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Incorporating oral bioaccessibility into human health risk assessment due to potentially toxic elements in extractive waste and contaminated soils from an abandoned mine site

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(Article begins on next page)

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Abstract: Oral bioaccessibility and solid phase distribution of potentially toxic elements (PTE) from extractive waste (EW) and soils from an abandoned mine site were investigated in northwest Italy. Mineralogical analysis using micro-X-ray fluorescence (micro-XRF) and scanning electron microscopy (SEM) were also performed to correlate the PTE bioaccessibility to the morphological properties of the EW and the soils. The oral PTE bioaccessibility data were further used to inform the potential human health risk posed by such site using total concentrations and bioaccessibility corrected values. In particular, the total concentrations of arsenic (As), cadmium (Cd), and zinc (Zn) in the waste rock fraction (<2 mm) were exceeding by 2.5, 89 and 493 times, respectively compared to the Italian soil permissible limits. The total concentrations of PTE were even higher in the <250 μm fraction of EW and soil samples (for e.g. in waste rock samples by 3, 6, and 2 folds for mean value of As, Cd and Zn respectively). Detailed analysis of the bioaccessible fraction (BAF) across all samples showed that Cd and Zn were highly bioaccessible ranging from 72-98%, while As had a lower BAF ranging between 5 and 34%. The presence of PTE in the alkaline calcareous rock can lead to high bioaccessibility of PTE, indicating there is potential risk to human health as hazard index (HI) was recorded to be >1 and a Cancer Risk (CR_{total}) >1 x 10⁻⁵. This study provides information for site-specific risk assessments and planning future research.

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Dear Editor,

Please find attached the manuscript of the article titled "**Oral bioaccessibility and solid phase distribution of potentially toxic elements from extractive waste and contaminated soils from mining activities: Potential implications for human health risk analysis**" submitted for publication in Chemosphere.

Please note that the corresponding author is Neha Mehta (n.mehta@qub.ac.uk Tel+44 07425249868), as reported in the manuscript.

Authors have consulted the Guide for Authors in preparing the submitted manuscript and they confirm that the manuscript has not been previously published, in whole or in part, and is not under consideration by any other journal. Moreover, the study does not involve human subjects.

Oral bioaccessibility analysis of potentially toxic elements (PTE) using Unified BARGE method, solid phase distribution using non-specific sequential extraction, and mineralogical analysis using micro-XRF and SEM were performed on extractive waste and soil samples. The study was undertaken in an abandoned mine site in Gorno, northwest Italy. Results showed that bioaccessible fraction was <35% for As due to entrapment by minerals. For Cd and Zn the bioaccessible fraction >75% due to alkaline calcareous geology. The extractive waste and soil resulted in potential human health risks considering total and bioaccessible concentrations at the site. The present study demonstrates use of bioaccessibility to inform the risk assessment of abandoned mine sites.

The research has provided evidence on contamination due to PTE and associated human health risks which matches with scope of Chemosphere. We look forward to your interest and kind reply.

Yours sincerely,

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Highlights

- Extractive waste and soil from abandoned mines pose risk to human health.
- As, Cd and Zn were present in high concentrations at Gorno site.
- Bioaccessible fraction (BAF) of As was <35% due to entrapment by minerals.
- BAF for Cd and Zn were >75% due to alkaline calcareous geology.
- Weathering and soil forming processes influence oral bioaccessibility.

1 **Oral bioaccessibility and solid phase distribution of potentially toxic elements from**
2 **extractive waste and contaminated soils from mining activities: Potential implications**
3 **for human health risk analysis**

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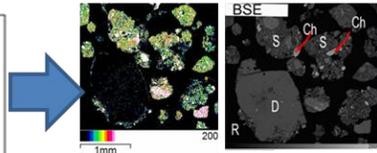
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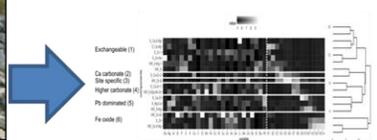
Abandoned Mine Site



Calcite, dolomite, sphalerite and Zn oxide are major minerals.



Oral bioaccessibility: High due to calcareous bedrock



Solid phase distribution: PTE associated with carbonates

Human health risk assessment

1 **Abstract**

2 Oral bioaccessibility and solid phase distribution of potentially toxic elements (PTE) from
3 extractive waste (EW) and soils from an abandoned mine site were investigated in northwest
4 Italy. Mineralogical analysis using micro-X-ray fluorescence (micro-XRF) and scanning
5 electron microscopy (SEM) were also performed to correlate the PTE bioaccessibility to the
6 morphological properties of the EW and the soils. The oral PTE bioaccessibility data were
7 further used to inform the potential human health risk posed by such site using total
8 concentrations and bioaccessibility corrected values. In particular, the total concentrations of
9 arsenic (As), cadmium (Cd), and zinc (Zn) in the waste rock fraction (<2 mm) were
10 exceeding by 2.5, 89 and 493 times, respectively compared to the Italian soil permissible
11 limits. The total concentrations of PTE were even higher in the <250 μm fraction of EW and
12 soil samples (for e.g. in waste rock samples by 3, 6, and 2 folds for mean value of As, Cd and
13 Zn respectively). Detailed analysis of the bioaccessible fraction (BAF) across all samples
14 showed that Cd and Zn were highly bioaccessible ranging from 72-98%, while As had a
15 lower BAF ranging between 5 and 34%. The presence of PTE in the alkaline calcareous rock
16 can lead to high bioaccessibility of PTE, indicating there is potential risk to human health as
17 hazard index (HI) was recorded to be >1 and a Cancer Risk (CR_{total}) >1 x 10⁻⁵. This study
18 provides information for site-specific risk assessments and planning future research.

19 **Keywords:** *Abandoned mine site; Bioaccessibility; Risk assessment; Solid phase distribution;*
20 *Potentially toxic elements (PTE); Triassic western southern Alps (Italy)*

1 1 Introduction

2 Mining contributes to the contamination of the ecosystem by generating extractive waste (EW) also
3 called as mining waste (Nakaona et al; 2019; Stewart, 2019). The high volume of EW with
4 potentially toxic elements (PTE) in abandoned mines has led to human health concerns as these
5 PTE tend to persist in the environment (Boente et al., 2020; Damian et al., 2018; Delil and Koleli,
6 2019; Edokpayi et al., 2016; Karlsson et al., 2018; Kasemodel et al., 2019; Kaupilla et al., 2018;
7 Khelifi et al., 2019; Petrella et al., 2019a; Petrella et al., 2019b; de Souza et al., 2015; Väänänen et
8 al., 2016; Yang et al., 2019).

9 A common approach used to assess the potential human health risks posed by contaminants is to
10 conduct human health risk assessment (HHRA) that entails determining the identity and extent of
11 the contaminants, characterising their toxicity and estimating the magnitude of exposure of local
12 population (Adimalla, 2019). Traditional HHRA methods use total concentrations of contaminants
13 to assess potential risks. However, recent studies have emphasised that human health risks are
14 associated with absorption of PTE in systemic circulation (blood) referred as bioavailable fraction
15 and consideration of total concentrations alone can result in overestimation of impacts on human
16 health (Boim et al., 2019; Du et al., 2020; González-Grijalva et al., 2019; Yu and Yang, 2019).

17 Maddaloni et al. (1998) conducted oral bioavailability studies to measure the absorption of
18 soilborne Pb into humans. Several other studies used *in vivo* animal studies analyse oral
19 bioavailable fractions (Bradham et al., 2016; Brattin and Casteel, 2013; Casteel et al., 2006; Juhasz
20 et al., 2010; Kang et al., 2016; Suh et al., 2019). Nevertheless, assessing bioavailability requires
21 complicated, lengthy and costly procedures that are also ethically constrained (Molina et al., 2013).

