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# Hemp Cultivation in Soils Polluted by Cd, Pb and Zn in the Mediterranean Area: Sites Characterization and Phytoremediation in Real Scale Settlement

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**Abstract:** Polluting activities affect, directly or indirectly, large areas of agricultural lands. Metal polluted soils could be managed by phytoremediation using hemp (*Cannabis sativa* L.). To know the phytoremediation capability of industrial hemp in metal polluted soils under semiarid environments, an experimental project with the support of local farmers was conducted in Sardinia (Italy). This work was carried out in three main steps: (i) identification and selection of the study sites, (ii) field trials, at local farms, both on contaminated and non-polluted sites, (iii) evaluation of heavy metals contents accumulated in the different parts of the plants. Five study sites were chosen. Three of them were severely polluted by heavy metals. Concentrations of Zn and Cd in plants generally were positively correlated with soil content and were different in each part of the plant. The higher values of Zn and Cd were detected in leaves of plants grown in polluted sites (Zn > 950 mg $kg^{-1}$ and Cd > 6.8 mg $kg^{-1}$). High values of Pb were also detected in plants grown in non-contaminated soils: this contamination may be due to atmospheric deposition related to polluting sources far to the cultivation.

**Keywords:** heavy metals; *Cannabis sativa*; soil pollution

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## 1. Introduction

The cultivated areas in the world represent a small proportion of the total land and their expansion is limited despite the needs of the growing world population. To meet the growing demand for food, there has already been an increase in cultivated areas equal to 12% between 1960 and 2010 at the expense of forests, grasslands and wetlands [1]. For the immediate future, it is estimated that the world population will need about 50% more food by 2050 [2]. Increases in food production should be supported both by improving crop management (e.g., breeding, fertilization, etc.) and by preserving and, where possible, recovering marginal and unusable polluted soils. Soil contamination compromises food security and furthermore the risk increases with the growing population [3]. Large amounts of agricultural areas in the world are polluted by anthropogenic activities, such as industrial, mining or intensive agricultural processes [4,5]. In the European Union, 2.8 million sites are potentially affected by polluting activities [6] and between these 137,000 $km^2$ of agricultural lands have been impacted by metal pollution [5].

Some metals and metalloids are potentially toxic for biological organisms at low concentration and could be accumulated in the food chain [7]. These elements that could be distinguished for their density and/or chemical characteristics, are generally present in the environmental matrices at very low concentrations and are identified by the terms potentially toxic elements (PTEs) [8], trace metals (TMs) [9,10], heavy metals and metalloids (HMs) [9,11,12]; this latter definition is widely used [7,8]. Heavy metal (loid)s can be released into the environment by anthropogenic activities or natural processes [13]

depending on the element [14]. Soils polluted by hazardous metals and metalloids such as As, Pb, Cd and Hg, are not suitable for food production because crops cultivated in these soils can contain harmful elements for human health [12]. In this context, the recovery of polluted soils appears to be a necessary strategy to support the production of both food and non-food agricultural productions. Traditional methods for remediation of metal contaminated soils ordinarily can include excavation, chemical stabilization, incineration, vitrification, and soil washing. These processes can be fast but are very expensive and could introduce secondary pollutants in the environment causing microflora disturbance and irreversible changes in soil chemical and physical properties [15,16].

Alternative solutions to deal with soil pollution could be phytotechnologies, which are processes that use plants to contain, sequester, remove, or degrade inorganic and organic contaminants in soils, sediments, surface waters, and groundwater [17]. These techniques are considered more economic and environmentally sustainable compared to the traditional methods [16]. The application of phytotechnologies to the profitable use of contaminated lands are also defined as phytomanagement [10].

In agricultural polluted soils, avoiding food or feed production, phytoremediation could be a suitable and sustainable option to obtain soil restoration/covering and, contemporaneously biomass for bioenergy [18,19].

Special care must be placed in crop selection and among the numerous species suitable both for phytoremediation and bioenergy production, industrial hemp, *Cannabis sativa* L., is an interesting option [20]. This crop is interesting for bioenergy production [21–23] and several studies have focused on hemp for phytoremediation purposes. Most studies on phytoremediation with hemp were made under controlled conditions such as hydroponics [24,25] or pots [26–30]. Some studies have been conducted in the field and mainly in northern areas [31,32], while very little knowledge exists about phytoremediation using hemp in semi-arid climates [33,34].

Many hemp cultivars are available and among these there are high differences in plant height, yield production capability of plant parts (e.g., fibres, seeds, oil seed; total above ground biomass, etc.), type of plant reproduction (monoecious or dioecious plants) or lengths of the growing cycle. The choice of an appropriate hemp cultivar in phytoremediation is an important key factor to obtain interesting biomass yields with appropriate metal contents in a sustainable production process.

In this framework the Regional Council of Sardinia (Italy), in 2015, promoted an experimental phytoremediation project for the cultivation of hemp in the polluted lands of the island. To lead this ambitious project the regional agricultural research agency, Agris Sardegna, proposed a multidisciplinary project, named CANOPAES (acronym for "CANapa: OPportunità Ambientali ed Economiche in Sardegna"—Hemp: Environmental and Economic Opportunities in Sardinia), that aimed to recover the areas subjected to severe pollution that are often underutilized or abandoned. The development of advantageous, innovative, economically, and ecologically sustainable production in these areas, through the cultivation of industrial hemp are the main objectives of CANOPAES project.

If the main goal of the CANOPAES project was to explore the sustainability of the cultivation of industrial hemp in metal contaminated soil in semiarid environments, as described above, several secondary aims were also pursued:

- to test the feasibility to apply a phytoremediation activity in the described conditions, private farms were involved;
- to evaluate the characteristics of the polluted soils and their availability for phytoremediation, a massive soil study was applied concerning pedological, chemical and physical features, Potentially Toxic Metal contents;
- to know how the metal content in soil affects the crop, the relationships between potentially toxic metals in soils and in hemp plants were explored.

