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


Published in:
Chemosphere

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2020 Elsevier.
This manuscript is distributed under a Creative Commons Attribution-NonCommercial-NoDerivs License (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Download date: 27. Nov. 2021
Incorporating oral bioaccessibility into human health risk assessment due to potentially toxic elements in extractive waste and contaminated soils from an abandoned mine site

Neha Mehta¹,⁲*, Sabrina Cipullo³, Tatiana Cocerva⁴, Frederic Coulon³, Giovanna Antonella Dino², Franco Ajmone-Marsan⁵, Elio Padoan⁵, Siobhan Fiona Cox⁴, Mark R Cave⁶, Domenico Antonio De Luca²

¹Queen’s University Belfast, School of Mechanical and Aerospace Engineering, Belfast, BT9 5AH, UK
²University of Torino, Department of Earth Sciences, Torino, 10125, Italy
³Cranfield University, School of Water, Energy and Environment, Cranfield, MK43 0AL, UK
⁴Queen’s University Belfast, School of Natural and Built Environment, Belfast, BT9 5AG, UK
⁵University of Torino, Department of Agricultural, Forest and Food Sciences, Grugliasco, 10095, Italy
⁶British Geological Survey, Keyworth, Nottingham, NG12 5GG, UK

*Corresponding author: Neha Mehta (n.mehta@qub.ac.uk)

Graphical Abstract

Highlights

➢ As, Cd, Pb and Zn were present in high concentrations at abandoned mine (Gorno).
➢ Oral bioaccessibility of potentially toxic elements was highly variable.
➢ Calcareous geology, weathering and soil forming process influenced bioaccessibility.
➢ Oral bioaccessibility inclusion avoids over-conservative human health risk assessment.
Abstract

The waste rock, tailings and soil around an abandoned mine site in Gorno (northwest Italy) contain elevated concentrations of potentially toxic elements (PTE) exceeding the permissible limits for residential uses. Specifically, the maximum concentrations of As, Cd, Pb, and Zn were 107 mg/kg, 340 mg/kg, 1064 mg/kg, and 148 433 mg/kg, respectively. A site-specific human health risk assessment (HHRA) was conducted for residential and recreational exposure scenarios, using an approach based on Risk Based Corrective Action (RBCA) method, refined by incorporating oral bioaccessibility data.

Oral bioaccessibility analyses were performed by simulating the human digestion process in vitro (Unified BARGE Method). Detailed analysis of oral bioaccessible fraction (BAF i.e. ratio of bioaccessible concentrations to total concentrations on <250 μm fraction) indicated BAF of As (5-33%), Cd (72-98%), Co (24-42%), Cr (3-11%), Cu (25-90%), Ni (17-60%), Pb (16-88%) and Zn (73-94%). The solid phase distribution and mineralogical analyses showed that the variation of BAF is attributed to presence of alkaline calcareous rocks and association of PTE with a variety of minerals.

The HHRA for ingestion pathway, suggested that bioaccessibility-corrected cancer risk reached up to $2.7 \times 10^{-5}$ and $0.55 \times 10^{-5}$ for residential and recreational scenarios respectively (acceptable level is $1 \times 10^{-5}$). The hazard index (HI) recalculated after incorporation of oral bioaccessible concentrations for a residential scenario ranged from 0.02 to 17.9. This was above the acceptable level (>1) for 50% of the samples, indicating potential human health risks. This study provides information for site-specific risk assessments and planning future research.

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

Mining contributes to the contamination of ecosystems by generating extractive waste (EW), also known as mining waste (Nakaona et al.; 2019; Stewart, 2019). The EW from mineral extraction and processing has been accumulating for many years and the global EW production is estimated to be $350 \times 10^9$ tonnes per year (Vallero and Blight, 2019). Despite several measures have recently been implemented to manage EW in a more sustainable manner, release of PTE into the environment remains a concern, especially in areas with a legacy of historic mining. The Bureau de Recherches Géologiques et Minières (BRGM, French Geological Survey) estimates that there are more than 5200 million tonnes of waste rock and 1100 million tonnes of tailings stored in abandoned mines in the EU (BRGM, 2001). The high volume of EW in abandoned mines has led to human health concerns as these PTE tend to persist in the environment (Boente et al., 2020; Damian et al., 2018; de Souza et al., 2015; Delil and Koleli, 2019; Edokpayi et al., 2016; Karlsson et al., 2018; Kasemodel et al., 2019; Kaupilla et al., 2018; Khelifi et al., 2019; Petrella et al., 2019a; Petrella et al., 2019b; Väänänen et al., 2016; Wang et al., 2020; Yang et al., 2019).

A common approach used to assess the potential human health risks posed by contaminants is to conduct a human health risk assessment (HHRA). This entails determining the identity and extent of contaminants, characterising their toxicity and estimating the magnitude of their exposure to the local population (Adimalla, 2019; Gerba 2009; Zorpas 2020). Traditional HHRA methods use total concentrations of contaminants to assess potential risks. However, recent studies have emphasised that human health risks are associated with absorption of PTE into the systemic circulation (blood) (referred to as the bioavailable fraction) and indicated that consideration of total concentrations alone can result in overestimation of impacts on human health (Boim et al., 2019; Chi et al., 2020; Du et al., 2020; Fernández-Caliani et al., 2019; González-Grijalva et al., 2019; Ruby et al., 2002; Yu and Yang, 2019).