22 This has resulted in the development of several *in vitro* bioaccessibility methods (Beauchemin et al.,
23 2014; Ruby et al., 2002) and among others, the Unified BARGE method (UBM) developed by the
24 Bioaccessibility Research Group of Europe (BARGE) is now referenced as an ISO standard method
25 (ISO 17924:2018). Oral bioaccessibility is defined as the fraction of a contaminant that is

26 solubilized in the human gastrointestinal tract and is available for absorption (Wragg et al., 2011).
27 The UBM method has been validated for *in vivo* studies for As, Cd, and Pb (Denys et al., 2009 and
28 2012) and is being widely applied by environmental consulting services in France and England
29 (Foulkes et al., 2017; Pelfrêne et al., 2012). Recently, Mehta et al. (2019) examined the BAF of
30 PTE in samples from an abandoned nickel (Ni) mine in Italy. This said, incorporation of oral
31 bioaccessibility results into HHRA, has not been widely performed in Europe especially in Italy.
32 Potentially toxic elements occur in soil as a complex mixture of solid phase chemical components,
33 e.g. mineral phases; pore-water; and organic matter. Consequently, the occurrence and relative
34 distribution of PTE among these components control PTE dissolution and hence bioaccessibility
35 (Ettler et al., 2018; Ettler et al., 2019; Vasiluk et al., 2019). Therefore, the relative distribution of
36 elements in soil, as measured through solid phase distribution (Schaidler et al., 2007) can provide a
37 line of evidence to understand bioaccessible concentrations (CIEH, 1999).
38 Solid phase distribution in the present study was performed by a non-selective method coupled to
39 the chemometric identification of substrates and element distributions (CISED) method (Cave et al.
40 2004; Cipullo et al., 2018). The CISED method prevents redistribution of elements and provides the
41 necessary information on presence of PTE within various matrices and their effect on *in vitro*
42 bioaccessibility (Cox et al., 2013; Palumbo-Roe and Klinck, 2007).
43 To this end, the objectives of this study were to: (1) assess the PTE bioaccessible fractions in the
44 waste rock, tailings and contaminated soils from an abandoned mine located in the northwest Italy;
45 (2) investigate how the mineralogical composition and the solid phase distribution influence the
46 bioaccessible PTE fractions; and (3) incorporate oral bioaccessibility into HHRA to inform the
47 potential human health risks posed by abandoned mine sites.

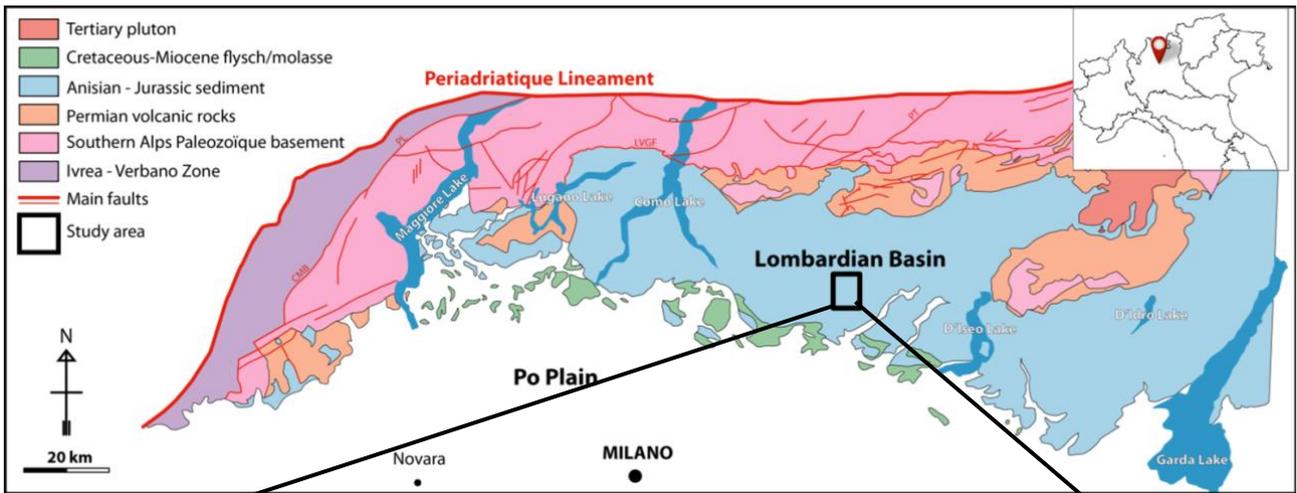
48 2 Methodology

49 2.1 Study site and sampling

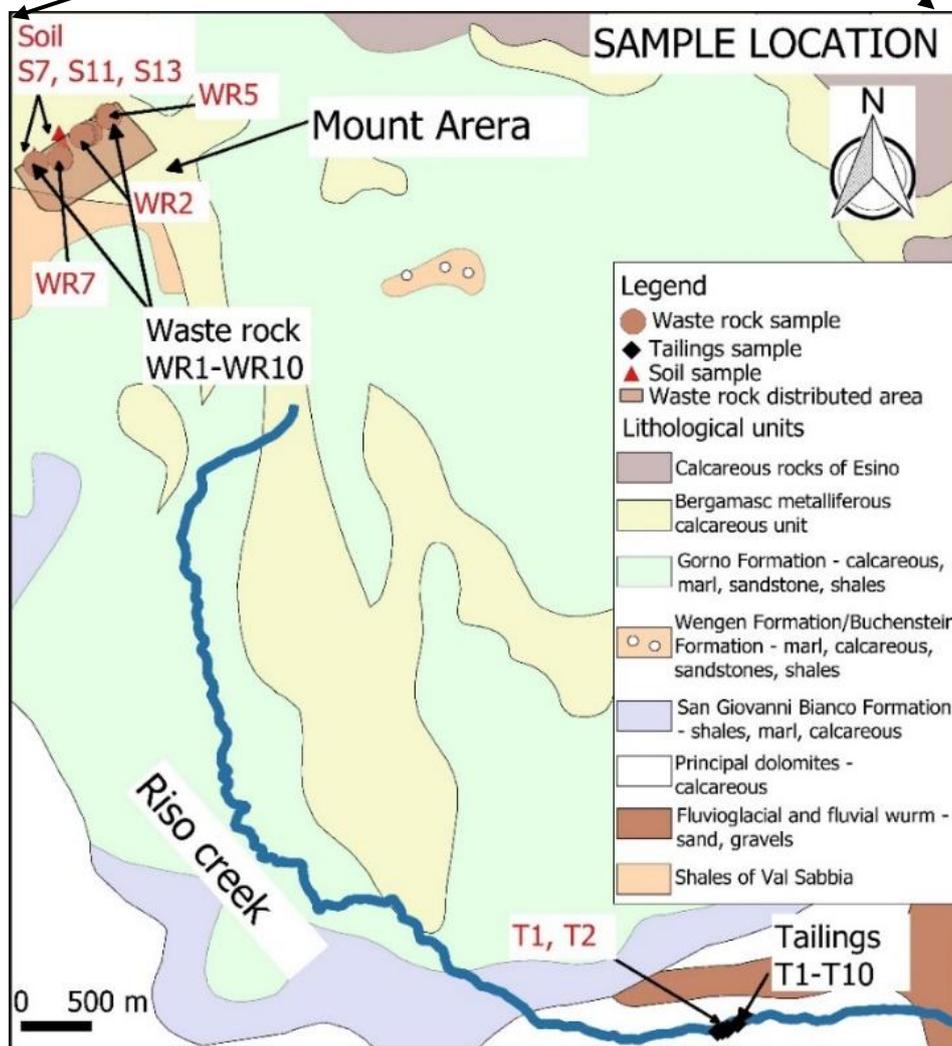
50 The abandoned mine site is located in the Gorno mining district comprising of the Seriana, Riso,
51 and Brembana valleys (Lombardy, northwest Italy). The site is composed of the Triassic carbonate
52 rocks of the central southern Alps (**Fig.1**) which has been formed due to crustal movements and
53 volcanic activities (Hou et al., 2016; Leach et al., 2010).

