

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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Abstract

Oral bioaccessibility and solid phase distribution of potentially toxic elements (PTE) from mining waste streams were investigated to assess the potential human health risk posed by abandoned mines. The solid phase distribution along with micro-X-ray fluorescence (micro-XRF) and scanning electron microscopy (SEM) analysis were also performed. The results showed that the total concentrations of PTE were higher in <250 μm size fractions of waste rock and soil samples in comparison to the <2mm size fractions. Mean value of total concentrations of chromium(Cr), copper (Cu) and nickel (Ni) in waste rocks (size fractions <250 μm) were found to be 1299, 1570 and 4010 mg/kg respectively due to the parent material. However, only 11% of Ni in this sample was orally bioaccessible. Detailed analysis of the oral bioaccessible fraction (BAF, reported as the ratio of highest bioaccessible concentration compared with the total concentration from the 250 μm fraction) across all samples showed that Cr, Cu, and Ni varied from 1 to 6%, 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 oral bioaccessibility, solid phase distribution and mineralogical analysis can provide insights into the distribution, fate and behaviour of PTE in waste streams from abandoned mine sites and inform human health risk posed by such sites.

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

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 potentially toxic elements (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).

There is growing awareness and concern about the harmful effects of elevated concentrations of toxic elements on human health (Golia et al., 2008). However, there is also increasing evidence that an elevated total concentration of elements may not be indicative of actual damaging effects to humans. Consequently, it has been proposed that bioavailable concentrations should be used to inform human health risk assessment (HHRA). In the context of the present research, the bioavailable concentration is the proportion of ingested contaminants that are absorbed from the gut into the body, reaching the systemic circulation (bloodstream) and targeting different organs (Oomen, 2000). However, measuring bioavailability *in vivo* is a difficult and lengthy procedure (Maddaloni et al., 1998). Therefore, a number of *in vitro* bioaccessibility methods have been developed to measure the oral bioaccessibility of a contaminant (Cox et al., 2013). The oral bioaccessible fraction is defined as the contaminant fraction that is released from soil during ingestion and digestion and therefore enters into solution in the gastrointestinal tract and is available for absorption. Bioaccessible concentration is therefore greater than or equal to the bioavailable concentration and can be used as a conservative measure to the bioavailability for HHRA (Paustenbach, 2000).

In vitro bioaccessibility methods measure the oral bioaccessibility of PTE by mimicking the stomach and intestine biochemical conditions. Over the last decade, several methods have been

developed and validated such as among others, the DIN method (RUB, Germany), the *In Vitro* Digestion model (RIVM, The Netherlands), the Physiologically Based Extraction Test (PBET), the Simulator of the Human Intestinal Microbial Ecosystem (SHIME; (LabMET/Vito, Belgium), and the Simplified Bioaccessibility Extraction Test (SBET) (Oomen et al., 2002; Cave et al., 2011). Differences between these bioaccessibility methods has led to differing values of bioaccessible concentration as the contaminant concentration released is dependent on : (1) the pH of the gastrointestinal compartment, (2) the residence time in the soil in solution, (3) the ratio of solid to liquid in the gastric solution, and (4) the bile concentrations and salts content (Oomen et al., 2002). Owing to the different procedures and associated variability in the results obtained, the unified BARGE method (UBM) for measuring the oral bioaccessibility of contaminants was developed by the Bioaccessibility Research Group of Europe (BARGE) in order to harmonize the use of oral bioaccessibility testing for contaminated soils (Denys et al., 2012).

Since then, several studies have used the UBM method to assess the distribution and bioaccessibility of PTE and therefore the potential risk posed by mining affected areas to human health. For example, Pelfrène et al., (2012) quantified the bioaccessible concentrations of cadmium (Cd), lead (Pb) and zinc (Zn) of smelter-contaminated agricultural soils in a coal mining area of northern France and showed that 78% of Cd, 32% of Pb, and 58% of Zn were bioaccessible and therefore posing a potential risk to human health. In another study, Foulkes et al., (2017) found that the bioaccessible concentrations of Pb, thorium (Th) and uranium (U) from solid wastes and soils from an abandoned uranium mine site in South West England were also posing a potential risk to human health, therefore, requiring some remedial action. However, inclusion of contaminant bioaccessibility in human health risk assessment (HHRA) is still not common practice across Europe and beyond, especially in Italy (Kumpiene et al., 2017). Therefore, the present study aims to provide evidence towards evaluating bioaccessibility to support the HHRA procedures for an abandoned mine site in Italy.

Potentially toxic elements (PTE) can be associated with the differing components in extractive waste (EW) and soils, and these differences 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 PTE bioaccessibility, such as pH, organic matter content, presence of clay, iron oxides and aluminosilicates (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, solid-phase distribution and mineralogical studies can be used as an additional lines of evidence to bioaccessibility testing during HHRA (CIEH, 2009).

Sequential selective chemical extractions are widely used for characterising the distribution of elements in the solid phase (Li et al., 2001). However, a number of limitations are often associated with such extractions especially the difficulty of finding a suitable method for all soil types and elements. To overcome this issue, Cave et al. (2004) developed a non-selective method coupled to chemometric analysis known as the chemometric identification of substrates and element distributions (CISED) method. The main advantages of CISED are: (1) rapid and simple extraction procedure, (2) overcoming non-selectivity and redistribution of trace elements, (3) partitioning of elements not based on their response to the extraction reagents, (4) results support directly the *in vitro* bioaccessibility tests (Palumbo-Roe and Klinck, 2007; Cox et al., 2013).

Studies have shown that the bioaccessibility of PTE can be affected by the mineral phases present in the EW and soil (Ruby et al, 1999; Meunier et al., 2010; Vasiluk et al., 2011; Cox et al, 2017). In particular, the encapsulation of PTE into the mineral matrices leads to change in the PTE-bearing surface area and (2) the association of PTE with different type of minerals result into the formation of alteration products. It is therefore imperative to investigate the geochemical composition of the mineral phases present in EW and soil in order to assess the factors influencing PTE bioaccessibility. Specifically, in this study, the total concentration, bioaccessible fraction (BAF) and the distribution of PTE were determined alongside mineralogical analysis of the extractive waste and

soil samples to inform the potential risk posed to human health by an abandoned mine at Campello Monti (Italy).