Maddaloni et al. (1998) conducted oral bioavailability studies to measure the absorption of soilborne Pb into humans. Several other studies have applied in vivo animal studies and analysed oral
bioavailable fractions (Bradham et al., 2016; Brattin and Casteel, 2013; Casteel et al., 2006; Juhasz et al., 2010; Kang et al., 2016; Suh et al., 2019). Nevertheless, assessing bioavailability requires complicated, lengthy and costly procedures that are also ethically constrained (Molina et al., 2013). This has resulted in the development of several in vitro bioaccessibility methods (Beauchemin et al., 2014) such as the Physiologically Based Extraction Test (PBET) by Ruby et al. (1996). The Unified BARGE method (UBM) was developed by the Bioaccessibility Research Group of Europe (BARGE), to harmonise oral bioaccessibility testing, which is now referenced as an ISO standard method (ISO 17924:2018). The oral bioaccessibility is defined as the fraction of a contaminant that is solubilised in the human gastrointestinal tract and is available for absorption (Wragg et al., 2011). The UBM method has been validated for in vivo studies for As, Cd, and Pb (Denys et al., 2009 and 2012) and is widely applied by environmental consulting services in France and England (Foulkes et al., 2017; Pelfrêne et al., 2012). Recently, Mehta et al. (2019) examined the BAF of PTE in samples from an abandoned nickel (Ni) mine in Italy. Although, many studies have demonstrated the importance of oral bioaccessibility in assessing human health risks, there is a paucity of research on the incorporation of oral bioaccessibility into HHRA at abandoned mine sites. Therefore, this study applies an oral bioaccessibility-correction for refining potential human health risks in an abandoned mine located in Gorno, northwest Italy. Specifically, the objectives were to: (1) determine the PTE bioaccessible fractions in waste rock, tailings and contaminated soils; (2) investigate the effects of mineralogy and solid phase distribution on the bioaccessible PTE fractions; and (3) perform bioaccessibility-corrected HHRA to calculate risks to human health via the oral pathway.

2 Methodology

2.1 Study site and sampling

The abandoned mine site is located in the Gorno mining district comprising of the Seriana, Riso, and Brembana valleys (Lombardy, northwest Italy). The site is composed of Triassic carbonate rocks of
the central southern Alps (Fig.1) that were formed due to crustal movements and volcanic activities (Hou et al., 2016; Leach et al., 2010). The study site for this investigation is a waste facility located in the vicinity of Mount Arera, one of the biggest and most accessible facilities in the region (Dino et al., 2018).

The site is rich in zinc-lead-silver stratabound ore deposits with mineralisation of Zn-Pb ± Ag ± baryte ± fluorite mostly occurring within the “Metallifero” (i.e. “ore-bearing”) formation, also known as the Gorno formation (Dino et al., 2018). The primary mineralisation consists of sphalerite (ZnS) and galena (PbS) (average Zn/Pb ratio = 5:1), with minor pyrite (FeS₂), marcasite (FeS₂), chalcopyrite (CuFeS₂) and argentite (Ag₂S). The dominant gangue minerals are calcite (CaCO₃), dolomite (CaMg(CO₃)₂), quartz (SiO₂) and ankerite (Ca(Fe,Mg,Mn)(CO₃)₂). The industrial exploitation for Zn and Pb took place between 1837 and 1982. A flotation process, using sodium based reagents and fuel oil, was applied for the treatment of valuable ores (information from historical documents maintained by the Municipal Corporation of Gorno). Hence, the two main types of EW present at the site are waste rock and tailings due to the separation and treatment processes. Samples of waste rock (n = 10), tailings (n = 10) and soil (n = 3) were collected (sampling procedure in Supplementary Material: S1).
Fig. 1. Geological setting with insert showing geographical location (modified from Beltrando et al., 2015) and the sample locations at Gorno. Sample numbers are shown in red font for samples analysed for bioaccessibility. WR: waste rock, T: tailings, and S: soil

2.2 Analytical methods

2.2.1 Physical, chemical and mineralogical characterisation
The waste rock, tailings and soil samples were dried in an oven at 80 °C until the weight remained constant. All samples were sieved to <2 mm and quartered to obtain a representative sample size of 10 g. The mineralogical analysis of waste rock, by Dino et al. (2018) showed presence of calcite,
dolomite, and sphalerite. Tailings contained calcite, dolomite, Fe sulphates and Pb sulphosalts. The soil samples were studied for mineral phases using micro-X-ray fluorescence (micro-XRF) Eagle III-XPL spectrometer equipped with an EDS Si (Li) detector and with an EdaxVision32 microanalytical system. Detailed micromorphology was conducted using a Cambridge Stereoscan 360 scanning electron microscope (SEM) coupled with energy-dispersive spectrometry on soil sample (Code – S7). All samples were analysed for pH using a 1:2.5 suspension of sample in water (ISO 10390:2005). The total concentrations of PTE in <2 mm fraction were determined using *aqua regia* (U.S. EPA 3051A, 2007; U.S. EPA 6010C, 2007). Briefly, 0.5 g of sample was digested using concentrated HNO₃ and HCl (1 HNO₃: 3 HCl). The concentrations of extracted PTE were measured using an Ametek Spectro Genesis Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) provided with an Ametek monochromator, a cyclonic spray chamber and a TeflonMira Mist nebulizer. All reagents used were of analytical grade. High-purity water from a Millipore Milli-Q Academic system was used. All samples were analysed in duplicate.

**2.2.2 Oral bioaccessible concentrations**

Following the basic physical-chemical-mineralogical characterisation, samples were selected for bioaccessibility analyses. Waste rock and soil samples were selected to ensure a representation of each dump and lithology from the Gorno site, so that results can be representative of the area. For tailings, the two shallowest samples were selected. The samples were sieved to <250 µm fraction and analysed for total and bioaccessible concentrations using *aqua regia* extractions and UBM, respectively (BARGE 2010; Denys et al., 2012; ISO 17924:2018). For quality control, 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 in *(Supplementary Material: S2)* summarises the measured and certified values of the BGS102 extractions. The extractions consisted of two phases: gastric and gastrointestinal. Bioaccessible concentration was considered as the highest concentration from gastric or
gastrointestinal phase, as this allowed HHRA estimations to be a “worst-case” scenario. Bioaccessible fraction is reported as the ratio of bioaccessible concentration compared with the total concentration from the 250 µm fraction (Eq. 1).

