+55.6%) than Fedora 17 (128.7 g plant⁻¹). For each plant biomass fraction, the Futura 75 variety produced significantly more straw (+1.6 times) and roots (+2.2 times), while no difference was observed in seed production (Figure 2B). Again, the Cr level and its interaction with genotypes did not statistically affect the whole biomass or the single fractions.

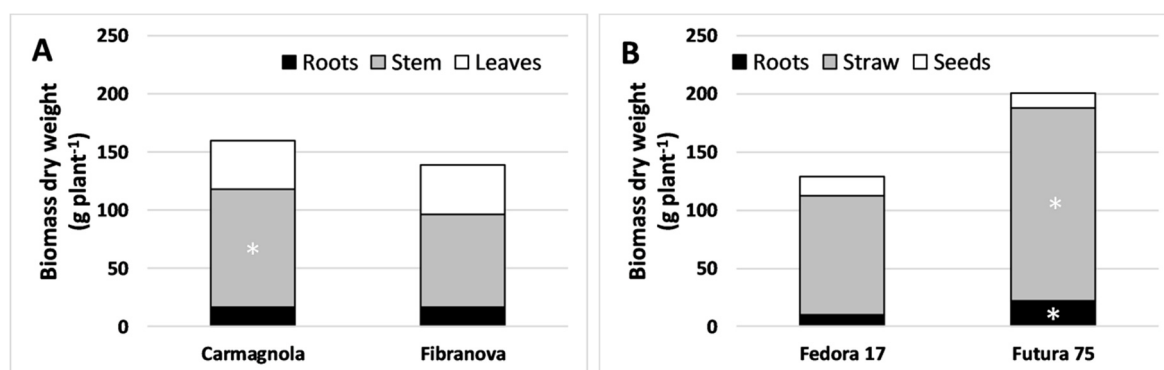


Figure 2. Biomass fractions dry weight of fiber (A) and seed (B) varieties at harvest time. Asterix indicates significant differences between the same fraction of the two varieties at $p < 0.01$ (Tukey's HSD test).

3.2. Chromium and Macronutrient Concentrations in the Biomass

The levels of tannery sludge application did not influence the Cr and macronutrient concentrations in the plants' aboveground biomass in either fiber or seed genotypes. In Table 2, the results are summarized for the two hemp types, as there were no significant differences between the two varieties of each hemp type.

In the root fraction of both variety types, the Cr concentration increased with the increase in the tannery sludge application; the significantly lowest and highest values were found in the Tmin and T200 treatments, respectively. The Cr concentration in the T100 treatment was instead not significantly different either from T50 and Tmin or T200 (Table 2). A positive linear regression between Cr content in the soil and Cr concentration in the root biomass was found for both fiber and seed genotypes. A significantly ($p < 0.05$) higher slope was observed for seed genotypes (Figure 3).

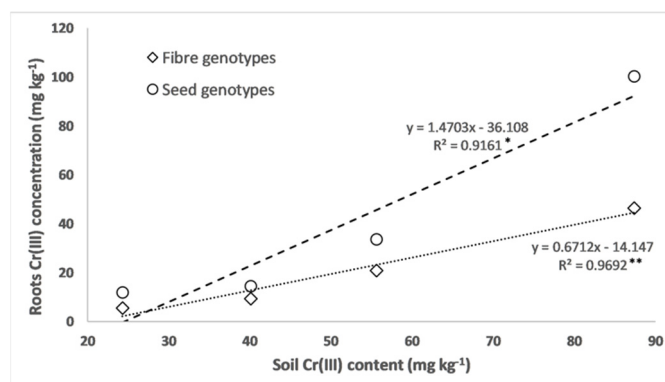


Figure 3. Linear regression between root Cr concentration and soil Cr content for both fiber and seed varieties as a function of hemp type. Significance level of the regression lines is shown at * $p < 0.05$ and ** $p < 0.01$.

Table 2. Chromium and macronutrient concentrations in the hemp biomass.

