water

MDPI

Article

Morpho-Physiological and Metal Accumulation Responses of Hemp Plants (*Cannabis Sativa* L.) Grown on Soil from an Agro-Industrial Contaminated Area

Fabrizio Pietrini, Laura Passatore, Valerio Patti, Fedra Francocci, Alessandro Giovannozzi and Massimo Zacchini *

Research Institute on Terrestrial Ecosystems, National Research Council of Italy, Section of Montelibretti, Via Salaria km 29.300, 00015 Monterotondo, Italy; fabrizio.pietrini@cnr.it (F.P.); laura.passatore@iret.cnr.it (L.P.); ittapoirelav@gmail.com (V.P.); fedrafrancocci@gmail.com (F.F.); a.giovannozzi@tiscali.it (A.G.)

* Correspondence: massimo.zacchini@cnr.it; Tel.: +39-0690672537

Received: 1 April 2019; Accepted: 15 April 2019; Published: 18 April 2019

check for updates

Abstract: Hemp is a promising plant for phytomanagement. The possibility to couple soil restoration to industrial crop cultivation makes this plant attractive for the management of contaminated sites. In this trial, *Cannabis sativa* L. plants were grown in a greenhouse on soils from two sites of "Valle del Sacco" (Lazio Region, Italy), a wide area contaminated by agro-industrial activities. One site was representative of moderate and diffuse metal(loid) multi-contamination, above the Italian concentration limit for agriculture (MC—moderately contaminated). The second site showed a metal(loid) content below the aforementioned limit, as a typical background level of the district (C—control). After 90 days, biometric and physiological parameters revealed satisfactory growth in both soil types. MC-grown plants showed a slight, but significant reduction in leaf area, root, and leaf biomass compared with C-grown plants. Chlorophyll content and chlorophyll fluorescence parameters, namely the quantum yield of primary photochemistry (F_v/F_m) and the Performance Index (PI_{ABS}), confirmed the good physiological status of plants in both soils. Metal(loid) analyses revealed that As, V, and Pb accumulated only in the roots with significant differences in MC- and C-grown plants, while Zn was found in all organs. Overall, preliminary results showed a satisfactorily growth coupled with the restriction of toxic metal translocation in MC-grown hemp plants, opening perspectives for the phytomanagement of moderately contaminated areas.

Keywords: chlorophyll fluorescence; industrial crop; metal tolerance; phytomanagement; phytoremediation; soil pollution

1. Introduction

In the agricultural soil, the overuse of fertilisers and waste release from industrial processes has resulted in a large number of contaminated sites over Europe requesting to be reclaimed, contributing to exacerbated land degradation problems [1]. A recent survey reported that in the European Union, approximately 340,000 contaminated sites are present, most of them polluted by metal(loid)s [2]. The awareness about the harmful effects produced by such contaminants on human health, through plant cultivation and animal breeding, is forcing the characterisation of different environmental restoration technologies, among which eco-sustainable ones are largely studied and successfully applied, especially in sites characterised by moderate and diffuse contamination [3,4]. In this regard, the phytomanagement is recognised as an effective approach to carry out a risk management strategy [5,6], being constituted by an array of gentle remediation options (GROs) technologies that can be applied as a part of integrated site risk management solutions. In this sense, the phytomanagement approach represents an application of the phytoremediation biotechnology, exploiting in a broader way the ecological benefits offered by plants. Specifically, phytomanagement relies on the choice of suitable plant species for the purpose of the management of the contaminated sites [7]. Among polluted sites, agricultural areas represent a particular concern for safe food production [8], forcing local administrations to restrict their exploitation with relevant loss of income for farmers. Therefore, particular attention is currently being paid to investigating the possibility of cultivating non-food crops on contaminated lands that, besides the capability to remediate soils, could satisfactorily grow and produce biomass and other bio-products for multiple profitable uses, avoiding the metal transfer to the food chain [9].

