

Article

Assessment of the Bioavailability and Speciation of Heavy Metal(loid)s and Hydrocarbons for Risk-Based Soil Remediation

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Received: 28 July 2020; Accepted: 15 September 2020; Published: 22 September 2020



Abstract: For the assessment of the environmental and sanitary risks deriving from contamination of agricultural soils, it is crucial to identify and characterize the contaminants and study the soil chemical properties influencing their mobility and bioavailability. This information is essential for the selection of the best site remediation and securing strategy. The study site of this work is agricultural land of 6 ha in the province of Naples (Italy) subject to the past illegal burial of industrial wastes, principally from tanneries. With the aim of identifying the contaminants and assessing their mobility and bioavailability, the soil of the site was characterized for the main chemical and physical properties and for the concentration of potentially toxic elements and hydrocarbons. The readily and potentially bioavailable fractions of the main metal contaminants and their distribution in the soil geochemical fractions were determined by extraction in 1 M of NH_4NO_3 , 0.05 M of ethylenediaminetetraacetic acid (EDTA) pH 7, and European Community Bureau of Reference (EU-BCR) sequential fractionation. Further, the speciation of heavy hydrocarbons and chromium was carried out. The agricultural soil was widely contaminated by chromium, zinc, and heavy hydrocarbons (up to 4487, 1846, and 1800 mg/kg, respectively). In some sub-areas, contaminations by cadmium, lead, and copper (up to 283, 417, and 1183 mg/kg, respectively) were also observed. The chromium was found to be scarcely mobile and bioavailable and was mainly associated with the oxidizable, residual, and reducible fractions of the soil (on average 56%, 25%, and 19% of the total, respectively). However, chromium speciation revealed the presence of a significant amount of highly toxic Cr(VI) (above the legal threshold of 2 mg/kg), despite the low oxidizing power of the soil. Zinc was more mobile and bioavailable than chromium and was mainly distributed among the acetic acid-extractable and reducible fractions of the soil (on average 28% and 47% of the total, respectively). Cadmium was found to be very mobile and bioavailable, and was mainly distributed in the acetic acid-extractable and reducible fractions of the soil (on average 40% and 45% of the total). The speciation of heavy hydrocarbons showed that they consist almost entirely of long-chain aliphatic hydrocarbons, considered not very toxic and immobile. These results suggest that the use of not-edible plant coverage might be the best securing and remediation action for the study site, with the potential to preserve the soil ecosystem services, contain the risk of soil erosion and particle dispersion, and phytoextract the bioavailable metals.

Keywords: agricultural soil; potentially toxic elements; heavy hydrocarbons; bioavailability; contaminant speciation

1. Introduction

Soil pollution by human activities is an increasing problem in the world, threatening human health and the environment [1–3]. Thus, exposure and risk assessment for humans and the environment is crucial [4], as well as the definition and selection of the most appropriate site-specific remediation objectives and strategies [5–7]. On the basis of the level of contamination, type of contaminants, soil properties, and destination use, the risks and the remediation objectives could be different, and also risk mitigation or progressive reduction in the bioavailable pool of contaminants could be considered [4,6,8,9]. The bioavailable fraction of a contaminant is considered as the portion of the contaminant's total content that is available and hence harmful to target organisms, or that is mobile and transferable to water or other sites [10–12]. Recently, the Italian legislation set threshold values (CSC) for inorganic and organic contaminants in agricultural soils (D.M. 46/2019), and also suggested the measurement of bioavailability of metals exceeding the CSC, but did not elaborate threshold values for bioavailable concentrations; instead, other European countries (Germany, Slovakia, and Austria) have elaborated these values for ammonium nitrate extraction to assess the risk of metal transfer to food plants in agricultural soils with chemical extractions [13,14].

In polluted soils, the bioavailability of potentially toxic elements (PTEs) is strictly linked to the nature of the contaminant(s), the chemical forms in which they occur in soil, and the soil chemical-physical properties controlling the repartition of the contaminant(s) between the various soil geochemical fractions [13,15]. Knowledge of these characteristics is necessary in order to optimize the management of a contaminated site. For organic contaminants, bioavailability in soil and chemical and biological methods of measurement were deeply reviewed by Ortega-Calvo et al. [16]. According to these authors and depending on the processes involved, bioavailability can be considered the potential of the contaminant for direct transport and interaction with the cell membrane (i.e., chemical activity), or can incorporate the interactions of the contaminant with the soil and the water phase (i.e., bioaccessibility). Usually, risk assessment approaches start with the use of total concentrations as a first estimation of exposure. More detailed information, such as the speciation, chemical activity, or bioaccessibility, is necessary if there is a clear need [17]. The inclusion of bioavailability measurement in regulation requires the development of standard operating procedures [8] and agreement between scientists and regulators regarding the incorporation of bioavailability knowledge into existing risk assessment structures [18].

The present study investigated the total load, speciation, and bioavailability of inorganic and organic contaminants (heavy metal(loid)s and hydrocarbons) in an agricultural land subject to the past illegal burial of industrial wastes (mainly tannery wastes) and located in the Campania Plain on the north-east side of the Campania region (southern Italy). Most of this territory was traditionally agricultural and known for producing popular traditional food products, all benefiting from protected designation of origin (PDO) status, such as water-buffalo mozzarella cheese; canned San Marzano tomatoes; Piennolo tomatoes; and Falerno, Asprinio, and Falanghina dei Campi Flegrei wines. Unfortunately, during the 1990s, part of the area was used for the illegal dumping of industrial wastes, which were partially burned in the open air, causing the development of fires [19]. The area, once known as “Campania felix” because of its highly fertile soils, was renamed as the “Land of Fires”, causing a deep crisis in the local economy. Fortunately, recent investigations have proved that the area actually contaminated by the illegal waste disposal is more limited than expected, reaching no more than 3% of the total surface [20,21]. The contamination is mainly due to potential toxic elements (PTEs) and, in some cases, organic compounds [21].

The choice of the remediation intervention in the area is very delicate. It must answer not only to the requisites of efficacy and efficiency, but also to the requisites of popular acceptability. A variety of soil remediation techniques exist [22,23], and many of them are complex, expensive, and sometimes environmentally destructive. In a global scenario of increasing attention to soil protection [24,25], the preservation of soil ecosystem services must be taken into account in the selection of a remediation strategy. The use of plants and microorganisms is among the most inexpensive and sustainable

remediation strategies, and through various mechanisms can help to clean and secure polluted sites, minimizing the risks, stabilizing pollutants, and at the same time preserving the soil ecosystem services [4,9,26–28].

The knowledge of the speciation and bioavailability of contaminants, as assessed in the present study, is crucial in order to assess the suitability of phytoremediation as an appropriate strategy for the management of the study site.

2. Materials and Methods

2.1. Study Site and Soil Sampling

The study site is agricultural land of 6 hectares, currently confiscated by the Italian Judiciary due to the past illegal burial of industrial wastes (probably mainly tannery sludges), situated in the province of Naples (Campania region, Italy); a peach orchard was planted in the field. From preliminary investigations carried out by the regional authorities, the soil was found to be severely contaminated by potentially toxic elements (PTEs), mainly Cr and Zn, and heavy hydrocarbons (HH-C > 10) listed and normed by Italian legislation (D.Lgs 152/2006).