In this study, were explained the results obtained by the massive soil characterization and were presented the first results of hemp cultivation in semiarid polluted fields.

## 2. Materials and Methods

### 2.1. Identification and Selection of Study Sites

The study was planned to be conducted in multiple sites representative of agricultural areas subjected to severe pollution in Sardinia. For this scope, a preliminary phase was carried out to identify suitable agricultural areas classified as contaminated or impacted by industrial and/or mining pollution. The survey was made using the Regional Environmental Information System—SIRA [35] and the Database of the Sardinian Soil—DBSS [36]. To involve farmers interested in participating in the CANOPAES project, public selection procedures were carried out in 2017 and 2018. Farmers who wanted to participate had to have an irrigable arable area (minimum 2500 $m^2$) available for at least three years for hemp cultivation, guarantee the ordinary cultivation (directly or through rental) and follow the recommendations of the researchers involved in the project. Experimental cultivations, in fact, were conducted in agricultural polluted areas directly by the farmers, whereas the researchers planned the experimental design and monitored crop growth and development during the growing seasons.

A database of farmers, with related information regarding the companies' equipment for crop management, irrigation availability and presence of metal pollution in their soil, was set. A Postgres server v. 13 [37] was used, connected with Postgis (v. 3.1) extension to QGIS software desktop v. 3.20.0 [38], in order to georeference both the farm locations, and the polluted and control sites. Then, several inspections were carried out to verify the possibility of conducting experimental hemp crops and the levels of metal pollution throughout preliminary fast soil chemical analyses.

### 2.2. Field Trials

Field trials were established in late spring 2017 and were cultivated in Site 3 (polluted) and in Site 5 (unpolluted). In each site, three industrial hemp monoecious cultivars (Uso 31, Felina 32, Futura 75) were cultivated (each plot about 500 $m^2$).

After a primary tillage with ripper and rolling harrow to obtain a good preparation of seedbed, industrial hemp was sown by Agris staff to guarantee a proper distribution of varieties following the layout of the experimental design. The experimental design applied was the randomized complete block design (RCBD) with three replications and the same randomization over the years. To guarantee the exact positioning every year, each plot trial was georeferenced using a global navigation satellite system (GNNS) receiver, the Geomax Zenith 35 Pro TAG with RTK correction for centimetre-scale accuracy.

To evaluate the potential phytoextraction of industrial hemp, samples of hemp were collected at seed maturity stages. The same day or the day after irrigation, samples of whole plants (including roots) were collected. Two samples of 25–250 whole plants for each plot were harvested depending on the dry matters (DMs) quantities required for the analyses. The whole plants sampled were separated into different parts: roots, stems, leaves, seeds, only the roots were rinsed with tap water and deionized water. Fresh weight and dry weight (after the samples dried at 65 °C for 72 h) were determined and finally, Zn, Pb and Cd tissue contents were analysed. For plant sample nitric acid and hydrogen peroxide digestion was applied [39], in the oven MARS 5, CEM and the matrix was analysed using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) OPTIMA 7300 DV, PerkinElmer.

### 2.3. Soil Samples and Chemical Analyses

To evaluate the spatial and vertical variability of the main pedological characteristics within the experimental sites, the pedological characterization was carried out through the description of a soil profile and four mini pits for each site. Each mini pit was placed near the four vertices of the field trial interested by the hemp cultivation and the soil was sampled. Soil samples were air-dried and analysed for texture (sand, silt, clay content) and main chemical parameters (pH in water, total and active calcium carbonates, organic carbon, extractable bases, total nitrogen, total and extractable phosphorus, total and exchangeable

potassium, total sodium, exchangeable sodium percentage, cation exchange capacity, base saturation). Soil chemical and physical analyses were carried out following the Italian official procedures [40].

For the heavy metal contents, the total extractable concentration of cadmium, lead and zinc was obtained by aqua regia mixture digestion (3:1 of HCl:$HNO_3$) according to the soil analysis Italian law [40]), in microwave oven (MARS 5, CEM). The analysis of the available metal fraction [40] was performed by extraction with diethylenetriaminepentaacetic acid (DTPA). The metal concentrations were determined using an ICP-OES (Optima 7300 DV, PerkinElmer, Inc., Waltham, MA, USA).

### 2.4. Statistical Analyses

Data obtained from soils and plants were statistically analysed according to the following steps. First the homogeneity of variance (Bartlett's Test) and the normal distribution (Shapiro-Wilk and Box-Cox tests) were checked. After that, when necessary, logarithmic transformation was applied to obtain the normality distribution of variables. Finally, data were analysed using mixed models in which sites, varieties and parts of plants were considered fixed factors.

For a further understanding of the relationships among the HM concentrations in the part of hemp plants and the HM contents in the soil, the Principal Component Analysis (PCA) was applied. Indeed, this analysis allows decomposition of a multivariate dataset, by reducing its dimension in only the two principal components, so that the data variation can be explained clearly [41]. Before performing the PCA, data standardization was made, and then PCA loadings and scores were used to construct the biplot that graphically summarized the main relationships existing between variables considered.

Statistical analyses were performed by the GenStat 20th software [42] and R [43]. The VM COMPARISON procedure was applied for the means comparison of the variables with fixed effects ($\alpha = 0.05$).

## 3. Results

### 3.1. Identification and Selection of Study Sites

The applications of interested farmers to participate in the project were 23 in 2017 and 30 in 2018. In 2017, most requests were from the Sardinian southwest region, called 'Sulcis-Iglesiente', while in 2018 the applications came from the rest of Sardinia without the prevalence of any area (Figure 1a).

Cross referencing these data with the georeferenced information available in the DBSS and SIRA systems [35,36], 18 candidates sites were selected. Based on the results of the first fast chemical analyses of the soil samplings made in candidate sites, (data not shown) eight candidates were chosen (Figure 1b).

