

# Accepted Manuscript

Hexavalent chromium quantification by isotope dilution mass spectrometry in potentially contaminated soils from south Italy

Antonio G. Caporale, Diana Agrelli, Pablo Rodríguez-González, Paola Adamo, J. Ignacio García Alonso



PII: S0045-6535(19)31132-4

DOI: <https://doi.org/10.1016/j.chemosphere.2019.05.212>

Reference: CHEM 23947

To appear in: *ECSN*

Received Date: 27 March 2019

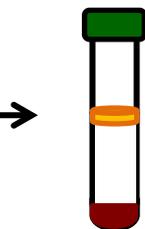
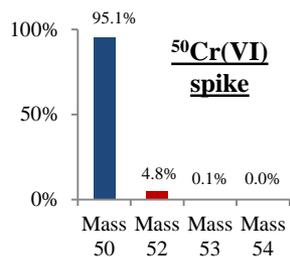
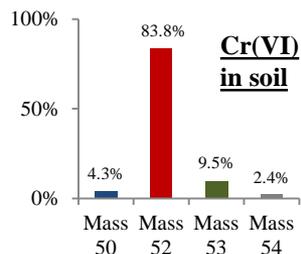
Revised Date: 17 May 2019

Accepted Date: 23 May 2019

Please cite this article as: Caporale, A.G., Agrelli, D., Rodríguez-González, P., Adamo, P., Alonso, J. Ignacio. Garcí., Hexavalent chromium quantification by isotope dilution mass spectrometry in potentially contaminated soils from south Italy, *Chemosphere* (2019), doi: <https://doi.org/10.1016/j.chemosphere.2019.05.212>.

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# Cr(VI) quantification in soil by IDMS



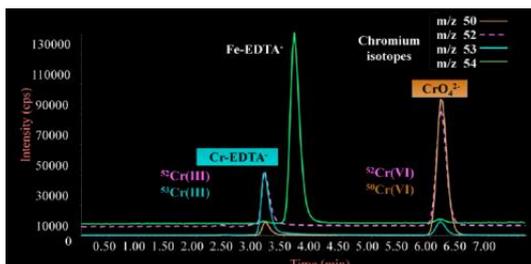
**50 mM Na<sub>2</sub>EDTA**



**5 min at 80 °C**



**IC hyphenated to ICP-MS**



1 **Hexavalent chromium quantification by isotope dilution mass spectrometry in potentially**  
2 **contaminated soils from south Italy**

3 \*Antonio G. Caporale<sup>1</sup>, Diana Agrelli<sup>1,2</sup>, Pablo Rodríguez-González<sup>3</sup>, Paola Adamo<sup>1</sup>, J. Ignacio  
4 García Alonso<sup>3</sup>

5 <sup>1</sup> *Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055*  
6 *Portici, Naples, Italy*

7 <sup>2</sup> *CIRAM - Interdepartmental Center for Environmental Research, University of Naples Federico II,*  
8 *Via Mezzocannone 16, 80134 Naples, Italy*

9 <sup>3</sup> *Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo,*  
10 *Julián Clavería 8, 33006 Oviedo, Spain*

11 \* Corresponding author: [ag.caporale@unina.it](mailto:ag.caporale@unina.it)

12  
13 **Abstract**

14 Due to carcinogenicity of hexavalent chromium [Cr(VI)], its accurate quantification in Cr-  
15 contaminated soils is of paramount importance. The aim of this work was to quantify Cr(VI) by  
16 species-specific IDMS in soil samples from two Italian case studies: A) farmland potentially  
17 contaminated by pseudo-total Cr and Zn and heavy hydrocarbons due to past illegal burial of  
18 tannery wastes; B) Solofrana valley where volcanic soils are potentially contaminated by pseudo-  
19 total Cr and Cu due to tannery activities. Hexavalent Cr extraction from soils was performed by  
20 focused microwaves (5 min at 80 °C) using 50 mM EDTA, followed by the separation of Cr species  
21 by IC and detection by ICP-MS. The Cr(VI) extracted from 20 soil samples of case study A ranged  
22 from 0.15 to 11.18  $\mu\text{g g}^{-1}$ , with 70 % of samples exceeding the Cr(VI) screening value set by Italian  
23 Parliament for residential/urban soil to assess their potential contamination. Higher levels of Cr(VI)  
24 (22.0-107.1  $\mu\text{g g}^{-1}$ ) were extracted from other 7 Cr-most-contaminated soil samples, which required

25 a pre-treatment with n-hexane to remove part of organic compounds from each sample, since these  
26 reducing agents made the quantification of Cr(VI) by IDMS more challenging because they caused  
27 an almost complete reduction of  $^{50}\text{Cr(VI)}$  used for IDMS quantification. Hexavalent Cr extracted  
28 from soil samples of case study B ranged from 0.70 to 5.79  $\mu\text{g g}^{-1}$ , with 42 % of samples exceeding  
29 the value set by Italian legislation. In both case studies, the Cr(VI) extracted from soil was  
30 significantly correlated to the pseudo-total Cr content.

31

32 **Keywords:** Cr(VI); IDMS; Cr speciation; soil contamination; tannery wastes; heavy hydrocarbons.

33

## 34 1. INTRODUCTION

35 Chromium mainly exists in two stable oxidation states, trivalent chromium [Cr(III)] and hexavalent  
36 chromium [Cr(VI)] (Jacobs and Testa, 2005; Lilli et al., 2015; Ščančar and Milačič, 2014; Shahid et  
37 al., 2017). Trivalent Cr can be considered an essential nutrient for human health, being involved in  
38 glucose, insulin and protein metabolism, while Cr(VI) is classified as Group A human carcinogen,  
39 also provoking severe health problems on the respiratory tract, liver, kidney, gastrointestinal and  
40 immune systems and possibly on the blood (IARC, 2012). Thus, several directives have been  
41 adopted at the European and international levels to perform Cr speciation in the environmental  
42 studies (Séby and Vacchina, 2018).

43 Chromium mobility and bioavailability in soil environments strongly depend on its chemical form  
44 (Avudainayagam et al., 2003). Trivalent Cr is undoubtedly the most thermodynamically stable form  
45 of Cr in soil, as it readily precipitates as chromium hydroxide [Cr(OH)<sub>3</sub>] and iron-chromium  
46 hydroxide [(Fe,Cr)(OH)<sub>3</sub>] or becomes immobilised after sorption onto soil colloids (Leita et al.,  
47 2009). On the other hand, Cr(VI) is thermodynamically metastable in the pore solution and much  
48 more mobile in soil, since Cr(VI) oxyanions (chromate CrO<sub>4</sub><sup>2-</sup>, hydrogen chromate HCrO<sub>4</sub><sup>-</sup>, and  
49 dichromate Cr<sub>2</sub>O<sub>7</sub><sup>2-</sup>) are not adsorbed onto soil colloids under sub-neutral to alkaline conditions  
50 (Becquer et al., 2003; Mpouras et al., 2017). At these soil pHs, Cr(VI) can be present in the form of  
51 solid minerals in association with different cations characterised by a wide range of solubility:  
52 barium, calcium, lead and zinc chromates are the most insoluble (Kotas and Stasicka, 2000; Pettine  
53 and Capri, 2005), while alkaline and some alkaline-earth chromates are highly soluble. Being a  
54 strong oxidant, Cr(VI) can be readily reduced to Cr(III) in the presence of various electron donors  
55 such as soil organic matter (Bolan et al., 2003; Saha et al., 2011); however, with favourable pedo-  
56 climatic conditions, even in organic soils, Cr(VI) may persist for prolonged periods of time (Leita et  
57 al., 2009). In most cases, the presence of Cr(VI) in the soil is due to anthropogenic sources, since it  
58 is used in a number of industrial applications such as electroplating, tanning, industrial water  
59 cooling, paper pulp production and petroleum refining (Choppala et al., 2013; Tchounwou et al.,

60 2012). The levels and forms of Cr(VI) in soils depend on several factors such as pH ( $\text{HCrO}_4^-$  and  
61  $\text{Cr}_2\text{O}_7^{2-}$  at pH 0.7-6.5; and  $\text{CrO}_4^{2-}$  at pH 6.5-14.0), the redox potential and the presence of natural  
62 oxidants (e.g., manganese oxide) or reducing agents [e.g., Fe(II), sulphide and organic matter]  
63 (Banks et al., 2006; Choppala et al., 2018).

