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Article

Metal(loids) bioaccessibility in road dusts from the surrounding villages of an active mine

Carla Candeias [1]*, Paula F. Ávila [2], Eduardo F. Silva [1] and Fernando Rocha [1]

[1] Geosciences Department, GeoBioTec - Geobiosciences Geotechnologies and Geoengineering Research Center, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal; candeias@ua.pt, eafsilva@ua.pt; tavares.rocha@ua.pt
[2] LNEG - National Laboratory of Energy and Geology, 4465-956 S. Mamede Infesta, Portugal; paula.avila@lneg.pt
*Correspondence: candeias@ua.pt

Abstract: Human activities, in general, cause a significant impact on the environment and human health. The present study aims to characterize road dusts of villages located near an active mine and to assess metal(loids) bioaccessible fractions. Collected road dusts samples (< 250 μm fraction) pseudototal, gastric (G) and gastrointestinal (GI) phases (UBM assay) concentration, mineralogical composition, Enrichment Factor (EF) and Risk for humans, were determined. Results obtained revealed that arsenic represents the higher Risk to humans, with mean pseudototal values higher than the maximum reference value range. Enrichment factor points to As significant to very high enrichment in all villages. Also, Cd presents maximum EF values in all villages classified as very high enrichment. Particles enriched in As, Ca, Fe, Cu, Al, Ti were identified by SEM-EDS in weathered agglomerates, linked to mine wastes and long-distance transport, both by wind and/or traffic. Arsenic bioaccessibility fraction (%BAF) presents low values in the studied samples, possibly due to low Fe complexes solubility with adsorbed As, limiting arsenic release and reducing its bioaccessibility. Concentrations of Cd bioaccessible G and GI phases are within the reference values range, while for Cu are above and for Pb lower, than reference value range. Results show that the pseudototal fraction risk is overestimated when comparing to BAF%, nevertheless, total G and GI risk are above the carcinogenic target risk (1.00E-06) in most samples. Carcinogenic risk of the bioaccessible contaminants shows that As is the element that represents higher risk for developing cancer over a lifetime, being ingestion the main risk route.

Keywords: road dusts; active mine; metal(loids); Arsenic; oral bioaccessibility; human health risk

1. Introduction

Mining and mineral processing are known for the impact on the environment by polluting air, waters and soils. Large amounts of rejected materials are deposited in tailings and dams, generating dust particles enriched in potentially toxic elements (PTEs) what might disturb health by entering the human body. Road dusts are considered to be good indicators of metal(loids) contamination by diverse sources, such as industrial activities and traffic [1,2]. Metal(loids) contamination in road dusts, e.g., As, Cd, Cu, Cr, Pb, Hg, is difficult to be removed, even after several years [3]. In mining areas, dust particles are dispersed (e.g., winds, traffic) and usually PTEs adsorbed, therefore there is a potential for these dust particles to be deposited and contaminate soils and plants and to be ingested, inhaled and/or adhere to the skin of humans [4]. Particles less than 250 μm adhere to hands and thus may be ingested through hand to mouth behaviors [5].

Several studies take in consideration the road dusts total (or pseudototal) metal(loids) for the health risk assessment [e.g., 6] where oral bioaccessibility is defined as the PTE concentration. PTE's will be released from is matrix in to the gastrointestinal tract becoming potentially available for absorption [7, 8], being variable for each contaminant and location. Thus, the PTEs pseudototal fraction will lead to the overestimation of the health risk assessment. Several in vitro assays are proposed to estimate the bioaccessible fraction of pollutants, such as Simplified Bioaccessibility Extraction Tests (SBET), a conservative method that only simulate the stomach settings. Presently the Solubility Bioaccessibility Research Consortium method (SBRC), Physiologically Based Extraction Test (PBET), in vitro Gastrointestinal method (IVG), and Unified Bioaccessibility Method (UBM) are the most used in vitro gastrointestinal digestion methods due to the recognized results [9]. This study aims to characterize the road dusts of four villages located in the surroundings of the Panasqueira mine and to assess As, Cd, Co, Cr, Cu, Fe and Pb bioaccessible fraction using both gastric and gastrointestinal phases by the UBM in vitro method in road dusts impacted mostly by mining activities.

2. Materials and methods

Study area, geology and mining activities: Panasqueira mine (central Portugal), is considered the biggest W–Sn deposit of the Western Europe. The history of this mine goes back to the end of the 19th century. Tungsten is widely used in metal-alloys, being well-known as a steel-hardener. The tungsten demand, and thus its price, rises inevitably during major armed conflicts. It was during the two World Wars and the Corean War that tungsten (W) extraction at the Panasqueira mine, was at its highest. A detailed description of the study area and geology is presented in Ávila et al. [10] and Candeias et al. [11]. Briefly, the deposit contains significant amounts of wolframite [$(Fe,Mn)WO_4$], cassiterite [SnO_2] and, as a minor accessory, chalcopyrite [$CuFeS_2$]; arsenopyrite [FeAsS] is an abundant rejected mineral, resulting in high As concentrations deposited in tailing and dams.