\[
BAF(\%) = \frac{\text{Bioaccessible concentration of element (mg kg\(^{-1}\))}}{\text{Total concentration of element (mg kg\(^{-1}\))}} \times 100
\]  

(1)

2.2.3 Non-selective sequential extractions

Potentially toxic elements occur in soil in association with a complex mixture of chemical components, e.g. mineral phases; pore-water; and organic matter. Consequently, the occurrence and relative distribution of PTE among these components control PTE dissolution and hence bioaccessibility (Ettler et al., 2018; Ettler et al., 2019; Gattullo et al., 2020; Vasiluk et al., 2019). Solid phase distribution in this study was performed by a non-selective method coupled to the chemometric identification of substrates and element distributions (CISED) method (Cave et al. 2004; Cipullo et al., 2018). The CISED method prevents resorption of elements to soil during extraction process due to the short time the extractant is in contact with samples. Moreover, it provides necessary information on the presence of PTE within various matrices. The mechanistic information about PTE solid phase associations can help in understanding leachability, solubility and mobility of PTE, elucidating the effects of mineralogical forms for in vitro bioaccessibility (CIEH, 1999; Cox et al., 2013; Palumbo-Roe and Klinck, 2007; Schaider et al., 2007).

The CISED extractions were carried out on selected samples (n = 2 each for waste rock, tailings, and soil) to determine the distribution of elements. Approximately 2 g of sample was sequentially extracted with 10 ml of extractant. The solution was mixed for 10 min in an end-over-end shaker, centrifuged (4350 g for 5 min) and the resultant leachate (liquid fraction) was collected in a clean sample bottle. The next extraction solution was added to the same sample and the process repeated. Seven different solutions were used twice (deionised water, 0.01M, 0.05M, 0.1M, 0.5M, 1.0M and
5.0M HNO$_3$), with progressive addition of H$_2$O$_2$ (0.25, 0.50, 0.75 and 1 ml) in the last four solutions for precipitation of oxides. The recovered liquid fraction was filtered with a 0.45 μm 25 mm nylon syringe filter and diluted four times with deionised water for elemental analysis using ICP-MS (NexION® 350D ICP-MS, Perkin Elmer). For quality control, acid blanks (1% nitric acid) and guidance material (BGS102) were included in extraction. The solid phase distribution of major and trace elements was determined using a self-modelling mixture resolution algorithm (SMMR) developed by Cave et al. (2004) in MatLab® Version R2015. The detailed analytical methods can be found in Mehta et al. (2019).

2.3 Human health risk assessment for ingestion

The Risk Based Corrective Action (RBCA) guidelines (ASTM 1995; ASTM 2015); and the exposure frequencies from the U.S. EPA (2002) and the APAT-ISPRRA (2008) (Italian databases to conduct risk assessment) were used to conduct HHRA for two scenarios: (1) a worst case scenario for residential area i.e. taking exposure frequency (EF) as 350 days a year for 24 hours a day (U.S. EPA 1991), and (2) a more realistic recreation scenario, with site-specific human activity patterns assumed, for 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-ISPRRA, 2008; Bharadwaj and Machibroda, 2008). The numerical estimation of risks due to ingestion of non-carcinogenic PTE was evaluated as hazard quotient (HQ) and for carcinogens as cancer risk (CR) (Eq. 4 and 5). The total PTE concentrations on <250 μm size fraction 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 <250 μm fraction without normalisation, for consistency. This is further supported by the U.S. EPA (2007 and 2008), approach which reported that the <250 μm fraction is the main fraction that adheres to
human skin and results in incidental ingestion. Average daily dose (ADD) was calculated by considering the receptor to be both children and adults, body weight (BW) 70 kg for adult and 15 kg for children – U.S. EPA (1991), (Eq. 2) and then adjusting it to \( \text{ADD}_{\text{adjusted}} \) following APAT-ISPR (2008) (Eq. 3).

\[
\text{ADD} = C_s \times \left( \frac{IR \times EF \times ED \times 10^{-6}}{BW \times AT} \right) \tag{2}
\]

\[
\text{ADD}_{\text{adjusted}} = \begin{cases} 
\text{ADD}_{\text{children}} + \text{ADD}_{\text{adults}} & \text{for carcinogens} \\
\text{ADD}_{\text{adults}} & \text{for non-carcinogens}
\end{cases} \tag{3}
\]

where, \( IR \) is ingestion rate (adults: 100 mg/day and children: 200 mg/day - U.S. EPA (2002)), \( ED \) is exposure duration (children: 6 years; adults: 24 years - U.S. EPA (1991)), \( AT \) is averaging time (for non-carcinogens: \( ED \), carcinogens: 70 years) and \( 10^{-6} \) is for unit conversion,

\[
HQ = \frac{\text{ADD}_{\text{adjusted}}}{RfD} \tag{4}
\]

\[
CR = \text{ADD}_{\text{adjusted}} \times CSF \tag{5}
\]

Reference dose (RfD) is 0.0003, 0.0005, 0.0003, 1.5, 0.04, 0.02, 0.0035, and 0.3 mg/kg-d for As, Cd, Co, Cr, Cu, Ni, Pb, and Zn, respectively. Cancer slope factors (CSF) for As and Pb are 1.5 and 0.0085 (mg/kg-d)^{-1} (U.S. EPA IRIS, 2019). These CSF and RfD values are also in accordance with Ministero dell'Amiente e della Tutela del Territorio e del Mare, 2006, decree no. 152/06 (The Ministry for Environment, Land and Sea Protection of Italy, 2006).