64 The determination of Cr(VI) in soil samples is often complicated, because quantitative extraction of  
65 the contaminant must be assured while avoiding Cr(III)-Cr(VI) redox interconversion reactions both  
66 during extraction and the subsequent chemical analysis (Pettine and Capri, 2005; Weibel et al.,  
67 2016). In addition to total Cr(VI) content, the easily-extractable and highly-mobile fractions of  
68 Cr(VI) are also determined, in soils where Cr contamination highly impacts on the surrounding  
69 environment (James et al., 1995; Zuliani et al., 2013).

70 Isotope dilution mass spectrometry (IDMS) is a calibration technique able to accurately quantify  
71 Cr(VI) from soil samples. When multiple spiking is applied Cr(III)-Cr(VI) redox interconversions  
72 during sample preparation can be corrected for (Fabregat-Cabello et al., 2012; Garcia Alonso and  
73 Rodriguez-Gonzalez, 2013; Guidotti et al., 2015; Rahman et al., 2005). The analytical procedure,  
74 standardised by USEPA as method 6800 (USEPA, 2007) requires the addition of known amounts of  
75 isotopically enriched Cr(III) and Cr(VI) to the samples before extraction. The selection of the most  
76 proper extracting solution plays an important role on the correct quantification of the levels of  
77 Cr(VI) in soils. The use of ethylenediaminetetraacetic acid (EDTA) as extracting solution, buffered  
78 at pH 10.0 (Korolczuk and Grabarczyk, 2005), shows two main advantages over the well-  
79 recognised USEPA 3060A (USEPA, 1995) extraction procedure at pH 12.0: i) it complexes Cr(III)  
80 preventing its oxidation to Cr(VI) throughout the analytical procedure; ii) it facilitates the extraction  
81 of Cr(VI) from the solid sample by complexation of other metals (e.g., Ba, Pb, Sr, Zn) forming  
82 insoluble chromates (Fabregat-Cabello et al., 2012; Guidotti et al., 2015). Therefore, since EDTA  
83 was able to prevent Cr(III) oxidation to Cr(VI) during extraction, the use of a single isotopically-  
84 labelled Cr(VI)-spike provides an accurate quantification of Cr(VI) in soil by IDMS (Fabregat-  
85 Cabello et al., 2012).

86 In the present study single-spike IDMS in combination with EDTA at basic pH (10.0) was applied  
87 to quantify Cr(VI) in soil samples from two rural areas of south Italy: A) 27 soil samples from six  
88 hectares of farmland potentially contaminated by pseudo-total Cr (up to 10,046  $\mu\text{g g}^{-1}$ ), Zn and  
89 heavy hydrocarbons ( $C>12$ ), due to past illegal burial of industrial wastes; B) 12 soil samples from  
90 the Solofrana valley where volcanic soils are potentially contaminated by pseudo-total Cr (up to 594  
91  $\mu\text{g g}^{-1}$ ) and Cu due to tannery activities.

92

## 93 **2. EXPERIMENTAL SECTION**

### 94 **2.1. Case studies, soil sampling and processing**

#### 95 *2.1.1. Case study A - Farmland contaminated by illegally burial of tannery wastes*

96 Case study A is a 60,000  $\text{m}^2$  farmland located in the Campania Plain (Province of Naples,  
97 Campania, south Italy). This area is currently confiscated by the Italian Judiciary (Caporale et al.,  
98 2018), due to a past illegal burial of industrial wastes of leather-tanning origin. From the  
99 geomorphological and geological points of view, case study A is located in a lowland environment,  
100 the Piana Campana graben, on the north of the Phlegrean Fields and NW of the Somma-Vesuvius  
101 complex, which are the main active volcanoes of the region. Due to the proximity to those volcanic  
102 centres, a preferential accumulation of pyroclastic deposits is found in these areas (Di Vito et al.,  
103 2013; Orsi et al., 2004) and then the soil parent material is mostly volcanic ash and pumice.  
104 According to the soil maps of Di Gennaro et al. (2002) and World Reference Base classification  
105 (FAO, 1998), the soils in the surroundings of case study A were classified as Pachi-Vitric Andosols.  
106 At the end of 2015, a soil contamination monitoring campaign, aimed to address the level and  
107 spatial variability of contamination, was carried out in case study A. Soil samples were collected at  
108 3 depths: 0-20 cm (d1), 30-60 cm (d2) or 70-90 cm (d3), from georeferenced points according to a  
109 sampling grid of 20x20 m. Also, soil samples were collected from pedological profiles and trenches  
110 (pt) opened in the field and well describing the complex distribution of the buried waste materials  
111 (Caporale et al., 2018). Prior to analysis, soil samples were firstly air-dried, then 2-mm sieved and

112 lastly pulverised (particles  $< 177 \mu\text{m}$  – 80 Mesh) by PM 200 ball mill (Reutch). On the basis of  
113 these analyses, site A was found to be potentially contaminated by pseudo-total Cr (up to  $10,046 \mu\text{g}$   
114  $\text{g}^{-1}$ ), Zn (up to  $1,846 \mu\text{g g}^{-1}$ ) and heavy hydrocarbons ( $\text{C}>12$ , up to  $2,150 \mu\text{g g}^{-1}$ ), mostly  
115 concentrated in the first metre of depth. Since the contaminant pseudo-total concentrations exceed  
116 the screening values set by Italian Parliament for residential/urban soil ( $150 \mu\text{g g}^{-1}$  for Cr and Zn;  $50$   
117  $\mu\text{g g}^{-1}$  for heavy hydrocarbons), according to Italian legislation (Italian-Parliament, 2006) the site is  
118 classified as potentially contaminated by pseudo-total Cr and Zn and heavy hydrocarbons and then  
119 in need of investigation to assess the factual risk for surrounding environment and human health.  
120 Pseudo-total Cr was quantified by *aqua regia* digestion (ISO 11466, 1995) and Inductively Coupled  
121 Plasma (ICP-MS) analysis at Acme Analytical Laboratories Ltd (Vancouver, Canada), while heavy  
122 hydrocarbons were analysed by UNI EN ISO 16703 (2011) at Chelab Srl (Resana, TV, Italy). The  
123 contents in soil organic carbon (C) and organic matter ( $\text{OM} = \text{C} \times 1.724$ ) were determined by wet  
124 digestion using the Walkley-Black procedure (Walkley and Black, 1937). The quantification of  
125 Cr(VI) by IDMS was carried out at the University of Oviedo (Spain) on selected soil samples  
126 ( $n=27$ ), chosen according to the overall level of contamination (pseudo-total Cr and heavy  
127 hydrocarbons) and the spatial distribution in the field.

128

### 129 2.1.2. Case study B - Farmland contaminated by floods with Cr-enriched river sediments

130 Case study B is located in the Solofrana river valley (Province of Avellino, Campania, south Italy),  
131 where volcanic soils with moderate to high andic properties are potentially contaminated by  
132 pseudo-total Cr and Cu due to tannery activities. Chromium contamination results from the long use  
133 of contaminated river waters for irrigation (prohibited since 1990) and frequent floods after intense  
134 rainfall, spreading Cr-enriched sediments on surrounding surfaces (Adamo et al., 2003; Adamo et  
135 al., 2006; D'Ascoli et al., 2006; Zampella et al., 2010). However, there is still no evidence that  
136 Cr(VI) occurs in these soils. The quantification of Cr(VI) by IDMS was carried out at the  
137 University of Oviedo in 12 air-dried, 2-mm sieved and pulverised (particles  $< 177 \mu\text{m}$  – 80 Mesh)

138 soil samples, collected from topsoil (0-20 cm) of areas affected by flooding (70 to 550 m far from  
139 the Solofrana riverbeds) in a past soil contamination monitoring campaign, object of several  
140 scientific studies (Adamo et al., 2003; Adamo et al., 2006; D'Ascoli et al., 2006; Zampella et al.,  
141 2010) and adequately refrigerated and stored over time.

