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**Linking oral bioaccessibility and solid phase distribution of potentially toxic elements in extractive waste and soil from an abandoned mine site: Case study in Campello Monti, NW Italy**

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(Article begins on next page)

1 **Linking oral bioaccessibility and solid phase distribution of potentially toxic elements in**  
2 **extractive waste and soil from an abandoned mine site: Case study in Campello Monti, NW**  
3 **Italy**

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12  
13 **Highlights**

- 14 • Extractive waste and soil at abandoned mines pose a risk to human health.
- 15 • Cr, Cu and Ni were present in high total concentrations at Campello Monti site.
- 16 • Potentially toxic elements were associated with clay and Fe oxide fractions.
- 17 • Mean BAF <11% for both Cr and Ni. Non-mobile form in Fe oxide fraction.
- 18 • PTE were found to be embedded in mineral grains of soil.

19  
20 **Abstract**

21 Mining activities have led to the introduction of high levels of potentially toxic elements (PTE) in  
22 soils. This has attracted governmental and public attention due to their non-biodegradable nature  
23 and hazards posed to human health and the environment. However, total concentrations of PTE are  
24 poor indicators of actual hazard to human health and can lead to overestimation of risk. In this  
25 study, oral bioaccessibility, the fraction available for absorption via oral ingestion, was used to  
26 refine human health risk assessment at an abandoned mine site from Campello Monti, north-west

Italy. The solid phase distribution was performed to characterise the distribution and the behaviour of PTE within the extractive waste streams and impacted soil nearby. Mineralogical information was obtained from micro-XRF and SEM analysis used to identify elemental distribution maps. The results showed that the total concentrations of PTE were high, up to 7400 mg/kg for Ni due to the presence of parent material, however, only 11% was bioaccessible. Detailed analysis of the bioaccessible fraction (BAF) showed that As, Cu and Ni varied from 7 to 22%, 14 to 47%, 5 to 21%, respectively. The variation can be attributed to the difference in pH, organic matter content and mineralogical composition of the samples. Non-specific sequential extraction also showed that the non-mobile forms of PTE were associated with the clay and Fe oxide components of the environmental matrices. The present study demonstrates how bioaccessibility, solid phase distribution and mineralogical analysis can help decision making and inform the risk assessment of abandoned mine sites.

Keywords: abandoned mine site, oral bioaccessibility, potentially toxic elements (PTE), risk assessment, solid phase distribution.

41

## 42 **1. Introduction**

Since the onset of the industrial revolution, mining and smelting activities have been at the forefront of economic development of many countries. Mining activities generate employment, while also producing a wide variety of minerals that can have countless uses in various contexts (Ono et al., 2016 ; Dino et al., 2018). Yet, mining and dressing activities have resulted in the generation of large quantities of waste and degraded soils. After the closure of mines, these waste dumps were abandoned. Further to this, the degraded soils, waste dumps and tailings are often geotechnically unstable and sources of contamination by PTE (Gál et al., 2007). As PTE tend to persist in the environment, these extractive waste dumps and soils often become a matter of concern for human health (Lim et al., 2009).

52           There is growing awareness and concern about the harmful effects of elevated  
53 concentrations of toxic elements on human health (Golia et al., 2008). However, there is growing  
54 evidence that an elevated concentration of elements may not be indicative of the actual damaging  
55 effects. Consequently, it has been proposed that bioavailable concentrations should be used to  
56 inform human health risk assessment (HHRA). In the context of the present research, the  
57 bioavailable concentration is the concentration of the contaminants reaching to the systemic  
58 circulation (bloodstream) and thus is able to reach all target organ sites (Oomen, 2000). However,  
59 measuring bioavailability in vivo is a difficult and lengthy procedure (Maddaloni et al., 1998).  
60 Therefore, a number of in.vitro bioaccessibility methods have been developed to measure the oral  
61 bioaccessibility of a contaminant (Cox et al., 2013). The oral bioaccessible fraction is defined as the  
62 fraction that, after ingestion, may be mobilized into the gut fluids (chyme). Bioaccessible  
63 concentration is greater than or equal to the bioavailable concentration and can be used as a  
64 conservative measure to the bioavailability for HHRA (Paustenbach, 2000).

65           The in vitro bioaccessibility methods measure the oral bioaccessibility of PTE by mimicking  
66 the stomach and intestine biochemical conditions. Some of the commonly used methods for  
67 measuring bioaccessibility are DIN method (RUB, Germany), In Vitro Digestion model (RIVM,  
68 The Netherlands), Physiologically Based Extraction Test (PBET), SHIME, LabMET/Vito,  
69 Belgium, Simplified Bioaccessibility Extraction Test (SBET) , etc. Difference in the  
70 bioaccessibility method can lead to difference in the value of the bioaccessible concentrations also,  
71 as the contaminant concentration released during a particular method depends on : (1) the pH of the  
72 gastrointestinal compartment, (2) the residence time of the soil in the solution, (3) the pH of the  
73 small intestinal solutions, (4) the ratio of solid to liquid in the gastric solution, (5) different bile  
74 concentrations and the different bile salts. These factors has been explained in detail in (Oomen et  
75 al., 2002). Owing to the different procedures and the results obtained the unified BARGE method  
76 (UBM) was developed by the Bioaccessibility Research Group of Europe (BARGE) for measuring  
77 the oral bioaccessibility of contaminants in order to harmonize the use of oral bioaccessibility in

contaminated soils of Europe The UBM method was used for the oral bioaccessibility evaluation in the present study as : (1) the method has been validated against in vivo studies for As, Cd and Pb (Denys et al., 2012) and (2) Hamilton et al. (2015) used the method to evaluate oral bioaccessibility of BGS 102 reference material and provided guidance data on a wider range of chemical elements making it feasible to control the quality of the data produced as the variations in the results can be checked due to inter-laboratory trials. Many studies have used the UBM method to assess contamination due to PTE in mining affected areas. For example, Pelfrène et al., (2012) quantified bioaccessible concentrations of Cd, Pb and Zn as 78%, 32%, and 58% respectively on smelter-contaminated agricultural soils in a coal mining area of northern France. Foulkes et al., (2017) applied the UBM method to measure bioaccessibility of Pb, Th, and U on solid wastes and soils from an abandoned uranium mine site in South West England. However, in Italy there is little to no attention towards inclusion of oral bioaccessibility in studies reporting HHRA (Kumpiene et al., 2017). Consequently, the present study provides evidence towards evaluating bioaccessibility to support the HHRA procedures for two abandoned mine sites in Italy.

Potentially toxic elements (PTE) are associated with the various components in EW and soils in different ways, and these associations can lead to variation in both mobility and availability (Cipullo et al., 2018). A wide range of EW and soil properties can thus lead to variation in bioaccessibility of PTE such as pH, organic matter content, presence of clay, iron oxides and alumino-silicates (Ruby et al., 1999; Peijnenburg and Jager, 2003; Martin and Ruby, 2004; Basta et al., 2005; Palumbo-Roe and Klinck, 2007; Denys et al., 2009; Reis et al., 2014; Palumbo-Roe et al. 2015). Therefore, determining the solid-phase fractionation in the matrix can be used as additional line of evidence to the in vitro bioaccessibility testing. Sequential selective chemical extractions are widely used for characterizing the distribution of elements in the solid phase (Li et al., 2001). However, a number of limitations are often associated with sequential chemical extraction schemes., most commonly, the difficulty of finding a suitable method for all soil types and elements. Cave et al. (2004) proposed a non-selective method coupled to chemometric analysis

104 that is called chemometric identification of substrates and element distributions (CISED). The main  
105 advantages of CISED method are simplicity of extraction procedure, and the partitioning of the  
106 chemical elements between the different components is not methodologically defined. The CISED  
107 is a useful methodology for understanding the results provided by in vitro bioaccessibility tests  
108 (Palumbo-Roe and Klinck, 2007; Cox et al., 2013).