As exposure to two or more contaminants may result in accumulative effects, HQ can be summed as hazard index (HI) (Eq. 6). A, HI <1 represents no significant risk of non-carcinogenic effects, whereas HI >1 represents a chance of non-carcinogenic effects, with an increase in probability as HI increases (U.S. EPA, 2011). Cancer risk (CR) of \( CR <1 \times 10^{-6} \) and \( CR_{\text{total}} <1 \times 10^{-5} \) is generally regarded as acceptable, where \( CR_{\text{total}} \) is the summation of cancer risks of individual contaminants as expressed in Eq. 7.
For incorporating oral bioavailability in HHRA (Eq. 8 and 9), $ADD_{\text{adjusted}}$ must be multiplied by
relative bioavailability (RBA) (i.e. the ratio of absolute bioavailability of the test material ($ABA_{\text{test}}$)
to the absolute bioavailability of the reference dose material ($ABA_{\text{reference}}$) (Vasiluk et al., 2011). However, as bioaccessibility $\geq$ bioavailability, BAF can be used as conservative proxy to replace
$ABA_{\text{test}}$. In this study, $ABA_{\text{reference}}$ was assumed equal to 1. Thus, exposure estimate (soil ingestion)
was corrected when estimating HQ and CR (Eq. 10). A similar methodology was applied by Luo et
al. (2012) and Cao et al. (2020) for incorporating oral bioaccessibility in HHRA due to PTE
contamination in urban parks of China and e-waste burning site in Accra, Ghana, respectively.

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

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

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

$$ADD_{\text{adjusted bio}} = ADD_{\text{adjusted}} \times \left(\frac{ABA_{\text{test}}}{ABA_{\text{reference}}}\right) \quad (9)$$

$$ADD_{\text{adjusted bio}} = ADD_{\text{adjusted}} \times \left(\frac{BAF \text{ in } \%}{100}\right) \quad (10)$$

3 Results and discussion

3.1 Mineralogical analysis

Semi quantitative analysis using micro-XRF demonstrated presence of calcite, dolomite, zinc oxide, and sphalerite as abundant minerals in soil. The minor minerals were galena, lead oxide, pyrite, and smithsonite. The results from SEM analysis on soil (sample code – S7) (Fig. 2) showed that As was associated with Fe rich grains, suggesting strong presence of As in Fe rich phases as the adsorption of As species occurs by ligand exchange of As for OH$_2$ and OH groups on Fe oxide hydroxide surfaces.
Arsenic was present within grains rich in Ca and Mg (dolomite rich), which can be explained by surface precipitation of small amounts of poorly ordered Fe (hydr)oxides with high specific surface area and adsorption capacity to dolomite rich minerals. Arsenic was also present in grains rich in clay minerals (indicated by presence of As-K-Mg silicates). Copper mainly occurred as chalcopyrite (CuFeS$_2$). Careful visual examination of the Cd map shows that Cd was also associated with chalcopyrite. Chalcopyrite tends to host trace elements such as Cd in sulphide ore deposits (Clark et al., 2001). Lead was present as galena, PbO, PbCO$_3$ and amorphous sulphides. Zinc was observed as zinc oxide, Cu-Fe-Zn-S phase, hemimorphite, sphalerite, smithsonite, and with clay minerals (Zn-K-Mg-Si).

**Fig.2.** Elemental distribution and composition of soil (sample code –S7) - Back scattered electron (BSE) micrograph showing Ch: chalcopyrite, D: dolomite, R: resin, S: sphalerite and zinc oxide and corresponding X-ray maps (SEM) for Cu, Si, As, Ca, Cd, Fe, K, Mg, Pb, S, and Zn.
3.2 Total concentrations of PTE in the 2 mm fraction of the waste rock, tailings and soil

The pH of waste rock samples varied between 7.4 and 7.9 (Fig.3). Zinc was present in high levels compared to Italian legislative limits with an average concentration of 73,900 mg/kg. This occurred due to early mining operations at Gorno where Zn was extracted from zinc oxide and zinc silicates and not from sphalerite (Dino et al., 2018), contributing to waste rock dumps with high concentrations of Zn from sphalerite (ZnS). The weathering of these waste rocks results in enrichment of the <2 mm fraction with secondary minerals of ZnS. Strong Cd enrichment (range 52-340 mg/kg) was observed due to the chemical similarity with Zn (Das et al., 1997). Strong geochemical associations between both elements have been found also in other mining sites e.g. lead-zinc mines in Upper Silesia (Poland) and Zawar (India) (Anju and Banerjee, 2011; Ullrich et al., 1999).

The concentrations of thallium (Tl) were found to be >1 mg/kg (limit for green and residential areas) as Tl is an accompanying element to Pb-Zn ores and can be introduced to the environment due to processing of these ores (Lis et al., 2003). Total As concentrations ranged from 13 to 68 mg/kg, with mean concentration of 45 mg/kg. The possible reason could be that pyrite (FeS$_2$), galena (PbS), sphalerite (ZnS), marcasite (FeS$_2$) and chalcopyrite (CuFeS$_2$) are commonly known to contain As as an impurity (Yan-Chu, 1994). The average concentration of antimony (Sb) was 30 mg/kg. This can be attributed to the fact that As and Sb share chemical features resulting in similar behaviour (Wilson et al., 2010). Copper (Cu) was found to vary from 30 mg/kg to 184 mg/kg, with an average concentration of 88 mg/kg. The samples showed trace concentrations of the ferromagnesian elements i.e. Cr-Ni-V-Co, and Se due to carbonatic platform (Bullock et al., 2019; McLennan, 2001).
Tailings and soil samples had alkaline pH values (Table 1). Tailings collected from 0-15 cm depth, had considerably less PTE compared to samples taken at increasing depths. The plausible reasons are: (1) top layer is essentially a soil layer while all other samples were tailings. These tailings were deposited in the area during processing of valuable ores; (2) leaching of PTE during rainfall events can result in greater contamination with increasing depth.