142

## 143 **2.2. Preliminary investigations: standard Cr net oxidation test and Cr(VI) quantification by** 144 **colorimetric-based techniques in soil samples from case study A**

145 The standard Cr net oxidation test (Bartlett and James, 1996) was performed on selected soil  
146 samples (n=10) from case study A, in a pseudo-total Cr concentration range of 34-4,487  $\mu\text{g g}^{-1}$ . The  
147 analytical procedure is based on the contact between the soil and a solution of Cr(III) ( $\text{CrCl}_3$  as  
148 source) and the subsequent measurement of the possible formed-Cr(VI) by colorimetric reaction of  
149 Cr(VI) with diphenylcarbazide and spectrophotometric detection at 540 nm. The number of  
150 potentially oxidisable moles of Cr(III) to Cr(VI) by soil manganese oxides present in the soil was  
151 calculated by dividing the number of moles of Mn from easily reducible manganese oxides by 1.5,  
152 being 1.5 the molar ratio between Mn and Cr(III) in the oxidation reaction (Chon et al., 2008). The  
153 moles of Mn from easily reducible manganese oxides were determined by analysing the extract of  
154 the second step of the three-stage sequential extraction protocol proposed by the Measurements and  
155 Testing Programme - MAT (formerly BCR) of the European Commission (Rauret et al., 1999).

156 The extraction of total Cr(VI) was carried out in a few representative samples according to USEPA  
157 3060A method (USEPA, 1995), which provides an extraction in a strongly basic environment (pH  
158 12.0) at 90 °C for 1 h. The determination of Cr(VI) in the extract was carried out according to the  
159 USEPA 7196A method (USEPA, 1992), which provides a specific colorimetric reaction between  
160 Cr(VI) and diphenylcarbazide in an acidic environment and a spectrophotometric detection at 540  
161 nm of the colored complex Cr(III)-difenylcarbazone. A Lambda 25, Perkin Elmer (Waltham, MA,  
162 USA) was employed for this purpose. Some modifications to the official analytical methods were  
163 made to minimise Cr(VI) reduction to Cr(III) during extraction: 1) extraction at ambient

164 temperature and 2) purification of the extract from organic matter in basic environment by  
165 aluminum sulphate (Ministerial-Decree, 1999). The determination of Cr(VI) in the extracts from  
166 USEPA 3060A was carried out even by USEPA 7199 method (USEPA, 1996), which provides a  
167 phase of purification of the extract from the organic matter with the aid of an ion chromatograph  
168 Dionex DX-120 (Sunnyvale, CA, USA), equipped with guard column (Dionex IonPac NG1) and an  
169 analytical column (Dionex IonPack AS7).

170

### 171 **2.3. Instrumentation**

172 The microwave-assisted extraction of Cr(VI) from the soil samples was carried out by an Explorer  
173 Hybrid focused microwave system (CEM, Mathews, NC). Focused microwaves were applied to the  
174 samples contained in 10-mL disposal glass vials. The system was equipped with temperature and  
175 pressure feedback control and magnetic stirring, so that the temperature and pressure of the vial was  
176 controlled throughout the extraction step. The chromatographic separation of the trivalent and  
177 hexavalent Cr species was performed on a Dionex DX-120 Liquid Chromatography (LC) system  
178 (Sunnyvale, CA, USA) using a Dionex Ion pack AS9-HC anion-exchange column (PEEK,  
179 4×250mm×9µm). The detection of the Cr(III) and Cr(VI) species was carried out by an Agilent  
180 7500c quadrupole ICP-MS instrument (Agilent Technologies, Tokyo, Japan) equipped with an  
181 octapole collision cell. Helium was used as the collision gas (Fabregat-Cabello et al., 2012; Guidotti  
182 et al., 2015) to minimise polyatomic interferences (e.g.  $^{35}\text{Cl}^{16}\text{O}^1\text{H}^+$ ,  $^{35}\text{Cl}^{17}\text{O}^+$  and  $^{40}\text{Ar}^{12}\text{C}^+$  for  $^{52}\text{Cr}^+$ ).  
183 The Cr(VI) extraction solution and the mobile phase for LC-ICP-MS analyses were prepared by  
184 dissolving 50 and 5 mmol, respectively, of ethylenediaminetetraacetic acid disodium salt dihydrate  
185 ( $\text{Na}_2\text{-EDTA}$ , Sigma, St. Louis, MO) in 1 L of ultrapure water at pH 10.0, adjusted with  $\text{NH}_4\text{OH}$  (30  
186 %) (Merck, Darmstadt, Germany).

187

### 188 **2.4. Reagents and Materials**

189 The  $^{50}\text{Cr}$ -enriched Cr(VI) spike solution was provided by ISC-Science (Oviedo, Spain). Table 1  
190 shows the isotopic composition (% abundance) and the concentration of  $^{50}\text{Cr}$ -enriched Cr(VI),  
191 quantified by reverse IDMS analysis. For this purpose, a natural abundance Cr(VI) standard was  
192 prepared dissolving at pH 10.0 potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) certified by the German Federal  
193 Institute for Materials Research and Testing (BAM Berlin). The standard reference materials SRM  
194 2700 (hexavalent chromium in contaminated soil, low level:  $14.9 \pm 1.2 \mu\text{g g}^{-1}$ ) and SRM 2701 (soil  
195 containing chromite ore processing residue, high level:  $551.2 \pm 34.5 \mu\text{g g}^{-1}$ ), purchased from NIST  
196 (National Institute of Standards and Technology, USA), were analysed for quality control purposes.  
197 The quantitative extraction of Cr(VI) from both reference materials required a two-cycle of  
198 microwave-assisted extraction at  $90^\circ\text{C}$  for 5 min, as reported Fabregat-Cabello et al. (2012).  
199 Unfortunately, these latter microwave-assisted extraction conditions were found not applicable to  
200 real soil samples from a contaminated area of northern Italy, because they caused the complete  
201 reduction of Cr(VI) (Guidotti et al., 2015). Thus, as assessed by Guidotti et al. (2015), in real soils  
202 the milder microwave-assisted extraction at  $80^\circ\text{C}$  for 5 min represents the best compromise  
203 solution to assure both accurate Cr(VI) quantification and minimisation of the reduction of the  
204 Cr(VI) during extraction. Accordingly, we applied in this work the extraction conditions proposed  
205 by Guidotti et al. (2015) to avoid Cr(VI) reduction in our soil samples, since they were set-up by the  
206 same lab equipments and chemicals employed in this work.

207

## 208 **2.5 Sample preparation procedures for the analysis of the soil samples**

209 The single-spike IDMS procedure requires the addition of known amount of isotopically-labelled  
210 Cr(VI) to the samples to reach isotopic equilibration (Guidotti et al., 2015). A total of 0.10 g of the  
211 soil samples was directly weighed into a 10-mL microwave glass vessel. Then, 5 mL of  $50 \mu\text{mol}$   
212  $\text{mL}^{-1}$   $\text{Na}_2\text{-EDTA}$  (pH 10.0) was added, and the sample was lately spiked with 0.10 g of a 3.4 or 34  
213  $\mu\text{g g}^{-1}$   $^{50}\text{Cr}$ -enriched Cr(VI) spike solution [according to the expected natural Cr(VI) concentration  
214 in the soil sample]. The amounts of sample and  $^{50}\text{Cr}$ -enriched Cr(VI) solution were accurately

215 weighted by an analytical balance with a precision of 0.01 mg. The ratio of concentrations between  
216 natural Cr(VI) in soil sample and  $^{50}\text{Cr}$ -enriched Cr(VI) always ranged from 0.1 to 10 to ensure  
217 accurate and precise results (Fabregat-Cabello et al., 2012).

218 The focused microwave assisted extraction was carried out at 80 °C for 5 min. After extraction, the  
219 samples were centrifuged at 5000 rpm for 5 min. An aliquot of 1 mL of the extract was diluted 1:10  
220 with ultrapure water. Finally 100  $\mu\text{L}$  of the diluted extract were injected into the LC-ICP-MS  
221 system. The use of a 1:10 dilution of the  $\text{Na}_2\text{-EDTA}$  extracting solution as chromatographic mobile  
222 phase avoided the need for neutralisation or acidification of the sample. Also, the centrifugation  
223 step avoided the need of sample filtering allowing a quicker sample preparation and a lower risk of  
224 contamination and/or interconversion of the Cr species (Fabregat-Cabello et al., 2012; Guidotti et  
225 al., 2015).