To verify the severity and the spatial distribution of the contamination, the soil sampling was carried out according to a sampling grid of 20 × 20 m at three depths, 0–20, 30–60, and 70–90 cm, for a total of 507 samples; furthermore, 15 soil samples were collected as background soil samples (BG) in five points surrounding the study field according to the three depths. A map of the study site showing the soil sampling georeferenced points is reported elsewhere (Figure 1a in [29]), in a study also providing some geomorphological and geological features of the area. The soil samples were named according to their position in the sampling grid (capital letters for columns and numbers for rows) and the depth of sampling (0–20, 30–60, 70–90). For instance, the soil sample A13/0–20 was taken on the sampling grid at the crossover between column A and row 13 at a depth of 0–20 cm.

After the sampling activity, the site was secured by explanting the peach orchard for food production purposes, then applying the ECOREMED protocol (LIFE11/ENV/IT/275; [30]) consisting of the planting of poplar trees and grass species for phytoremediation, avoiding as well the dispersion of soil particles in the atmosphere by the wind.

2.2. Soil Analysis

All the soil samples were analyzed for their content of heavy hydrocarbons with 10 or more carbon atoms (HH-C > 10) and 53 elements (Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Fe, Ga, Ge, Hf, Hg, In, K, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Pd, Pt, Rb, Re, S, Sb, Sc, Se, Sn, Sr, Ta, Te, Th, Ti, Tl, U, V, W, Y, Zn, Zr), among which there were the 14 potentially toxic elements (As, Be, Cd, Co, Cr, Cu, Hg, Ni, Pb, Sb, Se, Tl, V, Zn) normed by Italian legislation for agricultural, residential, public green, industrial, and commercial soils (D.M. 46/2019 and D.Lgs 152/2006). The determination of potentially toxic elements was carried out by the microwave-assisted aqua regia digestion and ICP-MS analysis (ICP-MS Elan 6000, Perkin Elmer) of the digested solutions [31,32]. The determination of heavy hydrocarbons (HH-C > 10) was carried out according to International Organization for Standardization (ISO) 16703 method [33]. This preliminary detailed mapping of contaminants served to identify the contaminants, the severity of contamination, and the spatial variability of the contaminants. A correlation analysis between the elements analyzed was followed to define if there were one or more sources of contamination. With these data available, it was decided to evaluate the mobility, bioavailability, and speciation of contaminants on a limited number of samples, having ascertained a single main source of contamination (apart from two field sub-areas, from which two samples were chosen). Therefore, a selection of 10 soil samples was analyzed for the main chemical and physical properties, the bioavailability and speciation of PTEs, the oxidizing power against chromium, and the total hexavalent chromium. The 10 soil samples were selected from the 522 field samples with the view to represent various total concentrations of PTEs and various depths. A further selection of nine

soil samples was analyzed for the hydrocarbon speciation. Again, 9 soil samples were selected from the 522 field samples with the view to represent the various total concentrations of hydrocarbons. The K_d determination was carried out on another five samples randomly selected from the 522 field samples. The main chemical and physical properties were analyzed according to the Italian official methods of soil analysis [34]. The soils were air-dried and sieved to 2 mm, then the pH was determined potentiometrically by applying a 1:2.5 soil:water ratio, organic carbon according to the Walkley and Black method, a cation exchange capacity (CEC) in $\text{BaCl}_2 + \text{TEA}$ solution at pH 8.1, carbonates by pressure Dietrich–Fruehling calcimeter, and a particle size analysis (Andreasen's pipette method) after wet sieving by sedimentation in aqueous medium.

The bioavailability and speciation of PTEs in the soil were analyzed following Rocco et al. [35]. Among the PTEs exceeding the CSC, we selected Cr, Zn, Pb, and Cu because of their greater diffusion in the field, and Cd for its high dangerousness and because it largely exceeds the CSC. In particular, extraction in 1 M of NH_4NO_3 (2 h of extraction in a soil:extractant ratio of 1:2.5; [36]) and in EDTA at 0.05 M and pH 7 (1 h of extraction in a soil:extractant ratio of 1:10; [37]) were carried out to determine the readily and potentially bioavailable amounts of PTEs, respectively. Sequential extractions with the European Community Bureau of Reference (EU-BCR) protocol [38] were carried out in 7 of the 10 samples to determine the distribution of PTEs among the soil geochemical fractions. Briefly, the procedure can be summarized as follows: Step 1—0.11 M of acetic acid; Step 2—0.5 M of hydroxylammonium chloride; Step 3—8.8 M of H_2O_2 at 85 °C/1 M of ammonium acetate; Step 4—aqua regia extraction; 16 h of extraction in a soil/extractant ratio of 1:40 for Step 1, 2, and 3. Extractions in water [39] were carried out to determine the potential mobility of PTEs in water; the soil/water partition coefficients (K_d) were calculated as the ratio between the concentration in the soil and the concentration extracted in water after 24 h of contact with the soil at a 1:2 soil:water ratio. The PTEs of interest (Cr, Zn, Cd, Cu, Pb) in the various extracts were determined by atomic absorption spectroscopy (AAS) (Analyst 700, Perkin Elmer).

The determination of the soil oxidizing power against chromium was carried out according to the official Italian method n° XII.6 (Supplemento Ordinario G.U. n° 248 del 21-10-1999).

The extraction of total hexavalent chromium was carried out according to the Environmental Protection Agency (EPA) 3060 A method [40], and the determination of Cr(VI) in the extract was carried out according to the EPA 7196 A and EPA 7199 methods [41,42]; the determination of Cr(VI) was carried out on seven samples, with the total Cr content ranging from 103 to 10046 mg/kg; four samples were taken from the 10 previously selected, and three were additional very highly contaminated samples collected from open trenches intercepting the buried sludges.

The hydrocarbon (HH-C > 10) speciation was carried out according to the Massachusetts Department of Environmental Protection (MADEP) approach [43].

3. Results and Discussion

3.1. Identification, Quantification and Spatial Distribution of Contaminants in the Site

As expected, the main contaminants detected in the study area were chromium, heavy hydrocarbons, and zinc, and were highly, widely, and disorderedly present in the field, with a tendency to a less widespread but more severe contamination in the 30–60 and 70–90 layers (Figure 1, Table 1). Chromium exceeds the Italian threshold (CSC) in 71% of the soil samples, with exceedances of up to 30 times the CSC; heavy hydrocarbons and zinc exceed the CSC in 60% and 37% of the soil samples, respectively, and reach levels 36 and 6.2 times higher than their respective CSCs. In addition to the expected contaminants, other normed contaminants were found to be enriched or exceeding the Italian contamination threshold concentration (CSC) for agricultural soil (above which a soil is defined as potentially contaminated) only in restricted sub-areas of the field, always together with the main contaminants mentioned above. In particular, we found two sub-areas: one contaminated by a very high level of Cd (up to 57 times the CSC; Table 1), and one contaminated by Pb, Cu, Co, Ni, and Se.

Furthermore, in the latter sub-area, Hg together with other not-normed elements such as Ca, Ag, Au, Pt, Pd, B, and S were found to be above the background values. Lead and copper occasionally exceeded the CSC also in other points of the field, in total exceeding in about 6% and 5% of the soil samples, reaching levels about 4 and 6 times higher than the CSC, respectively (Table 1). Additionally, Be exceeded the CSC in 12% of the samples, but these values were in line with the values detected in the background soil samples (Table 1) and therefore it can be considered as not contaminated. The exceedance of CSC for Be, as well as for As, in some background samples is due to the fact that some areas of the Campania region have baseline values of these elements higher than the CSC due to its volcanic origin and not easily quantifiable anthropic contribution [44]. In the same way, in this study the reference limits for Tl and V were set to 2.7 and 150 mg/kg, respectively, as defined by the “Agenzia regionale per la protezione ambientale”-“Istituto superiore per la protezione e la ricerca ambientale” (ARPA-ISPRA) [45] for the study area, to take into account the site-specific background values, which are higher than the CSC due to the volcanic and alluvial origin of the soils; the presence of geogenic Tl in the study area (exceeding the CSC) was also recently described by Duri et al. [46]. Other not-normed elements found well above the levels of the background samples were Ag and Au, with values on average 28 and 24 times higher than the average background soil values, and S, Ca, Nb, P, and Sn, with average values up to two times higher than the background soil values (Table 1).