The sites where the metal contents resulted above the contamination threshold established by the Legislative Decree 152/2006 [44] were considered "potentially polluted". Furthermore, the selection of candidate study sites was also made based on the presence of uncontaminated sites in adjacent areas available as "control".

Starting with the 53 applications from farmer who expressed an interest in participating in the project, eight planned experimental sites, that were highly polluted (3), moderately polluted (2) and non-polluted (3), were chosen for the project (Figure 1b). In fact, at the end of the selection phase, only three farmers signed up to join the CANOPAES project. The first farmer decided to participate in conducting three fields, hereafter named Site 2 and Site 3 (both polluted) and Site 4 (not polluted or control). Another farmer participated with a not polluted field (hereafter Site 5). Afterwards a farmer, that was interested in the project, but could not participate until 2019 due to administrative problems, was able to conduct an experimental field (hereafter named Site 1) that was moderately polluted. Finally, two other selected farmers who should have cultivated sites 6, 7 and 8 decided not to participate anymore.

For these reasons in this study the soils were described for all the 5 sites.

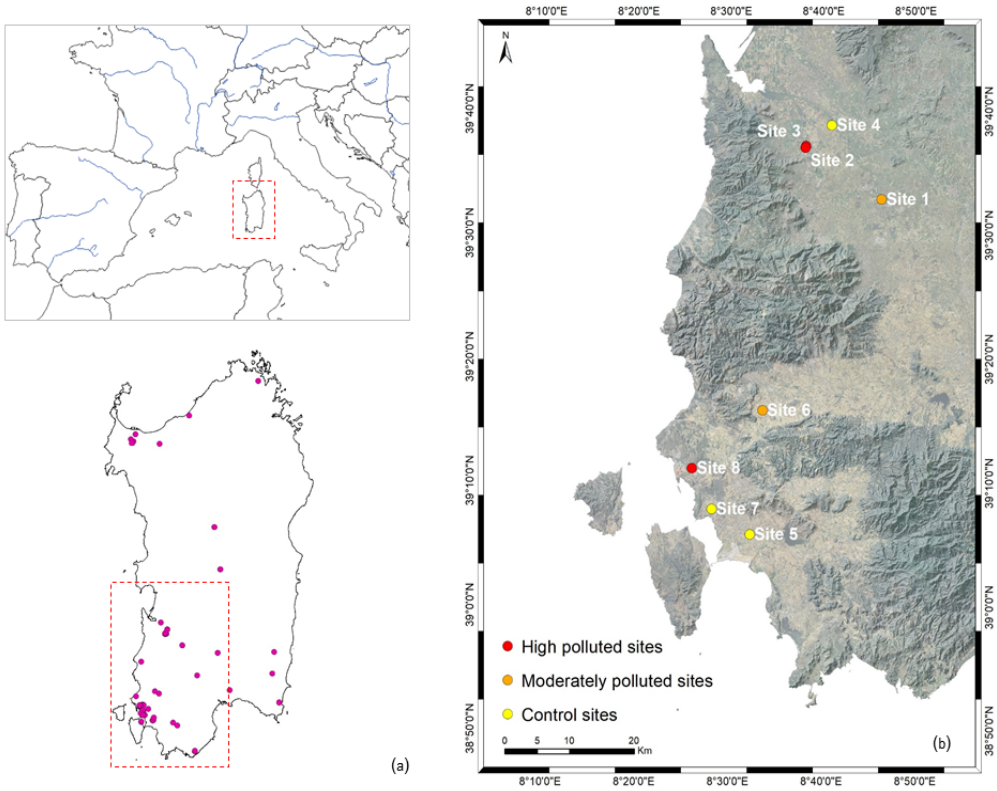


**Figure 1.** Case Study and selection of experimental fields: (**a**) polluted or mining georeferenced area, and request to participate by farmers in 2017 and 2018 (magenta symbol); (**b**) the Sulcis-Iglesiente study area and the five experimental fields, red ones are polluted sites, whereas yellow are control·sites.

### *3.2. Sites Pedological Description and HMs Distribution in Soil*

The main pedological characteristics of each experimental site were described and classified, by the soil profiles, according to the Soil Taxonomy [45].

For each horizon discovered in the profiles, physical and chemical properties and Zn, Pb, Cd contents, were described. Total and available Cd, Pb and Zn contents in the 5 soils are shown in Table 1 and more detailed data are reported in Supplementary Materials (S.M.).

**Table 1.** Total and available Cd, Pb, and Zn in soil of CANOPAES project: Sites 1–3 potentially polluted; 4,5 non-polluted.

| | Cd Total (mg kg$^{-1}$) | | Cd Avail. (mg kg$^{-1}$) | | Pb Total (mg kg$^{-1}$) | | Pb Avail. (mg kg$^{-1}$) | | Zn Total (mg kg$^{-1}$) | | Zn Avail. (mg kg$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Site 1** | 1.94 | c | 0.67 | c | 117.0 | c | 42.7 | b | 143.9 | c | 5.5 | c |
| **Site 2** | 4.17 | b | 2.46 | b | 308.0 | b | 74.8 | b | 572.5 | b | 115.5 | b |
| **Site 3** | 8.71 | a | 5.86 | a | 751.9 | a | 162.4 | a | 940.3 | a | 174.2 | a |
| **Site 4** | 0.84 | c | 0.19 | c | 52.0 | c | 6.3 | c | 146.1 | c | 2.7 | c |
| **Site 5** | 0.37 | c | 0.10 | c | 20.9 | c | 3.7 | c | 56.1 | c | 1.6 | c |

For any factor, means followed by the same letter do not differ significantly at $p \leq 0.05$ by LSD test.