226 A pre-treatment with n-hexane was applied in the analysis of 7 Cr-most-contaminated soil samples  
227 from case study A. Briefly, 0.1 g of each soil sample were pre-treated with 5 mL of n-hexane and an  
228 mechanically shaken for 15 min in an horizontal shaker at 150 rpm. Then the sample was  
229 centrifuged at 5000 rpm for 5 min. Finally the hexane was removed from the soil sample before  
230 microwave-assisted extraction (at 80 °C for 5 min).

231 The reduction (%) of the  $^{50}\text{Cr}$ -enriched Cr(VI) spike after microwave-assisted extraction was even  
232 calculated by IDMS, analysing Cr(III)-EDTA<sup>-</sup> and Cr(VI) peak areas by linear multiple regression,  
233 similarly to the approach followed by Pinel-Raffaitin et al. (2007), which considered the signals  
234 (peak areas) obtained for each tin isotope as a linear combination of the different contributing  
235 sources. The uncertainty on the amount of Cr(VI) extracted by each sample was calculated by the  
236 Kragten spreadsheet approach.

237

## 238 **2.6. Statistical analyses**

239 Box-plots, descriptive statistics and Pearson's correlation analysis (2-tails) were performed by the  
240 software IBM SPSS Statistics 20 (Chicago, IL, USA). Statistical differences were assumed at  $p <$   
241 0.05.

242

### 243 **3. RESULTS AND DISCUSSION**

#### 244 **3.1. Cr(VI) quantification in soil samples from case study A**

##### 245 *3.1.1. Preliminary investigations*

246 The potential of the soil from case study A to oxidise Cr(III) to Cr(VI), assessed on 10 samples by  
247 the standard Cr net oxidation test (Bartlett and James, 1996), was found to be very low (max: 0.4  
248  $\mu\text{mol}$  per 2 g of tested soil). According to the test guidelines and Italian regulations on the  
249 application of sewage sludge, a soil can be considered at risk of Cr(VI) formation when the amount  
250 of the formed-Cr(VI) obtained by the standard Cr net oxidation test is higher than 1  $\mu\text{mol}$  per 2 g of  
251 tested soil. The oxidation of Cr(III) to Cr(VI) in a soil can be essentially attributed to the presence  
252 of easily reducible manganese oxides (Palmer and Puls, 1994). The number of potentially  
253 oxidisable moles of Cr(III) by the manganese oxides present in the soil was calculated and ranged  
254 from 13 to 31  $\mu\text{g g}^{-1}$ . However, it should be stated that these calculated values might not represent  
255 the real condition in the soil, since the reduced manganese oxides can be re-oxidised and therefore  
256 become new reducing substrates. Also the new formed-Cr(VI) can be reduced by other soil  
257 components (Bartlett, 1991). In any case, some authors find a significant correlation in soil between  
258 the amounts of easily reducible manganese oxides and the soil capacity to oxidise Cr(III) (Chon et  
259 al., 2008). In our soil samples we did not observe this correlation (data not shown).

260 USEPA 3060A and 7196A methods are widely used for the determination of Cr(VI) in soil samples  
261 (Weibel et al., 2016), however, they were not able to provide reliable Cr(VI) concentrations in soil  
262 samples from case study A. In fact, the recovery of the Cr(VI) spike added to the soil was low  
263 (about 50 %) (Table 2), even when the Cr(VI) spike was added to the extractant solution during the  
264 procedure and not to the soil (data not shown). This was probably due to the reduction of Cr(VI) to

265 Cr(III) induced by the organic matter at the moment of extract acidification. The modification of the  
266 official procedure by cold extraction or by the purification of the extract from organic matter by  
267 means of aluminium sulphate led to an improvement in the recovery of the Cr(VI) spike in the  
268 samples previously analysed with the official procedure, but not always reach satisfactory results  
269 and this was especially true when the organic matter content of the samples increases (Table 2).  
270 Also, when applying cold extraction the risk of an incomplete Cr(VI) extraction from the soil  
271 increases. Even when USEPA 7199 was applied after USEPA 3060A extraction the recovery of the  
272 spike was unsatisfactory in most cases (spike reduction between 28 % and 96 %) (Table 2). The  
273 extent of spike reduction appeared higher when the soil organic matter content increased,  
274 reinforcing the hypothesis that organic compounds of the soil from case study A were the main  
275 electron donors readily reducing Cr(VI) to Cr(III). However, despite the underestimation of the real  
276 levels of Cr(VI) in the soils of case study A, concentrations of Cr(VI) between 4 and 14  $\mu\text{g g}^{-1}$  were  
277 found in the most contaminated samples. These values were higher than the legal threshold of 2  $\mu\text{g g}^{-1}$   
278 set by Italian Parliament for residential/urban soil (Italian-Parliament, 2006).

279

### 280 3.1.2. Cr(VI) quantification by single-spike IDMS

281 According to the preliminary results obtained using the USEPA methods, an alternative method  
282 based on IDMS was tested in the soil samples from case study A. Table 3 shows the concentration  
283 of Cr(VI) ( $\mu\text{g g}^{-1}$ ) obtained by species-specific single-spiking IDMS. The 27 analysed soil samples  
284 were classified in two different groups. The first group includes 20 soil samples (A1-A20), in which  
285 Cr(VI) quantification by IDMS was expected to be accurate due of the lower levels of spike  
286 reduction and did not require any soil pre-treatment. The second group comprises 7 soil samples  
287 (A21-A27), mostly from pedological profiles and trenches most-contaminated by pseudo-total Cr,  
288 in which Cr(VI) quantification by IDMS was only possible after a soil pre-treatment with n-hexane  
289 to remove reducing organic compounds.

290 In Italy soil quality standards for agricultural areas have not yet been established but the Italian  
291 Parliament has established screening values to assess potential contamination of residential/urban  
292 soils (Italian-Parliament, 2006). The amount of Cr(VI) extracted from the first group of samples  
293 (A1-A20) ranged from 0.15 to 11.18  $\mu\text{g g}^{-1}$  (median 2.98  $\mu\text{g g}^{-1}$ ), with 70 % of samples exceeding  
294 the Cr(VI) screening value (2  $\mu\text{g g}^{-1}$ ) and 85 % the pseudo-total Cr screening value (150  $\mu\text{g g}^{-1}$ ) set  
295 by Italian Parliament for residential/urban soil (Italian-Parliament, 2006) with concentrations up to  
296 6,831  $\mu\text{g g}^{-1}$ . Although the neutral to moderate-alkaline pH (7.4-8.0) and the sandy-loam texture of  
297 the soil may favour the occurrence of Cr(VI) in the soil, the medium to high content in organic  
298 matter (13-56  $\text{mg g}^{-1}$ ; Table 3) strongly promotes the reduction of the mobile Cr(VI) to the more  
299 thermodynamically stable and insoluble Cr(III). Part of this organic matter consists of heavy  
300 hydrocarbons (C>12), likely derived from leather-based industrial wastes illegally buried into the  
301 soil. In fact, the 80 % of the A1-A20 samples are potentially contaminated by heavy hydrocarbons  
302 (C>12) with concentrations exceeding the C>12 screening value (50  $\mu\text{g g}^{-1}$ ) set by Italian  
303 Parliament for residential/urban soil (Italian-Parliament, 2006). The Cr(VI) extracted from the A1-  
304 A20 soil samples is present in concentrations higher than 2  $\mu\text{g g}^{-1}$  and positively correlated with  
305 their own pseudo-total Cr content ( $r=0.828$ ; Table 4). However, Cr(VI) represents less than 1 % of  
306 the pseudo-total Cr in each of A1-A20 samples (Table 3 and Figure 1). Therefore, assuming a  
307 negligible occurrence of Cr(0), pseudo-total Cr in the soil is predominantly or almost exclusively  
308 present as Cr(III), namely the less toxic and immobile Cr species. Similar results were also  
309 observed by Moreira et al. (2018), who proved that Cr(VI) was always less than 0.2 % (0.7-2.0  $\mu\text{g}$   
310  $\text{g}^{-1}$ ) in contaminated soils of Amazon forest containing up to 2,346  $\mu\text{g}$  of pseudo-total Cr per g of  
311 soil. During microwave-assisted extraction, both organic matter [acting as an electron donor (Tian  
312 et al., 2010)] and, even more, heavy hydrocarbons (C>12) resulted to be powerful reducing agents  
313 of  $^{50}\text{Cr}$ -enriched Cr(VI) spike. The reduction of the  $^{50}\text{Cr}$ -enriched Cr(VI) spike occurred during  
314 extraction (Table 3, A1-A20), was basically moderate and significantly correlated to organic matter  
315 ( $r=0.605$ ; Table 4) and heavy hydrocarbons ( $r=0.780$ ; Table 4). In any case, Cr(VI) reduction is