The presence of huge tailings and other debris testify the long history of exploitation and ore treatment operations in Panasqueira mine (Figure. 1). The piles (Rio tailing > 1.2 Mm^3; Barroca Grande tailing > 7.0 Mm^3) and the open-air impoundments (Rio mud dam > 0.7 Mm^3; Barroca Grande with two mud dams > 1.2 Mm^3) are exposed, being altered by chemical, mineralogical, physical and geotechnical conditions. On the top of the Rio tailing, an arsenopyrite stockpile (~9,400 m^3) was deposited and remained exposed until June 2006 when was capped with geotextile and layers of clay. Detailed descriptions of the mining activity history and ore treatment process were presented in Ávila et al. [10, 12] and Candeias et al. [11].

Figure 1. Partial view of (a) active Barroca Grande tailing (7 Mm^3) and S. Francisco de Assis (SFA) village; and (b) abandoned Rio tailing and Zêzere river (in Portuguese rio) in dry season (1.2 Mm^3).

Samples collection and preparation: The study locations selected for the collection of road dust samples were: (a) S. Francisco de Assis (SFA; n=13) and Barroca (B; n=7) villages, both located within the mining operations area (Figure. 2), initially considered directly impacted by the mine; and (b) Casegas (C; NE of the mine; n=11) and Unhais-o-Velho (UV; SW of the mine; n= 6) villages, located on areas considered initially not impacted by the mine and out of the prevailing winds influence (main direction NW-SE) [13]. The number of samples collected was considered representative of the population density of each small village. Each sample was collected with new and clean plastic brooms and shovels on representative roads of each village. Samples were stored in individual polyethylene bags (research grade) for further treatment and analyses. On the laboratory samples were dried (≤ 40 ºC) until a constant weight was attained, disaggregated and wet sieved (250 µm). This fraction was selected for potentially toxic elements (PTEs) assessment once is considered a rational upper bond of the size range of ingested particles [14].

Figure 2. Panasqueira mine tailings (Barroca Grande and Rio) and the studied villages: São Francisco de Assis (SFA), Barroca, Casegas and Unhais-o-Velho (UV). Wind rose with data collected on the top of the Panasqueira mine [13] (image adap. Google Earth, 2021).

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Pseudototal chemical analysis: Samples 250 µm fraction was milled to assess the pseudototal concentration by multi-elemental analysis. A 0.5 g split was leached in hot (95 ºC) aqua regia (HCl–HNO_3–H_2O) for 1 h. After dilution to 10 ml with demineralized water, the solutions were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for As, Cd, Co, Cr, Cu, Fe and Pb (ACME labs, Canada). Detection limits: As = 0.01 mg kg^{-1}; Cd, Co, Cu, Pb = 0.1 mg kg^{-1}; Cr = 1 mg kg^{-1}; Fe = 100 mg kg^{-1}.

In vitro oral bioaccessibility: Oral bioaccessibility of As, Cd, Co, Cr, Cu, Fe and Pb was assessed using the widely employed and validated in vitro extraction method, the unified bioaccessible assay (UBM) [15]. The UBM assay was applied in the < 250 µm granulometric road dust fraction. The UBM test simulates the mouth, stomach (G) and gastrointestinal (GI) compartments chemical processes by means of synthetic digestive solutions and is fully described in Wragg et al. [16]. G and GI extractions were analyzed by ICP-MS for the same elements as the pseudototal analyses. The percentage of the bioaccessible contaminant (%BAF) concentration was calculated:

$$\%BAF = \frac{concentration\ of\ bioaccessible\ element\ (mg\ kg^{-1})}{concentration\ of\ total\ element\ in\ sample\ (mg\ kg^{-1})} \times 100$$

X-ray diffraction (XRD): The < 250 µm fraction of the eight samples that where selected based on the pseudototal concentration (see Results and discussion section) were submitted to XRD in order to identify the mineralogical constituents. The mineralogical composition (including efflorescence) was determined using a Philips X'Pert MPD machine equipped with CuKa radiation, graphite monochromator, automatic divergence slit and 0.5–receiving slit. A step size of 0.05º 2q, a scan setting of 2–70º 2q and a 10 s counting time were chosen.

Scanning Electron Microscopy with energy dispersive spectroscopy (SEM-EDS): The < 250 µm fraction of each sample was individually placed on an aluminum holder and coated with carbon (C) using an Emitech K950X carbon evaporator, after what were analyzed with an HITACHI SU-70 high resolution SEM equipped with a Bruker energy dispersive spectroscopy (EDS) detector, in order to identify the chemical elemental composition of the particles separately. The size and shape of each speck was also taken into consideration. Inorganic identification of insoluble particles was performed using a mix of protocols that allow to semi-quantify the mineralogy of each particle [17].

Granulometry: The < 250 µm fraction was sieved to obtain the < 106 µm fraction. The particle size distribution of road dusts was determined by X-ray sedimentation technique with a Sedigraph III Plus grain size analyzer from Micromeritics. This technique determines the relative mass distribution of a sample by particle size being based on two physical principles: sedimentation theory (Stokes' law) and the absorption of X-radiation (Beer-Lambert law).

The accuracy and analytical precision of the methods were determined using analyses of reference materials and duplicate samples in each analytical set. Results were within the 95% confidence limits of the recommended values given for certified material. The relative standard deviation was between 5% and 10%.