Variety Types	Plant Fractions	Elements	Tmin	T50	T100	T200
Fibre	Roots	Cr (mg Kg ⁻¹ dw)	5.68 ± 7.03 b	9.55 ± 5.26 b	20.98 ± 18.42 ab	46.41 ± 34.51 a
		N (% dw)	1.22 ± 0.32 a	0.87 ± 0.13 b	0.76 ± 0.23 b	0.89 ± 0.18 b
		K (mg Kg ⁻¹ dw)	12,951.48 ± 2704.58 a	5323.26 ± 1568.75 b	6641.23 ± 4162.18 b	7258.03 ± 3209.18 b
		P (mg Kg ⁻¹ dw)	1994.87 ± 436.56 a	1316.11 ± 150.19 b	1275.63 ± 210.54 b	1542.95 ± 300.87 b
	Stem	Cr (mg Kg ⁻¹ dw)	0.30 ± 0.10	0.27 ± 0.09	0.30 ± 0.13	0.41 ± 0.18
		N (% dw)	0.80 ± 0.13	0.66 ± 0.12	0.65 ± 0.07	0.75 ± 0.18
		K (mg Kg ⁻¹ dw)	10,163.56 ± 2557.41	9932.75 ± 1882.04	10,724.10 ± 2211.84	11,908.36 ± 4572.6
		P (mg Kg ⁻¹ dw)	1335.28 ± 318.24	1422.45 ± 281.29	1588.31 ± 442.97	1538.28 ± 351.57
	Leaves	Cr (mg Kg ⁻¹ dw)	0.55 ± 0.10	0.72 ± 0.18	0.58 ± 0.04	0.66 ± 0.11
		N (% dw)	2.50 ± 0.44	2.08 ± 0.41	2.11 ± 0.35	2.44 ± 0.38
		K (mg Kg ⁻¹ dw)	14,426.02 ± 1298.27	15,983.73 ± 3276.28	16,070.84 ± 2967.53	17,450.22 ± 4685.19
		P (mg Kg ⁻¹ dw)	2489.66 ± 445.04	2594.71 ± 392.26	2487.89 ± 327.08	2838.39 ± 545.75
Seed	Roots	Cr (mg Kg ⁻¹ dw)	11.92 ± 4.01 b	14.56 ± 8.94 b	33.57 ± 30.38 ab	100.46 ± 109.24 a
		N (% dw)	0.83 ± 0.20	0.86 ± 0.16	0.73 ± 0.20	0.86 ± 0.13
		K (mg Kg ⁻¹ dw)	5495.77 ± 2009.73	5239.02 ± 1932.79	5017.82 ± 1932.66	5724.70 ± 1379.44
		P (mg Kg ⁻¹ dw)	1778.97 ± 508.31	1708.05 ± 377.79	1573.90 ± 530.46	1649.56 ± 330.49
	Straw	Cr (mg Kg ⁻¹ dw)	0.74 ± 0.11	0.95 ± 0.33	0.84 ± 0.34	0.78 ± 0.16
		N (% dw)	1.33 ± 0.11	1.31 ± 0.34	1.08 ± 0.19	1.25 ± 0.29
		K (mg Kg ⁻¹ dw)	15,320.60 ± 2316.58	16,656.38 ± 5158.17	1338.93 ± 3832.62	15,116.10 ± 3688.81
		P (mg Kg ⁻¹ dw)	3820.72 ± 659.72	4505.96 ± 604.59	3912.06 ± 581.18	3669.96 ± 1100.12
	Seeds	Cr (mg Kg ⁻¹ dw)	33.70 ± 16.13	36.17 ± 16.45	49.30 ± 30.19	41.74 ± 22.87
		N (% dw)	2.62 ± 0.76	2.44 ± 0.65	2.68 ± 0.62	2.55 ± 0.67
		K (mg Kg ⁻¹ dw)	6621.09 ± 508.78	6296.05 ± 681.80	6445.32 ± 718.98	6865.66 ± 1368.48
		P (mg Kg ⁻¹ dw)	8510.30 ± 1079.98	8909.49 ± 1213.61	9097.48 ± 1132.58	9352.20 ± 1903.70

Different letters in rows highlight significant differences among the Cr application levels at $p < 0.01$ (Tukey's HSD test). dw: Dry weight.