316 corrected by the isotope dilution methodology applied. The addition of EDTA to the extractant  
317 solution enabled the formation of a stable complex - with the two most important cationic  
318 contaminants of the case study A [Cr(III) and Zn(II)]. Indeed, the formation of the Cr(III)-EDTA<sup>-</sup>  
319 was evident in all chromatograms acquired during the LC-ICP-MS running. Figures SM1 and SM2  
320 in the Supplementary Material also shows that EDTA forms a stable complex with Fe(III) detected  
321 at m/z 54 but its interference with <sup>54</sup>Cr is chromatographically resolved. Zinc complexation by  
322 EDTA was also desirable to correctly quantify Cr(VI) in these soils, since it forms chromates  
323 relatively insoluble in water (Saha et al., 2011). Differences in relative abundance of Cr(VI) (% of  
324 the pseudo-total content) in A1-A20 soil samples - collected at various depths (d1, d2 and d3) and  
325 from pedological profiles and trenches (pt, depth ranging from 30 to 180 cm) - have been found  
326 (Figure 1). The most oxidised soil layers [0-20 cm (d1), 30-60 cm (d2)] showed wider and greater  
327 boxes and upper whiskers in comparison to the deeper and less oxidised soil layer [70-90 cm (d3)].  
328 This indicates a higher Cr(VI) content in the more aerated soil horizons, probably due to a greater  
329 probability of re-oxidation of manganese oxides involved in the chromium cycle in the soil  
330 (Bartlett, 1991). As observed in Figure 1, the box of Cr(VI) (% of the pseudo-total content) of the  
331 samples collected from pedological profiles and trenches (pt; A1, A5-A7, A13, A20), although  
332 shows the highest median, is narrower and lower than d1 and d2 boxes, probably because the  
333 abundance of organic matter in these samples strongly promotes the reduction of Cr(VI) to Cr(III),  
334 thus minimising the presence of the toxic and mobile anion in the soil (Antoniadis et al., 2017;  
335 Kalcikova et al., 2016; Xiao et al., 2013). Indeed, Zhang et al. (2017) stated that humic substances  
336 can limit the mobility of Cr(VI) in the soil by complexation-coupled reduction mechanisms. On the  
337 other hand, Wu et al. (2017) demonstrated that humic substances from cattle manure reduced  
338 Cr(VI) in the soil, then the produced Cr(III) formed outer sphere complexes with -OH and inner  
339 sphere complex with carboxyl groups of humic acids.

340 In contrast to the A1-A20 soil samples, the quantification of Cr(VI) by IDMS in the second group  
341 of soil samples (A21-A27) was done after a soil pre-treatment with n-hexane, which removed part

342 of the abundant Cr(VI)-reducing organic compounds (see Table 3). These samples were mainly  
343 collected from pedological profiles and trenches and were most-contaminated by pseudo-total Cr  
344 ( $1,314\text{--}10,046\ \mu\text{g g}^{-1}$ ). The C<sub>>12</sub> content in these samples ranged from 509 to  $2,150\ \mu\text{g g}^{-1}$  and they  
345 were characterised by a high content in organic matter ( $47\text{--}287\ \text{mg g}^{-1}$ ). The complexity and  
346 abundance of organic compounds in these samples caused the complete reduction ( $\sim 99\%$ ) of the  
347  $^{50}\text{Cr}$ -enriched Cr(VI) spike during the microwave-assisted extraction (at  $80\ ^\circ\text{C}$  for 5 min). Our first  
348 attempts to stabilise the organic compounds, by adding 0.5 g of Alumina, Silica gel or C18 bulk  
349 resin or lowering the extraction time (from 5 to 2.5 min) and temperature (30, 40, 50, 60 or  $70\ ^\circ\text{C}$ ),  
350 did not provide any improvement. For instance, increasing amounts of Cr(VI) were extracted from  
351 soil sample A21 up to  $70\ ^\circ\text{C}$ , but contextually at  $70\ ^\circ\text{C}$  the overall reduction of the  $^{50}\text{Cr}$ -enriched  
352 Cr(VI) spike raised to  $\sim 92\%$  (Figure SM3, Supplementary Material). A soil pre-treatment before  
353 the microwave-assisted extraction (at  $80\ ^\circ\text{C}$  for 5 min) with n-hexane aiming to partially remove  
354 heavy hydrocarbons from soil, namely the most powerful Cr(VI) reducing agents, allowed us to  
355 quantify the Cr(VI) in the Cr-most-contaminated soil samples A21-A27 (Table 3). Even though the  
356 reduction of the  $^{50}\text{Cr}$ -enriched Cr(VI) spike was on average  $98.4\%$ , Cr(VI) could be quantified in  
357 these samples with low uncertainties ( $0.1\text{--}17\%$  of the mean value, except A24). Although the  
358 relative abundance of Cr(VI) (% of the pseudo-total content) is always lower than  $2\%$ , the Cr(VI)  
359 extracted by these 7 soil samples is very high, ranging from  $22.0$  to  $107.1\ \mu\text{g g}^{-1}$  (median  $40.7\ \mu\text{g g}^{-1}$ ;  
360 Table 3, A21-A27), with  $100\%$  of samples exceeding the Cr(VI) screening value of  $2\ \mu\text{g g}^{-1}$  set  
361 by Italian legislation for residential/urban soil (Italian-Parliament, 2006).

362

### 363 **3.2. Cr(VI) quantification in soil samples from case study B**

364 The concentration ( $\mu\text{g g}^{-1}$ ) of Cr(VI) in the 12 soil samples from case study B, quantified by single-  
365 spike IDMS, is given in Table 5. The Cr(VI) amount extracted from these samples ranges from  $0.70$   
366 to  $5.79\ \mu\text{g g}^{-1}$  (median  $1.81\ \mu\text{g g}^{-1}$ ), with  $42\%$  of samples exceeding the Cr(VI) screening value of  $2$   
367  $\mu\text{g g}^{-1}$  set by Italian legislation for residential/urban soil (Italian-Parliament, 2006). This is the first

368 evidence of the Cr(VI) occurrence in these widely investigated soils. Table 6 shows that the amount  
369 of Cr(VI) significantly increases with pseudo-total Cr content ( $r=0.984$ ). However, it represents less  
370 than 1 % of the pseudo-total Cr (Table 5). Therefore, in case study B, the pseudo-total Cr is almost  
371 exclusively present as Cr(III), namely the less toxic and immobile Cr species. The pseudo-total Cr  
372 determined in 100 % of the samples of case study B exceeds the screening value ( $150 \mu\text{g g}^{-1}$ ) set by  
373 Italian Parliament for residential/urban soil (Italian-Parliament, 2006) (Table 5), with concentrations  
374 up to  $594 \mu\text{g g}^{-1}$ . According to Adamo et al. (2003), soils of Solofrana valley are neutral (soil pH  
375 around 7.0) and fine to medium textured sandy-silty or clay-sandy soils with 50-60 % of the soil in  
376 the clay-silt fraction. These soils are also characterised by a high content in organic matter,  
377 conceivably raised by the periodic depositions of the river sediments. Indeed the 12 analysed soils  
378 show an organic matter content ranging from 70 to  $83 \text{ mg g}^{-1}$  (median  $75 \text{ mg g}^{-1}$ ; Table 5),  
379 particularly high for Mediterranean soils. The presence of this considerable pool of organic matter,  
380 acting as Cr(VI)-reducing agent (Barajas-Aceves et al., 2007; Jardine et al., 1999), justifies the low  
381 relative abundance of Cr(VI) (<1 % of the pseudo-total content; Table 5). The low occurrence of  
382 Cr(VI) (at least as % the pseudo-total content) is also in part due the low potential of these soils to  
383 oxidise Cr(III) to Cr(VI), as assessed by Adamo et al. (2003) through the standard Cr net oxidation  
384 test proposed by Bartlett and James (1996). Extracting these soils by BCR (Community Bureau of  
385 Reference, European Commission) sequential extraction procedure (Ure et al., 1993), Adamo et al.  
386 (2003; 2006) also demonstrated that the majority of the pseudo-total Cr is associated to the organic  
387 substances, which easily complex and stabilise the Cr(III) in the soil (Gustafsson et al., 2014).  
388 The high organic matter content in the analysed soils (Table 5) caused a significant reduction of the  
389  $^{50}\text{Cr}$ -enriched Cr(VI) spike during the microwave-assisted extraction. The reduction factor obtained  
390 in the analysis of these set of samples ranged from 19 to 46 %. Nevertheless the quantification of  
391 Cr(VI) by IDMS was characterised by a low uncertainty in all the samples (Table 5). In contrast to  
392 case study A,  $^{50}\text{Cr}$ -enriched Cr(VI) spike reduction during extraction was not significantly  
393 correlated with organic matter content in soil samples ( $r=0.507$ ; Table 6). This means that, instead

394 of the organic matter content,  $^{50}\text{Cr}$ -enriched Cr(VI) reduction was affected by the complexity, the  
395 heterogeneity and the quality of the organic compounds in the soil samples.