109 The bioaccessibility of PTE has also been known to be affected by mineral phases present in  
110 matrices of EW and soil. The PTE bioaccessibility in comparison to the total concentrations has  
111 been known to be constrained by mineralogical aspects such as : (1) encapsulation of PTE in the  
112 mineral matrices which leads to changes in PTE-bearing surface area and (2) the association of PTE  
113 with different type of minerals resulting into formation of alteration products. Therefore, in order to  
114 assess the bioaccessibility of PTE and understanding factors influencing bioaccessibility it is  
115 imperative that we study geochemical data and mineral phases present in EW and soil. Considering  
116 the challenges linked with evaluating bioaccessibility and, the present study focuses on extractive  
117 waste (EW) and soils from the abandoned mine site at Campello Monti, which was important for Ni  
118 exploitation from mafic formations in north-west Italy. The study is structured on five main  
119 sections: (1) measuring total concentrations of PTE, (2) assessing bioaccessibility of PTE using  
120 UBM, (3) determining solid phase distribution of elements in different components using CISED,  
121 (4) mineralogical analysis of soil samples and (5) relating bioaccessible concentrations and  
122 mineralogy and geochemistry of EW and soil samples.

123

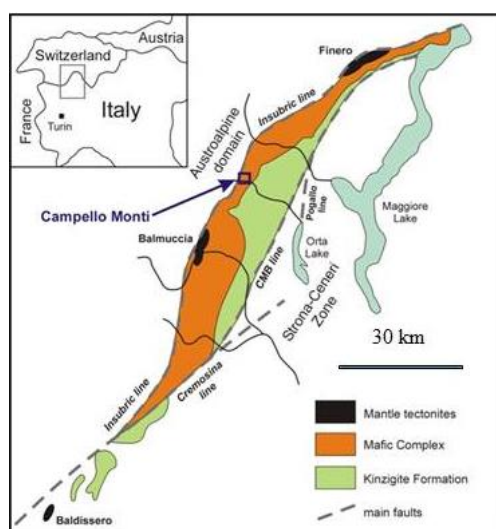
## 124 2. Methodology

### 125 2.1 Site description

126 Campello Monti is a small settlement in the village of Valstrona in the northern sector of Piemonte,  
127 Italy. Geologically, the site (**Figure 1**) is present in the ultramafic layers of the mafic complex of  
128 the Ivrea Verbano Zone. The Ivrea- Verbano zone is a tectonic unit which has preserved the  
129 transition from amphibolite to granulite facies (Redler et al., 2012). The mafic formation consists of

130 a sequence of cumulate peridotites, pyroxenites, gabbros and anorthosites, together with a large,  
 131 relatively homogeneous body of gabbro-norite, grading upwards into gabbro-diorite and diorite.  
 132 The Campello Monti area consists of lherzolites, in places with titanolivin, in large and smaller  
 133 masses.

134 The rocks in this area are rich in nickel, copper and cobalt. The area was exploited for nickel  
 135 production from Fe-Ni-Cu-Co magmatic sulfide deposits occurring from the Sesia to Strona valleys  
 136 from the 19th Century (1865) until the 1940s. The ore was extracted using underground mining  
 137 activities that left waste rocks near the mine tunnels (Mehta et al., 2018).

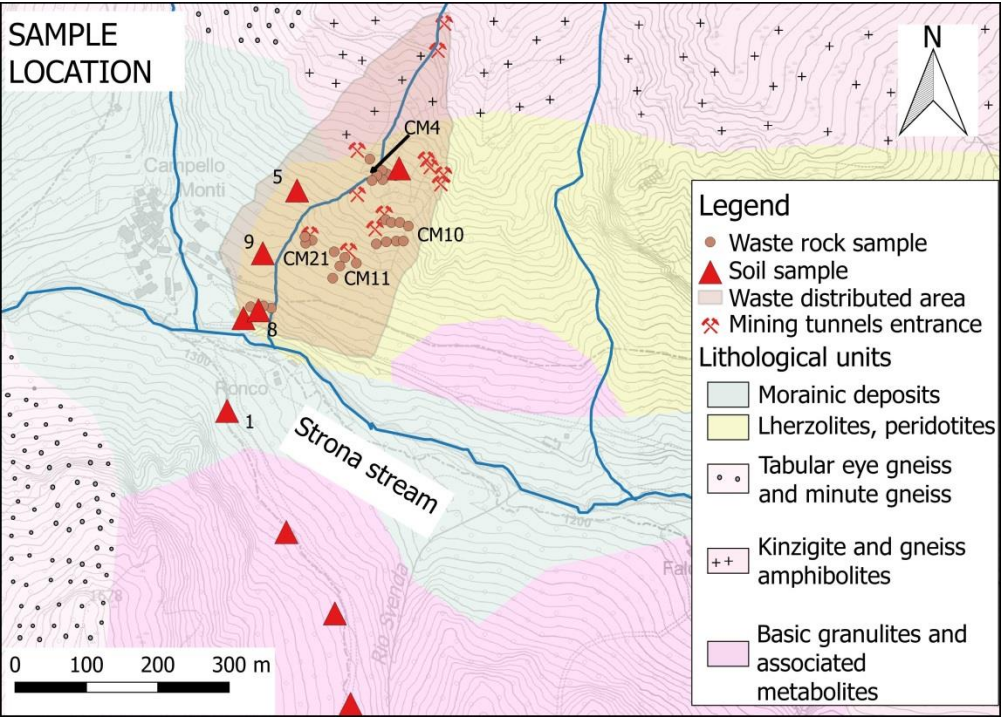


138  
 139 Figure 1. Geological setting of Campello Monti (modified from Fiorentini and Beresford, 2008).

## 140 2.2 Sample collection and preparation

141 A site investigation was performed to collect information about waste typology and location, in  
 142 order to ensure that the facilities are suitable for characterisation and sampling. The sampling site at  
 143 Campello Monti is composed of different waste rock dumps. These waste rock dumps were placed  
 144 on the north of the Strona stream and were formed by the dumping in vertical sequence of non-  
 145 valuable mineralisations and non-mineralised rocks. A systematic sampling strategy was adopted in  
 146 order to obtain representative data of the whole waste facility. Waste rock material was sampled  
 147 using hand shovel and a hammer (where necessary). Each sample (8-10 kg) was collected in an area

148 of 1.5 m<sup>2</sup>, by mixing 4 subsamples present at the vertices of the square and one subsample at the  
 149 centre of the square. after removing organic residues. In total, 26 samples of waste rock were  
 150 collected at the site in July 2016 (**Errore. L'origine riferimento non è stata trovata.**). Additionally, a  
 151 total of 9 soil samples were taken near the waste rock dumps to the north and south of the Strona  
 152 stream during the sampling campaigns in June 2016 and March 2017. In order to obtain  
 153 representative soil samples, the samples taken were formed by mixing 4 subsamples taken at the  
 154 vertices of a 1m x 1m square. All samples were taken at a depth of 0-15 cm. The extractive waste  
 155 samples and soil samples were dried in an oven for a period of 24 h to remove any moisture.  
 156 Samples were then sieved through 2 mm sieves and quartered to obtain a representative sample size  
 157 of 10 g. The pH was measured in a 1: 2.5 suspension of each sample in water (ISO 10390, 2005).



158  
 159 Figure 2. Waste rock and soil sample locations at Campello Monti. Sample numbers are shown for  
 160 the samples analyzed for bioaccessibility.

161  
 162 **2.3 Total concentrations measurement**

163 The samples were analyzed for their concentrations of chemical elements on the 2 mm fraction  
 164 using the method described in U.S. EPA, 3051 A, (2007) and U.S. EPA, 6010 C, (2007). Briefly,



0.5 g of sample was digested using 3 ml concentrated HNO<sub>3</sub> and concentrated HCl (1:3). The concentrations of As, Be, Cd, Co, Cr (total), Cu, Ni, Pb, Sb, Se, V and Zn were measured using an Ametek Spectro Genesis Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). The instrument was provided with an Ametek monochromator, a cyclonic spray chamber and a Teflon Mira Mist nebulizer. The instrumental conditions included a plasma power of 1.3 kW, sample aspiration rate of 30 rpm, argon nebulizer flow of 1 l/min, argon auxiliary flow of 1 l/min and argon plasma flow of 12 l/min. All the reagents used were of analytical grade. All metal solutions were prepared from concentrated stock solutions (Sigma Aldrich). High-purity water (HPW) produced with a Millipore Milli-Q Academic system was used throughout the analytical process. All samples were analyzed in duplicate.