The macronutrient (N, P, K) concentration was not affected by the treatments in any fraction of the plant of seed varieties, while in the fiber varieties, modifications were observed in the roots. In particular, the macronutrient concentrations detected in the root biomass of fiber varieties were significantly decreased by tannery sludge application regardless of the different quantities. In the fiber varieties, the increase in Cr concentration in the root fraction influenced the concentration of macronutrients. Specifically, when tannery sludge was applied, N, K, and P concentrations decreased by 31.1%, 50.5%, and 30.9%, respectively, compared to T_{min}. This uptake imbalance did not occur in the aboveground fractions.

3.3. Chromium and Macronutrients Plant Mass Balance

The T100 and T200 levels of tannery sludge significantly increased Cr accumulation in the root biomass in both fiber and seed varieties (Figure 4A,B). On the contrary, Cr accumulation in the aboveground biomass did not change with different Cr application levels. The Cr translocation factor (aboveground-to-belowground biomass) differed between fiber and seed varieties, being 0.48, 0.39, 0.14, and 0.10 for fiber varieties in the T_{min}, T50, T100, and T200, respectively, while it was 3.03, 2.54, 1.70, and 0.47 for seed varieties in the same treatments. Focusing on the marketable fractions of the two variety types, the average Cr uptake was 0.03 ± 0.04 mg plant⁻¹ in the stems of fiber varieties and 0.60 ± 0.17 mg plant⁻¹ in the seeds of seed varieties. The variety and interaction between variety and Cr application level were not statistically significant.

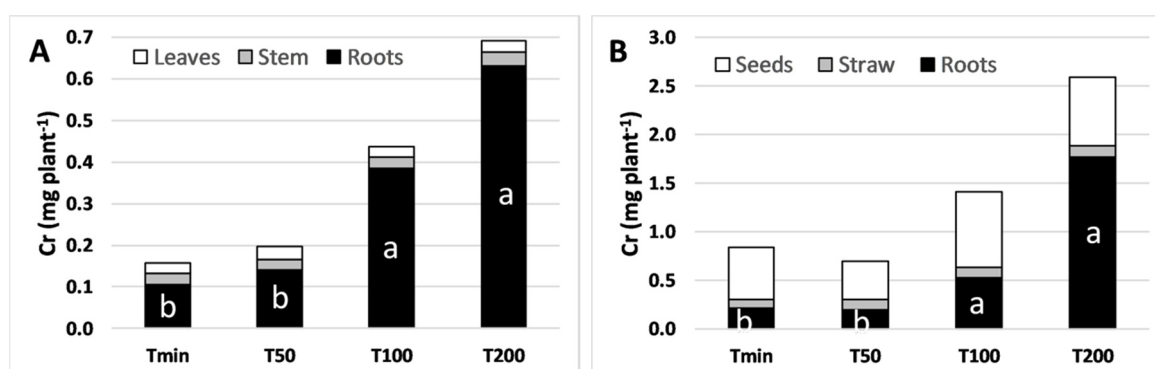


Figure 4. Plant chromium accumulation in each biomass fraction of fiber (A) and seed (B) varieties at harvest time. Different letters indicate significant differences among sludge treatments for the same biomass fraction at $p < 0.01$ (Tukey's HSD test).

Concerning Cr uptake, looking at the roots of both fiber and seed varieties, positive linear regressions were found between Cr uptake and its content in the soil (Figure 5A), with a higher significant ($p < 0.01$) slope for the seed varieties. Even though the aboveground biomass Cr uptake was found not to be related to Cr application level, looking at the whole plant biomass, the Cr content was positively related to soil Cr application level; in addition, in this case, seed genotypes had a significantly ($p < 0.01$) greater slope (Figure 5B). No significant differences were found between either of the two varieties of the fiber genotypes or the two varieties of the seed genotypes.

