



Assessment of optimal conditions for the restoration and recovery of agricultural soil



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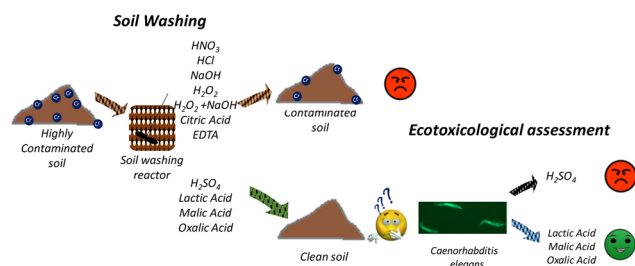
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GRAPHICAL ABSTRACT



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ABSTRACT

We assessed whether soil with high Cr contamination could be reclaimed by alkali, mineral, and organic acid-based ligands (OABs) washing. We tested HNO_3 , H_2SO_4 , HCl , NaOH , H_2O_2 , lactic acid (LA), malic acid (MA), oxalic acid (OA), and citric acid (CA), together with EDTA, obtaining the highest efficiencies in presence of 1 M sulfuric acid (98%). Nonetheless we noted that using OABs, we obtained a Cr(III) removal efficiency similar to the one obtained using mineral acids. Indeed 1 M of LA and MA and 0.8 M of OA allowed obtaining, respectively, 88%, 75%, and 67% removal percentage. The extraction process with OABs was strongly dependent on intraparticle diffusion of Cr-LA, Cr-MA, and Cr-OA complexes. We also determined the apparent diffusion coefficients. Residual toxicity of treated soils was tested with the nematode, *Caenorhabditis elegans*. The OAB washing generally allowed getting a soil without Cr and with reduced toxicity. However, the washing process also removed other cations that acted as nutrients. Consequently, we conducted toxicity tests on enriched soil and found that the mortality index improved. In some cases (LA and MA), mortality was comparable to that observed with uncontaminated control samples. In contrast, when contaminated soils were washed with sulfuric acid, in all conditions, we observed significant ecotoxicity. Therefore, we concluded that only the OAB treatment provided a non-toxic soil that could be reused for anthropic activities.

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1. Introduction

The consumption and the depletion of natural soils is a well-recognized emergency in agricultural systems [1]. The problem is likely to become even more serious in the future, because of climate changes, population growth, unusual floods, and desertification [2]. Differentiated strategies must be tested and deployed to address the problems and establish the premises for a sustainable development, consistent with the goals established by the United Nations in 2016 [3,4]. In that context, a valuable alternative can be individuated in the rehabilitation and in the recovery of the fertility of soils contaminated by anthropogenic activities. Unfortunately, quite often, soil contamination is related to the improper or illegal disposal of industrial waste [5,6]. In these cases therefore, soils are characterized by isolated zones, called hot spots [7], with high levels of contamination, where rehabilitation cannot be achieved in a straightforward manner. In most cases, after remediation, hot-spots cannot be returned to agricultural use, because the techniques adopted in these areas to remove the contaminants, drastically reduce the fertility of the soil, and sometimes, alter its original structure and composition [8,9].

Among other pollutants found in hot-spot areas trivalent chromium, plays a special role [10]. Cr(III)-contaminated soils are present in many countries, due to the wide use of Cr(III) in different industrial processes, including leather tanning, metallurgic electroplating, wood preservation, and textile dyeing [10,11]. Although Cr(III) does not pose a serious hazard to living organisms, due to its general inability to cross cell membranes, it can be environmentally converted to Cr(VI) [12–14], which, instead, is mutagenic and carcinogenic at low sub-ppm levels [15]. Moreover if Cr(III) enters the intracellular medium, through pinocytosis and/or endocytosis processes, it can directly affect the DNA, causing severe damages to the cells [16]. Finally, it is possible that Cr(III) is accumulated in the roots of plants, and generates reactive oxygen species (ROS), which exert toxic effects on vegetables [17,18].

The most frequently adopted technologies for the remediation of Cr(III)-contaminated sites are based on extraction or immobilization processes [19–21]. Among them the only one which is applicable to remove Cr(III) from the soil and prevent its migration into other environmental matrices is soil washing. Previous studies have tested the efficiencies of different washing agents (Table 1). As it can be easily

seen the highest efficiencies have been reached by using strong mineral acids (hydrochloric and sulfuric acids), which, unfortunately, partially dissolve the solid matrix. Thus, those agents are not appropriate for the reclamation of agricultural soils. Moreover, it is not clear whether the tested agents destroy microbial and enzyme activities i.e. have an ecotoxicological effect [22].

Cr(III) forms stable metal-organic complexes with several amines and carboxylates [31,32], which are common constituents of natural organic matter. These complexes are also found in numerous marine environments [33]. Therefore, some carboxylic acids used as chelating agents are expected to remove Cr(III) from soils without altering the natural equilibrium of the environment. Indeed some selected organic acid-based ligands (OABLs) have been tested for Cr(III) removal from contaminated soils [29,30], obtaining Cr removal rates higher than 70%. In aqueous solution, Cr(III) forms several stable complexes with some OABLs, such as citric acid, lactic acid (LA), and oxalic acid (OA) (Table S1). The relative concentrations of these complexes are affected by the pH of the solution [34]. Although no studies have reported the stability constants of stable complexes between Cr(III) and malic acid (MA), Cr(III)-MA complexes have been found to be benign, and even useful to treat human diseases (i.e. diabetes, treatment of high cholesterol levels) [35]. Other studies have shown that OABLs applied at intermediate or low concentrations can improve plant growth [36], although, at high concentrations, may pose non-negligible health risks [36]. Currently, it remains unknown whether OABLs are present in the soil after soil washing treatments.