54 The site is rich in zinc-lead-silver stratabound ore deposits with mineralisation of Zn-Pb ± Ag ±
55 baryte ± fluorite mostly occurring within the “Metallifero” (i.e., “ore-bearing”) formation also
56 known as the Gorno formation (Dino et al., 2018). The primary mineralisation consists of sphalerite
57 (ZnS) and galena (PbS) (average Zn/Pb ratio= 5:1), with minor pyrite (FeS₂), marcasite (FeS₂),
58 chalcopyrite (CuFeS₂) and argentite (Ag₂S). The dominant gangue minerals are calcite (CaCO₃),
59 dolomite (CaMg(CO₃)₂), quartz (SiO₂) and ankerite (Ca(Fe,Mg,Mn)(CO₃)₂). The industrial
60 exploitation for Zn and Pb took place from year 1837-1982. The waste facility is in the vicinity of
61 the Mount Arera which is one of the biggest and most accessible facilities (Dino et al., 2018), is
62 used as case study here. For the treatment of the valuable ores, flotation process was used using
63 sodium based reagensts and fuel oil (information from historical documents maintained by the
64 Municipal Corporation of Gorno). Hence, the two main types of EW present at the site are waste
65 rock and tailings due to separation and treatment processes. The samples of waste rock (n=10),
66 tailings (n=10) and soil (n =3) were collected from the site (sampling procedure in **supplementary**
67 **material**).

68



69



70

71

72

Fig.1. Geological setting (modified from Beltrando et al., 2015) together with: insert showing geographical location, and the sample locations at Gorno. Sample numbers are shown in red font for samples analysed for bioaccessibility.

73 2.2 Analytical methods

74 The waste rock, tailings and soil samples were dried in an oven at 80°C until constant weight. All
75 samples were then sieved to <2 mm and quartered to obtain a representative sample size of 10 g.
76 The mineralogical analysis of waste rock, by Dino et al. (2018) showed presence of calcite,
77 dolomite, and sphalerite. Whilst, tailings contained calcite, dolomite, Fe sulphates and Pb
78 sulphosalts. The soil sample was studied for mineral phases and micromorphology in the present
79 study, using micro-X-ray fluorescence (micro-XRF) and Cambridge Stereoscan 360 scanning
80 electron microscope (SEM) coupled with energy-dispersive spectrometry. The pH was measured in
81 a 1:2.5 suspension of sample in water (ISO 10390:2005). The total concentrations of PTE in the <2
82 mm fraction were determined using *aqua regia* (U.S. EPA 3051A and U.S. EPA 6010C, 2007), for
83 each sample in duplicate.

84 Following the basic physico-chemical-mineralogical characterisation, we selected samples for
85 bioaccessibility analyses. Waste rock and soil samples were selected to ensure representation of
86 dumps and lithologies. For tailings, the two shallowest samples were selected. The total and
87 bioaccessible concentrations were determined on the <250 µm fraction using the *aqua regia*
88 extractions and UBM (BARGE 2010; Denys et al., 2012; ISO 17924:2018) respectively. **Table S1**
89 **(supplementary material)** summarises the measured values of the BGS102 extractions.
90 Bioaccessible concentration was considered as the highest concentration from gastric or
91 gastrointestinal phase, as this allowed HHRA estimations to be the conservative (“worst-case”)
92 scenario. Bioaccessible fraction is reported as the ratio of bioaccessible concentration compared
93 with the total concentration from the 250 µm fraction (**Eq. 1**). A non-specific sequential nitric acid
94 extraction (CISED) (Cave et al., 2004) was carried out on selected samples (n=2 each for waste
95 rock, tailings, and soil) to determine the solid phase distribution of elements. The detailed analytical
96 methods can be found in Mehta et al. (2019).

$$97 \text{BAF (\%)} = \frac{\text{Bioaccessible concentration of element } \left(\frac{\text{mg}}{\text{kg}}\right)}{\text{Total concentration of element } \left(\frac{\text{mg}}{\text{kg}}\right)} \times 100 \quad (1)$$

2.3 Human health risk assessment for oral ingestion

The APAT-ISPRA, 2008 (Italian databases to conduct risk assessment) were used to conduct the HHRA for two scenarios: (1) worst case scenario based on a conservative approach for residential area (i.e. taking exposure frequency (EF) as 350 days a year for 24 hours a day), and (2) a more realistic site-specific human activity patterns assuming recreation exposure scenario, during which the number of outdoor weeks were limited to 32 weeks a year (mid March to mid November) and 3 hours a day of outdoor time (APAT-ISPRA, 2008; Bharadwaj and Machibroda, 2008). The numerical estimation for risks due to non-carcinogenic PTE was evaluated as the hazard quotient (HQ) and for carcinogens as cancer risk (CR) due to the ingestion (Eq. 4 and 5). The total PTE concentrations on the fraction (<250 µm) were used as the concentration at source (C_s). It should be noted that the Italian regulation suggests to use total concentration on <2 mm fraction normalised to weight of <20 mm fraction. However, as the oral bioaccessibility analysis using UBM was performed on <250 µm samples in this study, both the BAF and the risk calculations were determined for that fraction for consistency. This is further supported by the U.S. EPA (2000) approach which reported that the <250 µm fraction is the main fraction that adheres to human skin and results in incidental ingestion. The average daily dose ADD was calculated by considering receptor as children and adults, (BW, body weight for adult: 70 kg and children: 15 kg; Eq. 2) and then adjusting it to ADD_{adjusted} following APAT-ISPRA, (2008) (Eq. 3).

$$ADD = C_s \times (IR \times EF \times ED \times 10^{-6}) / (BW \times AT) \quad (2)$$

$$ADD_{adjusted} = \begin{cases} ADD \text{ children} + ADD \text{ adults} & \text{for carcinogens} \\ ADD \text{ children} & \text{for noncarcinogens} \end{cases} \quad (3)$$

where, IR is the ingestion rate (adults: 100 mg/day and children: 200 mg/day), ED is exposure duration (children: 6 years; adults: 24 years), AT is averaging time (for non-carcinogens: ED, carcinogens: 70 years) and 10⁻⁶ is for unit conversion,

$$HQ = ADD_{adjusted} / RfD \quad (4)$$

$$122 \quad CR = ADD_{\text{adjusted}} \times CSF \quad (5)$$

123 Ministero dell'ambiente e della tutela del territorio e del mare, 2006, decree no. 152/06 (The
124 Ministry for Environment, Land and Sea Protection of Italy) was referred for reference dose (RfD)
125 and Cancer Slope Factor (CSF).

126 As exposure to two or more contaminants may lead to accumulative effects, thus HQ can be
127 summed as hazard index (HI) (**Eq. 6**), $HI < 1$ is considered that there is no significant risk of non-
128 carcinogenic effects, whereas $HI > 1$ is considered that there is a chance of non-carcinogenic effects,
129 with a probability to increase as the HI value increases (U.S. EPA, 2011). Cancer risk, $CR < 1 \times 10^{-6}$
130 and $CR_{\text{total}} < 1 \times 10^{-5}$ (summation of cancer risks of individual contaminant as expressed in **Eq. 7**), is
131 generally regarded as acceptable.