Because of its biological characteristics, such as rapid growth, high biomass production, wide root system, high genetic variability, remarkable ability to adapt to different environmental conditions, and low susceptibility to disease and pests [10,11], hemp (*Cannabis sativa* L.) is a plant species of notable interest for phytomanagement. In fact, because of the multiple non-food uses [12], it can offer a good opportunity to integrate soil recovery with the cultivation of a commercially exploitable resource. Specifically, hemp plants involved in phytomanagement strategies can profitably produce fibres that could be commercialised as insulating or composite material, cellulose materials from stem (suitable for packaging industry), and seeds representing a source of oil for biofuel production. The hemp potential for phytotechnologies has been poorly explored so far; most studies have focused on tolerance and accumulation of metals [13–17] and few reports deal with plant behaviour versus organic contaminants such as chrysene and benzopyrene [18] and radio-compounds [19]. Therefore, a better characterisation of the growth and physiological responses of hemp plants in metal-contaminated soil under controlled conditions is requested. In this context, the application of the chlorophyll *a* fluorescence analysis, which represents a rapid and non-destructive technique to analyze the changes at the physiological and biochemical level in the photosynthetic apparatus under stress conditions, and the evaluation of the chlorophyll content, also performed with non destructive methods, are particularly useful. Specifically, OJIP fluorescence transient analysis, known as the JIP test [20], has been developed for the quantification of several phenomenological and biophysical expressions together with the energy flux parameters of photosystem II (PS II), and may be used to assess metal stress on plants in vivo [21]. This approach could be of relevance for a successful screening of plant materials for phytoremediation. In the present study, an ex situ pot experiment was performed with soil sampled from the “Valle del Sacco” area. It is a contaminated site recognised as a National Interest Site by the Italian Ministry of Environment (L. 248/2005) for its huge extension (7235 ha) and diffused contamination by metals and organics that prevents any food crop cultivation and grazing, which formerly characterised the area. This preliminary experiment, performed in a greenhouse, aimed to investigate the growth potential of hemp plants on moderately metal-contaminated soils, evaluating the presence of the pollutants in the biomass for possible exploitation as a bio-resource.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Seeds of *Cannabis sativa* L. (cv. Codimono, an Italian monoecious variety cultivated for fiber production) were placed in plates filled with moistened agriperlite in darkness at room temperature (R.T.) until germination occurred. Then, germinated seeds were transferred in 11 L pots filled with soil collected from the topsoil (up to 25 cm after removing 5 cm top layer) of the two sampling points (41°44.245′ N, 13°00.732′ E; 41°44.263′ N, 13°00.875′ E) within the area of “Valle del Sacco” polluted site (Municipality of Colleferro, Rome, Italy), characterised by different metal concentration levels.

The first soil was sampled as representative of the moderate and diffuse multi-contamination by metal(loid)s, specifically Pb, V, As, and Zn (as highlighted by a survey previously carried out within the activities of the Commissioner Officer for the “Valle del Sacco” National Interest Site, ref.

07384-022R01E03/2006 by Studio Geotecnico Italiano SRL), slightly above the Italian concentration limit for green areas, except for Zn (namely, 20 ppm for As, 100 ppm for Pb, 90 ppm for V, 150 ppm for Zn). The second one was chosen for its metal(loid) content below the aforementioned limit, as a typical background level of the district. The two soils were referred as moderately contaminated (MC) and control (C) soil, respectively.

Agronomic characteristics were analysed by a certified environmental analysis laboratory following the guidelines of the Italian Ministry of Agriculture and Forestry (DM 13/09/99) [22] as an Italian official reference for soil chemical analyses. The total nitrogen content was evaluated by dry combustion in an elemental analyser (Met. XIV.1, DM 13/09/99); the determination of available phosphorous was performed following the Olsen method, based on bicarbonate extraction and photometric detection (Met. XV.3, DM 13/09/99); potassium and magnesium content was determined by atomic-absorption spectrophotometry (Met. XIII.5, DM 13/09/99). Inorganic and organic contamination of soil samples was assessed by a certified environmental analysis laboratory based on a previous survey carried out within the activities of the Commissioner Officer for the "Valle del Sacco" National Interest Site (see above). Organic contaminant levels were far below the Italian legal limit (as for DL n.152/06) [23], in most cases even under the detection limits, and thus organic compounds were not considered in this study (data not shown). The experiment was set up in a completely randomized design with four replicates (pots) for each of the four variants (C-soil with plant, C-soil without plant, MC-soil with plant, MC-soil without plant). Pots were placed during summertime (June–August) in a greenhouse under natural photoperiod (about 14 h), with mean (night–day) temperatures of 21.1–28.8 °C and relative humidity of 50%–60%; four pots per treatment (C and MC) were prepared. Pots were sealed at the bottom to avoid metal leaching. Plants were irrigated daily by supplying the water loss for evapotranspiration to maintain 50% of the water holding capacity, evaluated before starting the experiment for each kind of soil. The water supply for each treatment was determined daily by weighing each pot using an electronic balance (SB 32,001 Delta Range; Mettler-Toledo Inc., Columbus, OH). Total cumulative transpiration was calculated by subtracting, for the whole cultivation period, the daily amount of water loss in C- and MC-soil without plant from that in C- and MC-soil with plant, respectively. No fertilisation and pest management was carried out. At the end of the growth period (90 days), during seed production, after measuring leaf area (LI 3100, LI-COR Inc., Lincoln, NE, USA) and other biometric and physiological parameters, plant organs (root, stem, leaves, and inflorescence) were harvested and stored in an oven at 60 °C until a constant weight was reached.