Tailings showed higher concentrations of As, Cd, Cu, Pb, Sb and Zn compared to the permissible limits in Italy. The Cd concentration was as low as 0.1 mg/kg at the surface but reached to 24.2 mg/kg at 125-175 cm depth. The concentration of Zn was 52 mg/kg at the sampling point close the surface and reaching 8887 mg/kg at 200 cm depth. The total As, Sb, and Cd concentrations in tailings ranged from 74 to 107 mg/kg, 17 to 319 mg/kg, and 14 to 88 mg/kg, respectively. Soil samples were also found to be contaminated by As, Cd, Pb, Sb, Tl, V, and Zn, which can result in environmental problems and cause damage to human health due to ingestion.
Table 1. pH and concentration of PTE in soil and tailings samples from Gorno in size fraction <2 mm.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Depth (cm)</th>
<th>pH</th>
<th>As</th>
<th>Be</th>
<th>Cd</th>
<th>Co</th>
<th>Cr</th>
<th>Cu</th>
<th>Ni</th>
<th>Pb</th>
<th>Sb</th>
<th>Tl</th>
<th>V</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit 1</td>
<td></td>
<td>20</td>
<td>2</td>
<td>2</td>
<td>20</td>
<td>150</td>
<td>120</td>
<td>120</td>
<td>100</td>
<td>10</td>
<td>1</td>
<td>90</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Limit 2</td>
<td></td>
<td>50</td>
<td>10</td>
<td>15</td>
<td>250</td>
<td>800</td>
<td>600</td>
<td>500</td>
<td>1000</td>
<td>30</td>
<td>10</td>
<td>250</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Tailings sampling point 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0-15</td>
<td>6.9</td>
<td>8.0</td>
<td>0.4</td>
<td>0.1</td>
<td>2.6</td>
<td>5.9</td>
<td>9</td>
<td>8.7</td>
<td>879</td>
<td>1</td>
<td>0.07</td>
<td>41.4</td>
<td>52</td>
</tr>
<tr>
<td>T2</td>
<td>15-50</td>
<td>7.2</td>
<td>42.2</td>
<td>0.4</td>
<td>20.3</td>
<td>2.3</td>
<td>4.2</td>
<td>246</td>
<td>3.7</td>
<td>1005</td>
<td>116</td>
<td>0.07</td>
<td>30.1</td>
<td>7554</td>
</tr>
<tr>
<td>T3</td>
<td>125-175</td>
<td>7.5</td>
<td>45.3</td>
<td>0.4</td>
<td>24.2</td>
<td>1.4</td>
<td>3.5</td>
<td>219</td>
<td>1.7</td>
<td>1008</td>
<td>110</td>
<td>0.07</td>
<td>24.4</td>
<td>8887</td>
</tr>
<tr>
<td>T4</td>
<td>225-275</td>
<td>7.5</td>
<td>40.2</td>
<td>0.4</td>
<td>19.3</td>
<td>1.3</td>
<td>3.6</td>
<td>132</td>
<td>1.5</td>
<td>695</td>
<td>70</td>
<td>0.07</td>
<td>24.7</td>
<td>6847</td>
</tr>
<tr>
<td>Tailings sampling point 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>0-40</td>
<td>7.8</td>
<td>28.9</td>
<td>0.6</td>
<td>14.2</td>
<td>3.1</td>
<td>5.7</td>
<td>161</td>
<td>5.8</td>
<td>665</td>
<td>72</td>
<td>0.06</td>
<td>31.9</td>
<td>5443</td>
</tr>
<tr>
<td>T6</td>
<td>40-65</td>
<td>7.8</td>
<td>38.0</td>
<td>0.1</td>
<td>22.7</td>
<td>2.0</td>
<td>1.2</td>
<td>247</td>
<td>1.1</td>
<td>1064</td>
<td>126</td>
<td>0.07</td>
<td>14.5</td>
<td>8560</td>
</tr>
<tr>
<td>T7</td>
<td>215-250</td>
<td>7.6</td>
<td>44.6</td>
<td>0.4</td>
<td>21.3</td>
<td>1.3</td>
<td>3.7</td>
<td>201</td>
<td>1.5</td>
<td>944</td>
<td>1</td>
<td>0.19</td>
<td>25.0</td>
<td>8600</td>
</tr>
<tr>
<td>Tailings sampling point 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T8</td>
<td>0-20</td>
<td>7.9</td>
<td>7.2</td>
<td>0.5</td>
<td>0.13</td>
<td>2.5</td>
<td>6.4</td>
<td>13</td>
<td>6.8</td>
<td>441</td>
<td>6</td>
<td>0.06</td>
<td>34.2</td>
<td>314</td>
</tr>
<tr>
<td>T9</td>
<td>60-100</td>
<td>8.1</td>
<td>38.1</td>
<td>0.3</td>
<td>19.2</td>
<td>1.2</td>
<td>3.3</td>
<td>156</td>
<td>1.3</td>
<td>759</td>
<td>81</td>
<td>0.07</td>
<td>24.2</td>
<td>6808</td>
</tr>
<tr>
<td>T10</td>
<td>220-240</td>
<td>7.8</td>
<td>43.4</td>
<td>0.5</td>
<td>20.4</td>
<td>1.6</td>
<td>4.3</td>
<td>183</td>
<td>1.7</td>
<td>882</td>
<td>97</td>
<td>0.53</td>
<td>28.7</td>
<td>8131</td>
</tr>
<tr>
<td>Soil samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S7</td>
<td>0-15</td>
<td>6.6</td>
<td>107.0</td>
<td>2.1</td>
<td>17.2</td>
<td>13.7</td>
<td>60.1</td>
<td>37.4</td>
<td>29.8</td>
<td>255</td>
<td>13.9</td>
<td>&lt;0.10</td>
<td>155.0</td>
<td>14730</td>
</tr>
<tr>
<td>S11</td>
<td>0-15</td>
<td>6.9</td>
<td>73.9</td>
<td>1.9</td>
<td>169.0</td>
<td>8.9</td>
<td>32.0</td>
<td>118.0</td>
<td>17.2</td>
<td>139</td>
<td>56.1</td>
<td>3.96</td>
<td>147.0</td>
<td>84300</td>
</tr>
<tr>
<td>S13</td>
<td>0-15</td>
<td>6.5</td>
<td>76.8</td>
<td>1.2</td>
<td>319.0</td>
<td>5.2</td>
<td>17.5</td>
<td>185.0</td>
<td>14.0</td>
<td>140</td>
<td>88.4</td>
<td>3.11</td>
<td>82.8</td>
<td>138482</td>
</tr>
</tbody>
</table>