2. Methodology

2.1 Site description

Campello Monti is a small settlement in the village of Valstrona in the northern sector of Piemonte, Italy. Geologically, the site (**Figure 1**) is present in the ultramafic layers of the mafic complex of the Ivrea-Verbano Zone. The Ivrea-Verbano zone is a tectonic unit which has preserved the transition from amphibolite to granulite facies (Redler et al., 2012). The mafic formation consists of a sequence of cumulate peridotites, pyroxenites, gabbros and anorthosites, together with a large, relatively homogeneous body of gabbro-norite, grading upwards into gabbro-diorite and diorite. The Campello Monti area consists of lherzolites, in places with titanolivine, in large and smaller masses (Mehta et al., 2018).

The rocks in this area are rich in nickel, copper and cobalt. The area was exploited for nickel production from Fe-Ni-Cu-Co magmatic sulphide deposits occurring from the Sesia to Strona valleys from the 19th Century (1865) until the 1940s. The ore was extracted using underground mining activities that left waste rocks near the mine tunnels (**Figure2**).

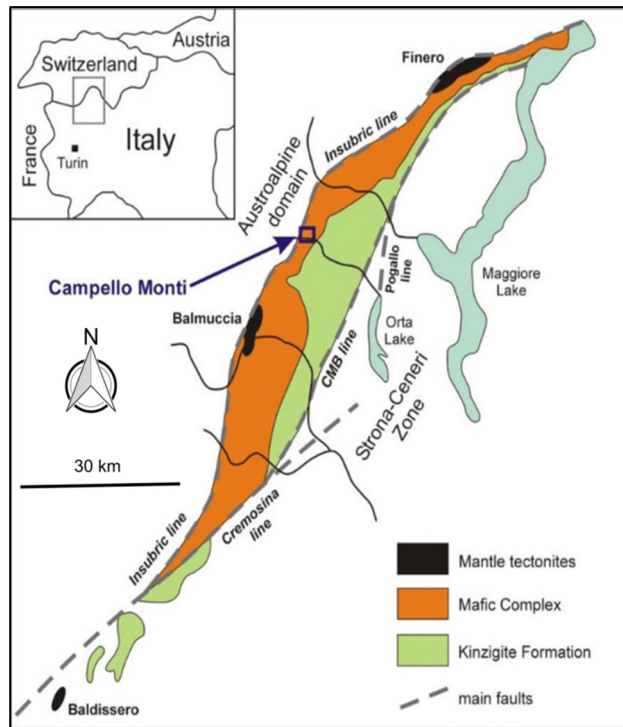


Figure 1. Geological setting of Campello Monti (modified from Fiorentini and Beresford, 2008).

2.2 Sample collection and preparation

The sampling site at Campello Monti is composed of different waste rock dumps. These waste rock dumps were placed on the north of the Strona stream (**Error! Reference source not found.**) and were formed by the dumping in vertical sequence of non-valuable mineralisations and non-mineralised rocks. A systematic sampling strategy was adopted in order to obtain representative data of the whole waste facility. Waste rock material was sampled using hand shovel and a hammer (where necessary). Each sample (8-10 kg) was collected by recovering four subsamples from the vertices of a 1.2m x 1.2m square and one subsample at the centre of the square, and mixing these together after removing organic residues. In total, 26 samples of waste rock were collected at the site in July 2016 (**Error! Reference source not found.**).

Additionally, a total of 9 soil samples were taken near the waste rock dumps to the north and south of the Strona stream during sampling campaigns in June 2016 and March 2017 (**Error! Reference source not found.**). In order to obtain representative soil samples, the samples taken were

formed by mixing 4 subsamples taken at the vertices of a 1 m x 1 m square. All samples were taken at a depth of 0-15 cm.

The extractive waste samples and soil samples were dried in an oven for a period of 24 h to remove any moisture. Samples were then sieved through 2 mm sieves and quartered to obtain a representative sample size of 10 g. The pH was measured in a 1: 2.5 suspension of each sample in water (ISO 10390, 2005).

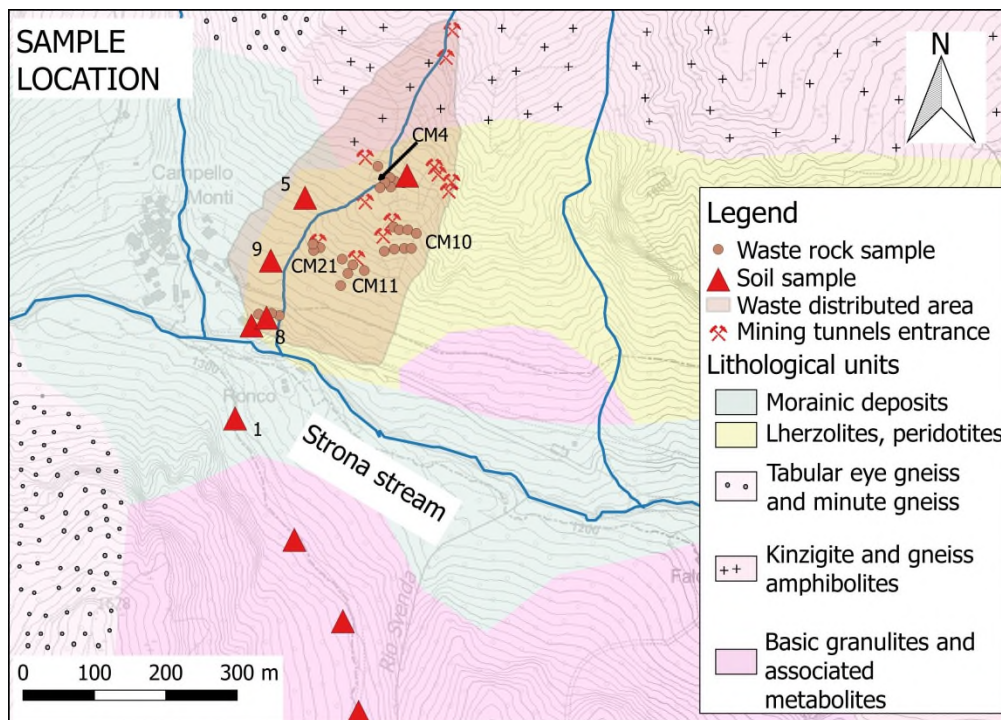


Figure 2. Waste rock and soil sample locations at Campello Monti. Sample numbers are shown for the samples analyzed for bioaccessibility.