2.2. Chlorophyll Content Determination and Chlorophyll Fluorescence Analysis

At the end of the experiment, total leaf chlorophyll content was estimated by a SPAD-502 Chlorophyll meter (Minolta Inc., Osaka, Japan), as reported by Pietrini et al. [24]. The measurements were taken from at least two fully developed leaves per plant. Four SPAD readings were taken from the widest portion of the leaf lamina, while avoiding major veins. The four SPAD readings were averaged to represent the SPAD value of each leaf. SPAD values were converted to chlorophyll content ($\mu g\ cm^{-2}$) using the equation by Cerovic et al. [25]; that is, chlorophyll content = (99 × SPAD value)/(144 − SPAD value).

On the same leaves chosen for SPAD readings, the chlorophyll fluorescence transient (OJIP transients) was measured using a plant efficiency analyser (PEA, Hansatech Instruments Ltd., King's Lynn, UK). The measurements were performed on leaves that were previously adapted to the dark for 60 minutes for the complete oxidation of the photosynthetic electron transport system, and the fluorescence intensity was measured for 1 s after the application of a saturating light pulse of 3000 $\mu mol\ m^{-2}\ s^{-1}$. For each experimental treatment, at least eight measurements were performed. The quantum yield of primary photochemistry (F_v/F_m) and performance index (PI_{ABS}) of photosystem II (PSII) were determined as described by Strasser et al. [20].

2.3. Metal(loid) Determinations

Soils and oven dried plant samples were finely ground (Tecator Cemotec 1090 Sample Mill; Tecator, Hoganas, Sweden), weighed, and mineralized. Mineralization was performed by microwave assisted acid digestion (U.S. EPA. 2007) by a certified environmental analysis laboratory. Specifically, the method EPA 3051A/2007 was used. Briefly, samples were dissolved in concentrated nitric acid and concentrated hydrochloric acid using microwave heating. The samples and acids were placed in fluorocarbon polymer (PFA) microwave vessels. The vessels were sealed and heated in the microwave unit (175 °C for 15 min). After cooling, the vessel contents were filtered, centrifuged, and then diluted to volume and analyzed. The total metal(loid) concentration was measured by ICP-MS in plant samples, following the UNI EN ISO 17294-2:2016 method, and by ICP-OES in soil samples, following the EPA 6010D/2014. Method detection limits (for both soil and plant samples) were as follows (mg/kg d.w.): As 0.5, Pb 1.0, V 0.5, Zn 1.0. As the QC procedure, a daily five-point calibration with multi elemental standard followed by a secondary standard calibration, analyses of a certified reference material (CRM) added to samples, and a matrix spike and matrix spike duplicate (MS/MSD) were performed. The bioconcentration factor (BCF) refers to the ratio between the metal(loid) concentration in plant organs and in the soil, as reported in Iori et al. [26].

2.4. Statistical Analysis

The data reported in the tables refer to four replicates (four pots per thesis). Normally, distributed data were processed by one-way analysis of variance (ANOVA), using the SPSS (Chicago, IL, USA) software tool, with the statistical significance of mean data assessed by t-test ($p \leq 0.05$).

3. Results and Discussion

As reported in Table 1, the analyses of agronomic parameters revealed a higher content of organic matter (SOM), total N, available K, and exchangeable Mg in C-soil. Both samples showed sandy loam ground, slightly alkaline pH, and a similar cationic exchange capacity (CEC). Metal (loid) concentrations of As, V, and Pb were below the threshold fixed by the Italian Law (for sites devoted to green areas as for Decree Law n.152/06 [23]) in C-soil and beyond the legal limit in the soil representing a moderate contamination (MC).

Table 1. Physico–chemical parameters and metal(loid) content of soil sampled in two different sites (C—control and MC—moderate contamination) in the "Valle del Sacco" area (Lazio Region, Central Italy).

Soil Type	Texture	pH	SOM (%)	N Total (‰)	P_2O_5 (ppm)	K_2O (ppm)	Mg (ppm)	CEC (meq/100g)	As (ppm)	Pb (ppm)	V (ppm)	Zn (ppm)
C	Sandy loam	7.93	3.7	2.56	78	779	101	16.9	17.3	77	76.5	67.4
MC	Sandy loam	8.07	2.2	1.43	87	667	77	15.7	22.6	115	106.7	92.8

Abbreviations: SOM, soil organic matter; CEC, cation exchange capacity.

In Table 2, some biometric parameters regarding hemp plants grown in pots filled with soils of the two sampled points are shown. Plant height and stem diameter were not affected by the different substrate composition, while leaf area was higher in plants grown in C-soil when compared with those grown in MC-soil. Anyway, the growth of plants (i.e., plant height, stem diameter, above ground, and root dry mass) can be considered as satisfactory with regards to data reported in field trials on different cultivars of fiber hemp [10,27], despite the notable differences in the growth conditions.