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 areas (Ministero dell' Ambiente e della Tutela del Territorio e del Mare, 2006, decree no. 152/06). Values above legislative limits are in bold.
3.3 Total and bioaccessible concentrations of PTE in the <250 µm fraction of the waste rock, tailings, and soil

The total concentrations of PTE for the <250 µm fraction were considerably higher than for the <2 mm fraction (Table 2) implying an increase in surface area and thus higher absorption of PTE to particles (Yao et al., 2015). Total As concentrations were found to be high with a maximum of 189 mg/kg. However, for all the samples, bioaccessible concentrations of As ranged from 5-33% of total concentrations. In contrast, samples recorded BAF of Cd ranging from 72-98%. Total concentrations of Co (5-12 mg/kg) and Cr (9-44 mg/kg) and bioaccessible concentrations (Co: 1-4 mg/kg and Cr: 1-2 mg/kg) were found to be low. The bioaccessible concentrations of Cu in waste rock, tailings, and soil samples recorded mean value of 115 mg/kg (BAF 54%), 145 mg/kg (BAF 49%), and 65 mg/kg (BAF 41%) respectively. Mean of total concentrations and BAF of Ni were 24 mg/kg and 33% respectively, for all the samples.

The bioaccessible fractions of Pb in waste rock, tailings, and soil samples recorded mean value of 32%, 84%, and 61%, respectively. The waste rock samples indicated high concentrations of Zn with mean value of 130 917 mg/kg. The mean bioaccessible concentration of Zn in waste rock was 119 461 mg/kg, resulting in BAF of 91%. Similar results were obtained for tailings and soil samples, with mean value of BAF as 75% and 80% respectively. Due to the limited number of samples, statistical methods were not used for prediction of oral bioaccessible concentrations based on total concentrations. However, the Pearson’s correlation coefficients conducted on samples (n = 8) showed strong correlation between total and bioaccessible concentrations of Cd, Co, Cu, Pb and Zn (Supplementary Material: S3).