2.3 Total concentrations measurement

The samples were analyzed for their concentrations of chemical elements on the 2 mm fraction 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 HNO₃ 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.

2.4 Bioaccessibility analysis (Unified BARGE method)

Following the analysis of total concentration of PTE on the <2 mm fraction, samples were selected for the measurement of bioaccessible concentrations. Waste rock and soil samples were selected to ensure representation of each dump and lithology. Total metal concentrations were measured on the <250 µm fraction using aqua regia extractions as described in section 2.3. The unified BARGE method (UBM) was followed for measuring bioaccessible concentrations of the <250 µm fraction (BARGE 2010; Denys et al., 2012). The <250 µm size fractions of EW and soil was analyzed for both total and bioaccessible concentrations as this is the particle size fraction which is believed to adhere to skin and to result in incidental ingestion exposures (U.S. EPA, 2000). To ensure quality control of the extraction process during oral bioaccessibility experiments, each batch of UBM extractions (n=10) included one procedural blank, six unknowns, one duplicate of two unknown samples and one soil guidance material (BGS102) (BARGE 2010; Hamilton et al., 2015). **Table 1** shows the comparison of the certified and measured values of the BGS 102 extractions. As pH plays an important role in controlling the leaching of the PTE from the matrix and overall extraction process, the pH meter was calibrated before extraction of every batch of samples.

UBM extractions were carried out using simulated digestive fluids including saliva, gastric fluid, bile and duodenal fluid, which were prepared from inorganic and organic reagents and enzymes one day prior to sample extractions. These fluids were used to represent the three main compartments of the human digestive system: mouth, stomach and small intestine. The extraction consists of two

phases, gastric (G) and gastrointestinal (GI) for which 0.4 ± 0.0005 g of sample was weighed in replicate in polycarbonate tubes (1 replicate for the gastric phase and 1 replicate for the gastrointestinal phase). For gastric phase extractions, saliva and gastric fluids were added to each tube (pH adjusted to 1.2 ± 0.05), followed by 1 h of end-over-end rotation, in an oven at a constant temperature of 37 °C. One of the replicates was extracted by centrifuging (4500 g for 15 min) (G phase), while the second replicate was retained for gastro-intestinal phase (GI phase) extraction. Simulated duodenal and bile fluids were added to this tube (pH adjusted to 6.3 ± 0.5) and rotated end-over-end for 4 hours at 37 °C. This was again followed by centrifuging (4500 g for 15 min) to obtain GI phase extracts. For both extractions, 10 ml of the supernatant was collected and preserved with 0.2 ml HNO₃ (15.9 M). Determination of PTE in extracts was performed by ICP-MS (Perkin-Elmer NexION 350X) using an internal standard (Rh). The bioaccessible fraction (BAF) for both the phases was calculated using Equation 1. To apply a conservative approach for human health risk assessment, BAF is reported as the ratio of highest bioaccessible concentration from gastric or gastrointestinal phase compared with the total concentration from the 250 µm fraction.

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

2.5 Chemometric identification of substrates and element distribution (CISED)

A non-specific sequential nitric acid extraction (CISED) (Cave et al., 2004) was carried out on selected samples (n=2 waste rocks, n=3 soil). Briefly, 2 g of sample was sequentially extracted with 10 ml of deionized water and a solution of increasing concentration of HNO₃ ranging from 0.01 M 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 5.0 M), with progressive addition of H₂O₂ (0.25, 0.50, 0.75 and 1 ml) in the last 4 extracting solutions to facilitate the precipitation of oxides. Each solution was mixed for 10 min in an end-over-end shaker

and centrifuged (4350 g for 5 min) to separate solid and liquid fractions. The solid fraction was then resuspended in the following extracting solution. The recovered liquid fraction was filtered with a 0.45 μm 25 mm nylon syringe filter and diluted 4 times with deionized water prior to analysis. Extracts were spiked with internal standards (Sc, Ge, Rh, and Bi) and the following elements Ca, Fe, K, Mg, Mn, Na, S, Si, P, Al, As, Ba, Cd, Co, Cr, Cu, Hg, Li, Mo, Ni, Pb, Sb, Se, Sr, V, Zn were measured using ICP-MS (NexION® 350D ICP-MS, Perkin Elmer). For data quality control, acid blanks (1% nitric acid) and reference material (BGS102) were included in the extraction procedure.

2.6 Modelling

The solid phase distribution of elements in the soil and waste rock samples was determined using a self-modelling mixture resolution algorithm (SMMR) developed by Cave et al. (2004) in MatLab (MatLab® Version R2015a). This modelling algorithm was used to identify (1) soil components with similar physical-chemical properties, (2) chemical composition data (single elements in each soil component expressed as percentage), and (3) the quantity of elements in each component (expressed in mg/kg). The algorithm was applied separately for waste rock and soil producing 7 and 8 distinct sets of physico-chemical phases for each of these respective runs. In order to categorise these physio-chemical phases into common distinct soil phases, hierarchical clustering was used in combination with geochemical profile interpretations. Briefly, heatmaps from hierarchical clustering were produced with a mean-centered and scaled matrix of profile and composition data using the Ward's method in R (v.3.4.1) and the results obtained were plotted with ggplot2, reshape2, grid and ggdendro packages (Wickham,2007; Wickham, 2009; Chang et al. 2013).

2.7 Mineralogical analysis

The mineralogical analysis of waste rock samples was performed in a previous study (Rossetti et al., 2017). Consequently, only the soil sample was analyzed for mineral phases in the present study. Micro-X-ray fluorescence (micro-XRF) was used to identify crystalline phases in one bulk soil

sample (sample code - 8). Element X-ray maps of soil samples were acquired using a micro-XRF Eagle III-XPL spectrometer equipped with an EDS Si (Li) detector and with an EdaxVision32 micro-analytical system. The operating conditions were 2.5 μ s counting time, 10 kV accelerating voltage and a probe current of 20 μ A. The spatial resolution was approximately 65 μ m in both x and y directions. Elemental maps were processed to determine mineral phases in soil using Petromod software (Cossio et al., 2002). The micromorphology and associated chemical analysis of solid phases in soil were analyzed with a Cambridge Stereoscan 360 scanning electron microscope (SEM) equipped with an energy-dispersive spectrometry (EDS) Energy 200 system and a Pentafet detector (Oxford Instruments). 10 kV accelerating voltage and 50 s counting time were used for analysis of the minerals. SEM-EDS quantitative data (spot size 2 μ m) were acquired and processed using the Microanalysis Suite Issue 12, INCA Suite version 4.01; natural mineral standards were used to calibrate the raw data and the $\phi\rho Z$ correction (Pouchou & Pichoir, 1988) was applied. Absolute error is 1% for all calculated oxides.