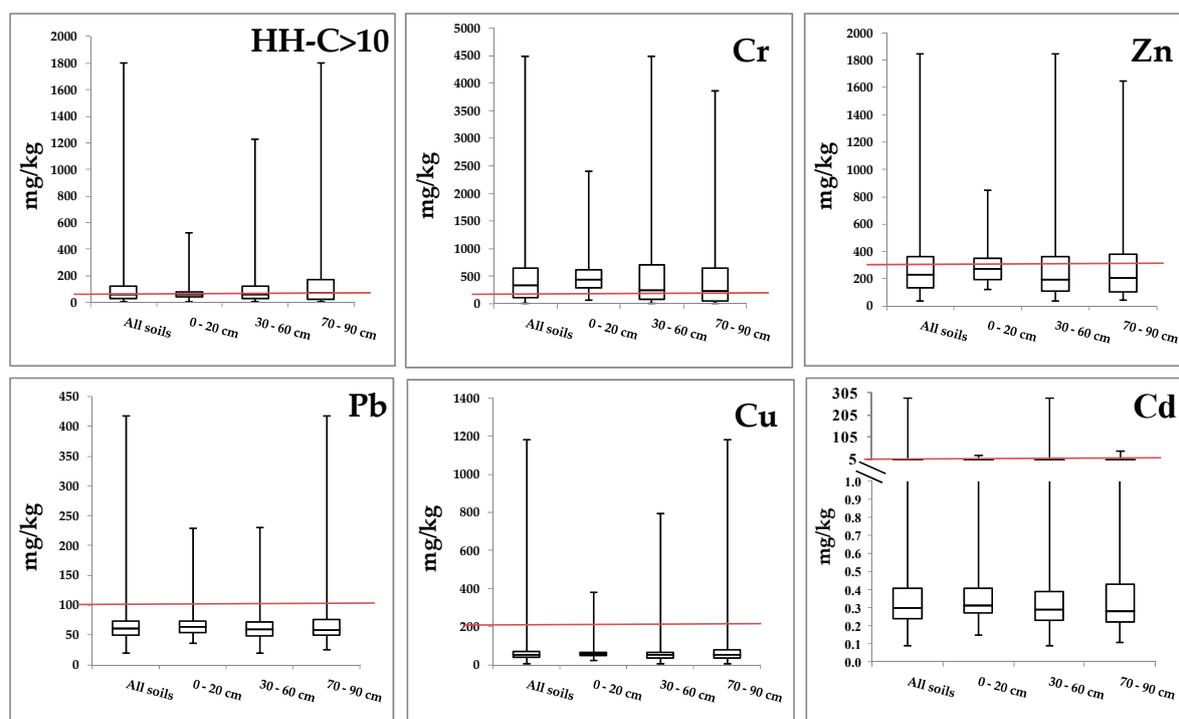


Figure 1. Total contents of HH-C > 10, Cr, Zn, Pb, Cu, and Cd (aqua regia digestion) in the soil samples ($n = 507$) collected in the study site. Each plot represents the minimum and maximum (whiskers) and median (bar) values; the box ranges from the 25^o to 75^o percentile. Red lines indicate the Italian contamination threshold concentration for agricultural soil set by D.M. 46/2019.

Significant correlations were found between the main contaminants (Cr, Zn, and HH-C > 10), suggesting a common origin (R^2 : Cr-Zn = 0.82; Cr-HH-C > 10 = 0.47; Zn-HH-C > 10 = 0.41). The correlations with HH-C > 10 are less clear than those between Cr and Zn, probably due to a partial natural degradation of hydrocarbons over the years [47]; moreover, these correlations increase from the surface soil layer to the deeper layers (R^2 Cr-HH-C > 10 = 0.25, 0.46, and 0.53 in the 0–20, 30–60, and 70–90 cm soil samples, respectively; R^2 Zn-HH-C > 10 = 0.20, 0.39, and 0.47 in the 0–20, 30–60,

and 70–90 cm soil samples, respectively), indicating that in the more aerated surface soil layers a higher natural attenuation by the microbial community took place [48].

Table 1. Ranges of elements and heavy hydrocarbons (HH-C > 10) concentrations in the soil samples ($n = 507$) in comparison with current Italian legal thresholds.

	CSC ⁺	BG [§] Soils	Site Soils [#]		Mean Site/Mean BG	% Samples > CSC ⁺	Max Value/CSC ⁺	
	(D.M. 46/2019)	(Min–Max)	(Min–Max)	Median				SD [£]
As (mg/kg)	30	15.9–33.2	4.9–26.7	17.1	3.9	0.8	0	-
Be (mg/kg)	7	4.9–9.7	0.6–10.0	5.8	1.3	0.9	12	1.4
Cd (mg/kg)	5	0.13–0.37	0.09–283	0.3	12.8	5.1	1.4	57
Co (mg/kg)	30	5.2–6.7	2.0–183	6.6	13.6	1.6	3.9	6.1
Cr (mg/kg)	150	3.1–38.8	8.4–4487	327	613	48	71	30
Cu (mg/kg)	200	13.4–65.5	6.25–1183	53.5	93.2	2.0	4.7	5.9
Hg (µg/kg)	1000	14–45	9–694	64	65	2.7	0	-
Ni (mg/kg)	120	2.3–6.9	2.1–205.6	8.3	20.8	2.7	1.2	1.7
Pb (mg/kg)	100	30.4–86.5	19.2–417	60.2	35.2	1.2	6.1	4.2
Sb (mg/kg)	10	0.37–0.73	0.16–29	0.54	1.47	1.5	0.2	2.9
Se (mg/kg)	3	0.1–0.5	0.1–6.0	0.4	0.7	3.3	1.6	2.0
Tl (mg/kg)	2.7 [*]	1.20–2.01	0.22–2.15	1.34	0.26	0.9	0	-
V (mg/kg)	150 [*]	45–56	19–75	51	8.9	1.0	0	-
Zn (mg/kg)	300	44.1–105	39.3–1846	230	242	4.5	37	6.2
HH-C > 10 (mg/kg)	50	5–43.2	5–1800	60.0	214	5.7	60	36
Ag (µg/kg)		49–159	45–60036	1202	4603	28		
Al (%)		3.40–7.82	1.00–6.98	4.25	0.78	0.9		
Au (µg/kg)		0.10–3.90	0.2–485.3	24.0	39.5	24		
B (mg/kg)		10.0–10.0	10.0–62.0	10.0	5.1	1.1		
Ba (mg/kg)		237.1–754.2	135.6–772.6	347.8	78.0	0.9		
Bi (mg/kg)		0.42–0.68	0.23–1.39	0.51	0.11	0.9		
Ca (%)		0.51–0.72	0.44–16.4	1.00	1.49	2.6		
Ce (mg/kg)		78.0–164.6	12.7–141.9	92.7	17.2	0.8		
Cs (mg/kg)		11.6–18.7	1.88–20.5	11.9	2.11	0.8		
Fe (%)		1.9–2.97	0.93–3.31	2.31	0.29	1.0		
Ga (mg/kg)		7.3–15.7	1.8–13.1	8.9	1.6	0.9		
Ge (mg/kg)		0.05–0.20	0.05–0.20	0.05	0.04	0.7		
Hf (mg/kg)		0.60–2.29	0.10–2.33	0.56	0.30	0.6		
In (mg/kg)		0.02–0.06	0.01–0.23	0.04	0.02	1.0		
K (%)		0.64–0.91	0.18–1.27	0.74	0.13	1.0		
La (mg/kg)		42.5–77.8	6.5–75.8	49.7	10.0	0.9		
Li (mg/kg)		18.0–42.8	2.9–38.4	19.8	4.6	0.8		
Mg (%)		0.19–0.33	0.15–0.58	0.26	0.04	1.0		
Mn (mg/kg)		448–742	262–1043	589	107	1.0		
Mo (mg/kg)		0.73–1.02	0.59–4.00	1.16	0.44	1.5		
Na (%)		0.089–0.249	0.107–0.476	0.199	0.06	1.3		
Nb (mg/kg)		1.19–4.75	0.48–17.97	3.98	2.95	2.3		
P (%)		0.027–0.089	0.018–0.483	0.0985	0.06	2.2		
Pd (µg/kg)		5.00–61.00	5.00–144.00	5.00	17.32	0.6		
Pt (µg/kg)		1.00–3.00	1.00–28.00	1.00	2.96	1.6		
Rb (mg/kg)		82.5–135.3	18.7–155.7	94.9	15.4	0.9		
Re (µg/kg)		0.50–0.50	0.50–2.00	0.50	0.21	1.1		
S (%)		0.01–0.03	0.01–2.13	0.04	0.24	9.0		
Sc (mg/kg)		1.6–3.3	0.8–2.9	1.8	0.3	0.9		
Sn (mg/kg)		2.5–8.3	2.1–47.2	6.5	5.9	2.0		
Sr (mg/kg)		132.0–204.8	84.6–244.5	152.5	21.7	1.0		
Ta (mg/kg)		0.025–0.025	0.025–0.025	0.025	0.00	1.0		
Te (mg/kg)		0.01–0.09	0.01–0.18	0.04	0.03	0.9		
Th (mg/kg)		16.1–34.6	1.3–28.6	16.9	4.0	0.8		
Ti (%)		0.134–0.242	0.021–0.245	0.153	0.03	0.9		
U (mg/kg)		4.1–8.1	1.8–10.2	4.9	1.2	0.8		
W (mg/kg)		0.9–1.3	0.5–6.8	1.0	0.5	0.9		
Y (mg/kg)		14.3–32.0	5.2–26.9	17.1	3.4	0.8		
Zr (mg/kg)		51.1–152.0	10.6–146.4	51.1	21.0	0.7		