Table 2. Biometric parameters and total transpiration measured in hemp plants (*Cannabis sativa* L., cv. Codimono) after 90 days of growth in a greenhouse in pots filled with soil sampled in two different sites (C and MC) in the "Valle del Sacco" area (Lazio Region, Central Italy). In each column, mean values (n = 4 ± SE) are reported. Different letters indicate significant different values (t-test, $p \leq 0.05$).

Soil Type	Plant Height (cm)	Leaf Area (cm^2)	Stem Diameter (cm)	Total Transpiration L/plant
C	158.8 (5.1) a	2507 (249) a	0.88 (0.02) a	16.58 (0.2) a
MC	154.3 (20.7) a	1657 (88) b	0.70 (0.05) a	14.44 (0.38) b

Biomass production data are reported in Table 3. The results revealed that the growth of hemp plants in MC-soil was reduced compared with that observed for plants cultivated in C-soil. In particular, the main differences were found at the root and leaf level. This latter result was consistent with previous reported data on leaf area in Table 2. Interestingly, stem and inflorescence biomass was not affected by the different soil characteristics. The calculation of the total amount of water loss for transpiration by plants revealed a higher total transpiration over the cultivation period in C-soil grown plants.

Taken together, results on growth performances highlighted a good adaptation of hemp plants to the soil sampled in the contaminated area. In this regard, it can be hypothesised that growth was supported mainly by the high SOM and nutrient content, especially with regards to C-soil grown plants. Moreover, it can be underlined that plant growth was not negatively affected by the presence of metal(loid)s in the soil, confirming previous data reported by several authors [14–16]. Notably, the presence of vanadium in the soil was also not deleterious for hemp plants, while in the literature, such metal is reported to be extremely toxic for plant growth at concentrations even lower than those tested in the present work [28,29].

Table 3. Dry biomass of different organs of hemp plants (*Cannabis sativa* L., cv. Codimono) obtained after 90 days of growth in a greenhouse in pots filled with soil sampled in two different sites (C and MC) in the "Valle del Sacco" area (Lazio Region, Central Italy). In each column, mean values (n = 4 ± SE) are reported. Different letters indicate significantly different values (t-test, $p \leq 0.05$).

Soil Type	Biomass (g dw plant $^{-1}$)			
	Root	Stem	Leaf	Inflorescence
C	3.73 (0.27) a	12.44 (1.42) a	9.33 (0.70) a	6.06 (1.19) a
MC	2.16 (0.41) b	8.14 (1.98) a	6.24 (0.39) b	3.79 (0.18) a

To investigate the responses of hemp plants grown in the two different soils at the photosynthetic level, measurements of leaf chlorophyll content and chlorophyll fluorescence were performed (Table 4).

Leaf chlorophyll content is one of the most important factors in determining photosynthetic potential and primary production [30]. Our results showed that a higher leaf total chlorophyll content was found in plants grown in C-soil compared with those grown in MC-soil. These data are in accordance with the higher leaf biomass and area found in plants grown in C-soil (Tables 2 and 3). The analysis of chlorophyll fluorescence was focused on two parameters—the quantum yield of primary photochemistry (F_v/F_m) and performance index (PI_{ABS}) of PSII. Among the chlorophyll fluorescence parameters, F_v/F_m is recognized as a good indicator for photo-inhibitory or photo-oxidative effects on PSII [31]. However, the most widely used parameter from the chlorophyll fluorescence OJIP transient is the PI_{ABS}, which provides quantitative information about the general state of plants and their vitality. PI_{ABS} is the product of three independent characteristics—the concentration of reaction centers per chlorophyll, a parameter related to primary photochemistry, and a parameter related to electron transport [20]. PI_{ABS} reflects the functionality of both PSI and II and produces quantitative information of the plant performance, especially under stress conditions [20,32]. Contrarily to chlorophyll content, the results showed that no differences between plants cultivated in both types of soil were observed concerning the F_v/F_m and PI_{ABS} parameters. Therefore, these data confirmed the good physiological status of hemp plants grown in both soil conditions, as also visually observed for the lack of damage symptoms such as chlorosis or necrosis. Our results are in line with those reported by other authors [33,34], who found a reduction of chlorophyll fluorescence parameters such as F_v/F_m, only under elevated metal concentrations, highlighting the ability of hemp plants to tolerate metal stress and to grow in contaminated soil.

Table 4. Leaf chlorophyll content and chlorophyll fluorescence parameter analysis (the quantum yield of primary photochemistry (F_v/F_m) and performance index (PI_{ABS}) of PS II) in hemp plants (*Cannabis sativa* L., cv. Codimono) after 90 days of growth in a greenhouse in pots filled with soil sampled in two different sites (C and MC) in the "Valle del Sacco" area (Lazio Region, Central Italy). In each column, mean values (n = 4 ± SE) are reported. Different letters indicate significantly different values (t-test, $p \leq 0.05$).