3. Results

3.1 Total concentrations of PTE

The pH and total concentrations of PTE in waste rock samples (no. of samples, n = 26) and soil samples (no. of samples, n = 9) are summarized in **Figure 3**. The value of pH in waste rock samples varied from 5.0 to 7.1 with a mean value of 5.9. Results showed that concentrations of Ni varied from 15.2 mg/kg to 2294 mg/kg with an average concentration of 640 mg/kg. The presence of slightly acidic samples and high concentrations of Ni can be attributed to the presence of ultramafic lithology rich in olivine and pyroxene in Campello Monti.

The concentration of Cr in waste rock samples varied from 39 mg/kg to 620 mg/kg with an average concentration of 299 mg/kg, while concentrations of Co ranged from 2.4 mg/kg to 77.8 mg/kg with a mean concentration of 32.1 mg/kg. The presence of Cr and Co is due to the fact that Ni in the earth's crust exhibits chalcophile and lithophile characteristics and is found to be associated with Cr

and Co. Copper was found to vary from 19 mg/kg to 806 mg/kg with a mean concentration of 284 mg/kg. The presence of Cu suggests that sulfide rich minerals (e.g. pyrite and chalcopyrite) that host both Ni and Cu may be present at the site. It should be noted that concentrations of Ni, Cr, Co and Cu in waste rocks are higher than Italian permissible limits for soils for recreational and habitation areas (Ministero dell'ambiente e della tutela del territorio, 2006, decree no. 152/06).

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

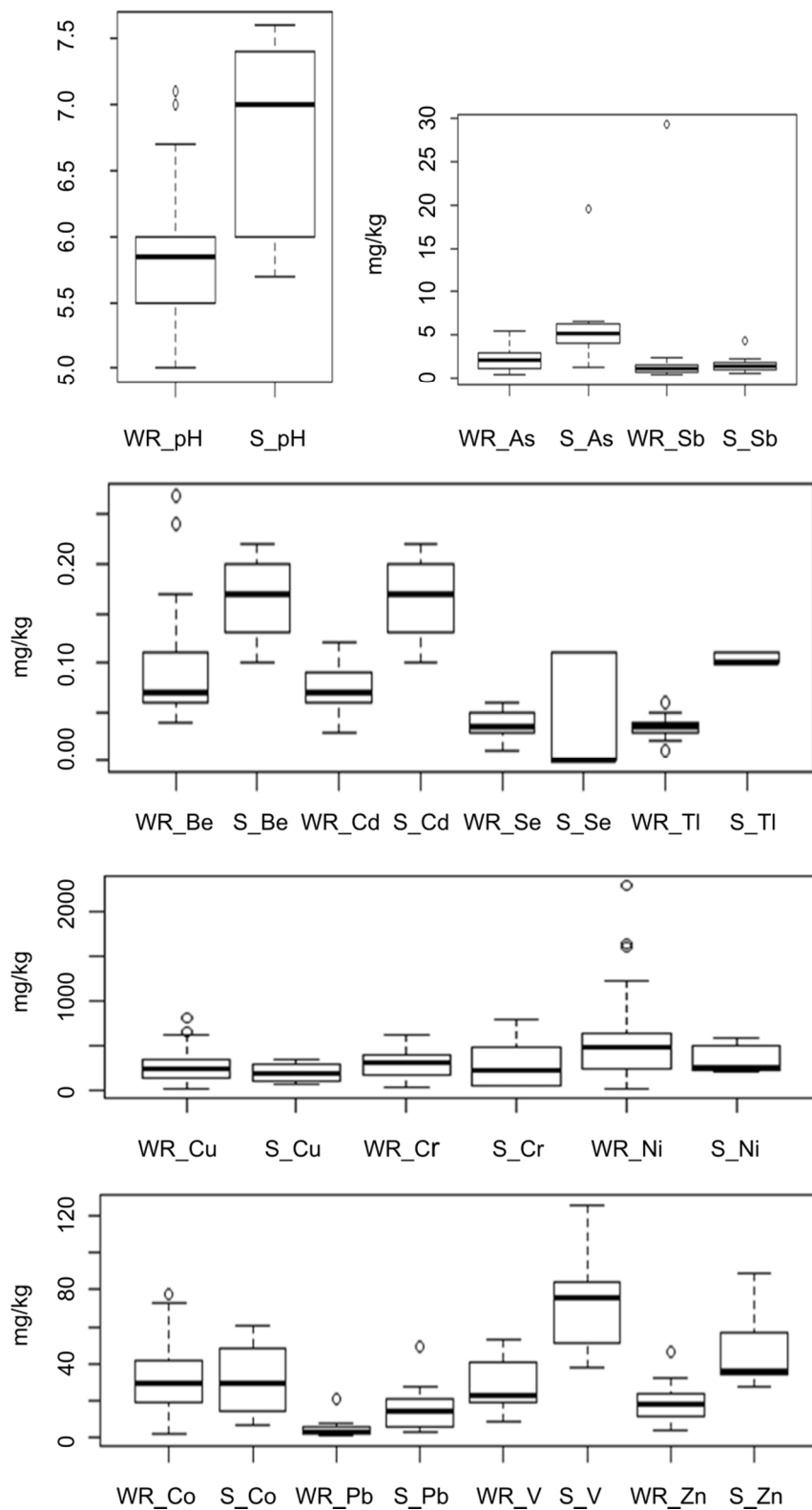


Figure 3. Box and Whisker plots showing pH and concentration of potentially toxic elements (PTEs) in mg/kg in waste rock (n=26) and soil samples (n=9) on <2 mm size fractions at Campello Monti. The pH and elements on X-axis are provided with sample identification code WR for waste rocks and S for soil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