Enrichment factor (EF): was applied to evaluate the degree of PTEs pollution and assess possible natural or anthropogenic sources:

$$EF = \frac{(C_i/Fe)_{sample}}{(C_i/Fe)_{crust}}$$

where C_i is the PTE pseudototal concentration in the sample and in the crust. Crustal Fe mean concentration was selected as a reference metal by assuming that the contribution of its anthropogenic sources can be considered negligible [18]. The EF results are classified as: (a) $EF < 2$ none to minimal enrichment; (b) $2 \leq EF < 5$ moderate enrichment; (c) $5 \leq EF < 20$ significant enrichment; (d) $20 \leq EF < 40$ very high enrichment; and (e) $EF \geq 40$ extremely high enrichment [19].

Non-carcinogenic and carcinogenic risks assessment: Human health risk assessment calculations were based on the assumption that residents, both children and adults, are directly exposed to dusts through three main pathways (a) ingestion; (b) inhalation and (c) dermal absorption of dust particles. Although particles <10 µm (PM10) are more relevant in inhalation process, coarser sizes are, probably, decomposed in the gastrointestinal track. The carcinogenic and non-carcinogenic side effects for each element were computed individually, considering reference toxicity levels for each variable, as extensively described in RAIS [20]. For each element and route (i.e., pathway), the non-cancer toxic risk was estimated by computing the Hazard Quotient (HQ) for systemic toxicity (i.e., non-carcinogenic risk). If HQ > 1, it indicates that non-carcinogenic effects might occur once exposure concentration exceeds the reference dose (RfD). Systemic toxicity is not expressed as a probability but as an evaluation, by comparing an exposure level over a period of time to a RfD derived for a similar exposure period. The cumulative non-carcinogenic hazard index (HI), corresponds to the sum of HQ for each pathway or variable. Similarly, HI > 1 indicate that non-carcinogenic effects might occur. The carcinogenic Risk, or the probability of an individual developing any type of cancer over a lifetime as a result of exposure to a potential carcinogen, was estimated by the sum of total cancer Risk for the three exposure routes. A Risk > 1.00E−6 is classified as the carcinogenic target Risk and if > 1.00E−4 it is considered unacceptable [20]. In this study, As, Cd, Co, Cu and Fe were considered to be chronic sources of non-carcinogenic risk, and As, Cd, Co and Pb were considered to pose a carcinogenic risk.

3. Results and discussion

Road dusts pseudototal As, Cd, Co, Cr, Cu, Fe and Pb concentrations are presented in Figure 3. These elements were selected due to their potential toxicity to humans and high concentrations found, when compared to guidelines. Road dust pseudototal concentrations ranged: 16 to 3530 mg kg^{-1} (As), 0.2 to 19.8 mg kg^{-1} (Cd), 2.5 to 11.8 mg kg^{-1} (Co), 7.1 to 45 mg kg^{-1} (Cr), 12.6 to 2379 mg kg^{-1} (Cu), 8600 to 35700 mg kg^{-1} (Fe) and 5.0 to 152.7 mg kg^{-1} (Pb). The same elements mean pseudototal concentrations: 404 mg kg^{-1} (As), 2.9 mg kg^{-1} (Cd), 5.2 mg kg^{-1} (Co), 16.9 mg kg^{-1} (Cr), 259 mg kg^{-1} (Cu), 19472 mg kg^{-1} (Fe) and 28.6 mg kg^{-1} (Pb). An ANOVA analysis showed that there are significant differences ($p < 0.05$) between As, Co, Cu, Fe and Pb samples concentrations in the four locations. The higher concentrations were found in SFA village.

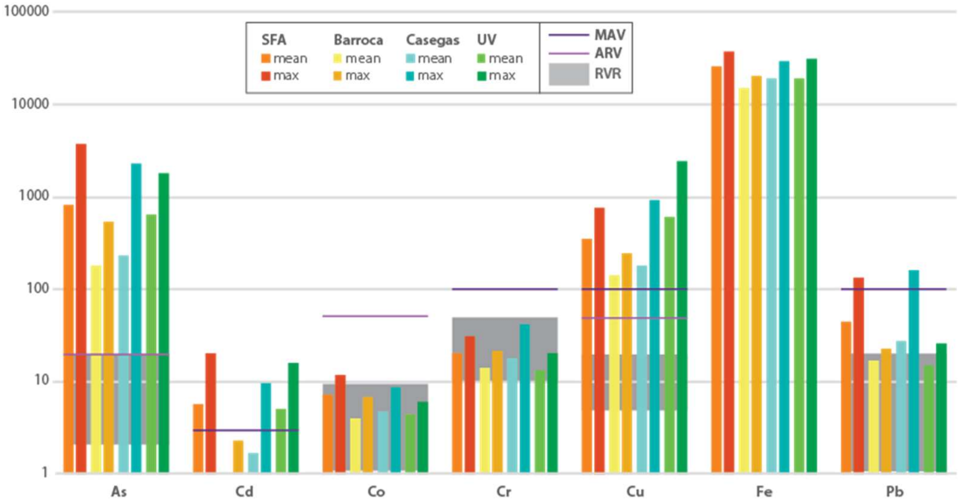

Figure 3. Mean and maximum (max) pseudototal concentrations determined in São Francisco de Assis (SFA; n=13), Barroca (n=7), Casegas (n=11) and Unhais-o-Velho (UV; n=6) road dust samples. Reference value range (RVR) interval, acceptable risk value (ARV), maximum acceptable value (MAV) [21] are disclosed.