Site 1 (39°32′52.74″ N–8°46′16.43″ E, 46 m a.s.l.) was located close to a lead smelter of the San Gavino city industrial area. The industrial site of San Gavino is subordinated to a specific Italian law for contaminated Sites that defines the Sites of National Interest (SIN). Specifically, the San Gavino site is included by the law into the SIN of Sulcis-Iglesiente-Guspinese [46]. The San Gavino lead smelter started in 1932, in the past processed Pb and Zn minerals from the Montevecchio mine [47]. After an inactive period, the San Gavino

thermal refinery is nowadays productive and decopperizes lead to obtain refined Pb as well as other products occurring with lead, silver, gold, and bismut. The highest levels of lead in the blood of people in the San Gavino population (adults and children) were found during the 1980–1981 monitoring years [48].

The area of interest for this study was classified for agricultural uses by the law, but indeed it was not utilized for a long time. Undisturbed vegetation, with predominantly herbaceous flora, covered the soil until the study started.

The soil of Site 1 was classified as Vertic Haploxerept, fine-loamy, mixed, superactive, thermic, with Ap, Bkss, Bkss2, C horizons. The contamination level was Pb > Zn > Cd with total values (in the topsoil) of 147, 125 and 2.4 mg $kg^{-1}$ respectively. The order of DTPA-extractable metal concentrations was Pb > Zn > Cd with 54.4, 4.86 and 0.95 mg $kg^{-1}$. The mobility of metals in the site, evaluated as the percentage of the total metal content extracted with DTPA, was 3.88% Zn, 37% Pb and 39.58% Cd.

Site 2 (39°37′5.65″ N–8°38′2.93″ E, 33 m a.s.l.) was located just 150 m outside of the areas amenable to the law for contaminated Sites of National Interest [46]. The entire area was affected by the flooding of the Sitzerri River that deposited, in its floodplain, high amounts of mining wastes from Montevecchio [47,49,50]. The Montevecchio mineral district activity, started in 1848 and closed definitively in 1991, was dedicated to extract and process PbS and ZnS. This experimental site was situated in irrigated land, and it was usually grazed or cultivated. The area was served by public irrigation infrastructure, but it was very damaged before the cultivation started.

The soil of the Site 2 was classified as Fluventic Dystroxerept, fine-loamy, mixed, superactive, with Ap1, Ap2, 2Bw, 3C1, 4C2, 5C3, 5C4, 6C5 horizons. The contamination level was Zn > Pb > Cd with total values (in the topsoil) of 441, 422 and 3.7 mg $kg^{-1}$ respectively. The order of DTPA-extractable metal concentrations was Zn > Pb > Cd with 115, 106 and 2.32 mg $kg^{-1}$ The mobility of metals in the site, evaluated as the percentage of the total metal content extracted with DTPA, was 26.07% Zn, 25.11% Pb and 62.70% Cd.

Site 3 (39°37′8.76″ N–8°38′6.48″ E, 32 m a.s.l.) was in the same area as Site 2. The main characteristics and management of the area are close to those described for Site 2.

The soil of the Site 3 was classified as Humic Haploxerept fine—loamy, mixed, superactive, thermic with Ap1, Ap2, Bw, C, 2C, 3C horizons. The contamination level was Zn > Pb > Cd with total values (in the topsoil) of 1076, 999 and 9.2 mg $kg^{-1}$ respectively. The order of DTPA-extractable metal concentrations instead was Pb > Zn > Cd with 258, 212 and 5.9 mg $kg^{-1}$. The mobility of metals in the site, evaluated as the percentage of the total metal content extracted with DTPA, was 19.7% Zn, 25.82% Pb and 64.13% Cd.

Site 4 (39°38′50.87″ N–8°40′53.13″ E, 21 m a.s.l.) was located in agricultural land, irrigated from decades, and it is usually cultivated with feed crops such as alfalfa, corn, sorghum. Public irrigation infrastructure serves this land.

The soil of the Site 4, one of the control sites, was classified as Fluventic Haploxerept, coarse—loamy, mixed, superactive, thermic with Ap, C, 2Bw, 2C, 3C horizons. The level of HMs was Zn > Pb > Cd with total values (in the topsoil) of 145, 50.7 and 1.01 mg $kg^{-1}$ respectively. The order of DTPA-extractable metal concentrations instead was Pb > Zn > Cd with 2.51, 5.2 and 0.21mg $kg^{-1}$. The mobility of metals in the site, evaluated as the percentage of the total metal content extracted with DTPA, was 1.73% Zn, 10.25% Pb and 20.79% Cd.

Site 5 (39°5′49.42″ N–8°32′15.62″ E, 5 m a.s.l.) was an agricultural area, that is the other control site, served by a public irrigation system for a long time and was usually cultivated by irrigated feed crops typical of the area, such as corn and alfalfa.

The soil of the Site 5 was classified as Pachic Calcixeroll coarse—loamy, mixed, semiactive, thermic with Apk, 2Bk, 3Bw, 4AB, 5Bwb horizons. The level of HMs was Zn > Pb > Cd with total values (in the topsoil) of 61.8, 24.1 and 0.4 mg $kg^{-1}$ respectively. The order of DTPA-extractable metal concentrations instead was Pb > Zn > Cd with 4.18, 2.25 and 0.12 mg $kg^{-1}$. The mobility of metals in the site, evaluated as the percentage of the total metal content extracted with DTPA, was 3.64% Zn, 17.34% Pb and 30% Cd.

To better understand the pollution levels in the experimental sites, Table 1 shows the distribution of the total and available HMs contents. The average values of HMs for each soil are reported in this table, while more detailed information is given in the Supplementary Materials (S.M.) file. The total HMs values shown in Table 1 were compared with the metal thresholds established by the Legislative Decree 152/2006 [44] that, for the case study were: 2 mg $kg^{-1}$ for Cd, 100 mg $kg^{-1}$ for Pb and 150 mg $kg^{-1}$ for Zn.