396

#### 397 **4. Conclusions**

398 Chromium speciation in soil is of paramount importance to assess the environmental and human  
399 health risks of potentially contaminated sites and to select and apply appropriate management and  
400 remediation strategies. This study investigated the extent of Cr(VI) occurrence in two Italian  
401 agricultural soils potentially contaminated by tannery-deriving Cr. The application of IDMS as  
402 calibration technique ensured the accurate Cr(VI) quantification in soil samples. The use of  
403 EDTA at pH 10 as extractant avoids Cr(III) oxidation and enables the separation of Cr species by  
404 anion exchange chromatography. In both case studies and in all of the analysed samples, it was  
405 found that the presence of medium-high level of organic matter in soil keeps the relative abundance  
406 (% of the pseudo-total content) of Cr(VI), below 2 %. However, in most samples the amount of  
407 Cr(VI) was found to be above the Italian screening value of  $2\ \mu\text{g g}^{-1}$ , suggesting that the toxic and  
408 carcinogenic anion may represent a potential risk for the surrounding environment and for human  
409 health. This is particularly important for case study B, as it is the first evidence of Cr(VI)  
410 occurrence in soils of Solofrana valley irrigated or flooded by river waters.

411 The chemical complexity of the most contaminated soil samples from case study A, a farmland  
412 potentially contaminated by pseudo-total Cr and Zn and heavy hydrocarbons, made the  
413 quantification of Cr(VI) very challenging for all the available analytical techniques; the similar  
414 critical issues in the extraction and quantification of Cr(VI) faced by classical USEPA 7196A and  
415 7199 methods and innovative IDMS method probably indicate a redox potential of these soil  
416 samples that does not allow a high occurrence of Cr(VI) in the soil. Further investigations are  
417 probably necessary considering a direct quantification of the Cr(VI) in the solid soil matrix of the  
418 Cr-most-contaminated samples by X-ray absorption near edge structure spectroscopy (XANES).

419 Lately, the findings of this study suggest that the maintenance of a medium-high content of organic  
420 matter in soil, promoting Cr(VI) reduction and lowering its bioavailability, along with no-tillage  
421 (i.e., no cyclic soil aeration) and possible supply of elemental sulphur [a Cr(VI)-reducing agent in  
422 soils impacted by leather tanneries (Shi et al., 2016)], might be appropriate actions to minimise the  
423 Cr(VI) occurrence in potentially contaminated soils as those investigated in this paper. The  
424 monitoring over time of the fate and stability of Cr(VI) in soil is also essential to manage and  
425 minimise the risks for environment and human health.

426

#### 427 **Acknowledgements**

428 Authors acknowledge the LIFE11/ENV/IT/275 EcoRemed project (2012-2017): *Implementation of*  
429 *eco-compatible protocols for agricultural soil remediation in Litorale Domizio Agro Aversano*  
430 *NIPS* and the After-LIFE Italian project on the application of EcoRemed protocol to assess and  
431 remediate soil contamination at case study A. Further, Antonio G. Caporale acknowledges  
432 University of Naples Federico II for allowing him the stay at the Department of Physical and  
433 Analytical Chemistry of the University of Oviedo (Spain).

434

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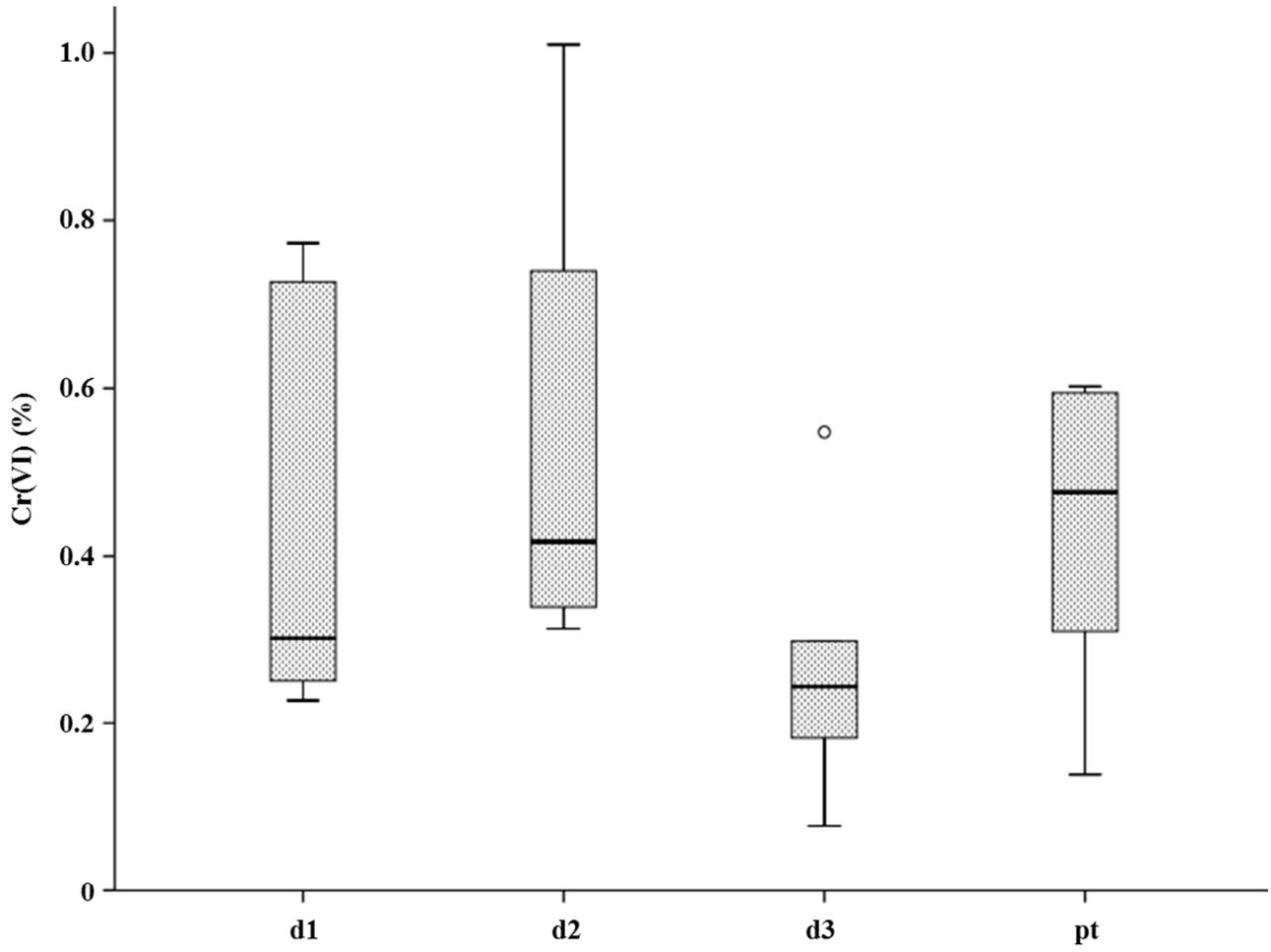
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Figure 1.



**Table 1.** Isotopic composition (% abundance) and concentration of the  $^{50}\text{Cr}$ -enriched Cr(VI) spike solution employed in this work.