175

#### 2.4 Bioaccessibility analysis (Unified BARGE method)

Following the analysis on total concentration of elements for the fraction under 2 mm, samples were selected for the measurement of bioaccessible concentrations. Waste rock and soil samples were selected to ensure representation of each dump and lithology. For tailings, the two samples closest to the ground surface were measured for bioaccessible concentrations. The total metal concentrations were measured on the <250 µm fraction using aqua regia extractions as described in section 2.3. Following the analysis on total concentration of PTE on the <2 mm fraction, samples of waste rock, soil and tailings were selected for measurement of bioaccessible concentrations, ensuring good representation of each matrix. For tailings, the two samples at the nearest depth from the ground were measured for bioaccessible concentrations. Each sample was sieved to <250 µm and total concentrations of PTE were measured using aqua regia extractions as explained in section 2.3. The Unified BARGE method (UBM) was also followed for measuring bioaccessible concentrations of the <250 µm fraction (BARGE 2010, Denys et al., 2012). To ensure quality control of the extraction process each batch of UBM extractions (n=10) included one procedural blank, six unknowns, one duplicate of two unknown samples and one soil reference material

191 (BGS102) (BARGE 2010; Hamilton et al., 2015). **Table 1** shows the comparison of the certified  
 192 and measured values of the BGS 102 extractions. As pH plays an important role in controlling the  
 193 leaching of the PTE from the matrix and overall extraction process, the pH meter was calibrated  
 194 before extraction of every batch of samples.

195 Unified BARGE method extractions were carried out using simulated digestive fluids  
 196 including saliva, gastric fluid, bile and duodenal fluid, which were prepared from inorganic and  
 197 organic reagents and enzymes one day prior to sample extractions. These fluids were used to  
 198 represent three main compartments of human digestive system: mouth, stomach and small intestine.  
 199 The extraction consists of two phases, gastric and gastro-intestinal for which  $0.4 \pm 0.0005$  g of  
 200 sample was weighed in replicate in polycarbonate tubes (1 replicate for the gastric phase and 1  
 201 replicate for the gastro-intestinal phase). For gastric phase extractions, saliva and gastric fluids were  
 202 added to each tube (pH adjusted to  $1.2 \pm 0.05$ ), followed by 1 h of end-over-end rotation. The  
 203 rotator was placed in an oven at a constant temperature of 37 °C. One of the replicates was  
 204 extracted through centrifugation at 4500 g for 15 min (G phase), while the second replicate was  
 205 retained for gastro-intestinal phase (GI phase) extraction. Simulated duodenal and bile fluids were  
 206 added to this tube (pH adjusted to  $6.3 \pm 0.5$ ) and rotated end-over-end for 4 hours at 37 °C. This  
 207 was followed by an identical centrifugation procedure to obtain GI phase extracts. For both  
 208 extractions, 10 ml of the supernatant was collected and preserved with 0.2 ml concentrated (15.9 M)  
 209 HNO<sub>3</sub>. Determination of PTE was performed by ICP-MS (Perkin-Elmer NexION 350X), while  
 210 using an internal standard (Rh). The bioaccessible fraction (BAF) for both the phases was  
 211 calculated using Equation 1. To apply a conservative approach for human health risk assessment,  
 212 BAF is reported as the percentage of highest bioaccessible concentration from gastric or gastro-  
 213 intestinal phase.

$$214 \quad \text{BAF} = \frac{\text{Concentration of bioaccessible element } (\frac{\text{mg}}{\text{kg}})}{\text{Total concentration of element } (\frac{\text{mg}}{\text{kg}})} \times 100 \quad (1)$$

## 217    **2.5 Chemometric identification of substrates and element distribution (CISED)**

218    A non-specific sequential nitric acid extraction (Cave et al., 2004) was carried out on selected  
219    samples (n=5) (n=2 waste rocks, n=3 soil). Briefly, 2 g of sample was sequentially extracted with  
220    10 ml of deionized water and a solution of increasing concentration of HNO<sub>3</sub> ranging from 0.01 M  
221    to 5.0 M. A total of 7 solutions were used twice (0.0 M, 0.01 M, 0.05 M, 0.1 M, 0.5 M, 1.0 M and  
222    5.0 M), with progressive addition of H<sub>2</sub>O<sub>2</sub> (0.25, 0.50, 0.75, and 1 ml) in the last 4 extracting  
223    solutions to facilitate the precipitation of oxides. Each solution was mixed for 10 min in an end-  
224    over-end shaker and centrifuged (4350 g for 5 min) to separate solid and liquid fractions. The solid  
225    fraction was then resuspended in the following extracting solution. The recovered liquid fraction  
226    was filtered with a 0.45 µm 25 mm nylon syringe filter and diluted 4 times with deionized water  
227    prior to analysis. Extracts were spiked with internal standards (Sc, Ge, Rh, and Bi) and the  
228    following elements Ca, Fe, K, Mg, Mn, Na, S, Si, P, Al, As, Ba, Cd, Co, Cr, Cu, Hg, Li, Mo, Ni,  
229    Pb, Sb, Se, Sr, V, Zn were measured using ICP-MS (NexION® 350D ICP-MS, Perkin Elmer). For  
230    data quality control, acid blanks (1% nitric acid) and certified reference material (BGS102) were  
231    included in the extraction procedure.

232

## 233    **2.6 Modelling**

234    Solid phase distribution of elements in soil and waste rock was calculated with MatLab (MatLab®  
235    Version R2015a) using a self-modelling mixture resolution algorithm (SMMR) developed by Cave  
236    et al. (2004). This modelling algorithm was used to identify (1) soil components with similar  
237    physical-chemical properties, (2) chemical composition data (single elements in each soil  
238    component expressed as percentage), and (3) amount of elements in each component (expressed in  
239    mg/kg). The algorithm was run separately for waste rock and soil producing 7 and 8 distinct sets of  
240    physico-chemical phases for each of these respective runs. In order to categorise these physio-  
241    chemical phases into common distinct soil phases hierarchical clustering was used in combination  
242    with geochemical profile interpretations. Briefly, heatmaps from hierarchical clustering were

243 produced with a mean-centered and scaled matrix of profile and composition data using the Ward's  
244 method in R (v.3.4.1) and the results obtained were plotted with ggplot2, reshape2, grid and  
245 gg dendro packages (Wickham, 2007; Wickham, 2009; Chang et al. 2013).

246

## 247 **2.7 Mineralogical analysis**

248 The mineralogical analysis of waste rock samples was performed in a previous study (Rossetti et  
249 al., 2017). Consequently, only the soil sample was analyzed for mineral phases in the present study.  
250 Micro-X-ray fluorescence (micro-XRF) was used to identify crystalline phases in the bulk soil  
251 sample (sample code - 8). Element X-ray maps of soil samples were acquired using a micro-XRF  
252 Eagle III-XPL spectrometer equipped with an EDS Si(Li) detector and with an EdaxVision32  
253 micro-analytical system. The operating conditions were 2.5  $\mu$ s counting time, 10 kV accelerating  
254 voltage and a probe current of 20  $\mu$ A. The spatial resolution was about 65  $\mu$ m in both x and y  
255 directions. The elemental maps were processed to determine mineral phases in soil using software  
256 program Petromod (Cossio et al., 2002). The micromorphology and associated chemical analysis of  
257 solid phases in soil were analyzed with a Cambridge Stereoscan 360 scanning electron microscope  
258 (SEM) equipped with an energy-dispersive spectrometry (EDS) Energy 200 system and a Pentafet  
259 detector (Oxford Instruments). 10 kV accelerating voltage and 50 s counting time were used for  
260 analysis of the minerals. SEM-EDS quantitative data (spot size 2  $\mu$ m) were acquired and processed  
261 using the Microanalysis Suite Issue 12, INCA Suite version 4.01; natural mineral standards were  
262 used to calibrate the raw data; the  $\phi\rho Z$  correction (Pouchou & Pichoir, 1988) was applied. Absolute  
263 error is 1 $\delta$  for all calculated oxides.