Based on soil chemical analysis and regulation, in Site 1, Site 2 and Site 3, the metal contents measured up to the Italian law thresholds [44] and they were considered "potentially polluted" with respect to the others that were considered "non polluted sites" (Site 4, Site 5). Non polluted sites were then used as "control" in comparison with the polluted ones.

### 3.3. Relationship between HMs Contents in Soil and in Plants

The analysis of the HM content in hemp plants revealed different behaviours depending on the metal analysed or the plant fraction observed.

To understand the distribution of the HMs in the different parts of hemp plants, Table 2 shows the metal concentrations observed, for each cultivated variety, in the two experimental sites.

In Table 2, as expected, differences in HM concentrations were significant between Site 3 (polluted site) and Site 5 (unpolluted site) for all metals considered. The hemp variety Uso 31 seems to be that which tends to accumulate the highest concentrations of Cd and Zn. The concentrations of metals in the different cultivars varied in the two sites considered and this behaviour is highlighted by the significant site × variety interaction. Once again, the trend of the contents of Zn and Cd were similar. The highest concentrations of HMs were observed in the variety Uso 31 followed by Felina 32, both in Site 3 (polluted), while the other highest value was found in Futura 75 of Site 5 (unpolluted). These different ranks determine the significant interaction observed.

Basically, no significant variety × parts and site × variety × parts interactions were observed but Site × parts of plants interaction were significant (Table 2).

The Pb value in leaves was 8.77 mg $kg^{-1}$ in Site 3, and 9.33 mg $kg^{-1}$ in Site 5, whereas Pb in seeds was significantly higher and almost double in Site 5 (2.80 mg $kg^{-1}$) that in Site 3 (1.54 mg $kg^{-1}$), that was classified as polluted. Comparable values in leaves and seeds between sites 3 and 5 were observed.

The biplot where scores and loadings obtained by the PCA were represented together were reported in Figure 2. The biplot graphically represents the main correlation existing between concentrations of HMs in part of the plants and the corresponding HMs contents in the soil where plants were cultivated.

The first two principal components account for 90.2% of the total variance and, hence, provide a good representation of the relations among the variables. The disposition and the magnitude of the blue vectors obtained by the PCA provide qualitative information on the correlation between the variables.

In Figure 2 the vectors, which represented available and total HMs content in the soil, are disposed essentially in the same direction of the scores representing the polluted site (Site 3). This arrangement indicates a greater presence of HMs in soil, both total and available concentrations, at Site 3.

The vectors of HMs in soil are arranged in the same direction as the vectors that represent the concentrations of the metals (Zn, Cd and Pb) contained in the different parts of the plant. This indicates essentially a greater presence of HMs in the different parts of the plants at Site 3.

Unlike other metals, different directions are observed for Pb content in seeds and leaves. The total Pb observed in soil in Site 5 (not polluted) was significantly lower than in Site 3 as already statistically observed (Table 1) and is contrary to what was observed for metals concentrations in parts of plants. Indeed, the observed Pb was statistically equal (leaves) if not higher (seeds) in plants of Site 5 compared with those of Site 3 (polluted also

for Pb). As shown in Figure 2, the same directions of metal in plants and in soils do not seem to distinguish between available and total metal contents in soils.