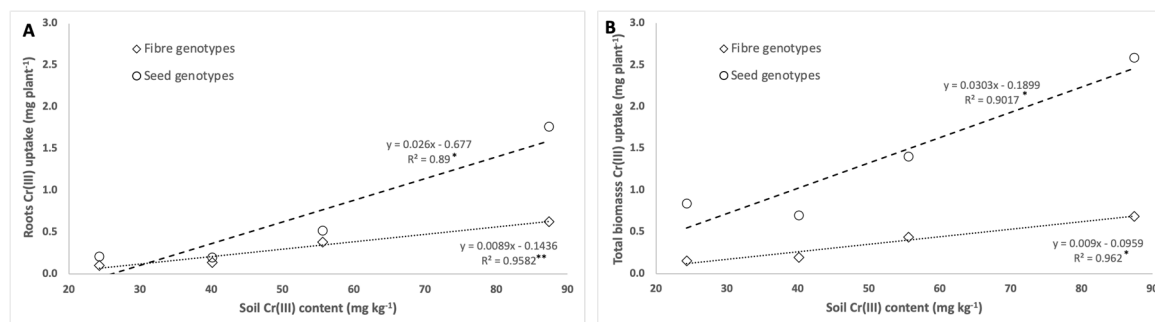


Figure 5. Regressions between soil Cr content and roots Cr uptake (A) and plant Cr uptake (B) as a function of hemp type. Significance level of the regression lines is shown at * $p < 0.05$ and ** $p < 0.01$

The Cr level significantly influenced the hemp N, P, and K uptake in the different plant fractions (Table 3).

In the fiber varieties, roots' N uptake was significantly lower when tannery sludge was applied, regardless of quantity (average value of 132.28 ± 46.57 g plant⁻¹), compared to Tmin (234.48 ± 70.83 mg plant⁻¹). The quantity of N accumulated in the stem biomass differed significantly between genotypes, with a higher value (+20.6%) found in Carmagnola compared to Fibranova (576.20 ± 148.39 mg plant⁻¹). The leaf N content was not influenced by experimental variables. In seed varieties, the tannery sludge application did not influence N accumulation in all biomass fractions. Instead, the Futura 75 variety showed a significantly higher N uptake in both root (2 times) and straw (+37.2%) fractions compared to Fedora 17 (87.63 ± 54.94 mg plant⁻¹ for roots and 1384.47 ± 462.85 mg plant⁻¹ for straw). Seeds' N accumulation did not change with experimental factors.

The plants' P uptake followed the same trend as N. In the fiber varieties, the roots' P uptake was significantly lower in the sludge treatments (average value of 21.63 ± 6.46 mg plant⁻¹) compared to Tmin (38.57 ± 10.52 mg plant⁻¹). In the stem biomass, Carmagnola accumulated a significantly higher P quantity (+28.7%) than Fibranova (114.82 ± 30.70 mg plant⁻¹). The leaf P content was not influenced by experimental factors. In the seed varieties, only genotype had a significant influence on P accumulation in root and straw fractions. Fedora 17 showed a significantly lower P uptake in both root (−55.5%) and straw (−31.1%) fractions than Futura 75 (37.84 ± 20.06 mg plant⁻¹ for roots and 628.70 ± 219.60 mg plant⁻¹ for straw). P content in the seeds did not change with experimental factors.

The K content in the root biomass of fiber varieties was significantly higher (2.4 times) in the Tmin compared to the sludge treatments (average value of 106.06 ± 73.47 mg plant⁻¹). The stem K uptake was not influenced by experimental variables, whereas the leaves' K content was significantly higher (+14.6%) in Carmagnola than in Fibranova (611.40 ± 102.77 mg plant⁻¹). In seed varieties, K accumulation in the straw and seeds was not significantly different either among treatments or between genotypes. Instead, the K content in the root biomass was significantly higher (2.9 times) in Futura 75 than in Fedora 17 (48.91 ± 33.08 mg plant⁻¹).

Table 3. Macronutrient uptake in the hemp biomass.