3.2 Bioaccessible concentrations

The total and bioaccessible concentrations of As, Cd, Co, Cr, Cu, Ni, Pb and V in waste rock and soil samples (<250 μm size fractions) at Campello Monti are presented in **Table 2**. This size fraction was analyzed for the total and bioaccessible concentrations as this is the particle size fraction which is more likely to adhere to skin and result in incidental ingestion exposures (U.S. EPA, 2000). Total concentrations for the <250 μm size fractions 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 (on size fractions <250 μm). 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 (G 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 As 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 of Cd 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. Co 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 Co 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 significantly lower than Cr total concentrations, Co

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

High total concentrations of Ni were found in waste rock samples with a variation from 1181 to 7408 mg/kg. However, the bioaccessible concentrations of Ni for waste rock samples were relatively low. Bioaccessible concentrations for gastric phase Ni varied from 119 to 776 mg/kg for waste rock samples, leading to a BAF of approximately 10%. A similar observation was made for soil samples. The total concentration and bioaccessible concentration for soil samples ranged from 59 mg/kg to 1504 mg/kg and from 12 to 280 mg/kg, respectively, thus leading to BAFs varying from 5% to 20%.

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

The range of bioaccessibility values reported for the soils were found to be comparable to those reported elsewhere, e.g. Barsby et al. (2012) conducted bioaccessibility analysis in ultramafic

geological setting of Northern Ireland using UBM and reported mean values of gastric phase of BAF of As, Co, Cr for soils as 14%, 18% and 1% respectively (here 13%, 26% and 3% respectively). The same study reported mean values of BAF for Cu as 31 % (here 31%), Ni as 12% (here 13%), V as 9% (here 7%). There was a marked difference in reported values of mean BAF of Pb as reported by Barsby et al. (2012) (33%) and this study (54%). However, the value obtained in this study was found to be more comparable with smelter contaminated agricultural soil of northern France, which showed a BAF of 58% (Pelfrêne et al., 2012).

Table 1. Results of the Unified Barge Method (UBM) digests of BGS 102 (n=3).

		As	Cd	Co	Cr	Cu	Ni	Pb	V
Gastric phase	Measured	3.17 ± 0.13	BDL ^b	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 ^a	3.90	0.02	9.50	36.70	8.60	13.00	15.30	6.10
Gastrointestinal 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

^aHamilton et al., 2015; ^bBDL- Below detectable limit.

Table 2. Total concentrations (mg/kg), bioaccessible concentrations (G and GI) (mg/kg) and bioaccessible fraction (BAF) (%) measured on <250 µm size fractions for 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

G = gastric phase and GI = gastrointestinal phase of Unified Barge Method UBM. Total represents total concentration of potentially toxic elements (PTEs) using *aqua regia*. Bioaccessible fraction is represented as BAF.

3.3 Interpretation of sequential extraction data

Identified physico-chemical components for the most representative samples of waste rock (sample code - CM 10) and soil (sample code - 8) at Campello Monti are highlighted in **Figure 4**. These samples were selected as the most representative samples as these were collected from the waste distributed area present in the major lithological unit of the area and were also present closest to the centre of the north-south stretch of all the collected samples. For these samples, the chemometric data analysis identified 7 components in the waste rock sample and 8 components in the soil sample. Each row represents a component identified by the algorithm, where the name is composed of the elements that make up >10% of the composition. The columns of the heatmap are based on model output showing the composition (%) on the left side and on the right side the extraction profiles (E1-E14).

Application of geochemical knowledge to data including the relative solubility of each component in the extracts, the major elemental composition of profiles, and the results of hierarchical clustering allowed 5 geochemically distinct clusters to be defined: pore-water, exchangeable, Fe oxide 1, clay related, Fe oxide 2. Heatmaps and dendrograms showing the results of hierarchical clustering for the remaining waste rock and soil samples are shown in the Supplementary Material (Figure S1).

Pore-water: In waste rock, the pore-water cluster was principally made up of S (*c.* 52.2%) and Mg (*c.* 24.7%). Other elements extracted were Ca (*c.* 7.4%) and Ni (*c.* 8.8%). The presence of nickel in the pore water component suggests mobility of Ni in the waste rock. The pore-water cluster of soil was predominantly composed of S (*c.* 64%) and Na, Mg, K which were all present at >5 %. These components in this cluster were extracted in water extractions and 0.01 M HNO₃ (E1-E4). This was the most easily extracted cluster suggesting it could be associated with the residual salts from the original pore water in the soil.

Exchangeable: In waste rock, the exchangeable component consisted of Cu (*c.* 36%), Mg (*c.* 17 %), S (*c.* 12%) and Ca (*c.* 12%). It was removed by the HNO₃ extracts over the range 0.01 M to 0.05 M. The presence of a Cu rich component could be due to the presence of Cu bearing ores, such as Cu Fe sulfides (chalcopyrite, CuFeS₂ and cubanite, CuFe₂S₃) at the site. The exchangeable cluster of soil was principally composed of Al (*c.* 48%), Ca (*c.* 27%), Cu (*c.* 7%) and S (*c.* 5%). It was removed by the HNO₃ extracts over the range 0.01 M to 0.1 M. High Ca and Al concentrations combined with removal on addition of relatively weak acid suggests that this cluster was associated with the presence of K-feldspar, which was found in micro-XRF analysis of the soil samples.

Clay related: This cluster was found only in soil and consisted of 4 different components (Al-Si, Al-Si1, Al-Si2 and Al-S). It was dominated by Al (*c.* 62%) and Si (*c.* 34%) and to a lesser extent by Fe (*c.* 3%). This component also consisted of the highest percentage of Co, Cr and Cu released during CISED extractions. These components were extracted with acid concentrations from 0.01 M HNO₃ to 1 M HNO₃, however, most elements were extracted in a narrower band of acid concentrations ranging from 0.1 M HNO₃ to 1 M HNO₃ (E7-E12). The high acid strength for extraction, predominance of Al, Si, and Fe, along with presence of trace elements suggests this cluster is likely to be clay related minerals and primary soil forming minerals such as olivine and pyroxene (Wragg 2005). Clay like minerals such as montmorillonite and kaolinite were identified during mineralogical analysis of soil sample using micro-XRF.