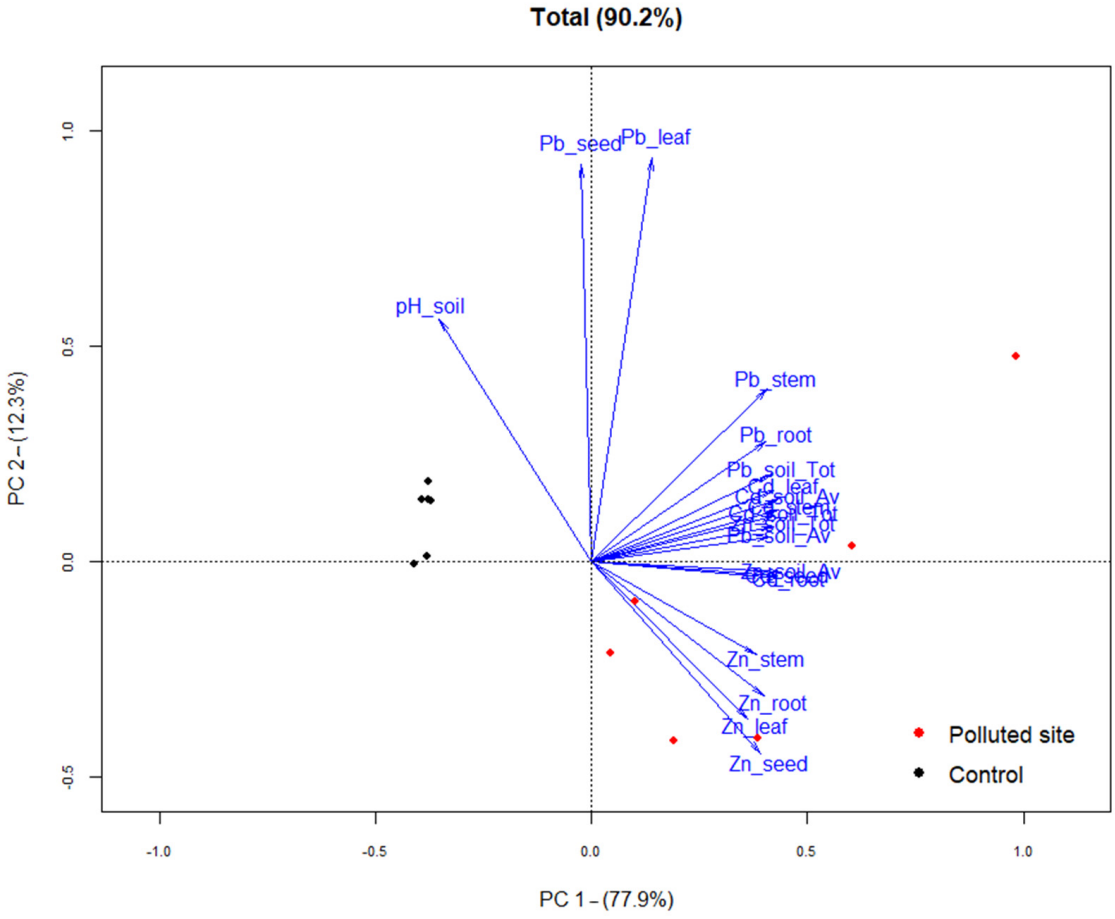


**Figure 2.** Biplot that represent relations among Cd, Pb and Zn in soil and hemp plants parts.

**Table 2.** Measurements of Cd, Pb and Zn in hemp plants, by variety and plant part, in different sites.

| Factor | Heavy Metals | | | | | |
|---|---|---|---|---|---|---|
| | Cd (mg kg$^{-1}$) | | Pb (mg kg$^{-1}$) | | Zn (mg kg$^{-1}$) | |
| **Site** | *** | | *** | | *** | |
| 3 | 4.15 | a | 6.75 | a | 281.3 | a |
| 5 | 0.06 | b | 3.03 | b | 31.6 | b |
| **Variety** | *** | | *** | | *** | |
| Uso 31 | 3.34 | a | 5.73 | a | 230.7 | a |
| Felina 32 | 0.46 | c | 3.32 | b | 58.7 | b |
| Futura 75 | 0.63 | b | 4.94 | a | 62.3 | b |
| **Parts** | *** | | *** | | *** | |
| Leaves | 1.80 | a | 9.05 | a | 278.75 | a |
| Stems | 1.00 | b | 3.65 | c | 45.53 | c |
| Roots | 0.94 | b | 5.87 | b | 43.66 | c |
| Seeds | 0.85 | b | 2.09 | d | 143.71 | b |
| **Site × Variety** | *** | | *** | | ** | |
| 3 Uso 31 | 11.42 | a | 10.40 | a | 766.9 | a |
| 3 Felina 32 | 3.32 | b | 4.13 | c | 223.4 | b |

**Table 2.** *Cont.*

| Factor | Heavy Metals | | | | | |
|---|---|---|---|---|---|---|
| | Cd (mg kg$^{-1}$) | | Pb (mg kg$^{-1}$) | | Zn (mg kg$^{-1}$) | |
| 3 Futura 75 | 1.70 | c | 7.06 | b | 129.8 | c |
| 5 Uso 31 | 0.74 | d | 3.06 | d | 69.2 | d |
| 5 Felina 32 | 0.01 | f | 2.65 | d | 15.2 | f |
| 5 Futura 75 | 0.18 | e | 3.42 | cd | 29.8 | e |
| **Site × Parts** | ** | | ** | | *** | |
| 3 Leaves | 6.80 | a | 8.77 | b | 952.3 | a |
| 3 Stems | 4.02 | b | 7.51 | b | 177.7 | c |
| 3 Roots | 3.52 | bc | 17.79 | a | 147.4 | d |
| 3 Seeds | 3.01 | c | 1.54 | d | 250.1 | b |
| 5 Leaves | 0.23 | d | 9.33 | b | 81.2 | e |
| 5 Stems | 0.00 | e | 1.65 | d | 11.4 | f |
| 5 Roots | 0.02 | e | 1.72 | d | 12.7 | f |
| 5 Seeds | 0.02 | e | 2.80 | c | 82.5 | e |
| **Variety × Parts** | **n.s.** | | **n.s.** | | **n.s.** | |
| **Site × Variety × Parts** | * | | * | | **n.s.** | |

*** Indicates significant at $p < 0.001$, ** Indicates significant at $p < 0.01$, * Indicates significant at $p < 0.05$, n.s. indicates non-significant. For any factor, means followed by the same letter do not differ significantly at $p \leq 0.05$ by the LSD test.

## 4. Discussion

The beginning of the CANOPAES project was complex, especially in the preliminary stages, due either to the difficulty in collecting information on the spread of polluted agricultural areas and to establishing fruitful collaborations with farmers. The results of the preliminary fast chemical analyses of the soil, realized during the starting selection of the sites, were confirmed by the obtained soil site characterization (Table 1).

According to the regulations in force to identify the polluted agricultural areas, the pollution threshold established by the L.D. 152/2006 [44] was used as reference. This law, "Norms Concerning the Environment", regulates the Italian waste management system and considers public, private, and residential green areas. Indeed, only in 2019, did Italy regulate agricultural polluted areas, with the Ministerial Decree 46/2019 [51]. The law, issued by the Environmental Ministry containing regulations relating restoration and environmental safety of the areas destined to agricultural production. This law defined contamination thresholds for agricultural soils that, for some metals, are slightly higher than the previous ones. In the Sulcis-Iglesiente, the area considered in this study, where numerous contaminated sites have been identified, a lack of irrigation availability was found. This factor made it extremely difficult, if not impossible, for farmers, to join the project because in southern environments, hemp is an irrigated crop.

For these reasons, and because of an awareness of the problems present in the polluted areas, to increase farmers participation, some information events were organized. Thanks to these events, 53 farmers applied to participate in the project but only three, who had the required requisites, finally joined the CANOPAES project.

What was observed during this phase highlights that the experimentation performed in modality "on farm trials" allows to evaluate further concrete problems in conducting phytoremediation in contaminated areas. In phytoremediation applied in the field, several factors can affect the efficiency of the treatment or the plant responses [52]. With respect to assessments made in an experimental station, or in a confined test environment, difficulties that can be underestimated or not considered at all, may arise in the real context of polluted areas.

By applying this method, bureaucratic or infrastructural problems (e.g., lack in efficient irrigation systems) could be revealed and, if possible managed, before the introduction of a new agricultural practice as phytoremediation. In the case of hemp, which is considered an irrigated crop, when evaluating the economic convenience of conducting phytoremedi-

ation in contaminated soils, it is therefore necessary to account for the costs of restoring irrigation systems.