Variety Types	Plant Fractions	Elements	Tmin	T50	T100	T200
Fibre	Roots	N (mg plant ⁻¹)	234.5 ± 70.8 a	128.7 ± 27.8 b	135.2 ± 61.9 b	132.9 ± 48.6 b
		K (mg plant ⁻¹)	253.6 ± 76.3 a	79.5 ± 28.3 b	124.0 ± 102.3 b	114.6 ± 70.1 b
		P (mg plant ⁻¹)	38.6 ± 10.5 a	19.6 ± 4.5 b	22.8 ± 8.0 b	22.5 ± 6.5 b
	Stem	N mg plant ⁻¹)	712.8 ± 103.8	630.9 ± 181.2	586.9 ± 126.7	613.7 ± 162.9
		K (mg plant ⁻¹)	894.2 ± 158.5	929.6 ± 204.6	954.3 ± 223.6	926.2 ± 214.4
		P (mg plant ⁻¹)	120.8 ± 38.9	136.4 ± 45.0	139.1 ± 29.0	126.1 ± 36.9
	Leaves	N mg plant ⁻¹)	1094.3 ± 141.4	871.3 ± 215.6	903.9 ± 163.0	918.3 ± 213.3
		K (mg plant ⁻¹)	635.9 ± 70.6	660.3 ± 117.5	684.0 ± 100.0	637.9 ± 138.1
		P (mg plant ⁻¹)	108.9 ± 15.9	108.1 ± 19.6	106.1 ± 10.7	107.3 ± 30.4
Seed	Roots	N (mg plant ⁻¹)	150.7 ± 90.0	101.6 ± 56.8	125.2 ± 118.1	142.3 ± 57.2
		K (mg plant ⁻¹)	108.8 ± 86.2	69.7 ± 53.4	100.7 ± 113.2	95.1 ± 43.1
		P (mg plant ⁻¹)	33.1 ± 22.2	20.5 ± 10.9	28.0 ± 27.6	27.3 ± 10.9
	Straw	N (mg plant ⁻¹)	1760.6 ± 600.3	1534.6 ± 524.9	1439.7 ± 506.2	1819.7 ± 811.0
		K (mg plant ⁻¹)	1987.0 ± 605.9	1908.0 ± 502.3	1835.1 ± 505.1	2157.2 ± 981.3
		P (mg plant ⁻¹)	498.4 ± 179.4	554.3 ± 226.0	517.1 ± 163.2	541.1 ± 277.9
	Seeds	N (mg plant ⁻¹)	422.6 ± 268.5	265.5 ± 154.0	397.4 ± 266.5	467.7 ± 226.5
		K (mg plant ⁻¹)	105.9 ± 49.9	65.8 ± 31.7	90.5 ± 54.0	120.7 ± 49.8
		P (mg plant ⁻¹)	135.1 ± 57.9	94.0 ± 49.0	130.3 ± 79.9	164.7 ± 63.7

Different letters in rows highlight significant differences among the Cr application levels at $p < 0.01$ (Tukey's HSD test). dw: Dry weight.

4. Discussion

The results obtained in this study show that hemp growth was not affected by Cr concentration in the soil, suggesting its suitability for phytomanagement at the studied Cr concentrations. Although our trial was conducted under greenhouse conditions, biomass yield was similar to the values usually obtained in open field conditions. For instance, the Carmagnola stem yield in our study was comparable to values already reported by Struik et al. [25], and Fibranova aboveground biomass was comparable to those of Cosentino et al. [26]. Considering seed cultivars Futura 75 and Fedora 17, their cumulative aboveground biomass (seed + straw) resulted in about twice the values obtained by Tang et al. [27] and Faux et al. [28], but they fertilized the plants at a lower rate (from 150 to 300 kg ha⁻¹ in our study and from 0 to 120 kg ha⁻¹ in Tang et al. [27] and Faux et al. [28]). Indeed, positive significant relationships between seed yield and N rate in hemp fertilization were found by Aubin et al. [24] and Stafecka et al. [29].