⁺ Contamination threshold concentration for agricultural soil; [§] BG soils defined as uncontaminated soil from fields surrounding the study area; [#] Soil samples collected from the studied field; [£] standard deviation; ^{*} Values set by ARPA-ISPRA [45].

High correlations were also observed between the main contaminants and other elements, such as phosphorus, calcium, and sulfur (data not shown), consistent with the presumed waste origin from tanneries [49–51].

3.2. Soil Properties

The background soil sample (BG4/0–20) showed a loamy sand texture (International Society of Soil Science (ISSS) classification), neutral pH, low electrical conductivity, no carbonate content, medium value of cation exchange capacity (CEC), and the usual content of organic carbon for an agricultural soil [52] (Table 2). It was conceivable that the chemical and physical characteristics of the soil samples collected from the studied field were similar to those of the BG samples. By contrast, the site soil samples showed a high variability in all the previously mentioned characteristics, with the exception of the texture, which ranged from sand to sandy loam. The pH ranged from neutral to sub-alkaline, the carbonate content varied from 0 to 79 g/kg, and the CEC ranged from medium to high values. It is likely that these differences stem from the heterogeneity of the soil samples variously mixed with the buried sludges, and the more severe contamination detected at the deeper layers likely derives from a lesser mixing of the buried sludges with the soil. Indeed, some significant correlations between the main contaminants (Cr, Zn, HH-C > 10) and the soil properties were observed (Table 3), in particular between the soil content of heavy hydrocarbons and organic carbon, total nitrogen, total carbonates, electrical conductivity, and pH in KCl. Correlations were also observed between the Cr and Zn and CEC and pH in KCl, although these are not significant for $p < 0.05$ (p value between 0.082 and 0.103). The correlation between the carbonate content and organic components (O.C., total N, and HH-C > 10) might be attributable to a high release of CO₂ from the decomposition of organic matter, leading to carbonate formation in a sub-alkaline and calcium-rich environment such as that of our contaminated soil samples [53–55]. On the other hand, the addition of tannery sludge amendments in soil is known to increase the soil pH and electrical conductivity [56].

The soil oxidizing power with respect to the chromium was always lower than the Italian threshold limit of 1 µmol, with a maximum detected value of 0.4 µmol.

3.3. Extraction in 1 M of Ammonium Nitrate

The amounts of the main soil inorganic contaminants (Cr, Zn, Pb, Cu, Cd) extracted by ammonium nitrate from the 10 selected soil samples are given in Table 4. These amounts can be considered indicative of the amounts of elements readily soluble and thus available to the plants [13]. With the exception of Cd, these amounts when expressed as the percentage of the total soil amount (aqua regia digestion) were very low, almost always below one percent. In terms of percentage, chromium was the least extracted element; slightly higher were the percentages of zinc and copper, while the percentages of cadmium extracted by ammonium nitrate were in the order of 10 to 18 percent. In terms of absolute amounts, the readily bioavailable fraction was always very low (in most cases, below one mg per kg), despite the very high total content of chromium and zinc. However, these small amounts extracted in ammonium nitrate in some cases were higher than the trigger values defined by some European countries adopting this extraction as an indicator of the metal transfer risk from soil to plant in agricultural soils (2, 0.1, 1, and 0.1 mg/kg for Zn, Pb, Cu, and Cd, respectively; [14]). The amounts of the main contaminants extracted in ammonium nitrate were found to be well correlated with the total amounts occurring in the soil (Figure 2). The correlation coefficients ranged from 0.74 for Cr to 0.99 for Cd. The different slopes of the interpolation lines can be considered indicative of the different tendencies of the various elements to be extracted; in fact, as expected, the more mobile Cd had the highest angular coefficient (0.0069), and the less mobile Cr the lowest (0.00009). Moreover, considering the interpolation equation of each element obtained in this specific study soil (see equations in Figure 2), the bioavailable concentrations for each level of total amount in the soil might be derived.

Table 2. Main chemical and physical properties and contaminant contents of the selected background (BG) and soil samples from the studied field.