132 For incorporating oral bioavailability in the HHRA (**Eq. 8, 9**), the ADD_{adjusted} was multiplied by the
133 relative bioavailability (RBA) (i.e. the ratio of bioavailable fraction from the soil and bioavailable
134 fraction from the dosing medium (NFESC, 2000)). However, as the bioaccessible fraction is \geq
135 bioavailable fraction, the BAF can be used to replace the bioavailable fraction from the soil
136 (ABA_{soil}). This said, in this study, we assumed the value of the bioavailable fraction from the dosing
137 medium (ABA_{medium}) equal to 1. Thus, the exposure estimate (soil ingestion) was corrected (**Eq. 10**)
138 when estimating the HQ and CR. Similar methodology was applied by Luo et al. (2012) and Cao et
139 al. (2020) for incorporating oral bioaccessibility in HHRA due to PTE contamination in urban parks
140 of China and e-waste burning site in Accra, Ghana, respectively.

$$141 \quad HI = \sum_{m=1}^i HQ_m \quad (6)$$

$$142 \quad CR_{\text{total}} = \sum_{m=1}^i CR_m \quad (7)$$

$$143 \quad ADD_{\text{adjusted bio}} = ADD_{\text{adjusted}} \times RBA \quad (8)$$

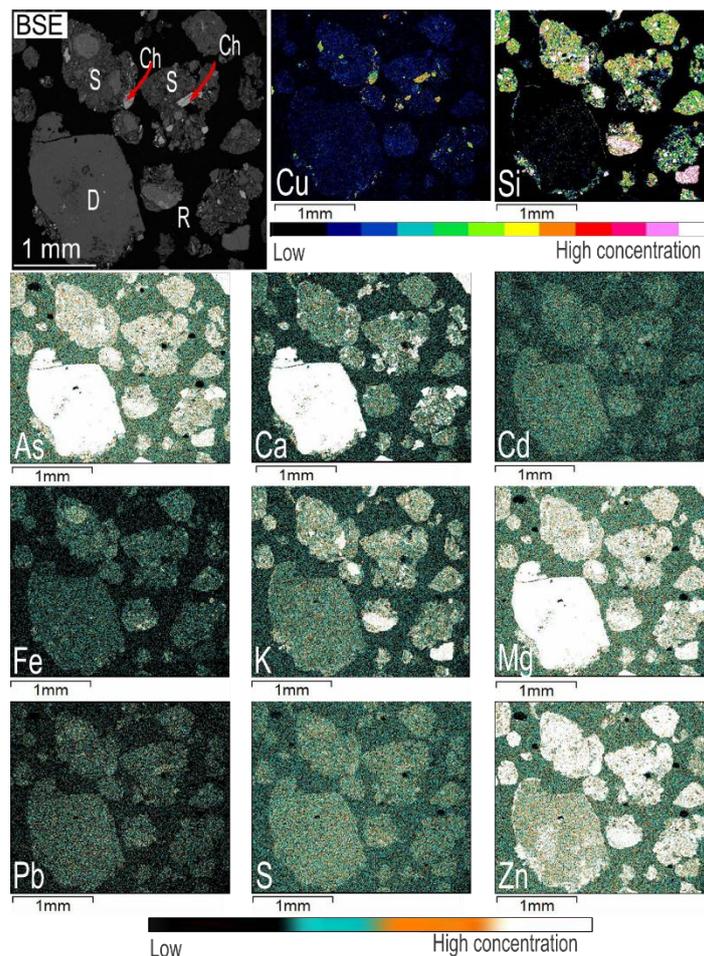
$$144 \quad ADD_{\text{adjusted bio}} = ADD_{\text{adjusted}} \times (ABA_{\text{soil}} / ABA_{\text{medium}}) \quad (9)$$

$$145 \quad ADD_{\text{adjusted bio}} = ADD_{\text{adjusted}} \times BAF \text{ (in \%)} / 100 \quad (10)$$

146 **3 Results and discussion**

147 **3.1 Mineralogical analysis**

148 Semi quantitative analysis using micro-XRF demonstrated presence of calcite, dolomite, zinc oxide,
149 and sphalerite as abundant minerals in soil (sample code – S7). The minor minerals were galena,
150 lead oxide, pyrite, and smithsonite. The results from SEM analysis (**Fig.2**) showed that As was
151 present in abundance in the grains rich in Ca and Mg (dolomite rich). Arsenic was also associated
152 with the grains rich in Fe, exhibiting strong presence of As in Fe rich phases, because the adsorption
153 of As species occurs by ligand exchange of As for OH₂ and OH groups on Fe oxide hydroxide
154 surfaces (Filippi et al., 2015; Jain et al., 1999). Arsenic was also present in grains rich in clay
155 minerals (indicated by presence of As-K-Mg silicates). Copper mainly occurred as chalcopyrite
156 (CuFeS₂). Careful visual examination of Cd map shows that it was sorbed on chalcopyrite.
157 Chalcopyrite tends to host trace elements such as Cd in sulphide ore deposits (Clark et al., 2001).
158 Lead was present as galena, PbO, PbCO₃ and amorphous sulphides. Zinc was observed as zinc
159 oxide, Cu-Fe-Zn-S phase, hemimorphite, sphalerite, smithsonite, and embedded in clay minerals
160 (Zn-K-Mg-Si).



161

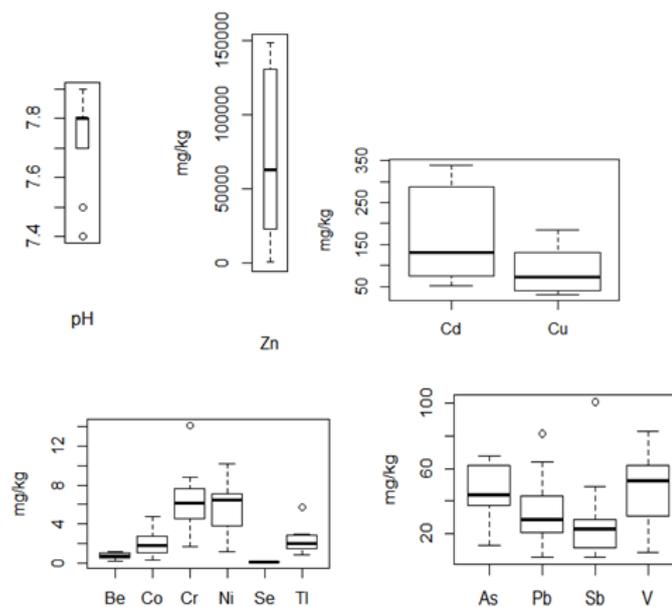
162 **Fig.2.** Elemental distribution and composition of soil (sample code -S7) - Back scattered electron
 163 (BSE) image showing Ch: chalcopyrite, D: dolomite, R: resin, S: sphalerite and zinc oxide and
 164 corresponding X-ray maps (SEM) for Cu, Si, As, Ca, Cd, Fe, K, Mg, Pb, S, and Zn.