The metal concentration in the soil sample of Site 1 (Table 1) were above the legal threshold levels for Pb [44]. Recently, the areas around the refinery have been monitored to define the pollution matrix levels [53] and in some cases, As, Hg, Pb concentration in soil were above the Italian law threshold, such as in the area occupied by the Site 1.

By the soil characterization carried out in this study, Site 1 was contaminated only with Pb on the soil surface, probably due both to the presence of the refinery nearby and by the geochemical background values [53]. The high amount of contaminant metals such as Cd, Pb and Zn, observed in the sites 2 and 3, was due to mine metal deposition [47,49,50,54,55]. Site 2 and Site 3 differ from each other despite being close and from the same geological matrix. Furthermore, Site 2 was expected to be more contaminated because it was closer to the source of the flood, but instead Site 3 was significantly more contaminated. Plants collected in Site 3 showed a presence of toxic metals (Cd, Pb, Zn) strictly related to the high contents observed in soils. The levels of toxic metals detected in soils of Sites 4 and 5 were much lower than the Italian law thresholds.

For Site 5, on the other hand, where contamination in the soil was not expected, this was confirmed by the soil chemical analysis but, when the plants were analysed, the presence of contaminants were revealed. It can be hypothesized that in the soil, the metals are diluted, while the concentration in the plants can be high due to atmospheric deposition. Metal dust originated from industries or by metalliferous mining with inadequate closure management, could be transported to other areas, by water and aeolian dispersion [28,33,56]. In southwest Sardinia, pollutants by industrial activities are correlated with environment and biota [57] and metal pollution affects people living near industrial sites [58]. In the Sulcis-Iglesiente area, included in San Giovanni Suergiu municipality (where Site 5 was located), remarkable levels of metal deposition were detected between the years 2008 and 2014 as reported by the Regional Environmental Protection Agency of Sardinia (ARPAS) in their technical reports [59].

Describing the chemical behaviour of HMs in soil, it can be observed that Zn is very similar to Cd. The mobilization of Zn is favoured by conditions of low pH and oxidizing environment, which make this element rather mobile in relation to other metals. Although Zn easily forms complexes in soils with organic matter and carbonates that can lead to an accumulation of the element in the surface horizons, its association with Fe and Al hydroxides and clays seems to be predominant. There is an inverse proportionality between the solubility and bioavailability of Zn and the presence in soils of Ca and P compounds, given the chalcophilia of this element. In general, it can be said that acidic, sandy soils poor in organic matter are those that mostly favour the mobilization of Zn [60]. The applications of Zn are many, but its dispersion into the environment originates mainly from the metallurgical industry and agriculture, which can lead to significant accumulations in the surface horizons, sometimes creating significant environmental problems [9,61,62].

Additionally, Pb has a marked chalcophilia, and as a result of weathering processes it is able to bind to carbonates, clays, organic matter and oxides of Fe and Mn present in the soils. Its affinity with calcium and generally with group 2 elements such as K, Ba, Sr, also allows it to replace these elements both in minerals and within the soil [60,61]. The solubility of Pb is inversely proportional to the pH values, as at high values it precipitates as carbonate, phosphate, and hydroxide, while at low pH value is weakly solubilized. All these characteristics make Pb one of the least mobile metals of all and therefore has very low natural concentrations. The typical presence of Pb in soils is concentrated in the surface horizons [56], favoured by the accumulation of organic substances, which also represents the most important "sink" of this element in polluted soils [63]. The increasing dispersion of Pb in the environment and its known toxicity to plants and animals has led to a multiplication of studies on the presence of the element in soils and its migration dynamics. The anthropogenic sources of diffusion are industrial, craft and agricultural activities. The danger of Pb lies, in addition to its intrinsic toxicity for organisms, to the

aforementioned tendency to stabilization, which makes its soil pollution practically irreversible. In fact, it is estimated that the natural removal of 10% of Pb from polluted soil takes about 200 years [62,64].

Several health and environmental problems could be caused by metals depending on their way in food chains, concentrations and chemical status [13].

The Cd does not perform essential biological functions for plants and animals and is, on the contrary, highly toxic [12,65,66]. The plants, once having absorbed the element, are unable to eliminate it and, therefore, tend to accumulate it mainly in the roots and, to a lesser extent, in the aerial parts of the plant; this phenomenon fortunately limits the movement of the Cd through the food chain [67]. Concentrations detected in the environment hardly lead to acute poisoning, but the greatest risk for human health is given by chronic accumulation [68]. The main sources of soil contamination can be: use in agriculture of sewage sludge and phosphate fertilizers deriving from phosphorites, rocks naturally rich in this element; industrial sources such as mines, Pb and Zn foundries [9]. The metal Cd has a remarkable affinity with organic matter and being easily bioavailable can enter the food chain through plants or migrate deep into the soil with the risk of contaminating groundwater. The mobility of the Cd is strongly influenced by the pH, increasing when the soil has an acid reaction, although migrations of Cd have also been reported in the literature in neutral and alkaline soils [63].

The preliminary results, obtained during the first experimental year of hemp cultivation, showed the capability of plants to grow until seed maturity in soils containing high concentrations of Cd, Pb and Zn and during environmental and agronomic disadvantageous conditions. In the polluted site, some heterogeneities in crop emergence were observed due to an imperfect seedbed preparation and to failures in the irrigation system.

Few studies have been realized to explore different phytoextraction capabilities among hemp varieties. Moreover, there did not exist a comparison using multi contaminated sites. In this study the differences in metal contents among varieties were significant. This result is in agreement with those observed by Shi and Cai [30] where 18 accessions tested for 45 days in soil contaminated by 25 mg $kg^{-1}$ of Cd showed interesting differences among varieties in Cd concentrations. According to Girdhar et al. [69], which compared the phytoextraction results obtained from some hemp varieties by different authors, the metal extraction in specific Cannabis varieties may be explored.