Soil Type	Chl Content (μg cm^{-2})	Chl Fluorescence Parameters (r.u.)	
		PI_{ABS}	F_v/F_m
C	60.4 (3.3) a	18.7 (1.7) a	0.793 (0.003) a
MC	44.4 (0.9) b	17.0 (1.6) a	0.791 (0.006) a

In Table 5, the concentration of the analysed metal(loid)s in the hemp plant organs is reported. Notably, no detection of the most toxic metal(loid) elements, namely, As, V, and Pb, was observed in the above ground organs of plants (stem, leaves, and inflorescence). Conversely, as expected for an essential micronutrient, Zn was found in all organs, especially in the inflorescence, followed by leaves and stem. Contrarily to above ground organs, the presence of metal(loid)s was detected at the root level. In this case, differences in plants were found for As, Pb, and V, with their concentrations being higher in plants cultivated in C-soil compared with MC-soil.

Table 5. Metal(loid) content in the organs of hemp plants (*Cannabis sativa* L., cv. Codimono) after 90 days of growth in a greenhouse in pots filled with soil sampled in two different sites (C and MC) in the "Valle del Sacco" area (Lazio Region, Central Italy). In each column, mean values (n = 4 ± SE) are reported. Different letters indicate significantly different values (t-test, $p \leq 0.05$); nd = not detected.

Plant Organs	Soil Type	Metal(loid) Concentration (ppm)			
		As	Pb	V	Zn
Root	C	1.9 (0.2) a	12.3 (2.7) a	11.4 (1.8) a	13.8 (1.7) a
	MC	0.8 (0.1) b	3.6 (0.2) b	4.1 (0.1) b	14.1 (3.9) a
Stem	C	nd	nd	nd	9.1 (2.2) a
	MC	nd	nd	nd	9.5 (1.1) a
Leaves	C	nd	nd	nd	21.1 (3.5) a
	MC	nd	nd	nd	35.2 (6.2) a
Inflorescence	C	nd	nd	nd	59.1 (1.7) a
	MC	nd	nd	nd	62.7 (5.5) a

The higher capability of C-soil-grown plants to accumulate the metal (loid) elements was in accordance with the higher growth performance highlighted by biomass production, leaf area, chlorophyll content, and leaf transpiration (Tables 2–4), associated with a better nutritional status (Table 1). Moreover, the hypotheses of a more recalcitrant soil pool in MC-soil or a more active excluder mechanism in MC-soil-grown plants cannot be ruled out.

The low ability of hemp plants to accumulate toxic metals from soils is consistent with previously reported works showing the preferential metal allocation in the root apparatus [14,17,35], even if a low metal translocation to shoots was reported in these investigations. Accordingly, a large variability for this trait, because of genetic and environmental factors, was reviewed [36]. In this regard, because of the remarkably higher Cd accumulation in the roots compared with the shoots, hemp was defined as a Cd-excluder plant [16]. Preliminary observations of this work suggest that hemp plants can restrict the uptake of other metal(loid) elements, namely, As, Pb, and V. Further investigations are needed to better evaluate this aspect.

The calculation of the bioconcentration factor (BCF) (Table 6) revealed that this index was higher in the roots of C-soil grown plants than MC-soil grown plants for all the metal(loid)s analysed, except for Zn, as a result of the different concentrations found in the plants and soils types, respectively. Anyway, the values of BCF found for hemp plants grown in both types of soils are to be considered very low following the literature on the matter [37]. According to the present data, low BCF for roots in hemp plants treated with Pb was also found [38–40]. A similar low BCF for As was also reported in hemp plants collected in a survey on a metal-contaminated site [40].

Table 6. Bioconcentration factor (BCF) calculated for roots (n = 4) of hemp plants (*Cannabis sativa* L., cv. Codimono) after 90 days of growth in a greenhouse in pots filled with soil sampled in two different sites (C and MC) in the "Valle del Sacco" area (Lazio Region, Central Italy). In each column, mean values (n = 4 ± SE) are reported. Different letters indicate significantly different values (t-test, $p \leq 0.05$).

Soil Type	Bioconcentration Factor			
	As	**Pb**	**V**	**Zn**
C	0.11 (0.008) a	0.16 (0.02) a	0.14 (0.01) a	0.21 (0.01) a
MC	0.03 (0.001) b	0.03 (0.005) b	0.03 (0.007) b	0.15 (0.02) a

Overall, results on metal(loid) accumulation evidenced that under our experimental conditions, hemp plants were able to exclude toxic metals—namely, As, Pb, and V—from entering the vascular tissues and being transported in the above ground organs. In fact, even though a high transpiration associated to a considerable biomass production was observed, metal loading by the rooting system was reduced and translocation to aerial parts was negligible. This aspect opens up intriguing questions about the possible toxic metal excluder mechanisms activated by hemp plants to cope with the metal presence in the soil to be addressed in future investigations. Contrarily, Zn was taken up and transported in stem, leaf, and inflorescence, where it is requested for physiological functions associated to its role as a micronutrient. Regarding the metal absorption capability of the hemp plants assayed in this trial, it should be taken into due account that the sub-alkaline condition of both sampled soils (Table 1) could have negatively affected the mobility of metals in the circulating soil solution, which is generally favored by a sub-acidic soil pH [41].