According to the literature [37], the removal efficiency of alkaline washing agents can be increased by adding H₂O₂. H₂O₂ might facilitate the degradation/dissolution of the organic substance, which results in release of the metal, or it might facilitate the leaching of Cr(III), because oxidized forms are more mobile than reduced forms. For example, Rock et al. [37] reported that the use of H₂O₂ increased Cr removal during soil washing processes, most likely because it enhanced oxidation of Cr(III) to Cr(VI), which is the most soluble form.

In this context, we investigated more thoroughly the efficiencies of recovering Cr(III)-contaminated soil through soil washing with alkali, mineral, and OABLs (HNO₃, H₂SO₄, HCl, NaOH, H₂O₂, LA, MA, OA, CA, EDTA). In the present study, we aimed to optimize the remediation process and maintain, simultaneously, the natural vocation of the site

Table 1
Removal efficiencies of washing reagents tested to remove Cr(III) for contaminated soils.

Soil	Soil characteristics	Reagent used	Removal efficiency (%)	Reference
Soil contaminated, a mixture of soil and industrial waste, central Scotland	pH: 10.3 CEC (meq/100 g): 15.4 TOC (%): 0.7 Cr (mg kg ⁻¹): 4940 Sand (%): 82.8 Silt (%): 14.5 Clay (%): 2.7	SDS (10 ⁻³ –0.1 M)	16–22	[23]
		NTA (10 ⁻³ – 0.1 M)	41–43	
		HCl (8% v/v)	100	
		EDTA (10 ⁻⁴ – 0.1 M)	20–22–36–54	
Artificially contaminated Soil	pH: 7.9 Organic Matter (%): 0.7 Cr (mg·kg ⁻¹): 1231 Sand (%): 71.1 Silt and Clay (%): 28.9	Water	19	[24]
		EDTA (0.1 M)	41	
Soil contaminated, North of Guangzhou City, China	pH: 5.50 Organic Matter (%): 5.98 Cr (mg·kg ⁻¹): 3912 Sand (%): 34.1 Silt (%): 59.2 Clay (%): 6.7	EDTA (10 ⁻³ M)	11	[25]
Soil contaminated at a deposition site for chromite ore, northeastern China	pH: 8.5 CEC (meq/100 g): 6.1 TOC (%): 1.32 Cr (mg·kg ⁻¹): 768.5 Sand (%): 18.8 Silt (%): 46.7 Clay (%): 34.5	Citric Acid/ Sodium Citrate (0.2 M)	49	[26]
		Citric Acid/ Sodium Citrate (0.2 M)	85	
Soil contaminated with metals and PCB, Montreal, Canada	pH: 7.5 CEC (meq/100 g): 9.5 Organic matter (%): 12.5 Cr (mg·kg ⁻¹): 436 Sand (%): 52 Silt (%): 33 Clay (%): 16	EDTA (0.002 M)	0	[27]
Artificially contaminated soil	pH: 4.7 CEC (meq/100 g): 3.8 Organic matter (%): 1.14 Cr (mg·kg ⁻¹): 270 Sand (%): 60 Silt (%): 30 Clay (%): 10	EDTA/NTA/DTPA (0.005 M), HNO ₃ , CaCl ₂ (0.5 M)	0	[28]
Contaminated soil	pH: 8.0 TOC (%): 2.3 Cr (mg·kg ⁻¹): 35.2 Sand (%): 64 Silt (%): 32 Clay (%): 4	H ₂ SO ₄ (1 N)	82	[29]
		HCl (1 N)	3	
		HNO ₃ (1 N)	73	
		Lactic Acid (25 %v/v)	71	
		Ethanol (50 %v/v)	2	
Soil samples from a chromium-contaminated agricultural field, central Taiwan	pH: n.d. CEC (meq/100 g): 27.91 Organic Matter (%): 4.89 Cr (mg·kg ⁻¹): 456.7 Sand (%): 57.0 Silt (%): 40.2 Clay (%): 2.8	HCl (0.5 M)	80	[30]
		Citric Acid (0.5 M)	77	

for agricultural use. To that end, we analyzed (i) Cr-removal efficiencies of OABs, based on various operative variables; (ii) the partitioning of Cr(III) into different soil fractions after the OABL remediation process; and (iii) the ecotoxicity of the soil before and after the OABL washing treatment.

2. Experimental

2.1. Materials

Detailed information on materials is presented in the Supporting Material.

2.2. Soil sampling

The soil samples were obtained in the Campania Region (southern Italy), in a site selected by the Italian government for urgent reclamation activities [38]. This area had been subjected to illegal industrial waste disposal for many years [39], and it harbors many hot spots that are highly contaminated with PTMs and organics. We collected samples from a large area that had been subjected to illegal tannery sludge disposal. We selected different trenches and manually collected 10 kg of soil, which we placed in hermetic plastic boxes. The samples were carefully mixed and homogenized, then dried at 40 °C until a constant weight was observed and stored at room temperature.