Considering the pseudototal results, As represents the higher risk to humans, with mean values 40.5 (SFA), 8.7 (Barroca), 20.6 (Casegas) and 30.7 (UV) times over the maximum reference value range (RVR_{As} = 2 to 20 mg kg^{-1}) and the acceptable risk value (ARV_{As} = 20 mg kg^{-1}) [21]. In fact, As mean and maximum values in Casegas and UV, villages considered as not impacted by the mining activities, are higher than in Barroca village, located near the Rio abandoned tailing. Similar tendency occurs with the Cd, Co, Cu, Fe and Pb pseudototal concentrations.

Enrichment factor (EF) was applied in order to determine the PTEs road dust pollution. Results (Table 1) are particularly high for As, with a maximum value in SFA very much above the EF classification for extremely high enrichment of 40. Also, As mean value in both impacted villages is above the very high enrichment classification, suggesting an As anthropogenic source in these dusts. Casegas and UV have minimum values of 5 and 15, suggesting a significant enrichment. The variables Co and Cr have all EFs below the minimal enrichment grade revealing a geogenic source. The SFA village is the one with higher As, Cd and Pb maximum values reflecting the impact of the Barroca Grande tailing located next to the village (Figure. 1). Considering these results, eight representative samples (higher As EF) were selected to conduct further analysis, being two from each one of the four villages.

Table 1. Enrichment factor maximum (max), minimum (min) and mean values on the samples collected on the four villages studied (in mg kg^{-1}).

EF		As	Cd	Co	Cr	Cu	Fe	Pb
SFA	max	699	77	1.4	0.5	40	11	24
	min	29	3.4	0.8	0.2	4.9	3.5	1.4
	mean	194	23	1.0	0.4	18	5.3	4.7
Barroca	max	189	19	1.2	0.6	21	7.5	3.2
	min	21	2.0	0.7	0.3	4.4	4.6	1.9
	mean	80	7.5	1.0	0.4	12	5.7	2.4
Casegas	max	572	68	1.3	0.8	74	9.3	19
	min	5	0.5	0.6	0.2	1.4	3.7	1.2
	mean	75	10	0.9	0.4	14	5.4	3.0
UV	max	494	63	1.2	0.7	119	15	3.5
	min	15	1.7	0.6	0.2	8.7	5.2	0.9
	mean	202	23	0.9	0.4	34	7.6	1.7

Previous studies identified diverse minerals on the tailings and dams rejected materials of the Panasqueira hydrothermal Sn–W deposit, e.g., quartz [SiO_2], arsenopyrite [AsFeS], chalcopyrite [$CuFeS_2$], sphalerite [(Zn,Fe)S], pyrrhotite [$Fe_{(1-x)}S$], siderite [$Fe^{2+}CO3$], chlorite group minerals, muscovite [$KAl_3Si_3O_{10}(OH)_2$] [22]. The XRD analysis of the samples < 250 μm fraction, identified quartz and clay minerals as more abundant ones and also scorodite [$Fe^{3+}AsO_4{\cdot}2H_2O$], yukonite [$Ca_3Fe^{3+}(AsO^4)_2(OH)_3{\cdot}5H_2O$] and/or arseniosiderite [$Ca_2Fe^{3+}{}_3(AsO_4)_3O_2{\cdot}3H_2O$], hematite [$Fe_2O_3$], clinochlore ferroan [(Mg, $Fe^{2+})_5Al(Si_3Al)O_{10}(OH)_8$], adularia [$KAlSi_3O_8$]. The mineralized dust materials are weathered, and Panasqueira occurring minerals, such as arsenopyrite, sphalerite and chalcopyrite, are mostly present as altered remains. Most of the As and metals are bound to secondary minerals such as, Fe(hydr)oxides, scorodite, arseniosiderite, and/or yukonite. Secondary Co, Cu and Pb mineral phases were not found in the dusts, possibly due to poorly crystalline and/or amorphous mineral phases are not possible to be identified by XRD analysis.

The observation and pseudo chemical analysis by SEM-EDS (Figure. 4) identified particles with similar composition in all of the studied locations. S. Francisco de Assis samples revealed weathered agglomerates rich in As, Ca, Fe, Cu, Al, Ti and sharp quartz particles, suggesting a source from the mining wastes deposited on the Barroca Grande tailing located in front of the village (Figure. 1a). Other particles with mixed composition of Si, Fe, Al, Pb, K, Ti were also found. In Barroca village, located near the abandoned Rio tailing, particles are also possibly linked to the rejected mine materials (enriched in Fe, Al, Cu, Si, As). As expected from the pseudototal chemical analysis results, samples from Casegas and Unhais-o-Velho also revealed particles enriched in Si, Al, Fe, Ti, Cu, As, possibly linked to the long-distance transport of mine wastes, both by wind or traffic. These rejected materials are transported to be used in roads and construction. Similar natural geological characteristics of the 4 studied locations is also a possible source.