165

166 3.2 Total concentrations of PTE in the 2 mm fraction of the waste rock, tailings and soil

167 pH of the waste rock samples varied between 7.4 and 7.9 (**Fig.3**). Zinc was present in high levels
 168 compared to the Italian legislative limits with an average concentration of 73 900 mg/kg. This is
 169 because during the earlier mining operations at Gorno, Zn was extracted from zinc oxide and zinc
 170 silicates and not from sphalerite (Dino et al., 2018). This contributed to the waste rock samples with
 171 high concentrations of Zn from sphalerite (ZnS). The weathering of these waste rocks results in
 172 enrichment of the <2 mm fraction with secondary minerals of ZnS. Strong Cd enrichment was
 173 observed and this is explained by the presence of Zn as both elements belong to the Group 12 of the
 174 periodic table, therefore sharing similar physical-chemical properties including similar electron
 175 structures and ionisation potentials (Das et al., 1997). Strong geochemical associations between

176 both elements have been found also in other mining sites e.g. lead-zinc mines in Upper Silesia
 177 (Poland) and Zawar (India) (Anju and Banerjee, 2011; Ullrich et al., 1999).
 178 The concentrations of thallium (Tl) were found to be >1 (limit for green and residential areas),
 179 because Tl is an accompanying element to Pb-Zn ores and is introduced to the environment due to
 180 the processing of these ores (Lis et al., 2003). Total As concentrations ranged from 13 to 68 mg/kg,
 181 with mean concentration of 45 mg/kg. The possible reason could be that pyrite (FeS₂), galena (PbS),
 182 sphalerite (ZnS), marcasite (FeS₂) and chalcopyrite (CuFeS₂) are commonly known to contain As as
 183 an impurity (Yan-Chu, 1994). The average concentration of antimony (Sb) was 30 mg/kg. This can
 184 be attributed to the fact that As and Sb are metalloids belonging to Group 15 of the periodic table.
 185 They both have identical s²p³ outer orbital electron configuration which results in same range of
 186 oxidation states in environmental systems (-3 to +5) and thus similar behaviour (Wilson et al.,
 187 2010). Copper (Cu) was found to vary from 30 mg/kg to 184 mg/kg, with an average concentration
 188 of 88 mg/kg. The samples showed trace concentrations of the ferromagnesian elements i.e. Cr-Ni-
 189 V-Co, and Se due to carbonatic platform (Bullock et al., 2019; McLennan, 2001).
 190



191 **Fig.3.** pH and concentration of PTE in mg/kg in waste rock samples (fraction <2 mm).
 192

193 The tailings and soil samples had alkaline pH values (**Table 1**). For the tailings, the 0-15 cm,
194 sample of point 1 had considerably less PTE compared to the samples taken at increasing depths.
195 The plausible reasons are: (1) the top layer is essentially a soil layer while all the other samples
196 were tailings. These tailings were deposited in the area during processing of valuable ores; (2) the
197 leaching of PTE during rainfall events can result in greater contamination with increasing depth.
198 The tailings samples showed higher concentrations of As, Cd, Cu, Pb, Sb and Zn compared to the
199 permissible limits in Italy. The Cd concentration was as low as 0.1 mg/kg at the surface, but reached
200 to 24.2 mg/kg in sample at 125-175 cm depth. The concentration of Zn was 52 mg/kg at the
201 sampling point close the surface and reaching 8887 mg/kg at depth of 200 cm. The total As, Sb and
202 Cd in tailings ranged from 74 to 107 mg/kg, 17 to 319 mg/kg, and 14 to 88 mg/kg, respectively.
203 Soil samples were also found to be contaminated by As, Cd, Pb, Sb, Tl, V, and Zn, which can lead
204 to environmental problems and cause damage to human health due to ingestion.
205

Table 1. pH and concentration of PTE in soil and tailings samples from Gorno in size fraction < 2 mm.

Sample	Depth (cm)	pH	As	Be	Cd	Co	Cr	Cu	Ni	Pb	Sb	Tl	V	Zn
Limit 1			20	2	2	20	150	120	120	100	10	1	90	150
Limit 2			50	10	15	250	800	600	500	1000	30	10	250	1500
Tailings sampling point 1														
T1	0-15	6.9	8.0	0.4	0.1	2.6	5.9	9	8.7	879	1	0.07	41.4	52
T2	15-50	7.2	42.2	0.4	20.3	2.3	4.2	246	3.7	1005	116	0.07	30.1	7554
T3	125-175	7.5	45.3	0.4	24.2	1.4	3.5	219	1.7	1008	110	0.07	24.4	8887
T4	225-275	7.5	40.2	0.4	19.3	1.3	3.6	132	1.5	695	70	0.07	24.7	6847
Tailings sampling point 2														
T5	0-40	7.8	28.9	0.6	14.2	3.1	5.7	161	5.8	665	72	0.06	31.9	5443
T6	40-65	7.8	38.0	0.1	22.7	2.0	1.2	247	1.1	1064	126	0.07	14.5	8560
T7	215-250	7.6	44.6	0.4	21.3	1.3	3.7	201	1.5	944	1	0.19	25.0	8600
Tailings sampling point 3														
T8	0-20	7.9	7.2	0.5	0.13	2.5	6.4	13	6.8	441	6	0.06	34.2	314
T9	60-100	8.1	38.1	0.3	19.2	1.2	3.3	156	1.3	759	81	0.07	24.2	6808
T10	220-240	7.8	43.4	0.5	20.4	1.6	4.3	183	1.7	882	97	0.53	28.7	8131
Soil samples														
S7	0-15	6.6	107.0	2.1	17.2	13.7	60.1	37.4	29.8	255	13.9	<0.10	155.0	14 730
S11	0-15	6.9	73.9	1.9	169.0	8.9	32.0	118.0	17.2	139	56.1	3.96	147.0	84 300
S13	0-15	6.5	76.8	1.2	319.0	5.2	17.5	185.0	14.0	140	88.4	3.11	82.8	138 482

207 Legislative limits currently adopted in Italy for PTE concentrations in the soil, Limit 1 is intended for green and residential areas, while Limit 2 for commercial and industrial
208 areas (Ministero dell'ambiente e della tutela del territorio, 2006, decree no. 152/06). Values above legislative limits are in bold.

209 **3.3 Total and bioaccessible concentrations of PTE in the <250 µm fraction of the waste**
210 **rock, tailings, and soil**

211 The total concentrations of PTE for the <250 µm fraction were considerably higher than for the <2
212 mm fraction (**Table 2**) implying an increase in surface area and thus higher absorption of PTE to
213 particles (Yao et al., 2015).

214 Specifically, total As concentrations were found to be very high. However, for all the samples, the
215 bioaccessible concentrations of As were no more than 34% of the total concentrations. In contrast,
216 samples recorded BAF of Cd ranging from 72-98%. The total and bioaccessible concentrations of
217 Co and Cr were found to be very low (**supplementary material: Table S2**). The bioaccessible
218 concentrations of Cu in waste rock, tailings and soil samples were found to have mean value of 115
219 mg/kg (BAF 54%), 145 mg/kg (BAF 49%) and 65 mg/kg (BAF 41%) respectively. The mean
220 values of BAF for Ni was <35% , for all the samples.

221 The mean values of BAF of Zn were recorded as 91%, 75% and 80% for waste rocks, tailings and
222 soil samples, respectively. The range of the BAF reported here for the PTE were in close range with
223 the values reported by Roussel et al. (2010) and Pelfrêne et al. (2012) from a smelter contaminated
224 urban and agricultural soil in northern France.

225

226 **Table 2.** Total and bioaccessible concentrations (mg/kg) and bioaccessible fraction (BAF in %)
 227 evaluated on <250 μm size fraction.

Sample	As				Cd			Cu		
	pH	G	Total	BAF	G	Total	BAF	G	Total	BAF
Waste rock										
WR2	7.8	8.5	115	7	462	476	97	100	350	29
WR5	7.8	11.0	189	6	431	442	98	135	150	90
WR7	7.4	8.6	98	9	388	394	98	110	260	43
Tailings										
T1	6.9	5.0	15	34	1	1	80	6	19	32
T2	7.2	20.1	68	30	28	39	72	284	440	65
Soil										
S7	6.5	15.7	142	11	394	440	89	124	234	53
S11	6.6	6.1	115	5	29	36	80	11	44	25
S13	6.9	8.7	78	11	179	210	85	58	132	44
Sample	Ni				Pb			Zn		
	pH	G	Total	BAF	G	Total	BAF	G	Total	BAF
Waste rock										
WR2	7.8	12	19	60	31	198	16	128 044	143 302	89
WR5	7.8	10	20	49	36	128	28	116 996	124 575	94
WR7	7.4	7	25	30	96	177	54	113 343	124 875	91
Tailings										
T1	6.9	3	19	17	22	25	87	149	193	77
T2	7.2	2	8	37	1567	1945	81	7139	9739	73
Soil										
S7	6.5	17	37	48	139	215	65	130 759	154 680	85
S11	6.6	4	37	12	141	248	57	5304	7304	73
S13	6.9	6	24	26	84	136	61	82 340	100 530	82

228 BAF: Bioaccessible fraction calculated using Eq. 1, G: concentration of PTE extracted in gastric phase of UBM; Total:
 229 total concentration of PTE using *aqua regia* on <250 μm fraction.