Isotope	Abundance (%)
50	$96.10 \pm 0.23$
52	$3.82 \pm 0.19$
53	$0.08 \pm 0.04$
54	Not detected
<b>Cr(VI) concentration (<math>\mu\text{g g}^{-1}</math>)</b>	
$134.83 \pm 3.44$	

**Table 2.** Preliminary Cr(VI) quantification in selected soil samples from case study A by USEPA 3060A extraction and colorimetric-based USEPA 7196A and USEPA 7199 methods.

Sample ID	Extraction/determination methods							
	USEPA 3060A/7196A		USEPA 3060A [Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> purification]/7196A		USEPA 3060A (cold extraction)/7196A		USEPA 3060A/7199	
	Cr(VI) ( $\mu\text{g g}^{-1}$ )	Spike reduction (%)	Cr(VI) ( $\mu\text{g g}^{-1}$ )	Spike reduction (%)	Cr(VI) ( $\mu\text{g g}^{-1}$ )	Spike reduction (%)	Cr(VI) ( $\mu\text{g g}^{-1}$ )	Spike reduction (%)
A2 (d2)	1.7 $\pm$ 0.1	46 $\pm$ 1	1.0 $\pm$ 0.1	24 $\pm$ 1	0.5 $\pm$ 0.1	10 $\pm$ 1	---	---
A13 (pt)	---	---	---	---	---	---	10.5 $\pm$ 0.4	83 $\pm$ 3
A16 (d3)	4.0 $\pm$ 0.2	52 $\pm$ 2	4.9 $\pm$ 0.2	44 $\pm$ 2	2.2 $\pm$ 0.1	21 $\pm$ 1	7.1 $\pm$ 0.3	28 $\pm$ 1
A18 (d3)	---	---	---	---	---	---	8.5 $\pm$ 0.1	42 $\pm$ 1
A20 (pt)	---	---	---	---	---	---	13.9 $\pm$ 0.6	88 $\pm$ 3
A27 (pt)	---	---	---	---	---	---	8.9 $\pm$ 0.1	96 $\pm$ 2
A28 (d2)*	---	---	---	---	3.2 $\pm$ 0.2	62 $\pm$ 2	5.2 $\pm$ 0.2	89 $\pm$ 4

\* Pseudo-total Cr content = 1458  $\pm$  30  $\mu\text{g g}^{-1}$ , organic matter content = 49  $\pm$  3  $\text{mg g}^{-1}$ . Pseudo-total Cr and organic matter contents of the A2, A13, A16, A18, A20 and A27 soil samples are shown in Table 3.

--- Analysis not performed

d2 and d3 indicate soil samples collected at 30-60 cm and 70-90 cm of depth, respectively; pt indicates soil samples collected from pedological profiles and trenches opened in the field.

**Table 3.** Pseudo-total ( $\mu\text{g g}^{-1}$ ) and hexavalent ( $\mu\text{g g}^{-1}$ ; % of the pseudo-total) Cr, organic matter ( $\text{mg g}^{-1}$ ) and heavy hydrocarbons (HC C>12,  $\mu\text{g g}^{-1}$ ) contents in soil samples from case study A. Samples A21-A27 were pre-treated with n-hexane, respectively, before microwave-assisted extraction. The reduction (%) of the  $^{50}\text{Cr(VI)}$ -enriched spike after microwave-assisted extraction is shown as well.

Sample ID	Pseudo-total Cr	Cr(VI)		Spike reduction	Organic matter	HC C>12
	( $\mu\text{g g}^{-1}$ )	( $\mu\text{g g}^{-1}$ )	(% of tot)	(%)	( $\text{mg g}^{-1}$ )	( $\mu\text{g g}^{-1}$ )
A1 (pt)	48 ± 1	0.15 ± 0.10	0.31 ± 0.22	1.6 ± 0.5	13 ± 1	20 ± 5
A2 (d2)	103 ± 4	1.04 ± 0.26	1.01 ± 0.26	2.1 ± 0.2	14 ± 1	19 ± 7
A3 (d1)	136 ± 3	0.99 ± 0.11	0.73 ± 0.08	50 ± 17	21 ± 1	27 ± 8
A4 (d1)	<u>298</u> ± 8	<u>2.30</u> ± 0.27	0.77 ± 0.09	10 ± 1	19 ± 2	45 ± 9
A5 (pt)	<u>331</u> ± 8	1.97 ± 0.10	0.59 ± 0.03	20 ± 2	54 ± 3	<u>68</u> ± 11
A6 (pt)	<u>426</u> ± 9	<u>2.36</u> ± 0.27	0.55 ± 0.06	17 ± 3	36 ± 2	<u>73</u> ± 10
A7 (pt)	<u>454</u> ± 11	<u>2.73</u> ± 0.10	0.60 ± 0.02	16 ± 3	34 ± 2	<u>143</u> ± 23
A8 (d1)	<u>546</u> ± 13	1.65 ± 0.34	0.30 ± 0.06	8.6 ± 0.8	26 ± 1	<u>69</u> ± 12
A9 (d2)	<u>955</u> ± 24	<u>3.48</u> ± 0.11	0.36 ± 0.01	23 ± 14	23 ± 1	<u>200</u> ± 29
A10 (d3)	<u>960</u> ± 22	<u>5.25</u> ± 0.34	0.55 ± 0.04	44 ± 8	43 ± 3	<u>307</u> ± 44
A11 (d1)	<u>1096</u> ± 27	<u>2.74</u> ± 0.10	0.25 ± 0.01	11 ± 1	42 ± 2	<u>59</u> ± 11
A12 (d2)	<u>1161</u> ± 29	<u>5.45</u> ± 0.36	0.47 ± 0.03	2.8 ± 0.2	26 ± 1	<u>106</u> ± 16
A13 (pt)	<u>1230</u> ± 25	<u>4.87</u> ± 0.12	0.40 ± 0.01	82 ± 2	55 ± 3	<u>680</u> ± 81
A14 (d1)	<u>1413</u> ± 34	<u>3.21</u> ± 0.11	0.23 ± 0.01	11 ± 2	34 ± 2	<u>79</u> ± 13
A15 (d3)	<u>1713</u> ± 45	1.31 ± 0.25	0.08 ± 0.01	17.9 ± 0.5	22 ± 1	<u>60</u> ± 11
A16 (d3)	<u>2372</u> ± 57	<u>7.08</u> ± 0.11	0.30 ± 0.01	13 ± 2	20 ± 1	<u>93</u> ± 15
A17 (d3)	<u>2802</u> ± 73	<u>6.81</u> ± 0.62	0.24 ± 0.02	7 ± 1	34 ± 2	<u>218</u> ± 32
A18 (d3)	<u>3456</u> ± 85	<u>6.28</u> ± 0.12	0.18 ± 0.01	11 ± 6	27 ± 1	<u>162</u> ± 24
A19 (d2)	<u>3572</u> ± 92	<u>11.18</u> ± 0.26	0.31 ± 0.01	6 ± 6	33 ± 2	<u>158</u> ± 24
A20 (pt)	<u>6831</u> ± 158	<u>9.45</u> ± 0.11	0.14 ± 0.01	57 ± 4	56 ± 3	<u>331</u> ± 42
Mean	1495	4.02	0.42	21	32	146
Min	48	0.15	0.08	2	13	19
P25	402	1.89	0.25	8	22	60
Median	1028	2.98	0.34	12	30	86
P75	1878	5.66	0.56	20	38	172
Max	6831	11.18	1.01	82	56	680
IT sc. val. *	<u>150</u>	<u>2</u>	---	---	---	<u>50</u>
Sam>ITs.v.	85%	70%	---	---	---	80%
A21 (d2)	<u>1314</u> ± 31	<u>22.5</u> ± 2.1	1.71 ± 0.16	97.7 ± 2.6	47 ± 3	<u>710</u> ± 98
A22 (d2)	<u>1946</u> ± 49	<u>22.0</u> ± 3.1	1.13 ± 0.16	98.3 ± 3.2	80 ± 5	<u>509</u> ± 72
A23 (d3)	<u>3857</u> ± 97	<u>26.2</u> ± 2.0	0.68 ± 0.05	97.5 ± 2.8	177 ± 9	<u>1240</u> ± 180
A24 (pt)	<u>4162</u> ± 86	<u>61.4</u> ± 33.3	1.47 ± 0.80	98.9 ± 2.1	272 ± 8	<u>960</u> ± 145
A25 (pt)	<u>4756</u> ± 112	<u>40.7</u> ± 0.1	0.86 ± 0.01	98.0 ± 2.5	260 ± 10	<u>1070</u> ± 129
A26 (pt)	<u>5563</u> ± 141	<u>107.1</u> ± 0.1	1.93 ± 0.01	99.2 ± 3.4	287 ± 12	<u>930</u> ± 151
A27 (pt)	<u>10046</u> ± 275	<u>46.7</u> ± 8.1	0.47 ± 0.08	99.5 ± 1.1	198 ± 8	<u>2150</u> ± 259
Mean	4521	46.6	1.18	98.4	189	1081
Min	1314	22.0	0.47	97.5	47	509
P25	2902	24.3	0.77	97.8	129	820
Median	4162	40.7	1.13	98.3	198	960
P75	5160	54.1	1.59	99.1	266	1155
Max	10046	107.1	1.93	99.5	287	2150
IT sc. val. *	<u>150</u>	<u>2</u>	---	---	---	<u>50</u>
Sam>ITs.v.	100%	100%	---	---	---	100%

\* Italian Legislative Decree No. 152 (2006). Attachments to the Part IV, Title V, Table 1, Column A.