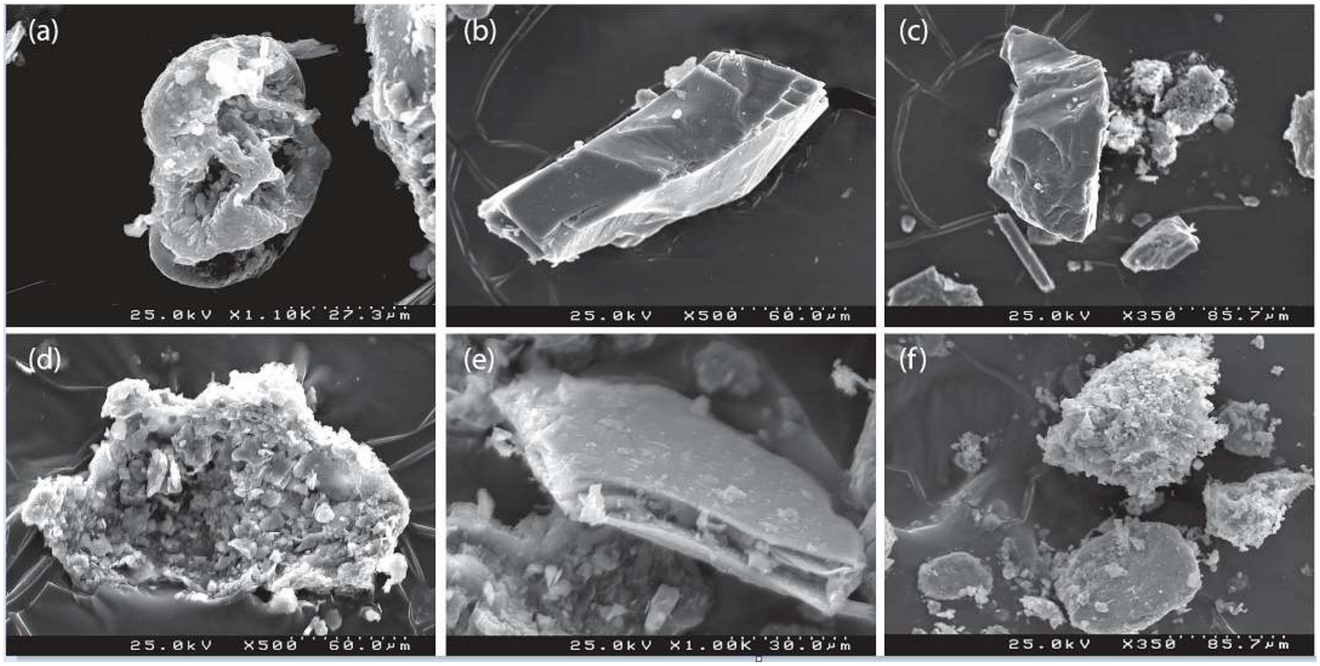

Figure 4. (a) Fe-Al-K-Si particle from Barroca; (b) and (c) on the center sharp quartz particle and on the left side of (c) Fe-Al-S and As-Fe-Ca rich weathered amorphous particles found in S. Francisco de Assis; (d) diverse particles mainly composed by Fe, As and Al; and (e) muscovite, both images from Casegas; and (f) Si-Al-Fe-Ti-Cu-As rich particles from Unhais-o-Velho.

Qin et al. [23], among others, established a link between the increase of gastric and gastrointestinal bioaccessible extracted concentrations and the decrease of particles grain size. Particles between 250 and 50 µm range 43.8 to 72.0 %, between 50 and 10 µm represent 17.1 to 31.2 % (Figure. 5), between 2 and 10 µm ranges 6.1 to 16.1 %, and clay fraction the less representative fraction (4.8 and 13.2 %). Several studies showed that < 2 µm is the most important fraction for a higher bioaccessible concentration of As and metals [24].

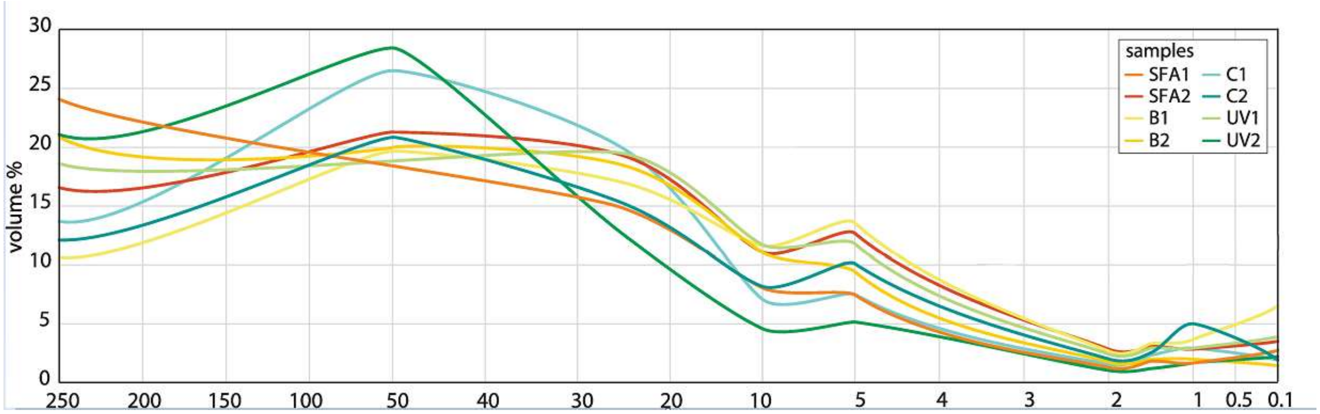

Figure 5. Size distribution (%) of dust particles, Ø < 250 µm.