	A13/0–20	B13/30–60	C5/30–60	D4/30–60	E10/30–60	F5/0–20	H2/70–90	A2-B2 16m/30–60	H3-I3 5m/70–90	BG4/0–20
Texture	From Sand to Sandy Loam									
pH—H ₂ O	7.5	7.9	8.0	7.7	7.9	7.9	7.4	7.5	7.6	7.2
pH—KCl	6.2	7.2	7.2	7.0	6.6	6.7	6.9	7.2	7.1	6.0
EC 1:5 (dS/m)	0.10	0.46	0.22	0.36	0.08	0.12	0.85	2.25	0.64	0.17
O.C. (g/kg)	12.0	28.6	13.1	16.1	8.0	15.2	11.7	27.0	15.4	16.8
Total N (g/kg)	1.51	7.09	3.07	2.85	1.03	1.99	1.75	7.03	2.18	1.95
Total carbonates (g/kg)	0	79	23	18	0	0	13	59	17	0
CEC (cmol ₍₊₎ /kg)	18.2	28.1	19.7	28.6	21.7	24.4	19.2	19.4	28.4	23.7
Oxidizing power for Cr (μmol)	0.39	0.05	0.10	0.04	0.17	0.14	0.03	0.04	0.06	0.09
Mn in reducible oxides (mmol/kg)	0.84	0.53	0.45	n.d.	n.d.	0.63	n.d.	0.64	0.49	0.90
HH-C > 10 (mg/kg)	26.9	647	200	313	18.9	69.0	93.0	710	162	43.2
Cr (mg/kg)	136	1458	955	4487	103	546	2372	1314	3456	34
Zn (mg/kg)	122	605	439	1846	129	360	1136	643	1648	105
Pb (mg/kg)	54.6	68.3	55.9	93.5	59.3	228.3	68.3	230	84.9	57.0
Cd (mg/kg)	0.26	0.61	0.41	0.51	0.23	21.9	0.31	1.41	0.38	0.37
Cu (mg/kg)	49.8	59.2	75.6	50.8	50.3	65.1	67.1	602	60.8	65.5

EC: electrical conductivity; O.C.: organic carbon; CEC: cation exchange capacity; N: nitrogen; n.d.: not determined.

Table 3. Pearson correlation coefficients between the main soil contaminants and the soil properties.

	HH-C > 10	Cr	Zn	pH—H ₂ O	pH—KCl	EC	O.C.	Total N	Total Carb.	CEC	Oxidizing Power
HH-C > 10	1										
Cr	0.260	1									
Zn	0.218	0.994	1								
pH—H ₂ O	0.143	−0.029	−0.064	1							
pH—KCl	0.664	0.560	0.546	0.524	1						
EC	0.702	0.222	0.241	−0.275	0.461	1					
O.C.	0.916	0.120	0.088	−0.034	0.434	0.590	1				
Total N	0.981	0.107	0.066	0.141	0.597	0.653	0.949	1			
Tot. carb.	0.954	0.199	0.159	0.196	0.683	0.580	0.891	0.966	1		
CEC	0.217	0.575	0.558	0.165	0.274	−0.215	0.316	0.165	0.227	1	
Ox. power	−0.494	−0.548	−0.552	0.058	−0.640	−0.453	−0.441	−0.440	−0.481	−0.443	1

The bold values are different from 0 at the alpha significance level = 0.05. EC: electrical conductivity; O.C.: organic carbon; CEC: cation exchange capacity; N: nitrogen.

Table 4. Absolute amount (mg/kg) and % of the total content (aqua regia digestion) of the Cr, Zn, Pb, Cu, and Cd extracted by NH_4NO_3 and EDTA from the 10 selected soil samples.

		Cr		Zn		Pb		Cu		Cd	
		mg/kg	% of Total	mg/kg	% of Total	mg/kg	% of Total	mg/kg	% of Total	mg/kg	% of Total
A13/0–20	NH_4NO_3	0.19	0.14	0.28	0.23	<0.1	<0.18	0.09	0.18	0.03	12.6
	EDTA	1.01	0.74	22.8	18.7	27.35	50.1	21.1	42.3	0.16	60.2
B13/30–60	NH_4NO_3	0.42	0.03	0.81	0.13	<0.1	<0.15	0.21	0.35	0.08	12.4
	EDTA	9.12	0.63	278	45.9	38.44	56.3	14.8	24.9	0.54	88.7
C5/30–60	NH_4NO_3	0.37	0.04	0.73	0.17	<0.1	<0.18	0.40	0.52	0.05	11.2
	EDTA	12.7	1.33	196	44.5	41.65	74.5	34.8	46.0	0.31	75.9
D4/30–60	NH_4NO_3	0.63	0.01	3.17	0.17	<0.1	<0.11	0.28	0.55	0.06	12.7
	EDTA	10.7	0.24	905	49.0	53.38	57.1	17.7	34.8	0.32	62.9
E10/30–60	NH_4NO_3	0.26	0.25	0.22	0.17	<0.1	<0.17	0.09	0.19	0.04	17.7
	EDTA	1.47	1.43	33.0	25.6	37.77	63.7	18.9	37.6	0.16	70.6
F5/0–20	NH_4NO_3	0.27	0.05	0.56	0.16	<0.1	<0.04	0.16	0.24	0.20	0.91
	EDTA	1.86	0.34	121	33.5	42.59	18.7	27.0	41.4	15.6	71.3
H2/70–90	NH_4NO_3	0.48	0.02	3.26	0.29	<0.1	<0.15	0.38	0.56	0.06	18.8
	EDTA	8.35	0.35	679	59.8	47.76	69.9	27.1	40.4	0.21	67.8
A2-B2/16m/30–60	NH_4NO_3	0.61	0.05	1.97	0.31	<0.1	<0.04	2.97	0.49	0.13	9.14
	EDTA	5.27	0.40	312	48.5	161.03	70.0	289	48.0	0.95	67.6
H3-I3/5m/70–90	NH_4NO_3	0.65	0.02	2.98	0.18	<0.1	<0.12	0.28	0.46	0.06	14.7
	EDTA	10.1	0.29	798	48.4	53.42	62.9	23.9	39.4	0.26	69.1
BG4/0–20	NH_4NO_3	0.30	0.90	0.25	0.24	<0.1	<0.18	0.13	0.19	0.04	10.3
	EDTA	1.12	3.29	20.5	19.5	32.30	56.6	26.8	41.0	0.16	43.9

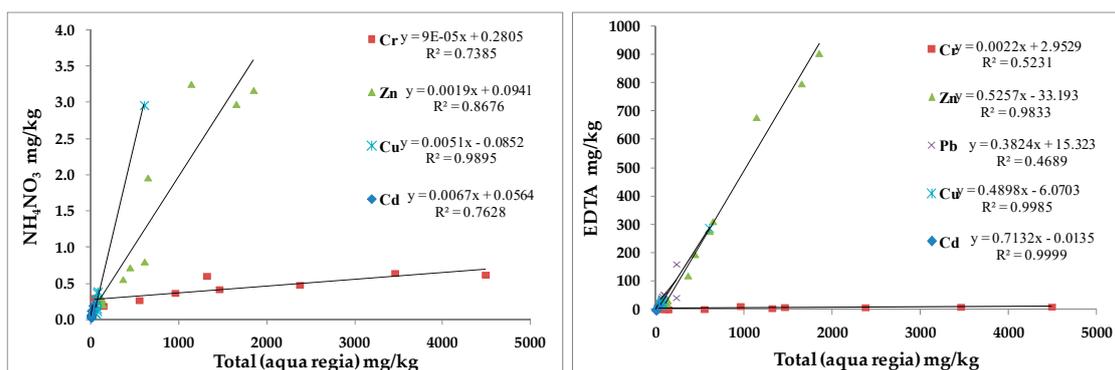


Figure 2. Correlation between the total amount (aqua regia digestion) of Cr, Zn, Pb, Cu, and Cd and the amounts extracted by NH₄NO₃ and EDTA from the 10 selected soil samples.