The trend of the BAF recorded in our study was Cd (72-98%) ≈ Zn (73-94%) > Cu (25-90%) > Pb (16-88%) > Ni (17-60%) > Co (24-42%) > As (5-33%) > Cr (3-11%). Mikutta et al. (2014) and Palumbo-Roe and Klinck (2007) reported BAF of As as 5-34% and 0.5-42% in mine impacted soils in Bulgaria and south England respectively, which is in close range with this study. On the other hand, samples in present study showed higher BAF for Cu and Ni compared to other mining areas (Darko
et al., 2019; Karadaş and Kara, 2011). Furthermore, BAF for Cd, Pb and Zn in our study is comparable to those reported by Roussel et al. (2010) on smelter contaminated urban soil in northern France. Pelfrène et al. (2012) recorded mean values of BAF in smelter contaminated agricultural soil in northern France for Cd, Pb, and Zn as 78%, 58% and 32% (here 85%, 61%, and 80%, respectively). Higher bioaccessibility of Zn was recorded in soil in Gorno compared to agricultural soil in northern France which could be due to difference in land use.
Table 2. Total and bioaccessible concentrations (mg/kg) and bioaccessible fraction (BAF in %) evaluated on <250 µm size fraction.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>G</th>
<th>Total</th>
<th>BAF</th>
<th>G</th>
<th>Total</th>
<th>BAF</th>
<th>G</th>
<th>Total</th>
<th>BAF</th>
<th>G</th>
<th>Total</th>
<th>BAF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Waste rock</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WR2</td>
<td>7.8</td>
<td>8.5</td>
<td>115</td>
<td>7</td>
<td>462</td>
<td>476</td>
<td>97</td>
<td>2.4</td>
<td>6.8</td>
<td>42</td>
<td>1.9</td>
<td>22</td>
<td>9</td>
</tr>
<tr>
<td>WR5</td>
<td>7.8</td>
<td>10.8</td>
<td>189</td>
<td>6</td>
<td>431</td>
<td>442</td>
<td>98</td>
<td>1.9</td>
<td>4.7</td>
<td>41</td>
<td>2.2</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>WR7</td>
<td>7.4</td>
<td>8.6</td>
<td>98</td>
<td>9</td>
<td>388</td>
<td>394</td>
<td>98</td>
<td>2.2</td>
<td>8.2</td>
<td>29</td>
<td>2.1</td>
<td>35</td>
<td>6</td>
</tr>
<tr>
<td><strong>Tailings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>6.9</td>
<td>4.9</td>
<td>15</td>
<td>33</td>
<td>0.8</td>
<td>1.0</td>
<td>80</td>
<td>2.5</td>
<td>5.5</td>
<td>40</td>
<td>0.6</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>T2</td>
<td>7.2</td>
<td>29.9</td>
<td>68</td>
<td>30</td>
<td>28</td>
<td>39</td>
<td>72</td>
<td>1.1</td>
<td>2.9</td>
<td>39</td>
<td>0.8</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td><strong>Soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S7</td>
<td>6.5</td>
<td>15.4</td>
<td>142</td>
<td>11</td>
<td>386</td>
<td>440</td>
<td>89</td>
<td>3.6</td>
<td>10.5</td>
<td>34</td>
<td>2.4</td>
<td>29</td>
<td>7</td>
</tr>
<tr>
<td>S11</td>
<td>6.6</td>
<td>6.1</td>
<td>115</td>
<td>5</td>
<td>28</td>
<td>36</td>
<td>80</td>
<td>2.8</td>
<td>11.9</td>
<td>24</td>
<td>1.5</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>S13</td>
<td>6.9</td>
<td>8.7</td>
<td>78</td>
<td>11</td>
<td>178</td>
<td>210</td>
<td>85</td>
<td>2.1</td>
<td>7.6</td>
<td>29</td>
<td>2.2</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td><strong>Cu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ni</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pb</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Zn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sample</strong></td>
<td><strong>pH</strong></td>
<td><strong>G</strong></td>
<td><strong>Total</strong></td>
<td><strong>BAF</strong></td>
<td><strong>G</strong></td>
<td><strong>Total</strong></td>
<td><strong>BAF</strong></td>
<td><strong>G</strong></td>
<td><strong>Total</strong></td>
<td><strong>BAF</strong></td>
<td><strong>G</strong></td>
<td><strong>Total</strong></td>
<td><strong>BAF</strong></td>
</tr>
<tr>
<td><strong>Waste rock</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WR2</td>
<td>7.8</td>
<td>100</td>
<td>350</td>
<td>29</td>
<td>12.0</td>
<td>20</td>
<td>60</td>
<td>31</td>
<td>198</td>
<td>16</td>
<td>128044</td>
<td>143302</td>
<td>89</td>
</tr>
<tr>
<td>WR5</td>
<td>7.8</td>
<td>135</td>
<td>150</td>
<td>90</td>
<td>9.8</td>
<td>20</td>
<td>49</td>
<td>36</td>
<td>128</td>
<td>28</td>
<td>116996</td>
<td>124575</td>
<td>94</td>
</tr>
<tr>
<td>WR7</td>
<td>7.4</td>
<td>110</td>
<td>260</td>
<td>42</td>
<td>7.5</td>
<td>25</td>
<td>30</td>
<td>96</td>
<td>177</td>
<td>54</td>
<td>113343</td>
<td>124875</td>
<td>91</td>
</tr>
<tr>
<td><strong>Tailings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>6.9</td>
<td>6</td>
<td>19</td>
<td>32</td>
<td>3.2</td>
<td>19</td>
<td>17</td>
<td>22</td>
<td>25</td>
<td>88</td>
<td>149</td>
<td>193</td>
<td>77</td>
</tr>
<tr>
<td>T2</td>
<td>7.2</td>
<td>284</td>
<td>440</td>
<td>65</td>
<td>2.0</td>
<td>8</td>
<td>37</td>
<td>1567</td>
<td>1945</td>
<td>81</td>
<td>7139</td>
<td>9739</td>
<td>73</td>
</tr>
<tr>
<td><strong>Soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S7</td>
<td>6.5</td>
<td>124</td>
<td>334</td>
<td>53</td>
<td>17.1</td>
<td>37</td>
<td>48</td>
<td>139</td>
<td>215</td>
<td>65</td>
<td>130759</td>
<td>154680</td>
<td>85</td>
</tr>
<tr>
<td>S11</td>
<td>6.6</td>
<td>11</td>
<td>44</td>
<td>25</td>
<td>4.3</td>
<td>37</td>
<td>12</td>
<td>141</td>
<td>248</td>
<td>57</td>
<td>5304</td>
<td>7304</td>
<td>73</td>
</tr>
<tr>
<td>S13</td>
<td>6.9</td>
<td>58</td>
<td>132</td>
<td>44</td>
<td>5.9</td>
<td>24</td>
<td>26</td>
<td>84</td>
<td>136</td>
<td>61</td>
<td>82340</td>
<td>100530</td>
<td>82</td>
</tr>
</tbody>
</table>

BAF: Bioaccessible fraction calculated using Eq. 1, G: concentration of PTE extracted in gastric phase of UBM; Total: total concentration of PTE using aqua regia on <250 µm fraction.
3.4 Solid phase distribution of the elements across waste rock, tailings, and soil matrices

3.4.1. Waste rock and soil

The physico-chemical components for the most representative sample of waste rock and soil (sample codes - WR2 and S7) are in Fig.4. Heatmap for sample codes – WR5 and S13 is shown in Supplementary Material: S4. Chemometric data analysis identified 7 components in waste rock sample and 9 components in the soil samples. Using the geochemical associations present at the site, six clusters were identified:

Exchangeable: This cluster in waste rock was extracted over the HNO₃ concentrations of 0.01 M to 0.05 M and consisted of S (72%), Mg (12%), Si (5%), and Zn (4%) suggesting dissolution of sulphates present at the site (Heidel et al., 2011). The exchangeable cluster in soil consisted of (Ca-S-Mg, Ca-Mg, Zn, Zn-Na components), with the majority of elements being released in distilled water and 0.01 M HNO₃ extractant indicating that the site is rich in carbonate. The elemental composition was Zn (38%), Ca (25%), Mg (19%) and S (6%). Elevated concentrations of Zn and Ca indicates presence of weathered products from minerals such as smithsonite (ZnCO₃), and/or hydrozincite (Zn₅(CO₃)₂(OH)₆) and hemimorphite (Zn₄(Si₂O₇)(OH)₁₂·H₂O) and Ca rich minerals like dolomite and calcite.

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

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

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

Pb dominated component consisted of S (54%), Mg (37%), Zn (4%) and Ca (3%) in waste rock. Lead concentration in this component was highest and equal to 77% of total CISED extracted Pb. This could be due to the occurrence of galena (PbS), which was present with sphalerite in minor association. The Pb dominated cluster in soil had high percentage of Ca (55%), Zn (27%), Mg (9%). The lead concentration in this cluster was 91%, while the other components in total consisted only 9% of Pb.