230 3.4 Solid phase distribution of the elements across waste rock, tailings, and soil matrices

231 3.4.1. Waste rock and soil

232 The physico-chemical components for the most representative sample of waste rock and soil
 233 (sample codes - WR2 and S7) are in Fig.4. The heatmap for sample codes – WR5 and S13 is shown
 234 in supplementary material (Fig.S1). Chemometric data analysis identified 7 components in the
 235 waste rock sample and 9 components in the soil samples. Using the geochemical associations
 236 present at the site, six clusters were identified as follows:

237 Exchangeable: This cluster in waste rock was extracted over the HNO₃ concentrations of 0.01 M to
238 0.05 M and consisted of S (72%), Mg (12%), Si (5%), and Zn (4%) suggesting the dissolution of
239 sulphates present at the site (Heidel et al., 2011).

240 The exchangeable cluster in soil consisted of (Ca-S-Mg, Ca-Mg, Zn, Zn-Na components), with
241 majority of elements being released in distilled water and 0.01 M HNO₃ extractant indicating that
242 the site is rich in carbonate. The elemental composition was Zn (38%), Ca (25%), Mg (19%) and S
243 (6%). The elevated concentrations of Zn and Ca indicates the presence of weathered products from
244 minerals such as smithsonite (ZnCO₃), and/or hydrozincite (Zn₅(CO₃)₂(OH)₆) and hemimorphite
245 (Zn₄(Si₂O₇)(OH)₂·H₂O) and Ca rich minerals like dolomite and calcite.

246
247 Ca carbonate component in soil composed of Ca (69%), Zn (27%) and Mg (9%) and was extracted
248 by concentrations of HNO₃ ranging from 0.01 M to 0.1 M indicating the presence of Zn rich
249 minerals and calcium carbonate from the Bergamasc calcareous metalliferous lithological unit.

250
251 Site specific: This component of waste rock comprised of Zn (74%), S (17%), Mg (6%) and Ca
252 (2%). Presence of Zn and S suggests the presence of oxidation products of sphalerite, zinc oxide,
253 and sulfur rich minerals.

254
255 Higher carbonate: The extraction of waste rock at 0.1 M HNO₃ resulted in a higher carbonates
256 component principally consisting of S (41%), Mg (30%), Mn (14%), Zn (12%), Ca (1%) and a
257 minor percentage of Fe. The presence of Ca-Fe-Mg-Mn implies dissolution of ankerite present as
258 gangue mineral in the site. The higher carbonate component in soil principally consisted of Ca
259 (59%) and Zn (30%) and was extracted at 0.1 M HNO₃.

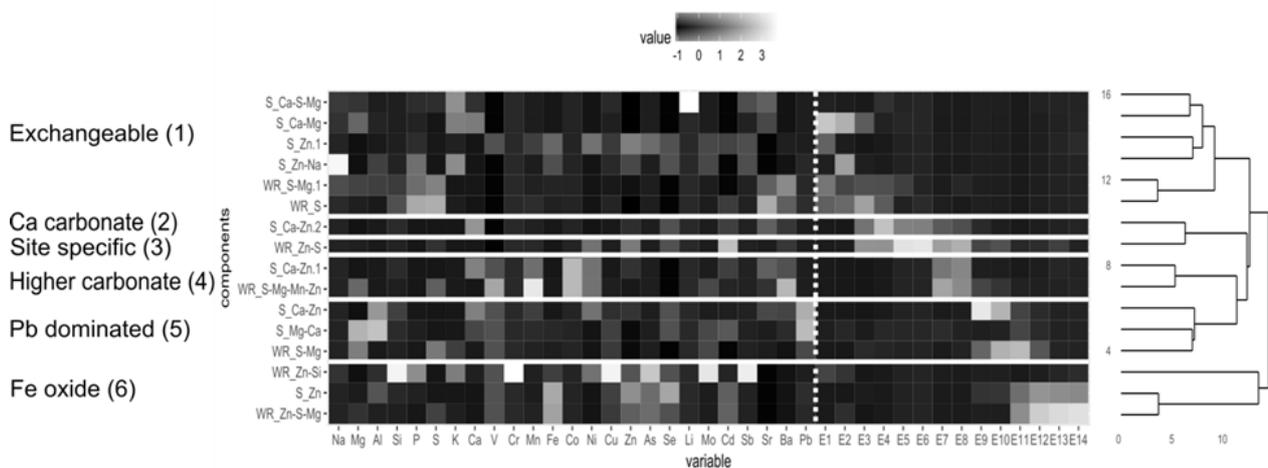
260
261 Pb dominated component consisted of S (54%), Mg (37%), Zn (4%) and Ca (3%) in waste rock.
262 Lead concentration in this component was highest and equal to 77% of total CISED extracted Pb.

263 This could be due to the occurrence of galena (PbS), which was present with sphalerite in minor
 264 association. The Pb dominated cluster in soil had high percentage of Ca (55%), Zn (27%), Mg (9%).
 265 The lead concentration in this cluster was 91%, while the other components in total consisted only
 266 9% of Pb.

267

268 Fe oxide: The Fe oxide cluster of waste rock comprised of Zn (57%), S (25%), Mg (12%) and Fe
 269 (3%) was extracted with acid concentration of 1 M to 5 M HNO₃ and H₂O₂. This component in soil
 270 consisted of Zn (85%), Fe (4%) and Mg (4%). The cluster recorded highest Fe concentrations and
 271 PTE extracted during the CISED extractions of both waste rock and soil samples. This was
 272 expected as the digestion in the last step results in dissolution of oxides.

273



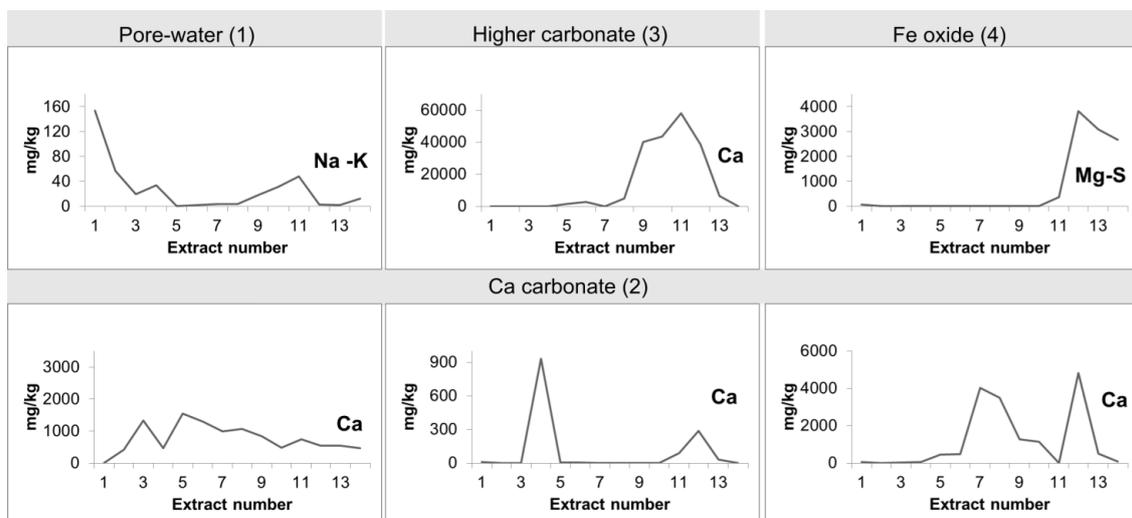
274 **Fig.4.** Heatmap and clustergram for CISED extracted WR and soil sample (sample code - WR2 and
 275 S7) of Gorno. The linkage between the components is shown in the dendrogram on the left hand
 276 side. Elemental composition data is on the left-hand side separated with a dashed vertical white line
 277 from the extraction number data (E1–14) on the right. The horizontal lines are used for dividing
 278 geochemical clusters. High concentrations are depicted by white/light grey and low concentrations
 279 by dark grey/black. Component names are provided with sample identification code (WR and S)
 280 along with the principal elements recorded for each component.