4. Conclusions

The results of this trial, obtained in a greenhouse pot experiment, put in evidence that hemp plants cv. Codimono can satisfactorily grow in soil moderately contaminated by metal(loid)s, producing biomass and inflorescence with seeds. Some growth parameters were slightly reduced in MC-soil grown plants compared with those grown in C-soil. Notably, the lack of toxic metal(loid)s in the above ground organs represents a valuable indication for the profitable cultivation of hemp plants in moderately metal contaminated sites. The slight alkaline soil pH reported in this trial is a factor to bear in mind to consider the uptake of metal(loid)s by plants, affecting the metal mobility and bioavailability. Taken together, the results of the present investigation, though preliminary, indicated that hemp plant cultivation could be exploited within a phytomanagement strategy on moderately contaminated areas, where the restriction for crop cultivation/grazing produces a loss of income for farmers. In such cases, the production of fibres, energy from biomass, and oil from seeds could be profitably achieved.

Author Contributions: M.Z. and F.P. conceived, designed, and supervised the experiments; F.F. managed the relations with the public and private bodies and organised the soil sampling procedures; L.P. supervised and performed the experimental work on plant growth; V.P. and A.G. performed the experimental work; all authors performed the biometric parameters analyses; F.P. performed chlorophyll content and chlorophyll fluorescence parameters analyses; M.Z. and F.P. performed the data elaboration; M.Z. wrote the paper with the contribution of F.P., L.P., and F.F.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Acknowledgments: Authors wish to thank Rachele Invernizzi (South Hemp Techno) for the seeds supply, the Municipality of Colleferro (Rome) and the Associazione Radicati for their support, Marco Giorgetti and Alberto Rinalduzzi for their technical assistance.

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

References

1. Panagos, P.; Borrelli, P. All that soil erosion: The global task to conserve our soil resources. In *Soil Erosion in Europe: Current Status, Challenges and Future Developments*; Soil Environment Center of the Korea: Seoul, Korea, 2017; pp. 20–21.
2. Panagos, P.; Van Liedekerke, M.; Yigini, Y.; Montanarella, L. Contaminated sites in Europe: Review of the current situation based on data collected through a European network. *J. Environ. Pub. Health* **2013**, *2013*, 158764. [CrossRef] [PubMed]
3. Vangronsveld, J.; Herzig, R.; Weyens, N.; Boulet, J.; Adriaensen, K.; Ruttens, A.; Thewys, T.; Vassilev, A.; Meers, E.; Nehnevajova, E.; et al. Phytoremediation of contaminated soils and groundwater: Lessons from the field. *Environ. Sci. Poll. Res.* **2009**, *16*, 765–794. [CrossRef]
4. Bianconi, D.; De Paolis, M.R.; Agnello, A.C.; Lippi, D.; Pietrini, F.; Zacchini, M.; Polcaro, C.; Donati, E.; Paris, P.; Spina, S.; et al. Field-scale rhyzoremediation of a contaminated soil with hexachlorocyclohexane (HCH) isomers: The potential of poplars for environmental restoration and economical sustainability. In *Handbook of Phytoremediation*; Golubev, I.A., Ed.; Nova Science Publishers, Inc.: New York, NY, USA, 2011; Chapter 31; pp. 1–12.
5. Evangelou, M.W.; Papazoglou, E.G.; Robinson, B.H.; Schulin, R. Phytomanagement: Phytoremediation and the production of biomass for economic revenue on contaminated land. In *Phytoremediation: Management of Environmental Contaminants*; Ansari, A.A., Gill, S.S., Gill, R., Lanza, G.R., Newman, L., Eds.; Springer International Publishing: Cham, Switzerland, 2015; Volume I, pp. 115–132.
6. Cundy, A.B.; Bardos, R.P.; Puschenreiter, M.; Mench, M.; Bert, V.; Friesl-Hanl, W.; Müller, I.; Li, X.N.; Weyens, N.; Witters, N.; et al. Brownfields to green fields: Realising wider benefits from practical contaminant phytomanagement strategies. *J. Environ. Manag.* **2016**, *184*, 67–77. [CrossRef] [PubMed]
7. Domínguez, M.T.; Maranón, T.; Murillo, J.M.; Schulin, R.; Robinson, B.H. Trace element accumulation in woody plants of the Guadiamar Valley, SW Spain: A large-scale phytomanagement case study. *Environ. Pollut.* **2008**, *152*, 50–59. [CrossRef] [PubMed]
8. Komínkova, D.; Fabbricino, M.; Gurung, B.; Race, M.; Tritto, C.; Ponzo, A. Sequential application of soil washing and phytoremediation in the land of fires. *J. Environ. Manag.* **2018**, *206*, 1081–1089. [CrossRef]
9. De Medici, D.; Komínkova, D.; Race, M.; Fabbricino, M.; Součková, L. Evaluation of the potential for cesium transfer from contaminated soil to the food chain as a consequence of uptake by edible vegetables. *Ecotoxicol. Environ. Saf.* **2019**, *171*, 558–563. [CrossRef] [PubMed]
10. Amaducci, S.; Zatta, A.; Pelatti, F.; Venturi, G. Influence of agronomic factors on yield and quality of hemp (*Cannabis sativa* L.) fibre and implication for an innovative production system. *Field Crops Res.* **2008**, *107*, 161–169. [CrossRef]
11. Prade, T.; Svensson, S.E.; Andersson, A.; Mattsson, J.E. Biomass and energy yield of industrial hemp grown for biogas and solid fuel. *Biomass Bioenergy* **2011**, *3*, 3040–3049. [CrossRef]
12. Salentijn, E.M.; Zhang, Q.; Amaducci, S.; Yang, M.; Trindade, L.M. New developments in fiber hemp (*Cannabis sativa* L.) breeding. *Ind. Crops Prod.* **2015**, *68*, 32–41. [CrossRef]
13. Linger, P.; Müssig, J.; Fischer, H.; Kobert, J. Industrial hemp (*Cannabis sativa* L.) growing on heavy metal contaminated soil: Fibre quality and phytoremediation potential. *Ind. Crop. Prod.* **2002**, *16*, 33–42. [CrossRef]