Fe oxide 1: The Fe oxide cluster was extracted only in waste rock. This cluster consisted of three different Fe dominated components (Fe-Mn-Si, Fe-Al-Cu and Fe-Mn-Al). These Fe dominated components were removed by acid concentrations ranging from 0.05 M HNO₃ to 0.5 M HNO₃ (E5-E10). The important elements extracted were Fe (*c.* 39%), Al (*c.* 16%), Mn (*c.* 12%), Cu (*c.* 7%), Ni (*c.* 6%), Si (*c.* 6%) and Mg (*c.* 5%). The presence of Fe, Cu, Ni in these components potentially suggests the presence of minerals like Fe Ni sulphide (pentlandite, (Fe,Ni)₉S₈) and Cu Fe sulphide

(chalcopyrite, CuFeS_2), which were found in mineralogical analysis of waste rocks from this site (Rossetti et al., 2017). The presence of Al and Si in this Fe oxide cluster showed that both these elements are more closely associated with iron in waste rock. In contrast, in the soil sample, Al was extracted in the clay related cluster.

Fe oxide 2: The Fe oxide 2 cluster in the waste rock sample, was principally composed of Fe (c. 65%). Other elements extracted were Al, Mg, Ni, Si and S with varying concentration from 2.6% to 12%. This cluster was removed by the HNO_3 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 the Fe sulphide mineral (pyrrhotite, $\text{Fe}_{(1-x)}\text{S}$) 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 2 cluster in soil included Fe (c. 75%), Al (c. 11%), Mg (c. 6%) and was removed by extracts containing HNO_3 over the range 1 M to 5 M and H_2O_2 (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.

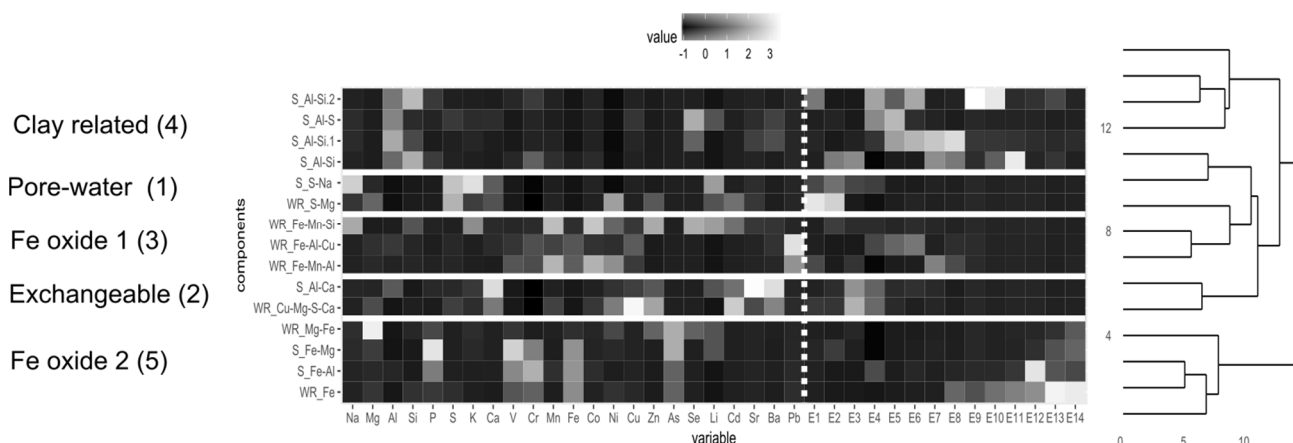


Figure 4. Heatmap and clustergram for CISED extracted waste rock and soil samples of Campello Monti (CM 10, and soil sample code - 8). The dendrogram on the right-hand side shows how hierarchical clustering of components. Elemental composition data is on the left-hand side of the image, separated with 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.