281 **3.4.2. Tailings**

282 The physico-chemical components present in selected sample from tailings (sample code - T2) are
283 presented in **Fig.5**. The tailings sample was subjected to separate chemometric data analysis, as
284 tailings were generated after processing of natural minerals, which led to variation in quantities and
285 mobility of elements when compared to waste rocks and soil. It was observed that CISED extracted
286 samples for tailings contained higher percentages of Na, as sodium based reagents were used for
287 flotation. The physico-chemical components of the tailings sample (sample code - T1) are in
288 **supplementary material (Fig.S2)**.

289 The pore-water component consisted of Na, K, and Pb and was mainly extracted by deionised
290 water. The Ca-carbonate cluster (Ca: 92%) was extracted from 0.5 M to 1 M, this is because the
291 carbonate form is a loosely bound phase and liable to change with environmental conditions
292 (Filgueiras et al., 2002). The higher carbonate consisted of Ca (96%). The presence of carbonates
293 could be due to dissolution of calcite and dolomite present at the site (Dino et al., 2018).

294 The Fe oxide component was principally formed by Mg, S, and Fe. It was extracted in
295 concentrations of HNO₃ ranging from 1 M to 5 M. The component consisted the majority of Fe
296 (94%) released during CISED extractions, due to presence of weathering product of Fe sulphate
297 observed in mineralogical analysis (Dino et al., 2018).



298
299
300
301

Fig.5. Extraction profiles obtained during CISED extractions of tailings (sample code – T2).

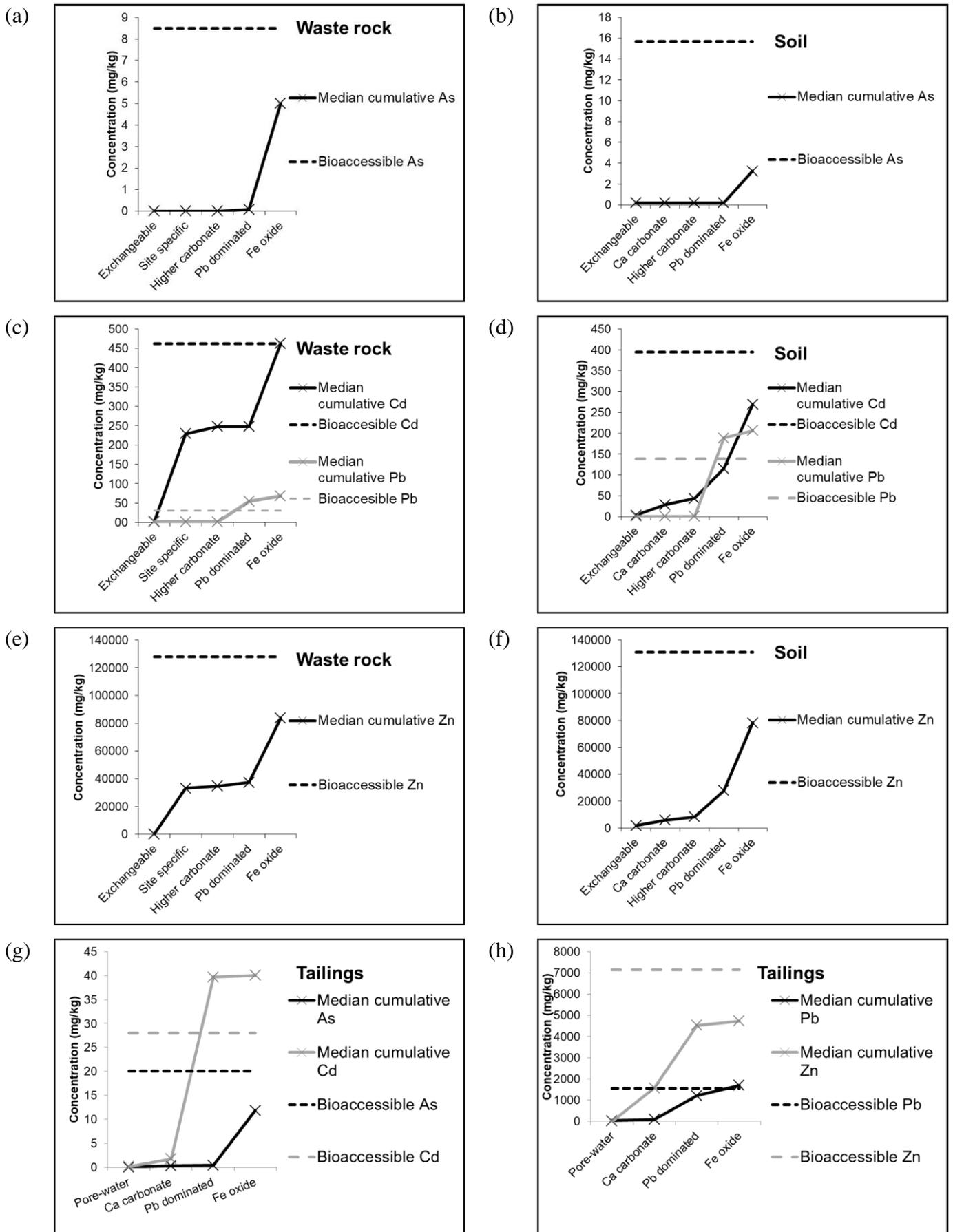
3.5 Comparing solid phase distribution and oral bioaccessibility for integrated assessment

Fig.6. exhibits the concentrations of As, Cd, Pb and Zn extracted in each CISED component and the bioaccessible concentration of elements. Dissolution of Fe oxide components contributed to the bioaccessible fractions of As. Bioaccessibility values of As in all samples were recorded to be low compared to total concentrations due to: (1) strong association between As and Fe oxides and Fe hydroxides, (2) presence of As with clay minerals, (3) co-occurrence of As and Ca rich minerals like calcite and dolomite (explained in **Section 3.1**). The high content of Ca^{2+} and high pH in waste rock, tailings and soil have a positive influence on the stability of Ca-Fe-As phases and thus these phases tend to be less labile and bioaccessible (Frau and Arda, 2004; Paktunc et al., 2004).

Dissolution of site specific and Fe oxide components led to the bioaccessibility fractions of Cd and Zn in waste rock. Whilst, dissolution of Pb dominated and Fe oxide components contributed to significantly high bioaccessible concentrations of Cd and Zn in soil. The Cd and Zn bioaccessible concentrations in tailings sample were contributed by higher carbonate components of CISED extractions. The bioaccessible concentrations of Cd and Zn were very high due to: (1) The calcareous geological context of area with high pH. Mineral phases that form under alkaline conditions tend to be less stable in the acidic conditions of the stomach and more bioaccessible (Ruby et al., 1999); and (2) Weathering of sulphide minerals in the presence of oxygen can result in speciation changes and the formation of secondary mineral phases that often have greater lability than the parent ore (Schneider et al., 2007). In contrast, the bioaccessible fraction for As was much less compared to Cd and Zn; demonstrating that even though all elements were present in the same geological context, the differences in BAF may occur due to associations of these metals with different minerals.