2.3. Analytical procedures

The analyses were conducted in triplicate, unless otherwise indicated, to reduce experimental error associated with sample heterogeneity. We performed a particle size distribution analysis according to the ASTM method, D 422-63 [40]. We used sieves of different sizes, including ISO (20 mm), ISO (10 mm), and ISO (5 mm), and different meshes, including n. 10, 18, 35, 50, 100, and 200. The fraction of soil particles smaller than 0.075 mm was analyzed with hydrometry. Only grain sizes smaller than 2.0 mm were used for subsequent experimental activities according to literature [41–44].

The total chromium concentration was determined according to the EPA method, 3051 [45], with atomic absorption spectrometry (AAS). We used a Varian instrument, Model 55 B SpectrAA (F-AAS) equipped with flame (acetylene/air). The Cr(VI) concentration was determined according to method 3060A [46]. Metal bioavailability was evaluated by sequential extraction, with the procedure proposed by the Community Bureau of Reference (BCR), slightly modified, as suggested by Pueyo et al. [47,48]. The element concentrations in the soil were evaluated using ICP-MS (Perkin Elmer Nexion 300, USA). A 1:2 soil-to-water ratio was used to measure the pH of the soil according to the EPA Method, 9045C [49]. The pH was measured with a HI 98,190 pH/ORP pH-meter (Hanna Instruments, USA). The loss-on-ignition index (LOI) at 550 °C was used to estimate the organic matter content of the soil [50,51]. The cation exchange capacity (CEC) was estimated, as described by Chapman [52], and electrical conductivity (CE) was estimated according to Violante and Adamo [53].

2.4. Soil washing procedure

Soil washing tests were performed in polyethylene bottles (50 ml) at room temperature, with a 10:1 liquid-to-solid ratio (L/S) [54]. The following inorganic and organic chemicals were used as washing agents: sulfuric acid (H₂SO₄), hydrochloric acid (HCl), nitric acid (HNO₃), sodium hydroxide (NaOH), hydrogen peroxide (H₂O₂), citric acid, DL-LA, OA, DL-MA, and EDTA. Detailed information on washing procedure and the test summary (Table S2) is presented in the Supporting Material.

2.5. Mathematical models adopted for data analysis

The extraction kinetics were analyzed with models previously adopted [55], which used three equations:

- (a) The Weber and Morris equation was used to verify the effect of intraparticle diffusion [56,57]:

$$q_t = k_D \cdot t^{0.5} + c \quad (1)$$

where q_t is the amount of chromium extracted per unit of soil mass (mg g⁻¹), at time t ; k_D is the rate constant of intraparticle diffusion (mg g⁻¹ min^{-1/2}); t is the contact time (min); and c is a constant (mg g⁻¹).

- (b) The Crank equation was used to study the kinetics of chromium extraction for short treatment times (generally, $\frac{q_t}{q_\infty} < 0.3$) [58]:

$$\frac{q_t}{q_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} e^{\left(\frac{-Dn^2\pi^2 t}{a^2}\right)} \quad (2)$$

where q_∞ is the amount of chromium extracted per unit of soil mass under equilibrium conditions (mg g⁻¹); a is the particle radius (mm); and D is the “apparent” diffusion coefficient (m² s⁻¹).

- (c) The Vermeulen equation was used to study the kinetics of chromium extraction for long reaction times ($\frac{q_t}{q_\infty} > 0.3$) [58]:

$$\frac{q_t}{q_\infty} = \left[1 - e^{\left(\frac{-\pi^2 D t}{a^2}\right)} \right]^{10.5} \quad (3)$$

The equations were solved with MatLab ODE45 to determine the apparent diffusion coefficient, D .

Deviations between the experimental data and the calculated values were estimated with the determination coefficient (R²).

2.6. Ecotoxicity tests

First, to correct the acidity of the soil and restore the elements removed during the soil washing process, we washed the soil with a solution that contained carbonate (CaCO₃, Na₂CO₃, MgCO₃, and K₂CO₃). Moreover, according to Udovic et al. [59], the aging process was simulated with five repetitive cycles, each comprising a 5-d exposure to 105 °C in an oven, followed by a 5-d exposure to -20 °C in a freezer. A summary of these tests is shown in Table S3 (Supporting materials).

For the ecotoxicological assessment, we used the nematode *Caenorhabditis elegans*. This species has been considered a useful model system for studying toxicity and toxicological mechanisms of various PTMs, including Cr(III) [60]. The wild-type nematode, strain N2, variant Bristol, was maintained as described previously by Liu et al. [61]. Briefly, nematodes were placed on Nematode Growth Media (NGM) plates seeded with *Escherichia coli* (strain OP50-uracil deficient) and stored at 20 °C.

The tests were performed with age-synchronous adult nematodes. The gravid nematodes were lysed with a bleaching mixture (10 g l⁻¹ NaOH, 10.5 g l⁻¹ NaOCl), followed by centrifuge washing with M9 buffer (3.0 g l⁻¹ KH₂PO₄, 6.0 g l⁻¹ Na₂HPO₄, 5.0 g l⁻¹ NaCl, 0.12 g l⁻¹ MgSO₄); then, the mixture was left overnight in NGM agar plates [62]. The nematode bioassay was carried out, with few modifications, according to the Standard Method, ASTM E2172 - 01 [63].

Soil samples (2.33 g wet weight) were hydrated with K-medium in centrifuge tubes. Ten worms were transferred to each test tube, and the soil was incubated at 20 °C for 24 h. All treatments were performed in four replicates, without feeding the worms. Nematodes were extracted from the soil samples with the centrifugation method, using a silica gel with a specific density of 1.13 g ml⁻¹ (Ludox TM 50, Sigma-Aldrich, St. Louis, MO, USA). After centrifugation (2000 rpm for 2 min), the

Table 2
Characteristics of the contaminated soil and European threshold values for residential use.