3.4 Mineralogical analysis

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

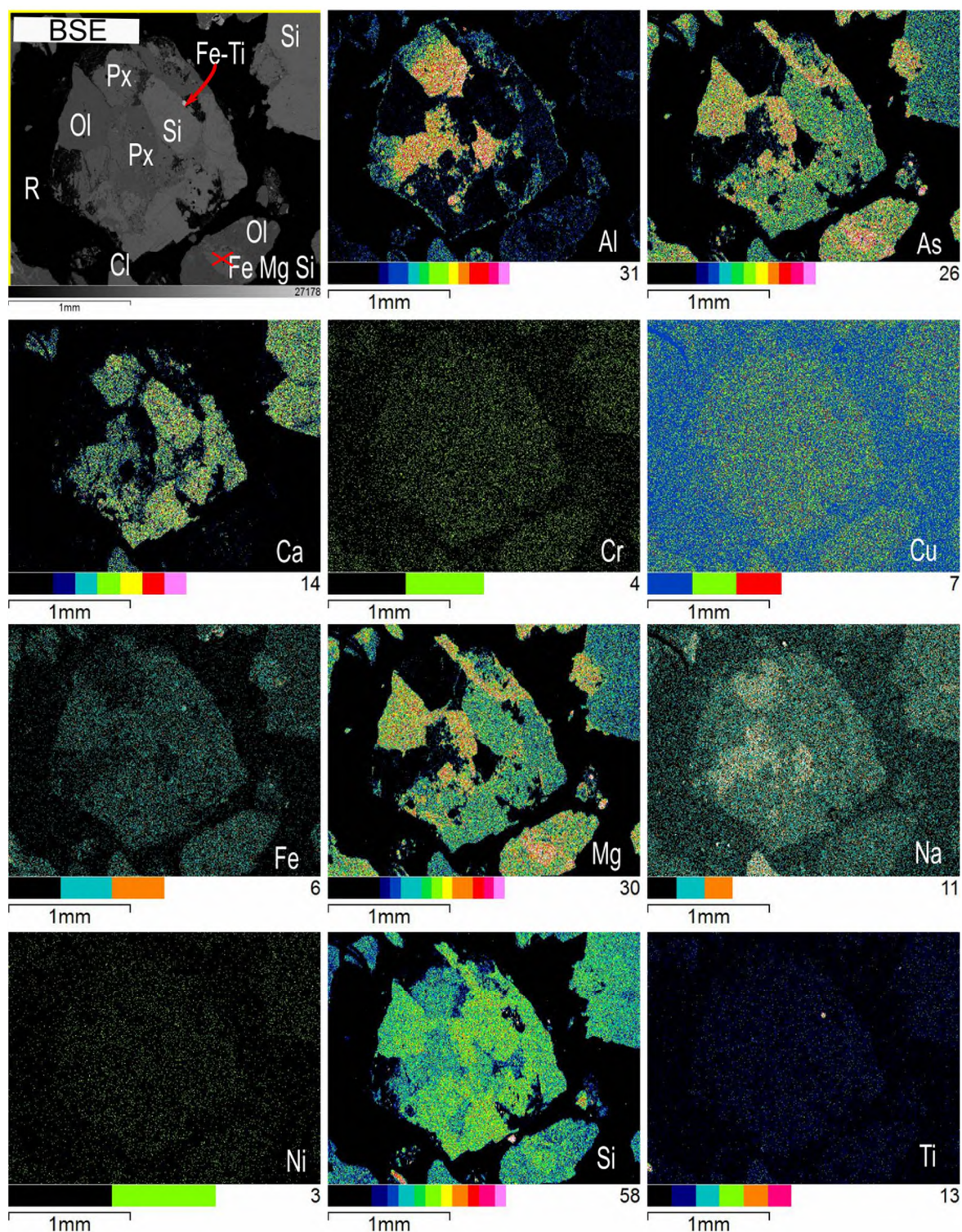


Figure 5. Detail of elemental distribution and composition of soil (sample code 8) - Back scattered electron (BSE) image showing Cl : Clay related mineral (montmorillonite), FeMgSi : Fe Mg silicates, Fe-Ti : Fe-Ti oxide, Ol : Olivine, Px : Pyroxene, R : resin, Si : Ca Mg Fe silicates and corresponding X-ray maps (SEM) for Al, As, Ca, Cr, Cu, Fe, Mg, Na, Ni, Si and Ti. The numbers on the bottom right of the elemental maps shows the highest number of colour contrasts present in each image. These color contrasts represent the difference in % of elements in the mapped area.

3.5 Relation of mineralogy and CISED to bioaccessibility

The extracted PTE and their bioaccessible fraction are plotted in **Figure 6**. The waste rock sample contained 11 mg/kg of As, but only 1 mg/kg of this was bioaccessible. The total concentration of As extracted by CISED was also 1 mg/kg, indicating that all the bioaccessible As in the soil was extracted by the CISED methodology. 97% of total CISED extracted As was associated with the Fe oxide 2 cluster. The Campello Monti site is rich in Fe bearing minerals suggesting that dissolution of Fe oxides/oxyhydroxides took place leading to As in extracted solutions. 9 mg/kg of As was present in the soil sample, while 1.8 mg/kg of this was bioaccessible and 1.2 mg/kg was extracted by CISED, suggesting that As could be present in mineral phases which were not dissolved through CISED but were dissolved in the gastrointestinal phase of bioaccessibility extractions. In fact, the SEM analysis of soil samples confirmed that As was locked within mineral phases in the soil samples. Higher dissolution of As enclosed in mineral grains during the UBM than CISED extractions could be due to the presence of organic reagents, body temperature conditions and/or the longer reaction time for UBM extractions. In fact, Yunmei et al. (2004) found that during dissolution of Fe-As-S rich mineral assemblages the concentration of As in solution tends to increase as temperature and time increase.

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

The bioaccessible concentration of Cu in waste rock was 157 mg/kg resulting in higher bioaccessible Cu concentrations than total Cu extracted during CISED extractions. However, in soil, the bioaccessible concentration was less than the CISED extracted concentration. Bioaccessibility of Cu in soil was extracted from the exchangeable clay related clusters, while Cu present in the Fe oxide 2 component did not contribute to bioaccessible Cu. The differences in bioaccessible Cu concentrations in waste rock and soil could be due to: (a) the association of Cu with metal sulfides in waste rock and clay related minerals rich in metal silicate phases in soils, as Cu tends to form a stable

and relatively inert complex with Si (Teien et al., 2006), which may be the cause of the reduced dissolution in soil compared to waste rock; or, (b) the difference in ratio of concentration of S/Fe extracted during CISED. It is worth mentioning that the ratio of concentration of S/Fe during CISED extraction in waste rock and soil was 12.8% and 7.6% respectively. Studies on dissolution reactions of Cu concluded that Cu is more chalcophile than siderophile and tends to dissolve faster with an increase in ratio of S/Fe in iron-sulphur based solutions (Holzheid and Lodders, 2001).

In waste rock samples, gastric phase bioaccessible concentrations of Cr and Ni increased with increase in total concentration, potentially suggesting that the majority of bioaccessible Cr and Ni is derived from phases which contribute to the total Cr and Ni in the sample (Cox et al. 2013). The total concentration of Cr in waste rock was 1,569 mg/kg while 51.2 mg/kg was extracted by CISED. The total concentration of Ni in waste rock was 4,586 mg/kg, however, only 661 mg/kg was removed during the CISED procedure. The extraction of 4% of total Cr and 14% of total Ni by CISED suggests that the majority of Cr and Ni was present in less reactive minerals such as olivine and pyroxene that are present as the primary minerals in the waste rock samples at the site (Rossetti et al., 2017) and are known to be relatively resistant to attack by HNO_3 .

Bioaccessible Cr in the waste rock arose from partial dissolution of Fe oxide 2 as shown in **Figure 6** whilst dissolution of pore-water, exchangeable and Fe oxide 1 components and partial dissolution of the Fe oxide 2 component contributed to bioaccessible Ni in waste rock. Higher concentrations of Ni than Cr in pore water and exchangeable components in waste rock suggests easier dissolution of Ni than Cr, potentially because Ni is primarily hosted by olivine in ultramafic rocks. Dissolution of olivine has been found to be rapid in comparison to most silicate minerals, due to its simpler structure (Pokrovsky and Schott, 2000). Venturelli et al. (2016) found that Ni tends to be more mobile than Cr and therefore was found in higher concentrations in weathered ultramafic rocks. Quantin et al., 2008 concluded that Ni tends to be more readily transferred to secondary minerals than Cr and Cox et al. (2017) found that Cr concentrations in basaltic soils were related to highly recalcitrant chrome spinel and primary iron oxides, while Ni was more widely dispersed

within the soils including in more extractable soil fractions. This led to higher BAF measurements being recorded for Ni than Cr.