It was observed that partial dissolution of Pb dominated clusters in waste rock and soil resulted in bioaccessible fractions of Pb. Lead, showed lesser concentrations during UBM extractions than CISED. It has been studied that, the dissolution of greater amounts of carbonates by the acidic

327 gastric solutions can result in an increase of hydroxy carbonate anions available in solution, and that
328 under such conditions, Pb can form insoluble compounds with the anions (Reis et al., 2014).



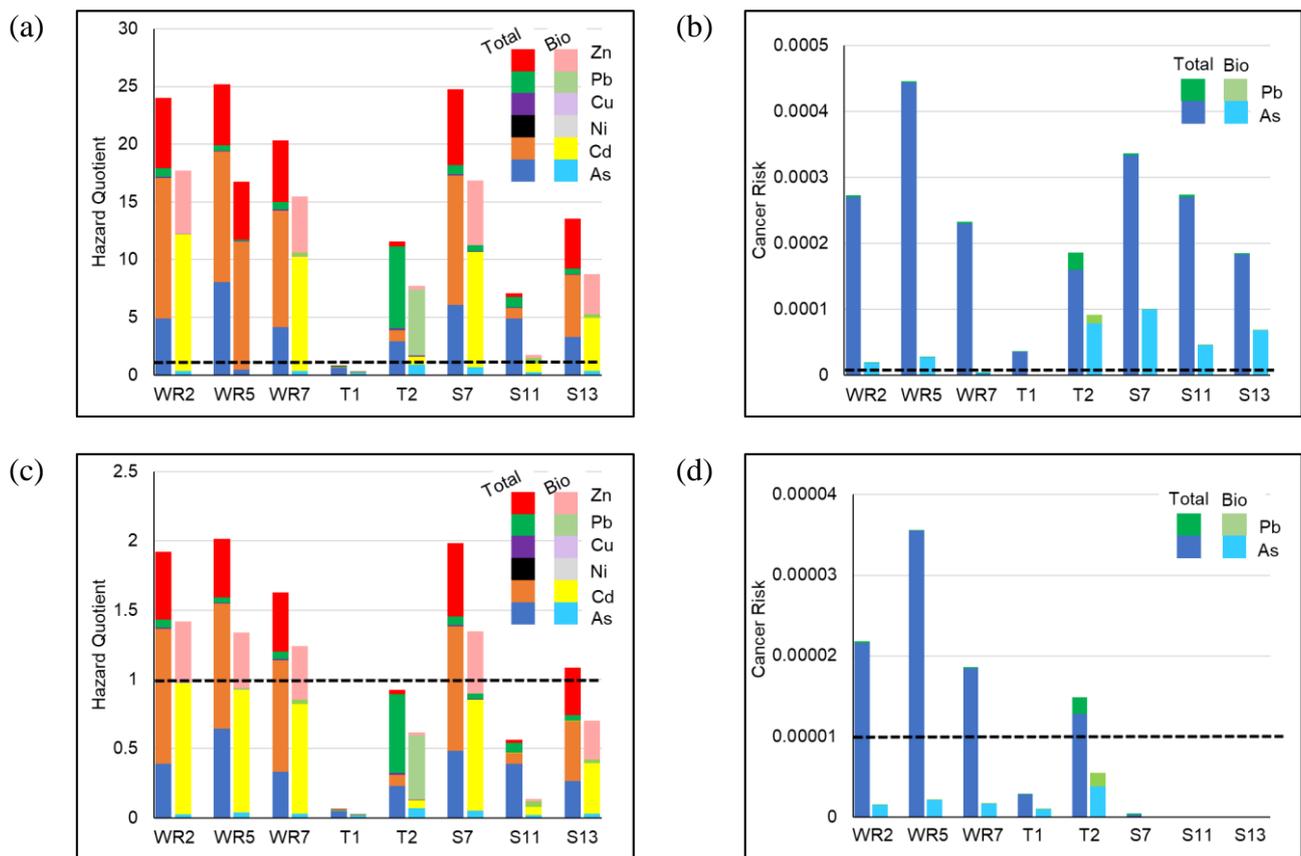
329 **Fig.6.** Concentration of PTE (mg/kg) in CISED extracted components and bioaccessible
 330 concentrations.

331 3.6. Indicative human health risk assessment

332 The noncarcinogenic HQ estimations were performed for As, Cd, Cu, Ni, Pb and Zn. The HI
333 defined as the summation of HQ, reached to a maximum of 25.2 considering the total
334 concentrations and to a maximum of 17.7 utilising bioaccessible concentrations, for the residential
335 exposure scenario (**Fig. 7a**). The carcinogenic risk evaluated for As and Pb implied that total cancer
336 risk (CR_{total}) reached a maximum of 4.4×10^{-4} which is greater than 1×10^{-5} , demonstrating
337 carcinogenic risk for total concentrations. For the bioaccessible concentrations the maximum value
338 was 2.7×10^{-5} .

339 For the recreational scenario, it was observed that the maximum HI for total and bioaccessible
340 concentrations was 2.0 and 1.4, respectively (**Fig. 7c**). The results also depicted that the site
341 surrounding T1, T2, S11, S13 had HI <1, for bioaccessible concentrations, implying no potential
342 non-carcinogenic risks. All the samples recorded $CR_{total} < 1 \times 10^{-5}$ using bioaccessible
343 concentrations. This indicates that incorporating bioaccessible concentrations in HHRA led to
344 absence of carcinogenic risks at all the sampling points and absence of potential noncarcinogenic
345 risks at more than half of the sampling points, as the HI and CR_{total} decreased by 25% and 90%
346 respectively.

347 It should be noted that all the risk calculations were performed using concentrations on size fraction
348 <250 μm for consistency, however the present study also reported lower total concentrations for
349 size fraction <2 mm than <250 μm (**Section 3.2 and 3.3**), implying that there could be
350 underestimation of potential risk, for oral pathway, if the calculations are based on size fraction <2
351 mm. Because of the influence of soil particle size on total concentrations, soil particle size should
352 be chosen properly for HHRA to avoid under- or overestimating human health risk (Cao et al.,
353 2020). Nevertheless, for robust decision making, the role of bioaccessibility in HHRA should not be
354 underestimated as noted by differences in potential risk due to total and bioaccessible
355 concentrations in the HHRA framework.



356 **Fig.7.** Human health risk for oral ingestion pathway, considering total and bioaccessible
 357 concentrations (sieved to <250 μm). Sample identification codes are on X-axis, and the potential
 358 risk values below the dashed black line on the Y-axis of the graph shows the risk values within
 359 acceptable levels. (a) Hazard quotient for residential scenario; (b) Cancer risk for residential
 360 scenario; (c) Hazard quotient considering recreation scenario; and (d) Cancer risk considering
 361 recreation scenario.

362 4 Conclusion

363 Waste rock, tailings and soil samples all had strong enrichment in PTE. The total PTE
 364 concentrations were higher in the <250 μm fraction than in the <2 mm fraction, implying higher
 365 specific surface area for the smaller size fractions. The mean value of the oral BAF across all the
 366 samples highlighted the following trend: Cd (87%) > Zn (83%) > Cu (48%) > Pb (56%) > Ni (35%)
 367 > As (14%). Determination of the solid phase distribution of the PTE along with mineralogical
 368 analysis and determination of the BAF allowed to assess the potential risks posed by abandoned
 369 mine sites. It further showed that pH, geological and lithological properties as well as the presence
 370 of carbonatic minerals, influenced strongly the PTE fractions. Finally, but not least, the study

371 demonstrated that role of bioaccessible concentrations on HHRA should not be underestimated and
372 therefore its inclusion is strongly recommended for future studies.

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382

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

none

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