ID	Clay (%)	Silt (%)	Sand (%)	pH	LOI (%)	CE (mS cm ⁻¹)	Al (mg kg ⁻¹)	As (mg kg ⁻¹)	Ca (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Cr(III) (mg kg ⁻¹)
Soil Sample	12	28	60	7.43	27	2.11	18361	11.2	42381	1.6	3051
European Limits range ^a								20–300		2–30	130–1000
Italian limit ^a								20		2	150

ID	Cr(VI) (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Pb (mg kg ⁻¹)	Sb (mg kg ⁻¹)	Se (mg kg ⁻¹)	Tl (mg kg ⁻¹)	V (mg kg ⁻¹)	α (μ m)
Soil Sample	3	102	17214	592	97	0.3	2.41	0.52	16.2	50
European Limits range ^a		120–1000			100–700	5–40	3–100	1–15	90–500	
Italian limit ^a	2	120			100	10	3	1	90	

^a [72].

supernatant of each sample was poured into 60-mm glass petri dishes and washed with K-medium. The extracted individuals were then counted under a microscope (40 \times magnification, MELJI). The measured endpoint was mortality. Test acceptability was 80% recovery and 90% control survival.

2.7. Statistical analysis

Statistical analysis was performed on the obtained data using full factorial analysis of variance (ANOVA) with post hoc Tukey test [64] to evaluate the differences of the different treatments and differences between the mean values of samples and controls in ecotoxicity tests. P-values ≤ 0.05 were considered significant. Statistical analyses were conducted in Microsoft® Excel 2013/XLSTAT®-Pro (Version 7.2, 2003, Addinsoft, Inc., Brooklyn, NY, USA).

3. Results and discussion

3.1. Soil characterization

Soil samples were characterized to verify their natural vocation and the initial level of contamination (Table 2). The moisture content at the sampling was equal to 31%. We found that the soil could be classified as a sandy-loam [65] with an average particle radius (α) of around 0.005 ± 0.001 cm. The texture was well balanced and allowed good water permeation, oxygenation, and root penetration. The CEC was 23 meq/100 g. This value was much higher than typically found in sand-based soils, where CEC normally ranges between 5 and 10 meq/100 g [66]. This high CEC value, coupled with the neutral pH, indicated that the soil was potentially rich in nutritive elements and had good fertility potential [67]. Another important indicator of soil fertility was the organic matter content (LOI). Once more, the measured LOI was rather high compared to the average LOI of natural soils (5–7%) [68,69], which suggested good potential for growing edible cultures. Therefore, we concluded that the natural vocation of the soil was agricultural. At the same time, of all the elements analyzed, only the Cr exceeds the regulatory thresholds of different European Countries. By comparing the values of all the elements with the background [70] have been verified, that only the Cr exceeds them. The measured concentration of total Cr indicated serious contamination due to anthropogenic activities. Almost all the chromium present was in the form of Cr(III). The small amount of Cr(VI) was likely due to the equilibrium between the two species [12,14] and the high level of organic matter, which spontaneously reduces Cr(VI) to Cr(III), even at pH values around and above neutrality. This reduction decreases Cr mobility [71].

After sequential extraction of the untreated soil, we found that the bioavailable fraction of chromium, which was the sum of the exchangeable (0%), reducible (33%), and oxidizable (66%) fractions, was approximately 99% of the total (Fig. 1, Supplementary materials). These results demonstrated that remediation treatment was needed to reduce the risks related to the presence of chromium. In addition, the high sand content favored the soil washing tests for remediation, according to the USEPA [73], which stated that sandy soil guaranteed good separation of the exhausted solution from the solid particles [19]. Furthermore, more than 60% of Mn is present in the reducible fraction. This confirms that the Cr can also be oxidized by the Mn in accordance with previous studies [74,75].

3.2. Soil washing with aqueous solutions containing inorganic acids or bases

Soil samples were first washed with inorganic acids (hydrochloric, nitric, and sulfuric acids) (Fig. 2a). We measured the percentage of Cr (III) removal at two different treatment times (3 h and 48 h). Two different concentrations of washing agent (0.1 M and 1.0 M) were tested for each solution, but no removal was observed at the lower concentration (0.1 M) (no statistically significant $p > 0.05$). This result

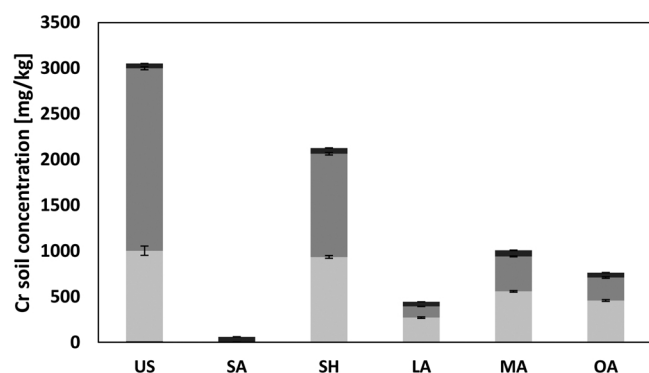


Fig. 1. Results of sequential extraction before and after the soil washing treatment Exchangeable and weak acid soluble fraction (□); Reducible fraction (▨); Oxidizable fraction (▩); Residual fraction (■). US: Untreated Soil; Treated soil at 96 h, 1:10 of S:L ratio. T = 25 °C, Initial concentration of the washing reagents: 1 M Sulfuric Acid (SA); 1 M Sodium Hydroxide (SH); 1 M lactic acid (LA); 1 M malic acid(MA); 0.8 M oxalic acid (OA).

could be explained by considering that only highly acidic solutions (1 M) could neutralize the soil buffer capacity to achieve strong acidic conditions (pH < 1). This finding confirmed that the chromium leaching process was affected by pH [76]. The extreme pH achieved in these tests certainly affected soil characteristics; they modified the soil structure and caused the loss of important mineral substances [77].