264

## 265 **3. Results**

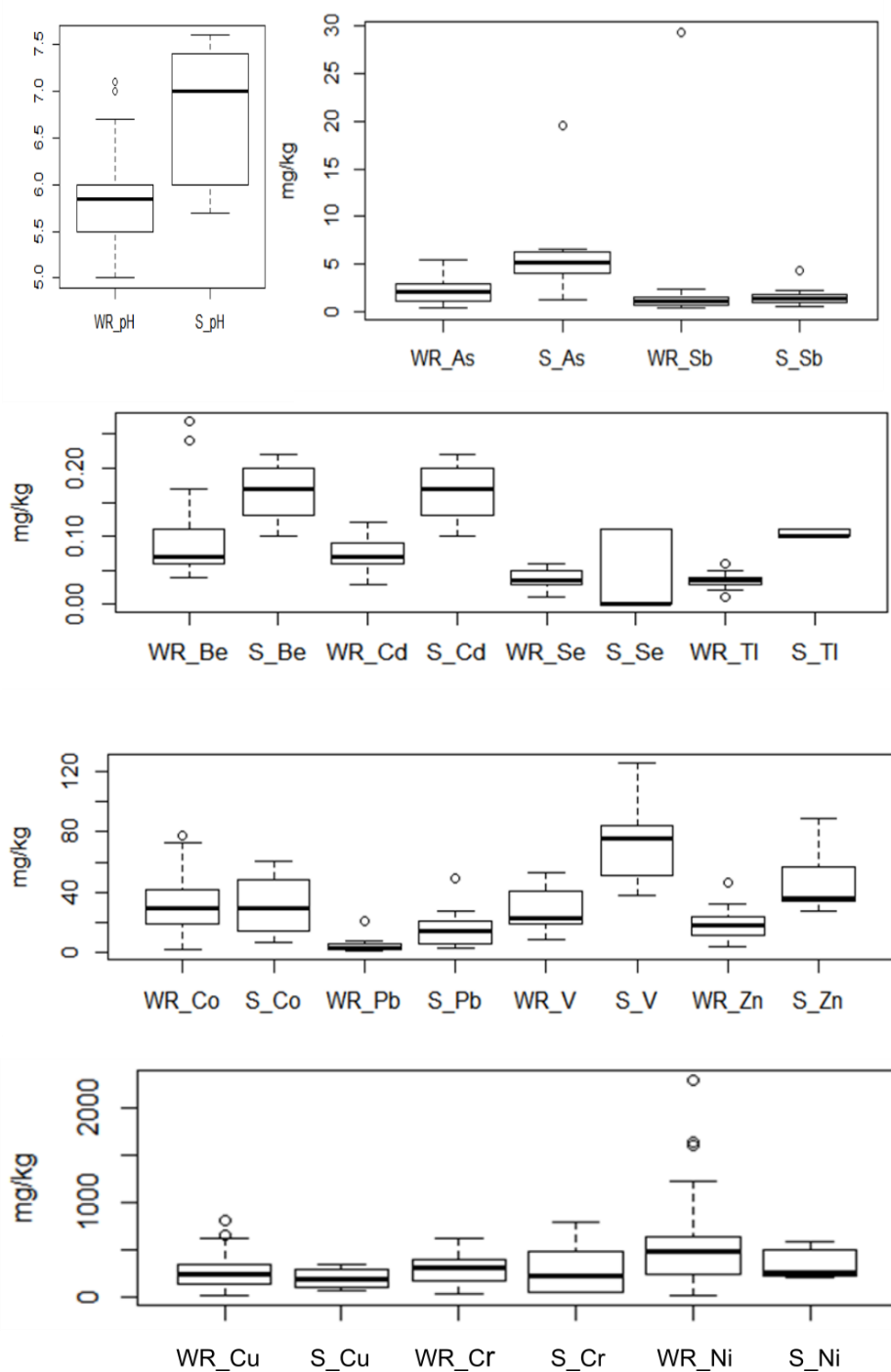
### 266 **3.1 Total concentrations of PTE**

267 The pH and total concentrations of PTE in waste rock samples (no. of samples, n = 26) and soil  
268 samples (no. of samples, n = 9) are summarized in **Figure 3**. The value of pH varied from 5.0 to 7.1

269 with a mean value of 5.9. The results showed that concentrations of Ni varied from 15.2 mg/kg to  
270 2294 mg/kg with an average concentration of 640 mg/kg. The presence of slightly acidic samples  
271 and high concentrations of Ni can be attributed to the presence of ultramafic lithology rich in  
272 olivine and pyroxene in Campello Monti.

273 The concentration of Cr varied from 39 mg/kg to 620 mg/kg with an average concentration  
274 of 299 mg/kg, while concentrations of Co ranged from 2.4 mg/kg to 77.8 mg/kg with a mean  
275 concentration of 32.1 mg/kg. The presence of Cr and Co is due to the fact that Ni in earth's crust  
276 exhibits chalcophile and lithophile characteristics and is found to be associated with Cr and Co.  
277 Copper was found to vary from 19 mg/kg to 806 mg/kg with a mean concentration of 284 mg/kg.  
278 The presence of Cu suggests that sulfide rich minerals (e.g. pyrite and chalcopyrite) that host both  
279 Ni and Cu, may be present at the site. It should be noted that concentrations of Ni, Cr, Co and Cu in  
280 waste rocks are higher than Italian permissible limits for soils for recreational and habitation areas  
281 (Ministero dell'ambiente e della tutela del territorio, 2006, decree no. 152/06).

282 Analysis of soil samples showed that pH values ranged from 5.7 to 7.6 with an average  
283 value of 7.0. The samples were found to be in near neutral conditions and less acidic than waste  
284 rock samples. Total Ni, Cr and Cu ranged from 212 to 594 mg/kg, 46 to 795 mg/kg and 66 to 345  
285 mg/kg respectively. Mean Ni, Cr, Cu concentrations, were 347, 296 and 200 mg/kg, an order of  
286 magnitude above the Italian permissible limits for soils for recreational and habitation areas.  
287 Concentrations of V were found to vary from 38 mg/kg to 126 mg/kg with a mean concentration of  
288 72 mg/kg. Concentrations of other elements were found to be within permissible limits. The  
289 presence of PTE in soil can be explained on the basis of lithogenic origin of soils and possible  
290 transport of PTE from extractive waste dumps.



291

292 Figure 3. Box and Whisker plots showing pH and concentration of PTE in mg/kg in waste rock  
 293 (n=26) and soil samples (n=9) on <2 mm size fractions at Campello Monti. pH and elements on X-  
 294 axis are provided with sample identification code WR for waste rocks and S for soil samples.

295

### 296 3.2 Bioaccessible concentrations

297 The total and bioaccessible concentrations of As, Cd, Co, Cr, Cu, Ni, Pb and V in waste rock and  
 298 soil samples at Campello Monti are presented in **Table 2**. Total concentrations for the <250  $\mu$ m size

fraction were considerably higher than total concentrations for size fractions under 2 mm (reported in Figure 3) potentially due to an increase in surface area and thus higher the absorption of PTE to particles (Yao et al., 2015). The bioaccessible concentrations were measured both for gastrointestinal and gastric phases. It was observed that for all PTE except As, metals were more bioaccessible in the gastric phase than the gastrointestinal phase. The bioaccessible fraction (BAF) was calculated as the ratio of the higher value of bioaccessible concentration (either gastric or gastrointestinal) to total concentration. The highest bioaccessibility value is used to ensure conservative values are used during risk assessment.

Total concentrations of As in waste rock and soil samples varied from 5.6 to 11.1 mg/kg and from 8.8 to 39.3 mg/kg respectively. The bioaccessible concentrations in gastrointestinal phase in waste rock and soil samples varied from 0.6 to 1 mg/kg and from 1.8 to 2.7 mg/kg respectively. Mean values of BAF were found to be 10.5% for waste rock samples and 12.8% for soil samples. Waste rock and soil samples showed mean total concentrations of Cd as 1.3 mg/kg and 0.5 mg/kg. The bioaccessible fractions were found to vary from 3% to 19% and from 20% to 85%, for waste rocks and soil, respectively.