To assess As, Cd, Co, Cr, Cu, Fe and Pb gastric (G) and gastrointestinal (GI) bioaccessible fractions, the UBM assay was applied to the selected samples. Previous studies (e.g. Pelfrêne and Douay [29]) suggest that As and metals are more soluble in the G fluid, due to more acidic conditions (pH ~1.2) when compared GI phase, with lower solubility under higher pH conditions (pH 5.8-6.8). Although G phase has slightly higher phase bioaccessible fraction (%BAF) when compared to GI %BAF, a t-student test did not reveal significant differences between the two phases ($p > 0.05$). It is difficult to understand contaminants bioaccessibility in mine-impacted dusts, once dissolution under gastric phases conditions is also influenced by mineralogy and particle size factors. Ore processing methodologies and environmental conditions will also impact particles behavior, e.g. formation of secondary minerals and morphology [25].

The As G phase bioaccessible fraction (%BAF_{AsG}) ranges 0.13 to 5.27 % and GI (%BAF_{AsGI}) 0.10 to 5.64 %, with lower values found in S. Francisco de Assis (Fig. 6). In fact, t-student test reveal that there are significant differences ($p = 0.027$) between %BAF_{As} in locations considered impacted by the mine (with lower mean concentrations) and villages considered as not impacted, in both G and GI phases. Similar low %BAF_{As} were reported in others mine impacted areas, e.g., 0.13% in Nova Scotia, Canada [26] and 0.6% in Tavistock, England [27]. Arsenic bounds to Fe might be mobilized due to dissolution of iron compounds. A low solubility of Fe complexes can limit the release of As reducing its bioaccessibility. Previous mineralogical studies in S. Francisco de Assis and Barroca villages [11] established a link between the tailings rejected materials and the collected dust samples, e.g. quartz [SiO_2], arsenopyrite [FeAsS], chalcopyrite [$CuFeS_2$] and sphalerite [ZnS]. Additionally, the tailings sulfides exposed to the atmospheric conditions induce the occurrence of secondary minerals, such as scorodite [$FeAsO_4.2H_2O$] and natrojarosite [$KFe^{3+}_3(SO_4)_2(OH)_6$]. Different arsenic minerals show different propensity for dissolution in low gastric and gastrointestinal pH conditions [26]. Antônio et al. [28] suggest that locations with the presence of high percentage of As secondary minerals tend to have lower As %BAF. Arsenopyrite and scorodite minerals present in dusts samples and their low solubility are consistent with the low As bioaccessible fractions, with similar percentages on the G phase and GI phases ($p > 0.5$). Maximum %BAF_{As} values were found in G and GI phases of sample C1, collected on a village considered not impacted by the mining activities. Previous studies found that As bioaccessible concentration decreases with increasing particle size, being particularly evident in the 0.3- to 5-µm fraction [29]. The influence of texture and grain size on As bioaccessibility may explain C1 behavior, a sample with a high percentage of finer grains, a fraction composed by particles with high surface areas, a factor promoting higher bioaccessibility.