The highest percentage of Cr(III) removal was achieved with 1 M H₂SO₄. The extraction kinetics reached a plateau in less than 3 h (97%). No significant increase in extraction efficiency was observed at longer treatment times. Indeed, after 3 h, the remaining Cr(III) was bound to the mineral fraction of the soil (92%), as shown with sequential extractions performed on treated samples (Fig. 1). The high extraction efficiency could be attributed to the stable complexes that Cr(III) formed with sulfates (i.e., CrSO₄⁺) [78].

The HCl washing efficiencies (0.1 M: 0% and 1 M: 66%) were lower than those reported in previous studies. Pichtel and Pichtel [23] reported complete Cr(III) removal with a 0.1 M HCl solution, after a 5-h extraction. Nonetheless, HCl may not guarantee good Cr(III) removal efficiencies, when the soil has very high organic matter content [30], because stable organic complexes form between Cr(III) and humic substances [19,79].

Sodium hydroxide washing efficiency was tested in the absence and presence of hydrogen peroxide, at varying treatment times and initial concentrations (Fig. 2b). The results indicated that, similar to the H₂SO₄ washing, the contact time did not significantly affect the efficiency of the process. In fact, the initial concentration of sodium hydroxide had a much more important effect on extraction efficiency. Cr(III) removal increased from 2–6% to 76–82% when we increased the NaOH from 0.1 M to 3 M, respectively. The pH values obtained at the end of the test were 8.6 and 13.4 for the solutions with 0.1 M to 3 M NaOH respectively. These results were ascribed to the dissolution of organic matter in the soil, which promoted the mobilization of the metal, as suggested by Impellitteri et al. [80]. Consistent with that hypothesis, a sequential extraction on treated samples indicated that only the oxidizable Cr fraction was removed; the total concentration of exchangeable, reducible, and residual Cr fractions remained unchanged from those found in the untreated soil (Fig. 1). Unfortunately, we found chromates (27%) in the spent solution, consistent with results reported by Zink et al. [81]. This finding suggested the possibility that chromates formed at high pH values.

High chromate concentrations (up to 30%) were also detected with NaOH washing, in the presence of hydrogen peroxide. The use of sodium hydroxide alone did not improve the removal efficiency. However, the formation of Cr(VI) represented additional potential

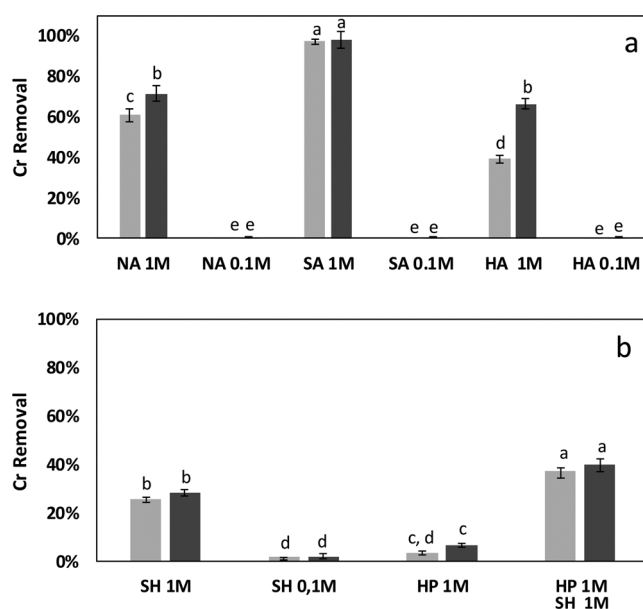


Fig. 2. Percentages of Cr(III) removal with different solutions and different exposure times during the soil washing tests. (a) Mineral acids; (b) basic solutions, with or without hydrogen peroxide. Light grey: 3-h exposure; black: 48-h exposure; Solid-to-liquid ratio = 1:10; Temperature = 25 °C; NA: nitric acid; SA: sulfuric acid; HA: hydrochloric acid; SH: sodium hydroxide; HP: hydrogen peroxide Data with different letters are significantly different (p < 0.05) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

environmental damage, which argued against adopting NaOH for soil remediation.

3.3. Soil washing with aqueous solutions containing OABLs

To avoid the negative effects of strong inorganic acids on soil properties [82] and the formation of chromates related to the use of alkaline solutions, we tested OABLs, which are weak natural acids. As stated in Section 1, several studies have estimated OABL efficiencies as washing agents [26,29,30], but few studies have investigated Cr(III) contamination in soils.

We first tested carboxylic acids for removing Cr(III) from soils (Fig. 3). We conducted some tests with EDTA and citric acid, but the Cr (III) removal efficiency was always below 6%. We did not perform tests at 1 M citric acid, because that condition would be impractical to realize at full scale, due to the large amount of reagent required (192 g l⁻¹). Moreover, the efficiency was not improved with EDTA (3%). Consistent with other studies [23,24], we hypothesized that the low removal efficiency of citric acid and EDTA may have been due to the presence of cation competitors, which have high stability constants in EDTA-metal complexes. Moreover, the EDTA was not biodegradable, and EDTA-metal complexes can be adsorbed to the soil, which results in toxicity [83]. Consequently, higher EDTA concentrations have not been investigated.