14. Citterio, S.; Santagostino, A.; Fumagalli, P.; Prato, N.; Ranalli, P.; Sgorbati, S. Heavy metal tolerance and accumulation of Cd, Cr and Ni by *Cannabis sativa* L. *Plant Soil* **2003**, *256*, 243–252. [CrossRef]
15. Arru, L.; Rognoni, S.; Baroncini, M.; Bonatti, P.M.; Perata, P. Copper localization in *Cannabis sativa* L. grown in a copper-rich solution. *Euphytica* **2004**, *140*, 33–38. [CrossRef]
16. Shi, G.; Liu, C.; Cui, M.; Ma, Y.; Cai, Q. Cadmium tolerance and bioaccumulation of 18 hemp accessions. *Appl. Biochem. Biotech.* **2012**, *168*, 163–173. [CrossRef] [PubMed]
17. Ahmad, R.; Tehsin, Z.; Malik, S.T.; Asad, S.A.; Shahzad, M.; Bilal, M.; Shah, M.M.; Khan, S.A. Phytoremediation potential of hemp (*Cannabis sativa* L.): Identification and characterization of heavy metals responsive genes. *Clean Soil Air Water* **2016**, *44*, 195–201. [CrossRef]
18. Campbell, S.; Paquin, D.; Awaya, J.D.; Li, Q.X. Remediation of benzo [a] pyrene and chrysene-contaminated soil with industrial hemp (*Cannabis sativa*). *Int. J. Phytorem.* **2002**, *4*, 157–168. [CrossRef]
19. Vandenhove, H.; Van Hees, M. Fibre crops as alternative land use for radioactively contaminated arable land. *J. Environ. Rad.* **2005**, *81*, 131–141. [CrossRef]
20. Strasser, R.; Tsimilli-Michael, M.; Srivastava, A. Analysis of the chlorophyll a fluorescence transient. In *Chlorophyll a Fluorescence*; Advances in Photosynthesis and Respiration; Papageorgiou, G., Govindje, E., Eds.; Springer: Cham, The Netherlands, 2004; pp. 321–362.
21. Singh, S.; Prasad, S.M. IAA alleviates Cd toxicity on growth, photosynthesis and oxidative damages in eggplant seedlings. *Plant Growth Regul.* **2015**, *77*, 87–98. [CrossRef]
22. Ministro per le Politiche Agricole. Approvazione dei "Metodi ufficiali di analisi chimica del suolo". Available online: http://ctntes.arpa.piemonte.it/Bonifiche/Documenti/Norme/13_Set_99.pdf (accessed on 17 April 2019).
23. Decreto Legislativo. Norme in Materia Ambientale. Available online: http://www.conou.it/wp-content/uploads/2015/11/Dlgs-152_2006-Norme-in-materia-ambientale.pdf (accessed on 17 April 2019).
24. Pietrini, F.; Iori, V.; Bianconi, D.; Mughini, G.; Massacci, A.; Zacchini, M. Assessment of physiological and biochemical responses, metal tolerance and accumulation in two eucalypt hybrid clones for phytoremediation of cadmium-contaminated waters. *J. Environ. Manag.* **2015**, *162*, 221–231. [CrossRef]
25. Cerovic, Z.G.; Masdoumier, G.; Ghozlen, N.B.; Latouche, G. A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. *Physiol. Plant* **2012**, *146*, 251–260. [CrossRef]
26. Iori, V.; Gaudet, M.; Fabbrini, F.; Pietrini, F.; Beritognolo, I.; Zaina, G.; Massacci, A.; Scarascia Mugnozza, G.; Zacchini, M.; Sabatti, M. Physiology and genetic architecture of traits associated with cadmium tolerance and accumulation in *Populus nigra* L. *Trees* **2016**, *30*, 125–139. [CrossRef]
27. Amaducci, S.; Errani, M.; Venturi, G. Plant population effects on fibre hemp morphology and production. *J. Ind. Hemp.* **2002**, *7*, 33–60. [CrossRef]
28. Chongkid, B.; Vachirapattama, N.; Jirakiattikul, Y. Effects of vanadium on rice growth and vanadium accumulation in rice tissues. *Kasetsart J. (Nat. Sci.)* **2007**, *41*, 28–33.
29. Vachirapatama, N.; Jirakiattiku, Y.; Dicinoski, G.W.; Townsend, A.T.; Haddad, P.R. Effect of vanadium on plant growth and its accumulation in plant tissues. Songklanakarin. *J. Sci. Technol.* **2011**, *33*, 255–261.
30. Dai, Y.J.; Shen, Z.G.; Liu, Y.; Wang, L.L.; Hannaway, D.; Lu, H.F. Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. *Environ. Exp. Bot.* **2009**, *65*, 177–182. [CrossRef]
31. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* **2000**, *51*, 659–668. [CrossRef]
32. Iori, V.; Pietrini, F.; Bianconi, D.; Mughini, G.; Massacci, A.; Zacchini, M. Analysis of biometric, physiological, and biochemical traits to evaluate the cadmium phytoremediation ability of eucalypt plants under hydroponics. *iFor. Biogeosci. For.* **2017**, *10*, 416–421. [CrossRef]
33. Linger, P.; Ostwald, A.; Haensler, J. *Cannabis sativa* L. growing on heavy metal contaminated soil: Growth, cadmium uptake and photosynthesis. *Biol. Plant.* **2005**, *49*, 567–576. [CrossRef]
34. Shi, G.R.; Cai, Q.S.; Liu, Q.Q.; Wu, L. Salicylic acid-mediated alleviation of cadmium toxicity in hemp plants in relation to cadmium uptake, photosynthesis, and antioxidant enzymes. *Acta Physiol. Plant.* **2009**, *31*, 969–977. [CrossRef]
35. Angelova, V.; Ivanova, R.; Delibaltova, V.; Ivanov, K. Bio-accumulation and distribution of heavy metals in fibre crops (flax, cotton and hemp). *Ind. Crops Prod.* **2004**, *19*, 197–205. [CrossRef]