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

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

The physico-chemical components present in selected sample from tailings (code - T2) are shown in Fig.5. Tailings sample was subjected to separate chemometric data analysis, as tailings were generated after processing of natural minerals, which led to variation in quantities and mobility of elements when compared to waste rocks and soil. It was observed that CISED extracted samples for tailings contained higher percentages of Na, as sodium based reagents were used for flotation. The components of tailings sample (code - T1) are in **Supplementary Material: S5**.

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

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

![Fig.5. Extraction profiles obtained during CISED extractions of tailings (sample code – T2).](image-url)
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 strong association between As and Fe oxides and Fe hydroxides (exposed in Section 3.1). This is also evidenced in Fig. 6a, where As is extracted in the Fe oxide fractions of CISED.

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 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 mobility than the parent sulphide minerals (Schaider 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 demonstrated that, the dissolution of greater amounts of carbonates by the acidic gastric solutions can result in an increase of hydroxy carbonate anions available in solution, and that under such conditions, Pb can form insoluble compounds with the anions (Reis et al., 2014).
Fig. 6. Median cumulative concentration in CISED extracted components (X-axis) and bioaccessible concentrations of potentially toxic elements (mg/kg). Bioaccessible: Bioaccessible concentrations, As: arsenic, Cd: cadmium, Pb: lead, and Zn: zinc

3.6. Human health risk assessment for ingestion

The non-carcinogenic HQ estimations were performed for As, Cd, Co, Cr, Cu, Ni, Pb and Zn. For residential exposure scenario, the HI defined as the summation of HQ, reached to a maximum of 25.4 considering total concentrations and to a maximum of 17.9 utilising bioaccessible concentrations (Fig. 7a). The carcinogenic risk evaluated for As and Pb implied that total cancer risk i.e. CR\text{total} reached a maximum of $4.4 \times 10^{-4}$ which is greater than $1 \times 10^{-5}$, demonstrating carcinogenic risk for total concentrations. For bioaccessible concentrations the maximum value was $2.7 \times 10^{-5}$.

For the recreational scenario, it was observed that maximum HI for total and bioaccessible concentrations were 2.0 and 1.4, respectively (Fig. 7c). The results also indicated that the site surrounding T1, T2, S11 and S13 had HI <1 (acceptable level), for bioaccessible concentrations, implying no potential non-carcinogenic risks. All the samples recorded CR\text{total} <1 $\times 10^{-5}$ (acceptable level) using bioaccessible concentrations. This indicates that incorporating bioaccessible concentrations in HHRA refined the risk assessment process, resulting in no unacceptable carcinogenic risks being identified at all the sampling points and absence of unacceptable potential non-carcinogenic risks at half of the sampling points, as the HI and CR\text{total} decreased by 34% and 93% respectively.

It should be noted that all the risk calculations were performed using concentrations on size fraction <250 µm for consistency and because this is the fraction that remains adhered to skin for accidental ingestion. However the present study also reported lower total concentrations for size fraction <2 mm than <250 µm (Section 3.2 and 3.3), implying that there could be underestimation of potential risk, for oral pathway, if the calculations are based on size fraction <2 mm. Because of the influence of soil particle size on total concentrations, soil particle size should be chosen properly for HHRA to avoid under- or overestimating human health risk (Cao et al., 2020). It is also recommend that for incorporation of oral bioaccessibility in HHRA, consistent particle size and samples should be
analysed. This is also in line with the approach used by Luo et al. (2012) and Cao et al. (2020) where samples were first analysed for total concentrations and then the same samples were used for bioaccessibility.

The limitations of the present study were that (1) the limited number of samples collected from the abandoned mine site hindered the use of a simple linear regression model for predicting bioaccessible concentrations of PTE; (2) all samples were not analysed for mineralogy and solid phase distribution. However, the conclusions drawn from the present study were in confirmation with oral bioaccessibility and mineralogy studies conducted elsewhere (Reis et al., 2014; Ruby et al., 1999; Schaider et al., 2007). Nevertheless, the present study demonstrated that for robust decision making, role of bioaccessibility in HHRA should not be underestimated as noted by differences in potential risks due to total and bioaccessible concentrations in the HHRA framework.

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

This paper has highlighted the importance of assessing site-specific oral bioaccessibility to provide improved estimates of human health risks from exposure to PTE in abandoned mine sites. Waste rock, tailings and soil samples had strong enrichment of PTE in Gorno, northwest Italy. The total PTE concentrations were higher in the <250 μm fraction than in the <2 mm fraction, implying higher specific surface area for smaller size fractions. The range of oral BAF across samples was observed as: As (5-33%), Cd (72-98%), Co (24-42%), Cr (3-11%), Cu (25-90%), Ni (17-60%), Pb (16-88%) and Zn (73-94%). Solid phase distribution and mineralogical analysis demonstrated that pH; geological and lithological properties; and presence of carbonatic minerals influenced oral bioaccessibility.

Oral bioaccessibility analysis concluded that PTE are not fully bioaccessible, which implies that use of total concentrations is not adequate in determining the actual risk associated. Incorporation of site-specific BAF values showed decrease in potential non-carcinogenic and carcinogenic risks by 34% and 93% respectively for ingestion pathway. Thus, bioaccessibility-corrected HHRA reduces the uncertainty associated with the human exposure estimates and the extent of required remediation efforts. The approach provided in this study, based on bioaccessibility-correction and RBCA guidelines, can be used in estimating hazards associated with exposures to PTE in soils near abandoned mine sites.

Acknowledgments

This work was completed as part of the REMEDIATE (Improved decision-making in contaminated land site investigation and risk assessment) Marie-Curie Innovation Training Network. The network has received funding from the European Union's Horizon 2020 Programme for research, technological development and demonstration under grant agreement n. 643087. Authors also express gratitude towards: Giorgio Carbotta for helping with sampling, Dr Jie Chen for analysing mineralogy using micro-XRF and SEM, Dr Christophe Real for providing bibliography related to geology of the
References