None of the tested organic compounds resulted in appreciable efficiency at low concentration (0.1 M). In contrast, with higher concentrations (1 M), we could achieve a substantial improvement in the extractive yield. At the highest concentrations of carboxylic acid (LA or MA at 1 M and OA at 0.8 M), the percentage of Cr(III) removal was similar to that obtained with mineral acids. The best performances (88%) were obtained with LA as the chelating agent; the removal efficiencies were comparable to those reported previously by Reynier et al. [29], who performed Cr(III) removal in naturally contaminated soil. Sequential extraction of our treated samples indicated that both

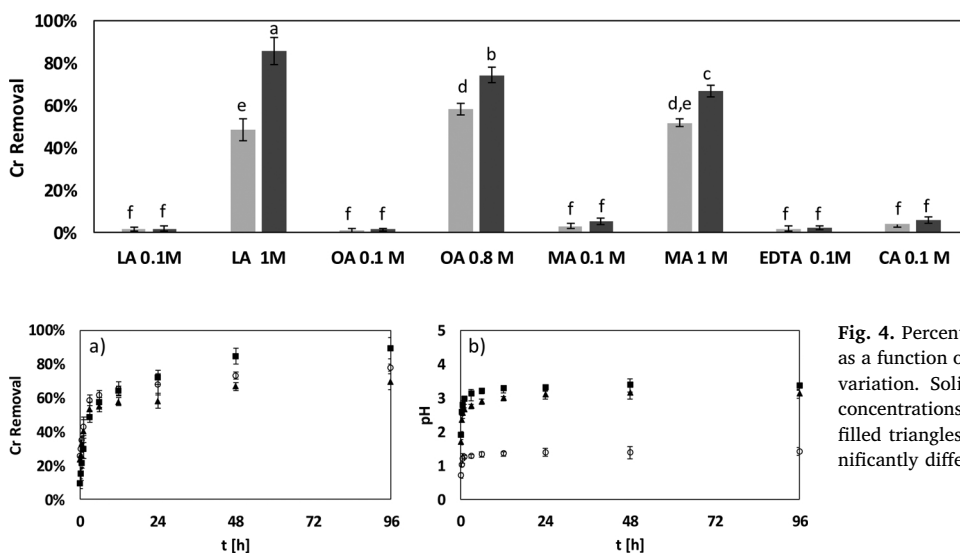


Fig. 3. Percentages of Cr(III) removal with different carboxylic acids and different exposure times. Light grey: 3 h; black: 48 h; Solid:liquid ratio = 1:10; Temperature = 25 °C; LA: lactic acid; MA: malic acid; OA: oxalic acid; CA: citric acid; EDTA: ethylenediamine tetra-acetic acid. Data with different letters (a–f) are significantly different ($p < 0.05$) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

reducible and oxidizable fractions could be removed (Fig. 1). Of note, a marked improvement in extraction efficiency was achieved by increasing the reaction time.

We also investigated soil washing with OABLs under various pH conditions. Indeed, the formation of Cr(III)-OABL complexes was favored by acid pH (Table S1). The solutions were pH 0.7 with 1 M OA, pH 1.7 with 1 M MA, and pH 1.9 with 1 M LA. We observed a trend in Cr removal, where rapid extraction was observed, in the first few minutes of treatment with LA, OA, and MA. This trend was ascribable to the strong acidic conditions (Fig. 4). The mechanism of Cr(III) removal appeared to be related to two processes: the ionic exchange between protons and Cr(III) and the formation of Cr(III)-based acid complexes. The first, more rapid process, was predominant in strong acid conditions; the second process was prevalent in weak acid conditions. Therefore, when soil washing was performed with weak acids, the pH rapidly increased due to the soil buffer capacity, and Cr(III) removal proceeded at a slower rate than when soil was treated with strong acids.

However, the buffer capacity was neutralized, due to the dissolution of carbonates and bicarbonates present in the soil (i.e., CaCO_3 , MgCO_3 , etc). Thus, we hypothesized that, in addition to removing the contaminant during treatment, some nutrients could be removed from the soil. Therefore, a complete treatment cycle might require enrichment before the soil could be reused.

3.4. Diffusion model

In the soil washing process, the rate of removing metals with organic compounds could be controlled by the rate of diffusion of the complexes [56,84]. In particular, Race et al. [55] found that intraparticle diffusion might be the main rate-limiting step. The presence of intraparticle diffusion can be detected when a linear relationship is observed in an evaluation with Eq. (1) (Fig. 5). The kinetics of Cr(III) extraction with carboxylic acids could be divided into three phases. The first phase, which lasted a few minutes, was governed by Cr(III) dissolution, due to acid pH. The following two phases were controlled by intraparticle diffusion. We observed multi-linearity in phases 2 and 3, which indicated that the diffusive process developed across pores of progressively smaller sizes [55]. In more detail, the first segment of the plot (phase 1) was ascribed to boundary layer diffusion effects, including external film resistance and superficial diffusion; the other linear segments (phases 2 and 3) were associated with the diffusive process across macropores and micropores, respectively [55]. Therefore, we excluded the experimental point obtained during the first 5 min of the process, when the extraction was mainly due to the strong

Fig. 4. Percentages of Cr removal with different carboxylic acids, as a function of exposure time. (a) Percentage Cr removal; (b) pH variation. Solid:liquid ratio, 1:10. Temperature = 25 °C. Initial concentrations: filled squares: 1 M LA; open circles: 0.8 M OA; filled triangles: 1 M MA. Data with different letters (a–f) are significantly different ($p < 0.05$).