36. Griga, M.; Bjelková, M. Flax (*Linum usitatissimum* L.) and Hemp (*Cannabis sativa* L.) as fibre crops for phytoextraction of heavy metals: Biological, agro-technological and economical point of view. In *Plant-Based Remediation Processes*; Springer: Heidelberg/Berlin, Germany, 2013; pp. 199–237.
37. Mattina, M.I.; Lannucci-Berger, W.; Musante, C.; White, J.C. Concurrent plant uptake of heavy metals and persistent organic pollutants from soil. *Environ. Pollut.* **2003**, *124*, 375–378. [CrossRef]
38. Kos, B.; Leštan, D. Soil washing of Pb, Zn and Cd using biodegradable chelator and permeable barriers and induced phytoextraction by *Cannabis sativa*. *Plant Soil* **2004**, *263*, 43–51. [CrossRef]
39. Zehra, S.S.; Arshad, M.; Mahmood, T.; Waheed, A. Assessment of heavy metal accumulation and their translocation in plant species. *Afr. J. Biotechnol.* **2009**, *8*, 12.
40. Varun, M.; D'Souza, R.; Pratas, J.; Paul, M.S. Metal contamination of soils and plants associated with the glass industry in North Central India: Prospects of phytoremediation. *Environ. Sci. Pollut. Res.* **2012**, *19*, 269–281. [CrossRef] [PubMed]
41. Houben, D.; Evrard, L.; Sonnet, P. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. *Chemosphere* **2013**, *92*, 1450–1457. [CrossRef] [PubMed]