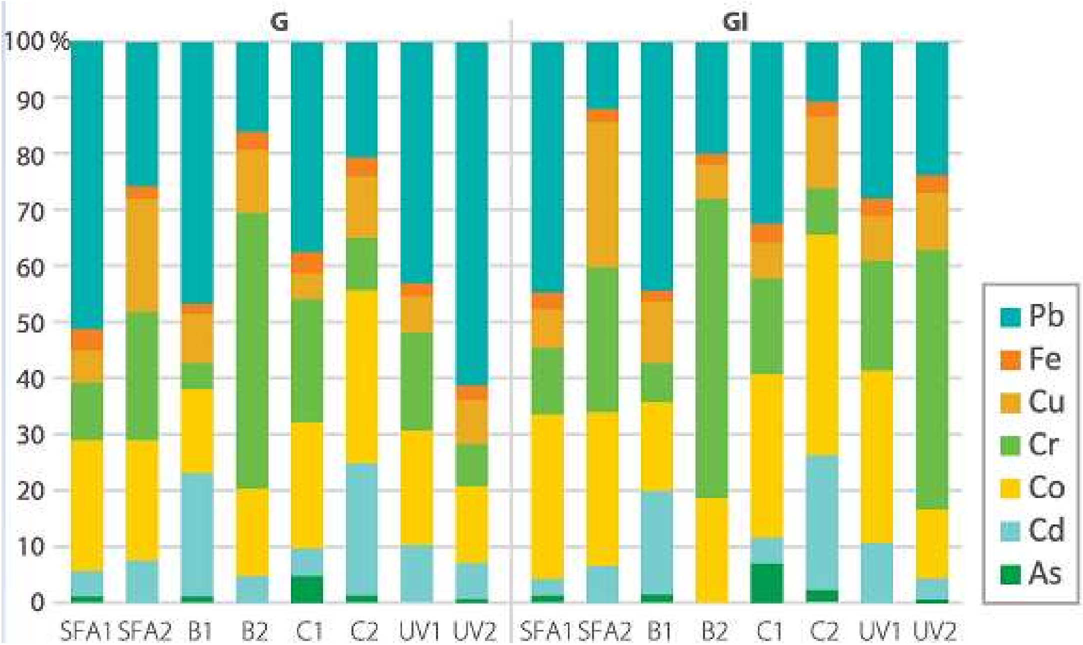

Figure 6. As, Cd, Co, Cr, Cu, Fe and Pb bioaccessible fractions (%BAF) for both Gastric (G) and gastrointestinal (GI) phases.

From a biological point of view, Ollson et al. [30] suggest that an association with Cd ions might interfere with the human gastric system leading to a reduction of As absorption. Nevertheless, Cd is considered a toxic metal and nondegradable in nature, with extensive application in different industries such as battery recycling and mining. Disorders in human health, such as bone disease, renal damages and several forms of cancer are linked to Cd exposure [31]. In this study $\%BAF_{CdG}$ ranges 0.52 to 37.35 % and $\%BAF_{CdGI}$ 0.07 to 21.96 % showing a higher tendency to be solubilized in the gastric phase, with the maximum percentages in both phases of B1 sample. Higher %BAF values can be attributed to the presence of montmorillonite $[(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2{\cdot}nH_2O]$ clay mineral is a possibility for the increase of Cd solubility, once it promotes Cd dissolution [32]. Concentrations of Cd bioaccessible G and GI phases are within the reference values range (0.1 – 1.0 mg kg^{-1}), with the highest concentration found in sample $UV1_G$ (1.0 mg kg^{-1}).

The transition element Co, has complex functions on the human body, being bound to vitamin B12, with a recommended daily intake of 0.1 g/day in adult's diet. Nevertheless, exposures to higher Co concentrations can cause health outcomes, such as genotoxicity and cancer [33]. The Co G fraction $\%BAF_{CoG}$ ranges 2.79 to 26.14 % and GI fraction $\%BAF_{CoGI}$ 2.58 to 22.73 %, with maximum bioavailable fraction in samples B1 (G) and UV1 (GI). Cobalt is adsorbed onto secondary Fe(III) (oxyhydr)oxides, characteristic of mining-affected environments such as Panasqueira. Chromium has been listed as a priority pollutant, being Cr^{3+} considered essential for a regular operation of human vascular and metabolic systems, while Cr^{6+} is its most dangerous form and able to induce human health outcomes, e.g. genotoxic, carcinogenic, and mutagenic effects [34]. However, plants or animals do not bioaccumulate Cr, being its impact dependent on its bioaccessible fraction. In this study Cr revealed a $\%BAF_{CrG}$ ranging 1.20 to 47.80 % and $\%BAF_{CrGI}$ 1.01 to 77.78 %, with maximum bioavailable fractions in samples $B2_G$ (5 mg kg^{-1}) and UV2 (7 mg kg^{-1}), both concentrations below the 10 mg kg^{-1} defined as the lower limit of the RVR [21]. Overall, bioaccessible results revealed a diversity of Cr solubility, with minimum extracted G and GI concentrations in sample SFA1 (0.3 mg kg^{-1}).

The geology is a common important factor for the presence of high Cu concentrations. Panasqueira deposit includes Cu rich minerals, such as chalcopyrite, what lead to high concentrations of Cu in old rejected materials deposited in tailings and dams due to less effective separation techniques used in the past [11]. In locations away from the exploration area, Casegas and UV villages, the Cu pseudototal concentration is higher than in SAF and Barroca. The Cu $\%BAF_{CuG}$ ranges 0.60 to 14.76 % and $\%BAF_{CuGI}$ 0.61 to 19.78 %. Sample $SFA2_{GI}$ have bioaccessible concentrations of G = 60 mg kg^{-1} and GI = 47 mg kg^{-1}, above RVR (5-20 mg kg^{-1}) in both extracted phases and ARV (50 mg kg^{-1}) in G phase. Lowest extracted concentrations were found in sample SFA1 (G = 4.4 mg kg^{-1}; GI = 4.5 mg kg^{-1}). Previous studies related a higher Cu bioaccessibility with a lower clay content, such as SFA1 sample, once clay fraction can cause a significant retention of metals [32]. Except for SFA1 and B2 samples, bioaccessible extracted concentrations are above the RVR.