Total concentrations of Co in waste rock and soil samples varied from 165 to 266 mg/kg and from 45 to 175 mg/kg respectively. The bioaccessible concentrations in waste rock and soil samples varied from 27 to 72 mg/kg and from 5 to 53 mg/kg respectively. Mean values of BAF were found to be 20% for waste rock samples and 26% for soil samples. The results on Co bioaccessibility showed that although total concentrations of Co were very less in comparison to Cr, the bioaccessible concentrations were present in the same range as Cr due to higher bioaccessible fractions of Co in comparison to Cr. Chromium in waste rock and soil samples was found to vary from 931 to 1569 mg/kg and from 79 to 1643 mg/kg respectively. Mean values of BAF of Cr for waste rock and soil samples was 1% and 2.75% respectively.

Total concentrations of Cu in waste rock and soil samples ranged from 953 to 2,006 mg/kg and from 85 to 848 mg/kg respectively. The bioaccessible concentrations in waste rock and soil

325 samples varied from 129 to 921 mg/kg and from 27 to 222 mg/kg respectively. Mean values of  
326 BAF were found to be 31% for waste rock samples and 26% for soil samples. Copper results  
327 showed higher bioaccessibility for soil samples compared to waste rocks, indicating a contrasting  
328 behavior with respect to the other PTE analysed. The results of Cu bioaccessibility showed that  
329 although total concentrations of Cu were not as high as Ni, the bioaccessible concentrations were  
330 almost of the same magnitude as nickel. This can be attributed to the higher BAF values of Cu  
331 when compared with Ni.

332 The samples were found to have a very high total concentration of Ni in waste rock samples  
333 with a variation from 1181 to 7408 mg/kg. However, the bioaccessible concentrations of Ni in  
334 gastric phase for waste rock samples was relatively low. The bioaccessible concentrations for  
335 gastric phase for Ni varied from 119 to 776 mg/kg for waste rock samples, thus leading to a BAF  
336 (ratio of bioaccessible concentration to total concentration) of approximately 10%. A similar  
337 observation was made for soil samples. The total concentration and bioaccessible concentration for  
338 soil samples ranged from 59 mg/kg to 1504 mg/kg and from 12 to 280 mg/kg, respectively, thus  
339 leading to BAFs varying from 5% to 20%.

340 Mean values of total concentration of Pb in waste rock and soil samples were found to be 25  
341 mg/kg and 18 mg/kg respectively. The bioaccessible fraction of Pb in waste rock and soil samples  
342 varied from 42% to 61%. Vanadium was found to vary from 34 mg/kg to 87 mg/kg for waste rock  
343 samples, with mean BAF of 4%. The soil samples recorded mean values of total concentrations and  
344 bioaccessible concentrations as 106 mg/kg and 7 mg/kg respectively.

345 The range of bioaccessibility values reported for the soils were found to be comparable to  
346 those reported elsewhere, eg. Barsby et al. (2012) conducted bioaccessibility analysis in ultramafic  
347 geological setting of Northern Ireland using UBM and reported mean values of gastric phase of  
348 BAF of As, Co, Cr for soils as 14%, 18% and 1% respectively (here 13%, 26% and 3%  
349 respectively). The same study reported mean values of BAF for Cu as 31 % (here 31%), Ni as 12%  
350 (here 13%), V as 9% (here 7%). There was a marked difference in reported values of mean of BAF



351 of Pb as reported by Barsby et al. (2012) 33% (here 54%). However, the value was found to be  
352 more comparable with smelter contaminated agricultural soil of northern France, which showed a  
353 BAF of 58% (here 54%) (Pelfrêne et al., 2012).

354

355 Table 1. Results of the UBM digests of certified reference material BGS 102 (n=3).

		As	Cd	Co	Cr	Cu	Ni	Pb	V
Gastric phase	Measured	3.17 ± 0.13	BDL <sup>b</sup>	9.57 ± 0.61	35.76 ± 0.58	8.66 ± 0.69	12.70 ± 0.51	15.35 ± 1.16	6.67 ± 0.40
	Reported <sup>a</sup>	3.90	0.02	9.50	36.70	8.60	13.00	15.30	6.10
Gastro-intestinal phase	Measured	2.54 ± 0.38		5.70 ± 0.75	6.19 ± 1.06		9.86 ± 0.82		2.23 ± 0.46
	Reported	3.30		5.50	13.10		10.50		3.40

<sup>a</sup>Hamilton et al., 2015; <sup>b</sup>BDL- Below detectable limit.

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358 Table 2. Total concentrations (mg/kg), bioaccessible concentrations (G and GI) (mg/kg) and BAF (%) measured on <250 µm size fractions for  
359 samples at Campello Monti.

Sample		As			Cd			Co			Cr		
		GI	total	BAF	G	total	BAF	G	total	BAF	G	total	BAF
Waste rock	CM4	0.6	5.6	11	0.1	0.9	6	27	188	14	25	1398	1
	CM10	1	11.1	9	0.3	1.4	19	69	266	26	20	1569	1
	CM11	0.6	7.5	9	0.2	1.9	13	58	295	20	26	1296	1
	CM21	0.7	6.3	13	0.0	1.1	3	30	165	18	9	931	1
Soil	5	1.8	15.3	11	0.2	1.0	20	53	175	31	54	1643	1
	1	2.9	39.6	7	0.6	0.7	85	23	68	34	3	79	3
	8	1.8	8.8	22	0.1	0.2	47	37	142	26	85	623	1
	9	1.2	9.4	12	0.2	0.2	73	5	45	10	124	701	6
		Cu			Ni			Pb			V		
		G	total	BAF	G	total	BAF	G	total	BAF	G	total	BAF
Waste rock	CM4	129	953	14	119	1181	10	10	21	49	2	87	2
	CM10	754	1955	39	502	4586	11	12	24	50	2	64	3
	CM11	921	2006	47	776	7408	10	10	25	42	2	34	6
	CM21	320	1367	23	256	2864	9	14	28	50	2	61	3
Soil	5	222	848	26	280	1504	19	8	15	51	9	149	5
	1	27	85	32	12	59	21	29	49	59	5	94	6
	8	135	441	31	73	1455	5	2	4	44	3	79	4
	9	45	256	17	38	763	5	2	4	61	12	101	12

360 G = gastric phase and GI = gastrointestinal phase of UBM. Total represents total concentration of PTE using *aqua regia*. Bioaccessible fraction is  
361 represented as BAF.

### 362 3.3 Interpretation of sequential extraction data

363 Identified physico-chemical components for the most representative samples of waste rock (sample  
364 code - CM 10) and soil (sample code - 8) at Campello Monti are highlighted in **Figure 4**. For these  
365 samples, the chemometric data analysis identified 7 components in the waste rock sample and 8  
366 components in the soil sample. Each row represents a component identified by the algorithm, where  
367 the name is composed of the elements that make up >10% of the composition. The columns of the  
368 heatmap are based on model output showing the composition (%) on the left side, and on the right  
369 side the extraction profiles (E1-E14).

370 A combination of geochemistry knowledge, relative solubility of each component in the  
371 extracts, major elemental composition, profile, and clustering obtained from the heat maps were  
372 used to define 6 geochemically distinct clusters: pore-water, exchangeable, Fe oxide 1, clay related,  
373 Fe oxide 2. The heatmap and clustergram for the remaining waste rock and soil samples are shown  
374 Supplementary Material (Figure 1).

375

376 Pore-water: In waste rock, the pore-water cluster was principally made up of S (c. 52.2%) and Mg  
377 (c. 24.7%). Other elements extracted were Ca (c. 7.4%) and Ni (c. 8.8%). The presence of nickel in  
378 the pore water component suggests mobility of Ni in the waste rock. The pore-water cluster of soil  
379 was predominantly composed of S (c. 64%) and Na, Mg, K which were all present at >5 %. These  
380 components in this cluster were extracted in water extractions and 0.01 M HNO<sub>3</sub> (E1-E4). This was  
381 the most easily extracted cluster suggesting it could be associated with the residual salts from the  
382 original pore water in the soil.