Underlined values of pseudo-total and hexavalent Cr and HC C>12 exceed Italian screening values set by L.D. 152/2006.

d1, d2 and d3 indicate soil samples collected at 0-20 cm, 30-60 cm and 70-90 cm of depth, respectively; pt indicates soil samples collected from pedological profiles and trenches opened in the field.

**Table 4.** Values of Pearson's correlation coefficient (r) for pseudo-total ( $\mu\text{g g}^{-1}$ ) and hexavalent ( $\mu\text{g g}^{-1}$ ; % of the pseudo-total) Cr, organic matter ( $\text{mg g}^{-1}$ ) and heavy hydrocarbons (HC C>12,  $\mu\text{g g}^{-1}$ ) contents in 20 soil samples (A1-A20, not pre-treated with n-hexane) from case study A.

Case study A	<b>Pseudo-total Cr</b> ( $\mu\text{g g}^{-1}$ )	<b>Cr(VI)</b> ( $\mu\text{g g}^{-1}$ )	<b>Cr(VI)</b> (% of <b>pseudo-total</b> )	<b>Spike reduction</b> (%)	<b>Organic matter</b> ( $\text{mg g}^{-1}$ )
<b>Cr(VI) (<math>\mu\text{g g}^{-1}</math>)</b>	0.828***				
<b>Cr(VI) (% of pseudo-total)</b>	-0.602**	-0.415			
<b>Spike reduction (%)</b>	0.221	0.160	-0.018		
<b>Organic matter (<math>\text{mg g}^{-1}</math>)</b>	0.385	0.380	-0.224	0.605**	
<b>HC C&gt;12 (<math>\mu\text{g g}^{-1}</math>)</b>	0.363	0.460*	-0.218	0.780***	0.643**

\*\*\*, \*\* and \* indicate statistically significant correlations (2-tails) at  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$ , respectively

**Table 5.** Pseudo-total ( $\mu\text{g g}^{-1}$ ) and hexavalent ( $\mu\text{g g}^{-1}$ ; % of the pseudo-total) Cr and organic matter ( $\text{mg g}^{-1}$ ) contents in soil samples from case study B. The reduction (%) of the  $^{50}\text{Cr(VI)}$ -enriched spike after microwave-assisted extraction is shown as well.

Sample ID	Pseudo-total Cr	Cr(VI)		Spike reduction	Organic matter
	( $\mu\text{g g}^{-1}$ )	( $\mu\text{g g}^{-1}$ )	(% of tot)	(%)	( $\text{mg g}^{-1}$ )
B1	<u>209</u> $\pm$ 4	0.72 $\pm$ 0.02	0.34 $\pm$ 0.01	40.1 $\pm$ 0.6	73 $\pm$ 3
B2	<u>212</u> $\pm$ 5	0.70 $\pm$ 0.02	0.33 $\pm$ 0.01	46.0 $\pm$ 0.6	83 $\pm$ 5
B3	<u>213</u> $\pm$ 4	0.75 $\pm$ 0.01	0.35 $\pm$ 0.01	41.1 $\pm$ 0.1	76 $\pm$ 4
B4	<u>229</u> $\pm$ 6	1.61 $\pm$ 0.02	0.70 $\pm$ 0.02	38.9 $\pm$ 0.5	75 $\pm$ 4
B5	<u>243</u> $\pm$ 6	1.59 $\pm$ 0.01	0.66 $\pm$ 0.01	41.8 $\pm$ 0.2	73 $\pm$ 2
B6	<u>244</u> $\pm$ 3	1.62 $\pm$ 0.02	0.67 $\pm$ 0.01	38.2 $\pm$ 0.2	76 $\pm$ 3
B7	<u>282</u> $\pm$ 9	<u>2.25</u> $\pm$ 0.02	0.80 $\pm$ 0.02	19 $\pm$ 1	75 $\pm$ 2
B8	<u>285</u> $\pm$ 7	2.00 $\pm$ 0.02	0.70 $\pm$ 0.01	19.1 $\pm$ 0.8	70 $\pm$ 3
B9	<u>285</u> $\pm$ 6	<u>2.17</u> $\pm$ 0.02	0.76 $\pm$ 0.02	36.7 $\pm$ 0.1	75 $\pm$ 2
B10	<u>543</u> $\pm$ 12	<u>4.99</u> $\pm$ 0.01	0.92 $\pm$ 0.03	27.6 $\pm$ 0.7	75 $\pm$ 3
B11	<u>573</u> $\pm$ 14	<u>5.79</u> $\pm$ 0.02	1.01 $\pm$ 0.02	27 $\pm$ 1	78 $\pm$ 4
B12	<u>594</u> $\pm$ 11	<u>5.11</u> $\pm$ 0.02	0.86 $\pm$ 0.01	24 $\pm$ 3	70 $\pm$ 3
Mean	326	2.44	0.68	33	75
Min	209	0.70	0.33	19	70
P25	225	1.38	0.58	26	73
Median	263	1.81	0.70	37	75
P75	350	2.94	0.81	40	76
Max	594	5.79	1.01	46	83
IT sc. val.*	<u>150</u>	<u>2</u>	---	---	---
Sam>ITs.v.	100%	42%	---	---	---

\* Italian Legislative Decree No. 152 (2006). Attachments to the Part IV, Title V, Table 1, Column A.

Underlined values of pseudo-total and hexavalent Cr exceed the Italian screening values set by L.D. 152/2006.

**Table 6.** Values of Pearson's correlation coefficient (r) for pseudo-total ( $\mu\text{g g}^{-1}$ ) and hexavalent ( $\mu\text{g g}^{-1}$ ; % of the pseudo-total) Cr and organic matter ( $\text{mg g}^{-1}$ ) contents in soil samples from case study B.

Case study B	<b>Pseudo-total Cr</b> ( $\mu\text{g g}^{-1}$ )	Cr(VI) ( $\mu\text{g g}^{-1}$ )	Cr(VI) (% of <b>pseudo-total</b> )	Spike reduction (%)
Cr(VI) ( $\mu\text{g g}^{-1}$ )	0.984***			
Cr(VI) (% of <b>pseudo-total</b> )	0.776**	0.865***		
Spike reduction (%)	-0.579*	-0.616*	-0.696*	
Organic matter ( $\text{mg g}^{-1}$ )	-0.192	-0.179	-0.283	0.507

\*\*\*, \*\* and \* indicate statistically significant correlations (2-tails) at  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$ , respectively

**Figure 1.** Comparison on hexavalent Cr (% of the pseudo-total content) extracted from 20 soil samples (A1-A20, not pre-treated with n-hexane) collected at case study A from: i) sampling grid 20x20 m at 0-20 cm (d1; 5 samples), 30-60 cm (d2; 4 samples) or 70-90 cm (d3; 5 samples) of depth; ii) pedological profiles and trenches (pt, 6 samples: depth ranging from 30 to 180 cm) opened in the field.

**Highlights**

Most of samples from two case studies exceed the legal Italian Cr(VI) screening value

The Cr(VI) is significantly correlated to the pseudo-total Cr content in both soils

The Cr(VI) extracted from both soils is less than 2 % of the pseudo-total Cr content

Both soils A and B show a low potential to oxidise Cr(III) to Cr(VI)

Organic compounds caused a partial reduction of Cr(VI)-spike during extraction phase