Focusing on the uptake and translocation of Cr, in all cultivars, the uptake was related to the concentration in the soil only within the roots, while no significant differences were observed for the amount of Cr in the stems, leaves, and seeds. The ability of hemp to compartmentalize Cr in the root system was already reported by Citterio et al. [12] in soil with a higher Cr concentration (126–139 µg g⁻¹) than our experiment (from 24.3 to 87.4 µg g⁻¹). Further, Ullha et al. [13] found a Cr translocation factor lower than 1 in soils with 0.93–4.48 µg g⁻¹, suggesting Cr accumulation in the roots. In our study, while the translocation factor decreased with the increase in Cr supply, reaching values lower than 1 only in the T200 treatment (87.4 µg Cr g⁻¹), the Cr content in the aboveground biomass remained stable among treatments. This plant behavior suggests that hemp can physiologically control Cr translocation in the aboveground biomass fractions. However, the maximum Cr concentration in aboveground biomass was reached in the seeds (average value of 40.4 mg kg⁻¹). As reported by Evangelou et al. [15], a plant is currently defined as a Cr hyperaccumulator if it is able to reach concentrations of at least 1000 mg kg⁻¹. Therefore, the results of our study, although limited to one-year observations, suggest that all the genotypes evaluated cannot be considered as hyperaccumulators. On the contrary, Baker et al. [30] defined excluders as those species that can grow in soils with a wide range of heavy metal concentrations without accumulating significant quantities of metals and maintaining their concentration constant and low in the aboveground biomass. In our experiment, regardless of genotype, as the Cr concentration in the soil increased from 24.3 to 87.4 µg g⁻¹, plants' Cr accumulation increased by 7.2 and 1.2 times in the roots and aboveground biomass, respectively. As a consequence, hemp acts as an excluder species for Cr. The plants' Cr uptake is not mediated by specific mechanisms, and it is absorbed by plants together with essential metals [31]. The reason for the high accumulation in the roots of the plants could be related to their ability to immobilize Cr in the vacuoles of the root cells [32,33], as already reported for other species [34,35].

Gardea-Torresdey et al. [36] reported that the absorption of macronutrients and microelements in *Salsola kali* was, in general, lower when the plants were grown in a medium containing Cr(III). Wyszowski and Radziemska [37] found that increasing doses of Cr(III) resulted in an approximate threefold reduction in N content in *Zea mays*. Our results only partially agree with these studies. Indeed, the content of N, P, and K decreased when the Cr content increased only in the root biomass of fiber varieties. This behavior suggests that the macronutrient response to Cr presence in the soil depends also on the variety and the portion of plant.

Hemp is, therefore, almost useless for restoring Cr-contaminated soils [12], but it is suitable for the economic exploitation of these soils (phytomanagement). Cr-contaminated land, which is not suitable for food production, could be used for other economic productions (hemp fiber and seeds), offering an alternative income to farmers living near such areas who perhaps lost their livelihood because of the contamination [15]. In our experiment, we demonstrated this potential in soils with an average Cr content higher than the majority of European countries [38], even though it is lower than the European threshold value of 100 mg Cr kg⁻¹ of soil [39].

Considering the low Cr concentration observed in this study for the hemp marketable fractions (especially seeds), the quantity is so low that it could be used in products for human consumption as well. Indeed, although there are no maximum levels for Cr in food, the European Food Safety

Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) advises a tolerable daily intake (TDI) of 300 µg Cr kg⁻¹ body weight per day [40]. This value means that a person of 80 kg, considering the seeds' Cr concentration reported in this study, needs to eat about 594 g of seeds daily to reach the TDI.

5. Conclusions

The low Cr content in the total and marketable aboveground biomass, despite the different availability in the soil, suggests that hemp can be considered a Cr excluder plant. This behavior indicates that hemp is a valid candidate for Cr-contaminated soil phytomanagement, which offers an economical and sustainable use of Cr-polluted lands.

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

Funding: Research was supported by the Department of Agronomy, Food, Natural Resources, Animals, and Environment FINA project “Genetic and agronomic hemp improvement to develop new varieties through conventional and molecular methods”.

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

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