3.4. Extraction in 0.05 M EDTA pH 7

On the other hand, the EDTA extraction was considered indicative of the element amount potentially available to the plants. Additionally, in this case the extracted amounts of PTEs were well correlated (with the exception of lead) with the total amounts of elements in the soil (Figure 2). The correlation coefficients ranged from 0.47 for Pb to 0.99 for Cd and Cu, and also in this case the interpolation equation of each element was obtained, and Cd had the highest angular coefficient (0.71) and Cr the lowest (0.0022). However, with the exception of chromium, the extracted percentages of the considered elements were in the order of several dozen percent (Table 4). Additionally, by EDTA chromium was extracted in a very low amount, both in terms of percentage and in absolute terms (mg/kg), although at a higher extent in comparison to ammonium nitrate. In the study site, a consistent pool of potentially available zinc which reached potential bioavailable concentrations of around 900 mg/kg in one of the most contaminated analyzed samples (D4/30–60) was evident. The potentially bioavailable concentration of Pb in the two analyzed Pb contaminated samples differed greatly despite having approximately the same level of contamination (43 mg/kg in F5/0–20 and 161 mg/kg in A2-B2/16m/30–60), suggesting a different origin of the contaminant in the two samples. Copper in the only Cu-contaminated soil samples (A2-B2/16m/30–60) reaches potential bioavailable concentrations of around 290 mg/kg. Cadmium in sample F5/0–20 reaches potential bioavailable concentrations of around 16 mg/kg.

3.5. Sequential Extractions with EU-BCR Protocol

The BCR sequential extraction procedure is usually considered indicative of the distribution of the contaminants between the various presumed geochemical phases occurring in the soil. The soil was extracted in sequence by extractants of increasing strength, and thus the first step represents the most mobile and bioavailable element pool, the other steps representing progressively less mobile and bioavailable element pools. The presumed geochemical fractions of PTEs extracted in sequence are: I step—soluble, exchangeable, and associated with carbonates; II step—associated with amorphous Fe and Mn oxides; III step—bound to organic matter and sulfides; IV step—occluded in non-siliceous minerals [35]. This procedure highlighted the difference in mobility of the two main soil contaminants, chromium and zinc. Chromium was mainly extracted in the third and second steps (Figure 3), and therefore was presumably associated with the soil oxidizable and reducible phases (likely organic matter and iron and manganese oxides, respectively), while the percentage of chromium extracted in the first step was very small (about 1% or less); in contrast, zinc was extracted mainly in the first two steps of the procedure, indicating the higher mobility and potential bioavailability of the elements. For both elements, the percentage extracted in the first step tended to increase with the total metal contents. The distribution of chromium among the soil geochemical fractions observed in this study—i.e., mainly associated with organic matter and Fe and Mn oxides—was in agreement with

that reported in other studies dealing with chromium in soils amended with tannery sludges [57]. Moreover, chromium from the tannery process should be predominantly in the Cr(III) species, and its retention in the organic fraction (generally high in tannery sludge, as in our case) limits its mobility and bioavailability, and, hence, plant toxicity [58]. Lead was extracted mainly in the second step of the procedure both in contaminated and not-contaminated soil samples, except for the sample F5/0–20, in which lead was mainly found in the residual fraction (Figure 3). The F5/0–20 soil sample corresponds to the unique hot spot of cadmium in the study field, and this together with the different distribution of lead among soil geochemical fractions and the different extractability in EDTA (Table 4) suggests a different source of contamination from the rest of the field. Copper was mainly extracted in the second and third steps both in the contaminated and not-contaminated soil samples, but in the only enriched sample the amount extracted in the first step differed considerably (from about 2 to 45 mg/kg, and from about 2–3% to 8%). Cadmium in all the samples was mainly extracted in the first and second steps of the BCR procedure, while the residual fraction was negligible, confirming the larger mobility of this element in the soil compared to all the other analyzed elements [59,60].

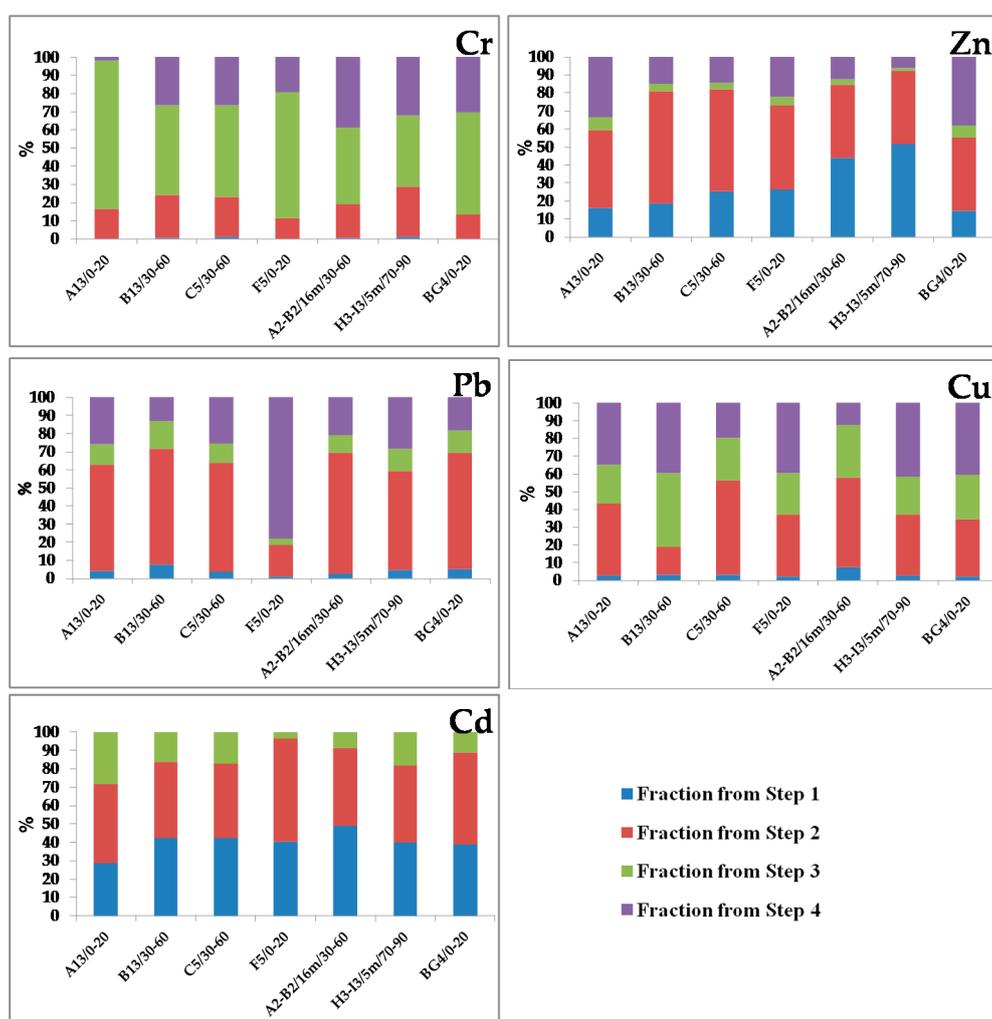


Figure 3. BCR sequential extractions of Cr, Zn, Pb, Cu, and Cd from 7 of the 10 selected soil samples. For each element, the distribution is shown in percentage terms.