383

384 Exchangeable: In waste rock, the exchangeable component consisted of Cu (c. 36%), Mg (c. 17 %),  
385 S (c. 12%) and Ca (c. 12%). It was removed by the HNO<sub>3</sub> extracts over the range 0.01 M to 0.05 M.  
386 The presence of a Cu rich component could be due to the presence of Cu bearing ores, such as Cu  
387 Fe sulfides (chalcopyrite, CuFeS<sub>2</sub> and cubanite, CuFe<sub>2</sub>S<sub>3</sub>) at the site. The exchangeable cluster of

388 soil was principally composed of Al (*c.* 48%), Ca (*c.* 27%), Cu (*c.* 7%) and S (*c.* 5%). It was  
389 removed by the HNO<sub>3</sub> extracts over the range 0.01 M to 0.1 M. High Ca and Al concentrations  
390 combined with removal on addition of relatively weak acid suggests that this cluster was associated  
391 with the presence of K-feldspar, which was found in micro-XRF analysis of the soil samples.

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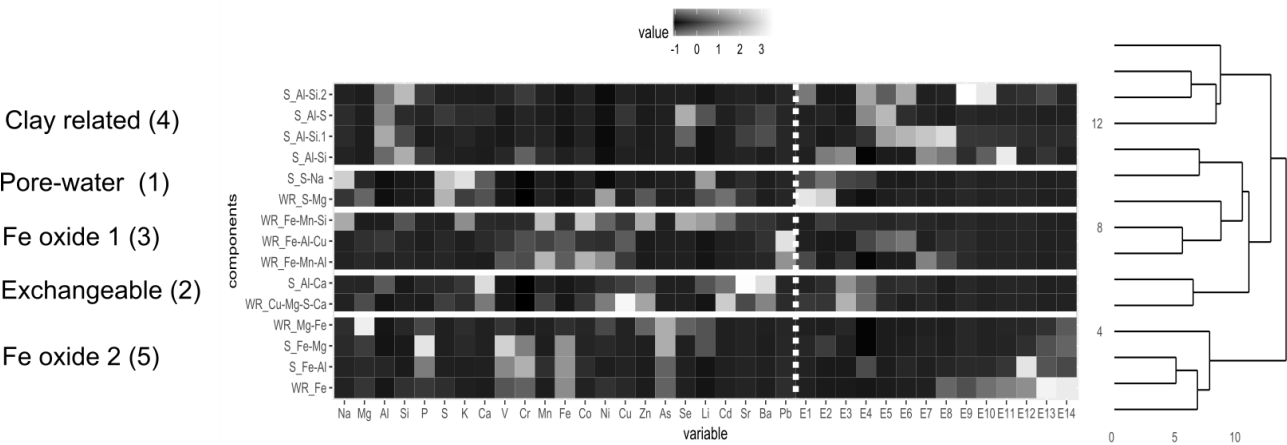
393 Clay related: This cluster was found only in soil and consisted of 4 different components extracted  
394 (Al-Si, Al-Si1, Al-Si2, Al-S). It was dominated by Al (*c.* 62%) and Si (*c.* 34%) and to a lesser  
395 extent by Fe (*c.* 3%). This component also consisted of the highest % of Co, Cr and Cu released  
396 during CISED extractions. These components were extracted with acid concentrations from 0.01 M  
397 HNO<sub>3</sub> to 1 M HNO<sub>3</sub>, however, most elements were extracted in a narrower band of acid  
398 concentrations ranging from 0.1 M HNO<sub>3</sub> to 1 M HNO<sub>3</sub> (E7-E12). The high acid strength for  
399 extraction, predominance of Al, Si and Fe, along with presence of trace elements in this cluster are  
400 likely to be extracted from clay related minerals and from the primary soil forming minerals such as  
401 olivine and pyroxene (Wragg 2005). Clay like minerals such as montmorillonite and kaolinite were  
402 identified during mineralogical analysis of soil sample using micro-XRF.

403

404 Fe oxide 1: The Fe oxide cluster was extracted only in waste rock. This cluster consisted of three  
405 different Fe dominated components (Fe-Mn-Si, Fe-Al-Cu, Fe-Mn-Al). These Fe dominated  
406 components were removed by acid concentrations ranging from 0.05 M HNO<sub>3</sub> to 0.5 M HNO<sub>3</sub> (E5-  
407 E10). The important elements extracted were Fe (*c.* 39%), Al (*c.* 16%), Mn (*c.* 12%), Cu (*c.* 7%), Ni  
408 (*c.* 6%) and Si (*c.* 6%), Mg (*c.* 5%). The presence of Fe, Cu, Ni rich components can be due to the  
409 presence of minerals like Fe Ni sulphide (pentlandite, (Fe,Ni)<sub>9</sub>S<sub>8</sub>) and Cu Fe sulphide (chalcopyrite,  
410 CuFeS<sub>2</sub>), which were found in mineralogical analysis of waste rocks from this site (Rossetti et al.,  
411 2017). The presence of Al and Si in this Fe oxide cluster showed that in waste rock, both these  
412 elements are more closely associated with iron unlike the soil sample, where Al was extracted in the  
413 clay related cluster.

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Fe oxide 2: In the waste rock sample, the Fe oxide cluster was principally composed of Fe (c. 65%). Other elements extracted were Al, Mg, Ni, Si, S with varying concentration from 2.6% to 12%. It was removed by the HNO<sub>3</sub> extracts over the range 0.5 M to 5 M (E9-E14). The presence of Fe,S rich components could be due to presence of Fe sulphide mineral (pyrrhotite, Fe<sub>(1-x)</sub>S) observed in microscopic images of waste rock from this site (Rossetti et al., 2017). The dominance of Fe and high acid extracts required to extract these components could be due to the presence of hematite occurring naturally in the site (Rossetti et al., 2017). The presence of two different Fe containing components for waste rock suggests the presence of different Fe oxide forms (such as amorphous and crystalline), that are being dissolved at different rates (Cave et al. 2004). The Fe oxide cluster in soil included Fe (c. 75%), Al (c. 11%), Mg (c. 6%) and was removed by extracts containing HNO<sub>3</sub> over the range 1 M to 5 M and H<sub>2</sub>O<sub>2</sub> (E11-E14). The Fe oxide 2 cluster was rich in Fe and Mg which suggests that the important Fe and Mg bearing minerals of the olivine group were mainly extracted at very high acid concentrations. The cluster was also found to have concentrations of As, Cr and Ni.



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Figure 4. Heatmap and clustergram for CISED extracted waste rock and soil samples of Campello Monti (CM 10, and soil sample code - 8). The dendrogram on the right-hand side shows how components link together. Elemental composition data is on the left-hand side separated with a dashed vertical white line from the extraction number data (E1–14) on the right. The horizontal white lines divide the map into clusters. High concentrations are depicted by white/light grey and low concentrations by dark grey/black. Component names comprise a sample identification code (WR and S) followed by the principal elements recorded for each component.

### 437    **3.4 Mineralogical analysis**

438    Semi quantitative analysis using micro-XRF showed that the dominant minerals present in soil  
439    (sample code - 8) were clay related (kaolinite and montmorillonite), Fe-Al (Mg) silicates, olivine,  
440    plagioclase and pyroxene. The secondary minerals determined during the analysis were Fe oxides,  
441    K-feldspar, Mn phases and sulfides. The results from SEM analysis (**Figure 5**) showed that As, Cr,  
442    Cu and Ni were locked within mineral grains. Arsenic was present in the minerals that did not  
443    contain Al. A reason could be that in primary rock forming silicate minerals, As can be incorporated  
444    in minerals through replacement of Al. It was also observed that As occurred in the mineral phases  
445    rich in Fe-Mg, showing strong association of As with Fe-Mg in the soil. This was also recorded in  
446    CISED analysis of soil samples where As was extracted in very high percentage in the Fe-Mg  
447    component. Chromium, Cu and Ni were found to be associated with both Al rich and Fe-Mg silicate  
448    minerals.