The total concentration of Cr in soil was 623 mg/kg with a bioaccessible Cr concentration of 85 mg/kg. The CISED method extracted 108 mg/kg of Cr. Differences in total, bioaccessible and CISED extracted concentrations suggest the non-mobile nature of Cr in soil. Dissolution of clay related clusters and partial dissolution of Fe oxide 2 led to the bioaccessible forms of Cr. The total concentration of Ni in soil was 1,455 mg/kg, however only 73 mg/kg was bioaccessible in gastric phase extractions. The bioaccessible form of Ni was likely to come from both the exchangeable and clay related clusters, and to a lesser extent from the Fe oxide 2 cluster, identified by the CISED extraction (**Figure 6e**). The possible reason could be that the clay related cluster consisted of weathered minerals, while the Fe oxide 2 cluster includes recalcitrant mineralization at the site in the form of pyrrhotite ($\text{Fe}_{(1-x)}\text{S}$), pentlandite ($(\text{Fe,Ni})_9\text{S}_8$) and chalcopyrite (CuFeS_2) (Rossetti et al., 2017).

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

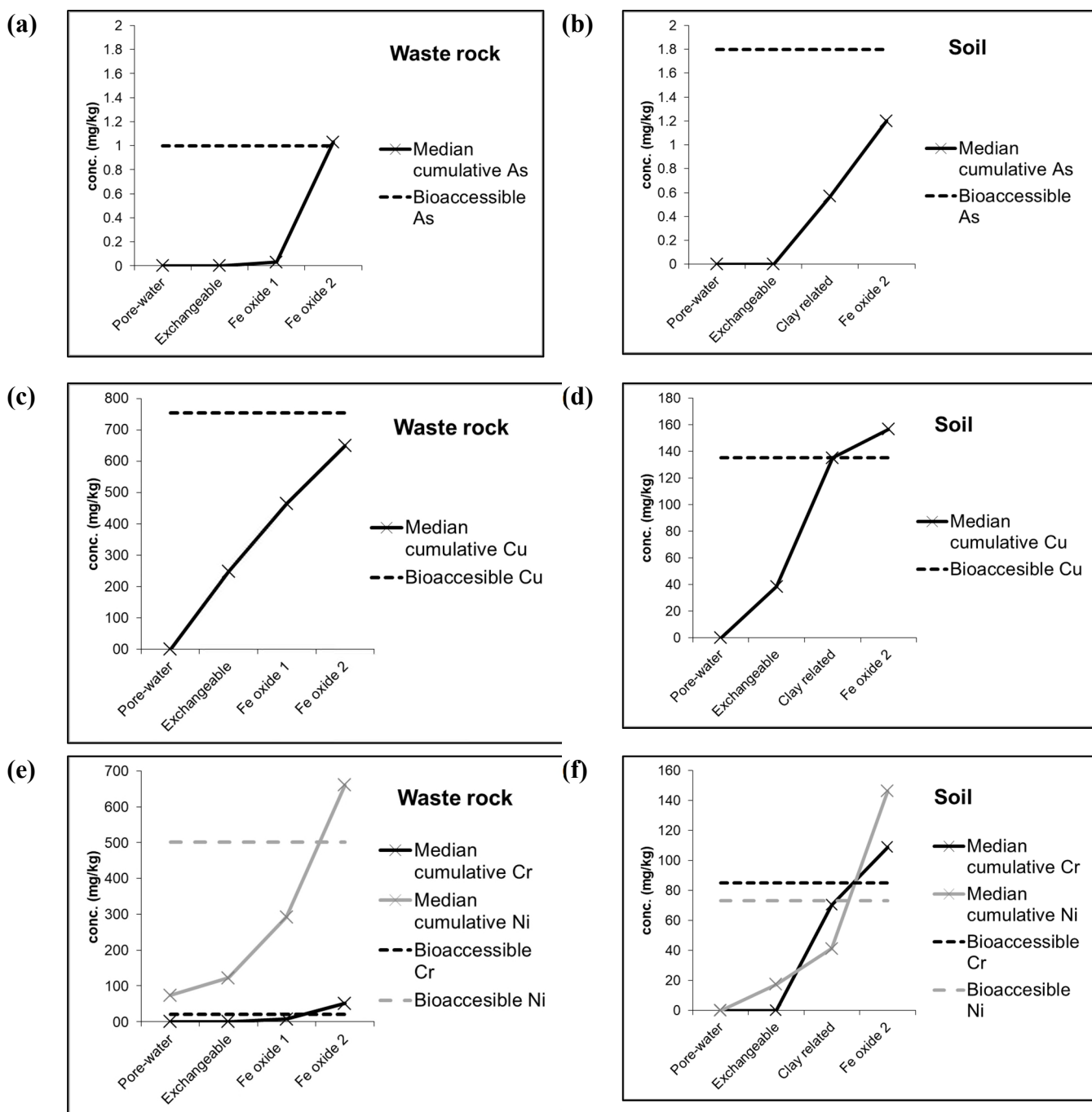


Figure 6. Median cumulative concentration of potentially toxic elements (PTE) in different components of CISED compared with bioaccessible concentrations in samples of Campello Monti (mg/kg).

4. Conclusions

This study investigated total concentrations and oral bioaccessible concentrations of PTE at an abandoned mine site in 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 sieved to <250 μm compared to samples sieved to <2 mm, potentially due to higher specific surface area in the former case.

However, not all PTE were bioaccessible. The mean value of the oral bioaccessible fraction (calculated as the ratio of bioaccessible concentration to total concentration, measured on <250 μm size fractions) was significantly less than 100% (11%, 1%, 31%, 10% and 4% for As, Cr, Cu, Ni and V respectively in waste rocks and 31%, 3%, 26%, 10% and 9% for soils). 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 therefore potential risks to human health strongly depends on soil pH, soil phases, solubility of Fe-rich phases and presence of clay like minerals. This research highlights how geological and lithological structures together with rock weathering and soil formation processes can lead to variations in PTE oral 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 oral bioaccessibility evaluations lead to more informed site based risk assessment.

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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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