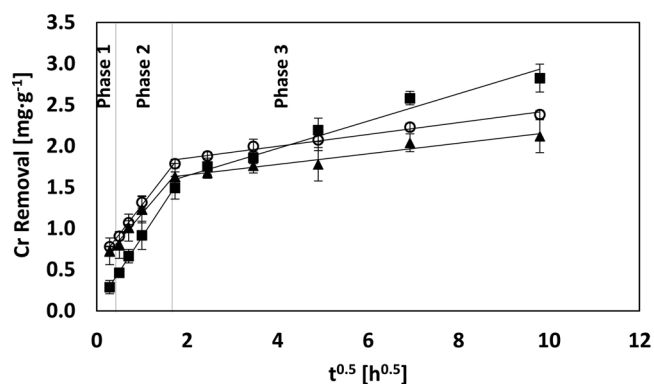


Fig. 5. Weber and Morris (Eq. (1)) results at different time points demonstrate intraparticle diffusion during soil washing. Cr removal was evaluated over time at a solid:liquid ratio of 1:10. Temperature = 25 °C. Filled squares: 1 M LA; open circles: 0.8 M OA; filled triangles: 1 M MA. Phase 2 - R^2 values are: LA = 0.99; OA = 0.93; MA = 0.95; Phase 3 - R^2 values are: LA = 0.98; OA = 0.97; MA = 0.97. LA: lactic acid; OA: oxalic acid; MA: malic acid.

acidic condition.

The remaining data were modeled with the Crank equation, for the first 3 h of treatment, and with the Vermeulen equation for the remaining time. To the best of our knowledge, no information was available in the literature on the diffusion coefficient for Cr(III)-organic acid complexes in soil. In Table 3, we report the best estimated values for the pore diffusivity of Cr(III)-organic acid complexes, compared to previously published values of D and k_D . Our estimated values of the diffusivity coefficient appeared to be consistent with those found in the literature, although the matrices and their porosities could influence the process.

3.5. Ecotoxicological assessment

We performed an ecotoxicological assessment to determine whether soil washing affected soil toxicity. We selected several treated samples for the ecotoxicological tests, in addition to samples of untreated contaminated soil and uncontaminated (control) soil (Table S3). We compared treated samples that exhibited the best results after OABL washing (1 M LA, 0.8 M OA, 1 M MA), in terms of Cr(III) removal, to soil samples washed with the most efficient inorganic acid (1 M H_2SO_4 ; Fig. 6). The Table S4 shows the chemical characteristics of the soils after extraction process. In general it was observed for all samples a decrease of the main cations (i.e. Ca, Fe, Mn). Sulfuric acid makes the

Table 3Ranges and best estimated values of the “apparent” diffusion coefficient (D) and the rate constant of intra-particle diffusion (k_D).

Compound	Solid Matrix	D [$\text{m}^2 \text{s}^{-1}$]	k_D [$\text{mg g}^{-1} \text{min}^{-1}$]	References
Lactic acid	Resins	$12.25 \cdot 10^{10}$ – $17.89 \cdot 10^{10}$	–	[85]
Cr(III)	Membrane	$3.02 \cdot 10^{10}$ – $4.67 \cdot 10^{12}$	–	[86]
Cr(III)	Waste sludge	–	0.98–1.80	[87]
Oxalic Acid	Mesoporous material	–	3.47	[88]
Malic Acid	Membrane	$4.87 \cdot 10^{10}$ – $7.17 \cdot 10^{10}$	–	[89]
Lactic Acid	Gel	$3.33 \cdot 10^{10}$ – $11 \cdot 10^{10}$	–	[90]
Lactic Acid	Ion exchanger	$0.22 \cdot 10^{11}$ – $4.3 \cdot 10^{11}$	–	[91]
Cr(III)-Lactic Acid	Contaminated soil	$1.27 \cdot 10^{12}$ – $24.2 \cdot 10^{12}$	0.166–0.793	this study
Cr(III)-Oxalic Acid	Contaminated soil	$4.98 \cdot 10^{12}$ – $61.2 \cdot 10^{12}$	0.072–0.669	this study
Cr(III)-Malic Acid	Contaminated soil	$5.37 \cdot 10^{12}$ – $36.2 \cdot 10^{12}$	0.064–0.646	this study

soil particularly acidic ($\text{pH} = 0.2$) and determines a higher removal of metal cations from soils than OABL.

Tests were conducted both without (Fig. 6a) and with (Fig. 6b) nutrient-enrichment; we also tested freshly treated samples and treated samples after they had undergone simulated aging to observe short-term and long-term effects, respectively.

Synchronized adult worms survived well after 24-h incubations in the control soil (mortality < 15%). In contrast, the untreated soil was highly toxic: *C. elegans* mortality was above 90% (Fig. 6a). In the un-enriched washed soils, *C. elegans* mortality strongly depended on the washing solution. Mortality was reduced by washing with MA (42%), OA (62%), and LA (46%) ($p < 0.01$, compared to untreated soil), but washing with sulfuric acid did not significantly change mortality (88%; $p > 0.05$, compared to untreated soil; Fig. 6a). However, in all artificially aged samples, mortality was above 40% (typically taken as a threshold value); therefore, these changes in soil toxicity were insufficient to consider the washed soil non-toxic.