3.6. Comparison between the Extractions

The absolute amounts of chromium, zinc, and cadmium extracted from the contaminated samples by the first step of the BCR procedure were much higher than the amounts extracted by NH_4NO_3 , but in the same order of magnitude as the amounts extracted by EDTA (Figure 4). By contrast, the absolute

amounts of lead and copper extracted by the first step of the BCR procedure were in the same order of magnitude as the amounts extracted by NH_4NO_3 , but much lower than the amounts extracted by EDTA (Figure 4). In the BCR procedure, 0.11 M of acetic acid solution is supposed to remove from the soil mainly water-soluble, exchangeable, and carbonate-bound metals. Only a small number of the analyzed soil samples contained carbonates, which likely originated from the decomposition of organic matter in a calcium-rich environment. Water-soluble metal forms are expected to make only a small contribution to the total metals extracted during this step, so it seems that most of the acetic acid-extractable metals must be sorbed specifically and non-specifically on soil exchange sites. More of the total Zn and Cd (on average 28% and 40% of the total, respectively) was extracted during the first step than the Cr, Pb, and Cu (on average 0.5%, 4%, and 3% of the total, respectively). It seems that in the studied soil, the complexing ability of acetate increases the proportion of Zn and Cd removed from organic sites and oxide material. Similar results were observed by Adamo et al. [61] during the fractionation of Cu in the Sudbury soils. Acetic acid was observed to displace greater quantities of Cu than were present on exchange sites. This increase was attributed to the greater desorption of Cu from organic matter or release from Fe and Mn oxides. The extraction of soil by 1 M of NH_4NO_3 solution is used to determine the readily soluble trace element contents [62]. In Germany, this method has been published as an official standard [36], and is used to estimate the transfer of heavy metals and arsenic from soils to plants in the German Federal Soil Protection and Contaminated Sites Ordinance [63]. Soil extraction with 1 M of NH_4NO_3 is performed at a pH value comparable to the soil solution. The extraction of metals is mainly caused by the desorption of cations, while the formation of colloids and soluble metal-organic complexes, which are hardly taken up by plants, is strongly suppressed due to the high ionic strength of the 1 M NH_4NO_3 solution. In the present study, very low amounts of analyzed metals were extracted from the soil with the 1 M NH_4NO_3 solution (on average 0.15%, 0.20%, <0.13%, 0.37%, and 12% of the total for Cr, Zn, Pb, Cu, and Cd, respectively). By contrast, the 0.05 M EDTA solution at pH 7.0 is supposed to determine the potentially bioavailable content of PTEs in soil [37]. EDTA is a well-known strong chelating agent which can form stable chelates with many metal ions and can remove organically bound metals, along with part of the metals occluded in oxides and secondary clay minerals. The Measurement and Testing Programme of the European Commission has standardized this extraction method, and certified reference materials (BCR-700) were produced to assess the quality of the data obtained using the procedure in question [13]. The amounts of metals extracted by 1 M NH_4NO_3 and 0.05 M EDTA solutions are consistent with the above considerations concerning the metal amounts extracted by the 0.11 M of acetic acid.

3.7. Extraction in Water

The solid-liquid partition coefficient (K_d) was indicative of the potential mobility of the contaminants in water, and therefore of the risk of leaching and transfer to other environmental compartments. Moreover, K_d is a parameter usually applied in the site-specific analysis of human health risk prescribed for potentially contaminated sites by Italian legislation (D.Lgs 152/2006). In this study, the PTE amounts extracted from the soil by water were very low—almost an order of magnitude lower than those extracted by ammonium nitrate (Table 5). Consequently, the measured K_d was high, in particular for chromium and zinc, indicating a low potential of migration in water. On the other hand, the contaminants do not seem to have spread into the soil, given the great inhomogeneity of the contamination levels, more than a decade after the sludge burning. Moreover, the preliminary investigation carried out by the regional authorities did not reveal the contamination of deep soil (>5 m deep).

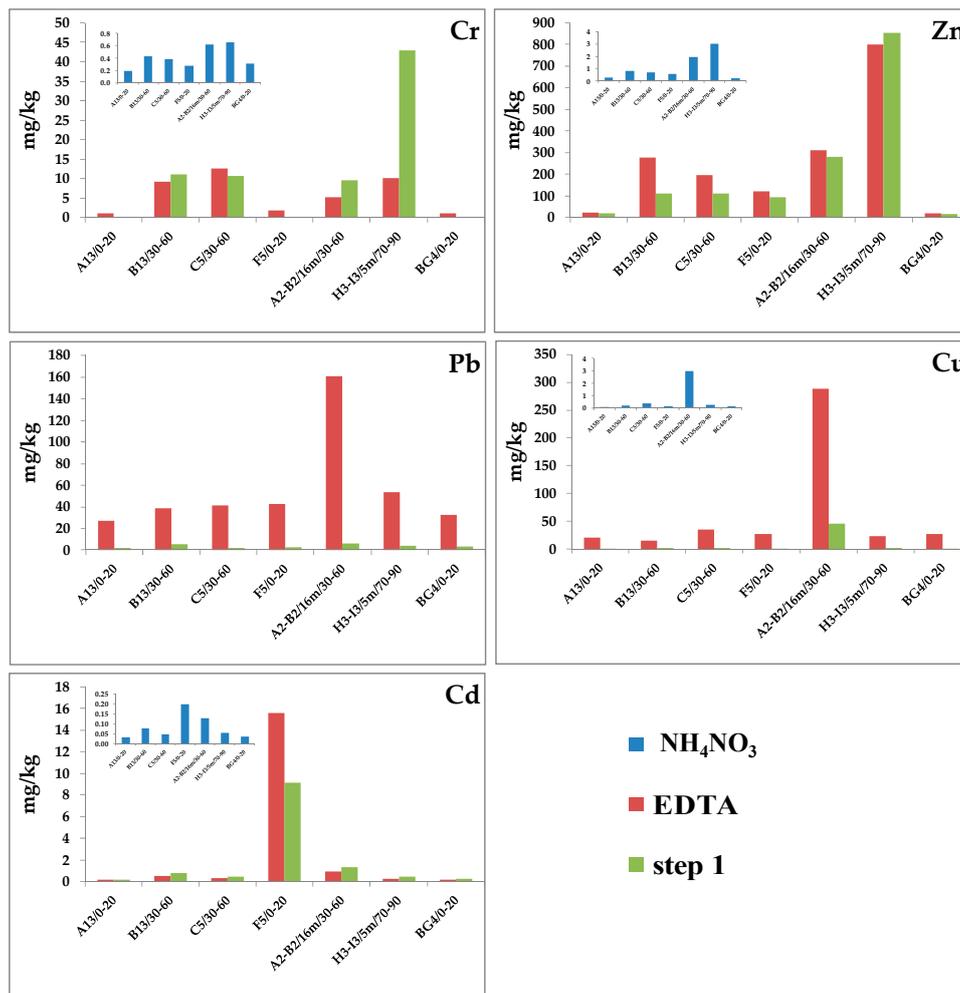


Figure 4. Comparison of the Cr, Zn Pb, Cu, and Cd amounts extracted by NH₄NO₃ (small graphs within the main graphs), EDTA, and Step 1 of the BCR sequential extraction. For Pb, the NH₄NO₃ extracted amounts were for all samples < 0.1 mg/kg.

Table 5. Amounts of the Cr, Zn, Pb, Cu, and Cd extracted by water as % of the total content (aqua regia digestion), and the calculated K_d value for five random samples. $K_d = C_{soil}/C_{water}$.