The metal distribution in different parts of the plants varied in the two sites. As can be observed in Table 2 the highest HMs values were observed for Cd and Zn in leaves and the values found for Site 3 were statistically higher than those of Site 5 (Table 2 and Figure 2). In the polluted soil, Zn in hemp was very higher than that observed by Angelova et al. [31] and Pietrini et al. [29]: in leaves, the part of the plant with the highest values observed, Zn exceeded 950 mg $kg^{-1}$.

Analyses revealed that Pb in tissues of plants collected in the polluted site has higher values for roots (17.79 mg $kg^{-1}$) followed by leaves and stems with significantly lower values for seeds (about 1.5 mg $kg^{-1}$). The Pb values described above are similar to those described by Ćaćić et al. [26] and Angelova et al. [31] for leaves. On the other hand, these latter authors reported lower contents in roots, stems, and seeds. Low contents of Cd and Pb were observed also by Saastamoinen et al. [32] in seeds an hemp oil of Finola cultivar obtained in field trial.

In the unpolluted site, Pb values in roots and stems were similar to contents observed in Site 3 whereas higher values were observed especially for leaves: in the latter case Pb did not differ between site 3 and 5. The higher value of Pb observed in the aerial parts of plants in Site 5, especially in leaves and seeds, cannot be explained by the translocation into the plants from the soil (phytoextraction), but probably was due to the atmospheric deposition. Indeed the presence of HMs from the atmosphere was confirmed by previous monitoring by the local regional environment agency [59] that reported remarkable deposition of air polluting metals as Pb and Zn.

The Cd concentration in tissues of plants grown in the polluted site was higher in leaves than stems and roots. Significant lower values were observed for seeds. The Cd values detected for stems, roots and seeds (3.0–4.0 mg $kg^{-1}$) were similar to those reported by Angelova et al. [31] and Zhang et al. [70] but lower than concentrations reported for *Cannabis sativa* by others [27,30,71]. Except for the leaves, Cd values in tissues of plants grown in the unpolluted site were low and often below the detection limits.

In general, in this study, higher values of Zn were observed in plants than for Cd and Pb. The highest concentrations of Zn relative to those of Pb were observed in the aerial parts of plants in this study, in agreement with that reported by Pietrini et al. [29]. The Cd in soil was the pollutant contained in lowest values, while in plants the concentrations were similar to those of Pb. In contrast, the Pb values in soil were 60–80 times higher than those of Cd. The plant uptake of Cd and Pb could be limited by the low mobility in soil of those elements. The uptake of Zn, as well as their mobility, could be favoured by the biological utilization. Several factors related to plant and soil, such as genetic, edaphic or climatic factors, could be involved in the plant metal uptake and translocations [7,15]. In this regard, to explore the capability of bioconcentration and translocation of the *Cannabis* species, and of the cultivars, the cultivation should be monitored during several repeated experimental years in the field.

The results showed *Cannabis sativa* had a limited capacity to accumulate Cd and Pb in its biomass.

The low values of these metals in marketable plant fractions, as stems, seeds, or flowers, to the exclusion of feed or food uses, make it interesting to explore the use of the biomass obtained from polluted soils for several supply chains. A more complete examination of these aspects can be carried out in the next years of the CANOPAES project activities. Among the various sectors that may be interested for the use of the biomass produced in these areas, excluding the food sector, the energy sector (for example the use in anaerobic digestion plants) could be one of the most promising [21]. The energy production by hemp could allow, at the same time, the reduction of waste and cost disposal in landfills. This study reported the first results of the CANOPAES project that aims to enhance the cultivation of hemp in soil polluted by metals in the Sardinia Region (Italy). The results obtained by the intensive soil characterization, integrated previous information, and boosted new knowledge for stakeholders interested in cultivation of metal polluted agricultural soil. By the study of pollutants in plant and soil, a close relationship between them was observed in some cases, but an unknown source of metal deposition in non-contaminated soils was also suggested. These first results could support the cultivation of industrial hemp in agricultural soils polluted by metals under semiarid climates.

## 5. Conclusions

A shared project of phytoremediation using hemp was started in agricultural areas polluted by metals, under a semiarid climate. It was preceded by characterization of the soil and several results were available to support the production capability and/or security in the mapped areas. The CANOPAES project, organized through field activities carried out in collaboration with the farmers, has highlighted numerous problems of the socio-economic and structural context of the studied area. Many of these problems emerged during the start-up phase, and the project management, made possible to highlight concrete problems (e.g., availability of irrigation systems) that could limit the success of a phytoremediation activity carried out with hemp in large, contaminated areas beyond the actual or observed metal phytoremediation capacity. The results suggest that hemp does not seem to behave as a hyper-accumulating plant towards Pb, Cd and Zn. However, hemp also shows an appreciable capability to grow until the seed matures in heavily contaminated soils and in environmental and agronomic unfavourable conditions. This study suggests interesting relationships between the metal content in plants and soil: the total or available metal content in the soil cannot alone explain the high plant contents as observed for Pb. Indeed, in this case the high metal content in plants could be related only to atmospheric pollution.

This research highlights the different phytoextraction capabilities among hemp varieties. This last aspect needs to be investigated further, to explore the capability of different hemp varieties to translocate metals from the soils to the different parts of the plants. The results that will be collected in the following years in the CANOPAES project could allow investigation of these issues and the environmental and economic sustainability of the cultivation of hemp as a phytoremediation crop in contaminated sites in the Mediterranean area. The evaluation of the ways for disposal of the biomass produced in such conditions and the economic sustainability of this activity is one of the main issues to tackle in the following years.

Monitoring hemp cultivation for several years in areas polluted by metals in the field could provide the data needed to resolve this last issue. This will be one of the main objectives in the continuation of the CANOPAES project.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12073548/s1.

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