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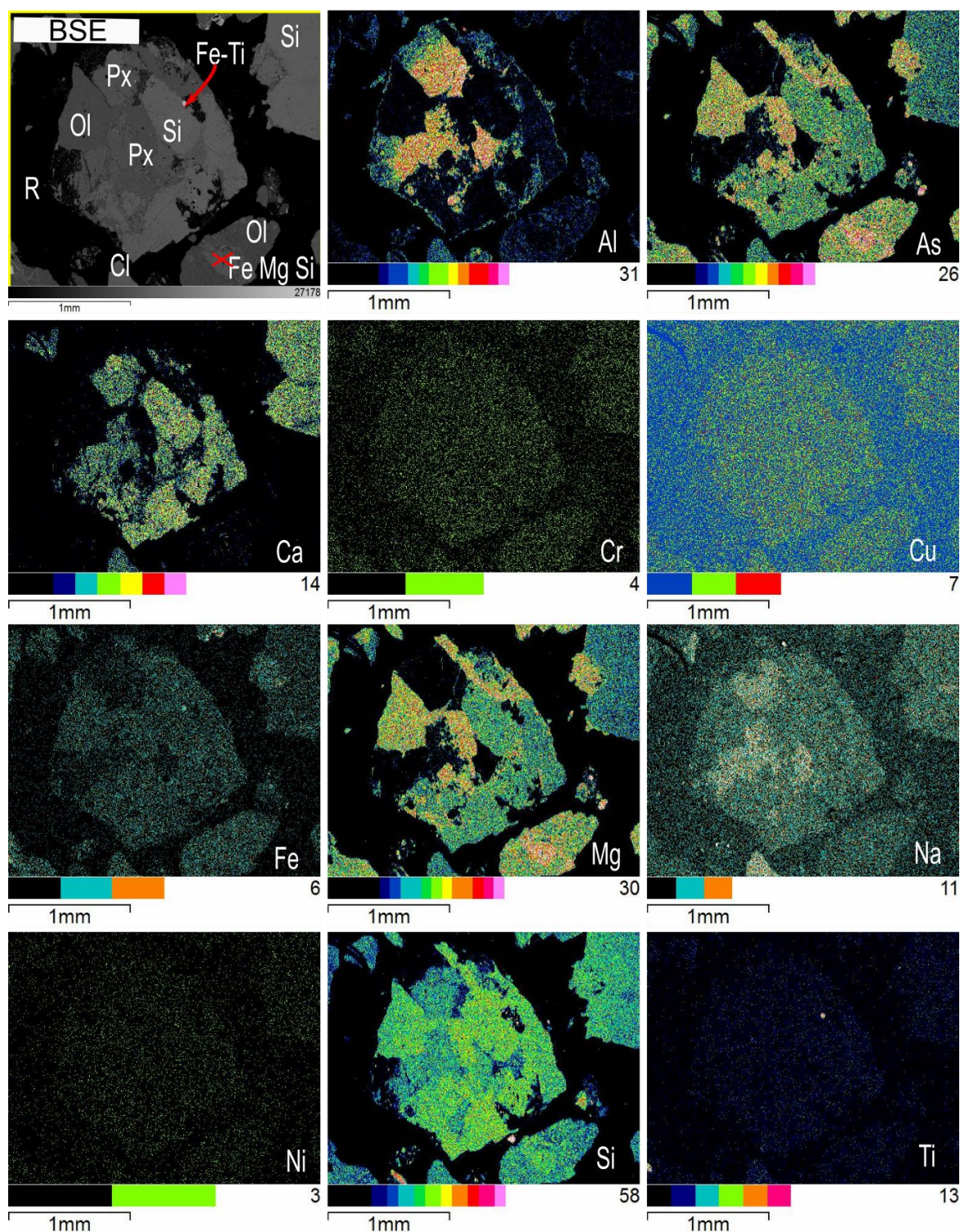


Figure 5. Detail of elemental distribution and composition of soil (sample code 8) - Back scattered electron (BSE) image showing Cl : Clay related mineral (montmorillonite), FeMgSi : Fe Mg silicates, Fe-Ti : Fe-Ti oxide, Ol : Olivine, Px : Pyroxene, R : resin, Si : Ca Mg Fe silicates and corresponding X-ray maps (SEM) for Al, As, Ca, Cr, Cu, Fe, Mg, Na, Ni, Si and Ti.

### 3.5 Relation of mineralogy and CISED to bioaccessibility



457 The extracted PTE and their bioaccessible fraction are plotted in **Figure 6**. The waste rock sample  
458 contained 11 mg/kg of As and only 1 mg/kg of this was bioaccessible. The total concentration of As  
459 extracted by CISED was also 1 mg/kg, indicating that As extracted in both the methods was similar.  
460 80% of total CISED extracted As was associated with the Fe oxide 2 cluster. The Campello Monti  
461 site is rich in Fe bearing minerals suggesting that dissolution of Fe oxides/oxyhydroxides took place  
462 leading to As in extracted solutions. 9 mg/kg of As was present in the soil sample, while 1.8 mg/kg  
463 of this was bioaccessible and 1.2 mg/kg was extracted by CISED, suggesting that As could be  
464 present in mineral phases which were not dissolved through CISED but were dissolved in the  
465 gastrointestinal phase of bioaccessibility extractions. In fact, the SEM analysis of soil samples  
466 confirmed that As was locked in mineral phases of soil samples. Higher dissolution of As enclosed  
467 in mineral grains during UBM than CISED could be due to the presence of organic reagents, body  
468 temperature conditions and/or the longer reaction time for UBM solutions. In fact, Yunmei et al.  
469 (2004) found that during dissolution of Fe-As-S rich mineral assemblages the concentration of As  
470 in solution tends to increase with increase in temperature and time.

471 The total concentration of Cu in waste rock was 1955 mg/kg while only 650 mg/kg of Cu  
472 (35%) was extracted by CISED extractions. Similar observations were made for Cu present in soil  
473 where 33% of Cu was removed in CISED extractions with total concentration and total CISED  
474 extracted concentrations of 441 mg/kg and 135 mg/kg, respectively.

475 The bioaccessible concentration of Cu in waste rock was 157 mg/kg resulting in higher  
476 bioaccessible Cu concentrations than Cu concentrations recorded during CISED extractions. It  
477 suggests that Cu associated with Fe and S present in the Fe oxide 1 cluster, which did not get  
478 extracted in CISED extractions, was extracted in bioaccessibility experiments. However, in soil, the  
479 bioaccessible concentration was less than the CISED extracted concentration. Bioaccessibility of Cu  
480 in soil was due to exchangeable, Fe oxide 2 and dissolution of clay related clusters, while Cu  
481 present in the Fe oxide 2 component did not contribute to bioaccessible Cu. The differences in  
482 bioaccessible Cu concentrations in waste rock and soil could be due to (a) the association of Cu

483 with metal sulfides in waste rocks while in soil Cu was present in clay related minerals rich in metal  
484 silicate phases in soil. It has been found that Cu tends to form a stable and relatively inert complex  
485 with Si (Teien et al., 2006), leading to reduction in dissolution in soil compared to waste rock, (b)  
486 the difference in ratio of concentration of S/Fe extracted during CISED. It is worth mentioning that  
487 the ratio of concentration of S/Fe during CISED extraction in waste rock and soil was 12.8% and  
488 7.6% respectively. Studies on dissolution reactions of Cu concluded that Cu is more chalcophile  
489 than siderophile and tends to dissolve faster with an increase in ratio of S/Fe in iron-sulfur based  
490 solutions (Holzheid and Lodders, 2001).

491 In waste rock samples it was observed that the gastric phase bioaccessible concentrations of  
492 Cr and Ni increased with increase in total concentration potentially suggesting that the majority of  
493 bioaccessible Cr and Ni is derived from phases which contribute to the total Cr and Ni in the sample  
494 (Cox et al. 2013). The total concentration of Cr in waste rock was 1,569 mg/kg while 51.2 mg/kg  
495 was extracted by CISED. The total concentration of Ni in waste rock was 4,586 mg/kg, however  
496 only 661 mg/kg was removed during the CISED procedure. The extraction of 4% of total Cr and  
497 14% of total Ni by CISED suggests that the majority of Cr and Ni was present in less reactive  
498 minerals such as olivine and pyroxenes that are resistant to attack by HNO<sub>3</sub>. Pyroxenes and olivine  
499 are present as the primary minerals in the waste rock samples at the site (Rossetti et al., 2017). The  
500 source of bioaccessible Cr in the waste rock with the partial dissolution of Fe oxide 2 is shown in  
501 Figure 6E. For Ni, it was observed that the same fraction was the source of bioaccessibility, in  
502 addition to dissolution of pore-water, exchangeable and Fe oxide 1 components. Higher  
503 concentrations of Ni than Cr in pore water and exchangeable components suggests easy dissolution  
504 of Ni. It could be because Ni is primarily hosted by olivine in ultramafic rocks. Dissolution of  
505 olivine has been found to be rapid in comparison to most silicate minerals as it has a simpler  
506 structure (Pokrovsky and Schott, 2000). Venturelli et al. (2016), while studying weathering of  
507 ultramafic rocks, found that Ni tends to be more mobile than Cr and was found in higher  
508 concentrations in weathered rocks. Another study reporting Cr and Ni mobility concluded that Ni

509 tends to be more readily transferred to secondary minerals (Quantin et al., 2008). Cox et al. (2017)  
510 found that Cr concentrations in basaltic soils were related to highly recalcitrant chrome spinel and  
511 primary iron oxides, while Ni was more widely dispersed within the soils including in more  
512 extractable soil fractions which led to higher BAF measurements being recorded for Ni than Cr.