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
	pH	7.8	7.6	7.4	7.6	7.7
Cr	total content	1458	2399	2372	3857	3572
	extr. in water (% of total)	0.006	0.004	0.010	0.007	0.016
	K _d	33,515	47,728	20,816	28,259	12,656
Zn	total content	605	846	1136	625	1357
	extr. in water (% of total)	0.014	0.025	0.028	0.035	0.021
	K _d	14,408	8095	7080	5790	9695
Pb	total content	68.3	63.3	68.3	56.8	96.2
	extr. in water (% of total)	0.080	0.088	0.16	0.36	0.13
	K _d	2525	2288	1232	551	1528
Cu	total content	59.2	48.9	67.1	120.2	91.7
	extr. in water (% of total)	0.16	0.26	0.20	0.092	0.32
	K _d	1287	770	1017	2165	626
Cd	total content	0.61	0.51	0.31	0.91	0.28
	extr. in water (% of total)	0.16	0.20	0.81	1.21	0.89
	K _d	1220	1020	388	167	350

3.8. Contaminant Speciation

Chromium may occur in soil in trivalent (CrIII) and hexavalent (CrVI) forms characterized by their different mobility and toxicity [64]. Chromium from tannery residues should be totally in the trivalent form [65]. However, taking into consideration the high Cr total content in the studied soil and the possibility of the interconversion of the two forms in the studied soil due to the high content of organic matter and the presence of reducible manganese oxides (shown by the Mn amounts extracted in the second step of the BCR procedure, Table 2) [57,65–67], we decided to measure the soil oxidizing power with respect to the chromium and the soil hexavalent chromium content. In Italy, the analysis of the soil oxidizing power is required for soils that receive amendments containing chromium to verify the risk of oxidation to hexavalent chromium. The oxidizing power of the studied soil was approximately an order of magnitude below the Italian threshold value of 1 micromole (Table 2). The results of the Cr(VI) analysis were not fully satisfactory because the recovery of the hexavalent chromium spike was very low (between 4% and 73%), with both methods of Cr(VI) determination in the extract (EPA 7196 A and EPA 7199) indicating a high soil reducing power against Cr(VI), in particular in the most contaminated soil samples where there was more organic matter, likely the main factor enhancing the reducing power of the soil. Indeed, the amount of recovered spike decreased with the increasing content of soil organic matter (Figure 5). Despite the difficulties and inaccuracies of the analyses, the concentrations of hexavalent chromium detected in the analyzed samples, although probably underestimated and corresponding to a very low percentage of total chromium (0.09–1.6%), were higher (from 4 to 13.9 mg/kg) than the threshold value of 2 mg/kg set for agricultural, residential, and public green soils (D.M. 46/2019 and D.Lgs 152/2006). There was one exception to this behavior, and it was the soil containing 103 mg/kg of total Cr (the only analyzed soil sample with an amount of total Cr below the Italian contamination threshold concentration (CSC) for agricultural soil). In this sample, the detected Cr(VI) content was 1.7 mg/kg. Hexavalent chromium quantification by isotope dilution mass spectrometry confirmed the great difficulties in determining accurately the Cr(VI) in the studied soil, as well as the non-negligible occurrence of this form in the soil [68].

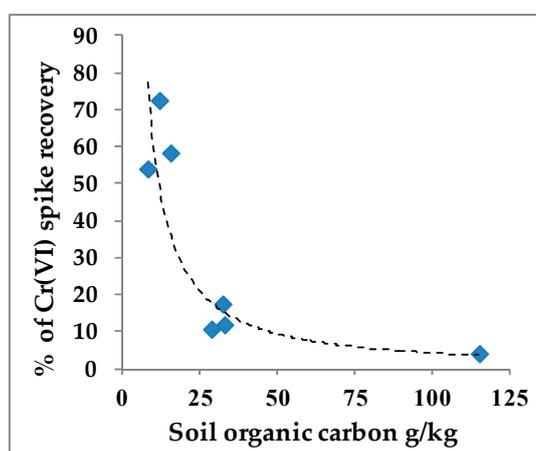


Figure 5. Percent of Cr(VI) spike recovery in the function of the soil organic carbon content.

The speciation of the heavy hydrocarbon fraction was performed to evaluate the risk of the mobility and toxicity of these contaminants. Heavy hydrocarbons were found to consist almost entirely of long-chain aliphatic hydrocarbons (>90%, Table 6), a class of hydrocarbons considered not very toxic and immobile because they are hydrophobic and non-volatile, and therefore with a low risk for human health and no risk of dispersion in the environment [69–71]. Given the origin of the contamination, they could probably arise from the skin fat or other phases of the leather process [69,72,73].

Table 6. Hydrocarbon speciation according to the MADEP approach on nine randomly selected soil samples.

	HH-C > 10 (mg/kg)	Aromatic Hydrocarbons C9–C10 (mg/kg)	Aromatic Hydrocarbons C11–C22 (mg/kg)	Aliphatic Hydrocarbons C5–C8 (mg/kg)	Aliphatic Hydrocarbons C9–C18 (mg/kg)	Aliphatic Hydrocarbons C19–C36 (mg/kg)
Sample 1	30.7	<0.011	<0.1	<2.2	<10	27
Sample 2	76	<0.011	<0.1	<2.2	<10	70
Sample 3	101	<0.011	<0.1	<2.1	<10	88
Sample 4	112	0.033	<0.1	<2.1	<10	100
Sample 5	134	0.033	<0.1	<2.3	<10	123
Sample 6	464	0.175	0.709	<2.1	36	390
Sample 7	487	0.05	<0.1	<2.2	18.6	460
Sample 8	564	0.182	0.129	<2.6	17.7	520
Sample 9	1580	0.121	0.153	<2.3	20.2	1510

4. Conclusions

In the studied agricultural site subjected to the past illegal burial of industrial wastes (mainly tannery wastes), the soil was found to be diffusely contaminated by chromium, zinc, and heavy hydrocarbons and locally by cadmium, lead, and copper. According to selective chemical extractions, the chromium in soil was found to be almost immobile and the only real concern was linked to the possible formation of hexavalent chromium, detected in a significant amount despite the low accuracy of the analytical methods for the studied soil. However, given the high reducing power of the soil, it seems unlikely that mobile hexavalent chromium has a sufficient lifetime to reach other environmental compartments. This aspect requires further study.

Cadmium and zinc were more bioavailable than chromium, and therefore the risk of transfer of these elements to plants and then to the food chain was higher, but the greater mobility and potential bioavailability were also promising for the application of phytoremediation to the site. All the metal contaminants were almost immobile in water, and hence no action seems necessary to avoid ground water and deep soil contamination. An analysis of ground water samples would be desirable to confirm this hypothesis.

The results of this work highlight how crucial the assessment of the bioavailability and the speciation of inorganic and organic contaminants is for the evaluation of the real risks associated with soil contamination. This approach supports phytoremediation as a good cost saving strategy to remediate and secure a wide contaminated area such as ours, preserving the soil ecosystem services. The use of not-edible plants, such as poplar trees, might be the best choice given the high bioavailability of a highly toxic contaminant such as cadmium; the addition of grass coverage would contribute to containing the risk of soil erosion and particle dispersion by the wind.

The results also suggest that the addition of organic matter—e.g., compost—could help to maintain a strong reducing environment in the soil that, in turn, reduces the risk of hexavalent chromium formation and transfer. In the studied soil, the natural attenuation of HH-C > 10 hydrocarbons, though non-hazardous as demonstrated by speciation, could be promoted by soil management practices that stimulate microbial activity.

Author Contributions: Conceptualization, P.A.; methodology, D.A., A.G.C. and P.A.; formal analysis, D.A. and A.G.C.; investigation, D.A., A.G.C. and P.A.; resources, P.A.; data curation, D.A.; writing—original draft preparation, D.A.; writing—review and editing, D.A., A.G.C. and P.A.; supervision, P.A.; project administration, P.A.; funding acquisition, P.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by EC LIFE11/ENV/IT 275 Ecoremed and Italian MIUR PRIN2017BHH84R.

Acknowledgments: This study was carried out within the program “Programma di riqualificazione funzionale e restituzione all’ordinario uso agricolo dell’area di San Giuseppepiello mediante applicazione e validazione del protocollo di risanamento LIFE-ECOREMED (LIFE11 ENV/IT/000275)”.

Conflicts of Interest: The authors declare no conflict of interest. All the authors read and approved the final manuscript.

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