We reasoned that the residual toxicity might be related to either the presence of toxic compounds or the removal of micronutrients combined with an acidic pH; both of these conditions could strongly affect the survival of *C. elegans* [92]. Therefore, we repeated all tests with nutrient-enriched soil (Fig. 6b). We found that the toxicity significantly decreased in soil samples washed with OABLs, which indicated that the remediation process lowered or eliminated soil toxicity. The mortality rate of nutrient-enriched soil washed with OA was around 30%, and it decreased to 20% and 21% for soils washed with MA and LA, respectively. Moreover, the two latter acids produced results similar to those obtained in the control soil, which indicated the absence of toxicity. Therefore, we concluded that the toxicity of unenriched soils was due exclusively to the leaching of micronutrients and the unfavorable pH value. In contrast, the toxicity found in soil samples washed with sulfuric acid persisted in nutrient-enriched soil samples. *C. elegans* mortality was above 50%, comparable to that observed in the aged untreated soil. Because the Cr(III) concentration was equivalent or less than the residual concentration in the samples washed with OABL, we

concluded that the sulfuric acid treatment mainly deprived the soil of natural metabolic activities, to such an extent that even adding nutrients could not remediate the soil. In addition, although the soil was nutrient-enriched, given the large amount of sulfuric acid used, a portion could have remained adsorbed to the soil. The results were similar in aged samples and fresh samples. In particular, only in the non-enriched samples there were marked improvement using SA and LA; indeed, the mortality decreased from 88% to 54% when SA was used, and decreased from 49% to 38% when LA was used. However, the mortality rate after washing with sulfuric acid remained greater than the rates observed after washing with the OABLs.

These results confirmed that washing with OABLs could restore contaminated soils, and we demonstrated that soil quality could be ensured when adequate nutrient-enrichment was performed. The highest efficiency, in terms nematode mortality, was observed in nutrient-enriched soil treated with LA, which significantly reduced the toxicity (Fig. 6b). Our statistical analyses showed that, in enriched soils, the reduction in soil toxicity was not significantly different in fresh or aged soils ($p > 0.05$). Thus, nutrient enrichment could restore soil quality by significantly reducing its toxicity, which rendered it suitable for anthropic activities.

4. Conclusion

The present study was conducted with a potentially fertile soil, which had been highly contaminated with Cr(III), due to anthropogenic activities. Our results showed that acid washing treatment may represent a promising solution for the complete rehabilitation of the site, provided that the washing solution is carefully chosen, and that the washed soil is adequately treated to equilibrate the pH and restore the micronutrient content. Based on our findings, the following conclusions could be drawn:

- based on the yields obtained in this study and other studies, the choice of appropriate washing solution depended on both the

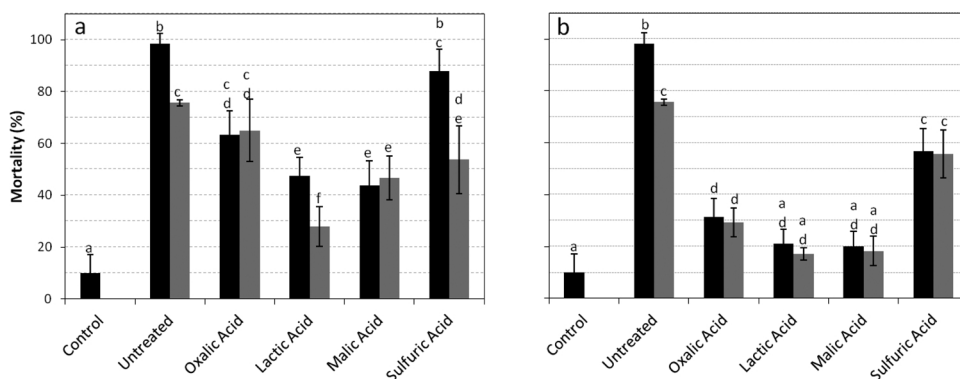


Fig. 6. Mean mortality of *C. elegans* after exposure to treated or untreated soil. (a) Soil without nutrient enrichment; (b) nutrient-enriched soil. Black: Fresh soil; light grey: aged soil. Data with different letters (a–f) are significantly different ($p < 0.05$) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

contaminant and the soil characteristics;

- OABL soil washing successfully treated fertile soils contaminated by Cr(III) and rehabilitated them for agricultural use. The maximum Cr removal efficiency of OABL (88%) was slightly less than the removal efficiency obtained by using inorganic acids (98%);
- among the three OABLs tested, the highest efficiencies were obtained, respectively, with LA (1 M), MA (1 M), and OA (0.8 M) for 88%, 75%, and 67% efficiencies;
- Cr(III) removal obtained by using OABLs was regulated by intraparticle diffusion mechanisms. The best estimated values of the “apparent” diffusion coefficient (D) and the rate constant for intraparticle diffusion (k_p) appeared to be consistent with those described in previous studies;
- treating contaminated soil with OABLs provided a non-toxic soil, suitable for anthropogenic activities.

It has to be finally highlighted that the washing treatment produces a contaminated spent solution that needs to be carefully treated. The presence of Me-OABL complexes requires the destruction of the complex before Cr removal. Further investigations are therefore needed to deepen the knowledge of the optimal treatment technique.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jhazmat.2019.04.007>.

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