513         The total concentration of Cr in soil was 623 mg/kg with a bioaccessible Cr concentration of  
514 85 mg/kg. The CISED method extracted 108 mg/kg of Cr. Differences in total bioaccessible and  
515 CISED extracted concentrations suggest the non-mobile nature of Cr in soil. Dissolution of clay  
516 related clusters and partial dissolution of Fe oxide 2 led to the bioaccessible forms of Cr. The total  
517 concentration of Ni in soil was 1,455 mg/kg, however only 73 mg/kg was bioaccessible in gastric  
518 phase extractions. The bioaccessible form of Ni was likely to come predominantly from the  
519 exchangeable and clay related clusters, and to a lesser extent from the Fe oxide 2 cluster, identified  
520 by the CISED extraction (**Figure 6e**). The possible reason could be that the clay related cluster  
521 consisted of weathered minerals, while the Fe oxide 2 cluster belongs to recalcitrant mineralization  
522 at the site in the form of pyrrhotite ( $\text{Fe}_{(1-x)}\text{S}$ ), pentlandite ( $(\text{Fe,Ni})_9\text{S}_8$ ) and chalcopyrite ( $\text{CuFeS}_2$ )  
523 (Rossetti et al., 2017).

524         For As, Cr and Ni it was observed that the BAF was higher for soil samples compared to  
525 waste rock samples. This could be because (a) elements in ultramafic lithologies are more tightly  
526 bound in the mineral lattice of the waste rocks compared to soils, (b) waste rock samples were more  
527 acidic than soil samples, which can cause some PTE to remain immobile (Ruby et al., 1999), (c)  
528 elements with particle binding abilities may become immobilised in rocks but can be released  
529 during weathering. However, the mean value of bioaccessible fractions in soil for all PTE analyzed  
530 was less than 54%. The possible reason could be the embedment of PTE within mineral grains of  
531 soil as observed in SEM analysis.

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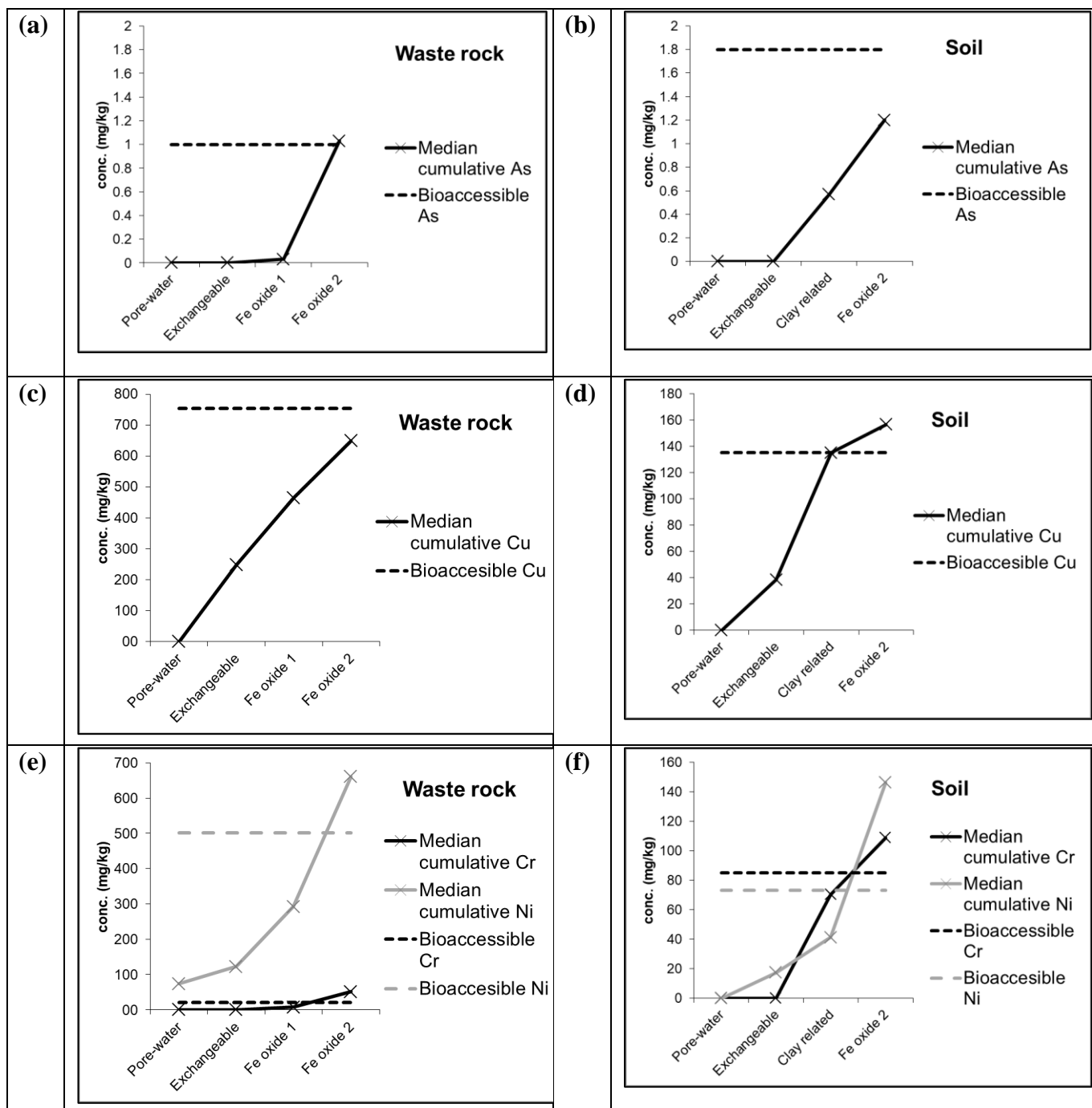


Figure 6. Median cumulative concentration of elements in different components of CISED compared with bioaccessible concentrations in samples of Campello Monti (mg/kg).

#### 4. Conclusions

This study investigated total concentrations and bioaccessible concentrations of PTE at an abandoned mine site of Campello Monti. The results showed that extractive waste facilities and local soils around the old mining areas are strongly enriched in PTE. This study also provided

evidence that total concentrations of PTE were higher in samples with particle size  $<250\ \mu\text{m}$  compared to samples ( $<2\ \text{mm}$ ), due to higher specific surface area in the former case. However, not all of these elements were bioaccessible. The mean value of the bioaccessible fraction (ratio of bioaccessible concentration to total concentration) was observed to be significantly less than 100 % (11%, 1%, and 31% for As, Cr, Cu respectively in waste rocks and 31%, 3%, and 26% for soils). The mean value of BAF of Ni was 10%. Mean values of BAF of V in waste rock and soil were observed to be 4% and 9% respectively. These results show that risk assessment of the site on the basis of total concentrations of PTE alone would significantly overestimate the potential risks to human health at the site. It is clear that the release of PTE and potential risks to human health strongly relies on pH, soil phases, and solubility of Fe-rich phases and presence of clay like minerals. The research conducted highlights how geological and lithological structures together with rock weathering and soil formation processes can lead to variations of bioaccessibility. Traditionally, criteria for the assessment and intervention strategies of contaminated sites have been derived using concentration-based standards and assuming that 100% of the contaminant is bioavailable. However, the results outlined in this research clearly indicate that bioaccessibility evaluations lead to more informed site based risk assessment.

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