Lead is a common environmental pollutant being its exposure via ingestion and inhalation a public health hazard, especially in children due to Pb poisoning via hand-to-mouth exposure. Chronic exposure to Pb might induce e.g., autism, brain and kidney damage, psychosis [35]. The bioaccessible fraction of Pb reveal $\%BAF_{PbG}$ ranging 5.97 to 84.95 % and $\%BAF_{PbGI}$ 3.95 to 53.45 %. In both phases, bioaccessible extracted concentrations are lower than RVR (1-20 mg kg^{-1}) with minimum values in sample SFA1 (G 3.0 mg kg^{-1}; GI 2.0 mg kg^{-1}). According to Ávila et al. [10], Barroca Grande tailing and dams have higher concentrations of As, Cd, Cu, and Pb in the coarser rejected materials, what might explain the variable Pb pseudototal concentrations in the < 250 μm fraction subject to UBM assay, with lower %BAF SFA1 and C2. Pb bioaccessibility is controlled by physical properties, higher amount of adsorption on clay minerals, organic matter and/or oxides, being nevertheless, difficult to predict. Furthermore, bioaccessibility of an element depends on its chemical speciation [24, 36].

Human health risk assessment

There are several studies suggesting that road dusts contaminated with PTEs can directly impact human health via oral ingestion, inhalation and dermal contact. Most of health risk assessment studies are based pseudototal concentrations. Nevertheless, not all PTEs concentration present in road dusts are available to absorption and the use of total contents would somehow overestimate the risk comparing with the use of bioaccessible contents [37].

Table 2 presents the pseudototal (PT), bioaccessible G and GI fractions risk assessment results for arsenic. Results show that the risk represented by the PT fraction is overestimated comparing to BAF. Nevertheless, total G and GI risk is above the carcinogenic target risk (1.00E-06) in all samples, except for SFA2 and B2. Additionally, carcinogenic risk of the BAF exposed that As represents the higher risk of developing cancer over a lifetime, being the other variables considered negligible, with Pb representing a maximum of 3.0% of the total Risk for both G and GI phases. Also, the ingestion route is the one that represents higher risk. Except for samples SFA1 and SFA2, in other samples the dermal exposure route represents ≤ 12.0 % and inhalation ≤ 0.2 %, for the sum of the three exposure paths.

Table 2. Arsenic carcinogenic risk on Pseudototal (PT), Gastric (G) and Gastrointestinal (GI) phases, taking in consideration the Ingestion + Inhalation + Dermal exposure routes (G_{Total}, GI_{Total} and PT_{Total}) and only Ingestion route (G_{Ing}, GI_{Ing}, PT_{Ing}). In orange, values above the carcinogenic target risk (> 1.00E-06) and in red considered as inadmissible (> 1.00E-04).

ID	G_{Ing}	G_{Total}	GI_{Ing}	GI_{Total}	PT_{Ing}	PT_{Total}
SFA1	**1.34E-06**	**1.62E-06**	**1.19E-06**	**1.44E-06**	**8.83E-04**	**1.07E-03**
SFA2	7.18E-07	8.71E-07	5.45E-07	6.61E-07	**5.61E-04**	**6.80E-04**
B1	**2.72E-06**	**3.30E-06**	**2.48E-06**	**3.00E-06**	**1.29E-04**	**1.56E-04**
B2	2.23E-07	2.70E-07	2.23E-07	2.70E-07	**7.08E-05**	**8.59E-05**
C1	**8.42E-06**	**1.02E-05**	**8.92E-06**	**1.08E-05**	**1.60E-04**	**1.94E-04**

C2	**4.95E-06**	**6.01E-06**	**5.20E-06**	**6.31E-06**	**5.67E-04**	**6.87E-04**
UV1	**1.66E-06**	**2.01E-06**	**1.88E-06**	**2.28E-06**	**4.40E-04**	**5.34E-04**
UV2	**2.38E-06**	**2.88E-06**	**2.48E-06**	**3.00E-06**	**2.29E-04**	**2.78E-04**

Toxicity of inorganic arsenic (As_i) varies with its valence state and with physical and chemical properties. Soluble As_i is absorbed through the GI tract being primarily distributed to the liver, kidney, aorta, and skin. Nevertheless, As_i can be excreted by the human body, mainly in urine, at rates as high as 80%. Acute lethal dose to humans was estimated to be ~0.6 mg/kg/day. Common identified symptoms of chronic As poisoning in humans are, e.g., weakness, general debility and lassitude, loss of appetite, energy, hair and weight, and mental disorders. Several studies have shown that As increases the risk of lung, skin, bladder, liver, kidney, and prostate cancer [38].

The systemic toxicity (i.e., non-carcinogenic hazard) of the < 250 µm fraction bioaccessible fraction, was below the target value of 1 in all samples in opposition to the pseudototal concentration where As presented HI = 8.5 in sample SFA1. Nevertheless, these dusts bioaccessible fraction do not represent a probability of non-carcinogenic effects to occur in both children and adults by ingestion, inhalation or dermal contact.

4. Conclusions

The mining and beneficiation process at Panasqueira mine produces mine wastes considered responsible for the high levels of metal(loids) noticeable on the road dusts collected in four villages located on the surroundings of the mine. The pseudototal contents identified in the analyzed dust samples, reflect the influence of the waste deposits materials (e.g., scorodite, yukonite, arseniosiderite among others) due to long-distance transport and dispersion of mine wastes, both by wind or traffic. These dust samples As pseudototal concentration exceed 40.5 (SFA), 8.7 (Barroca), 20.6 (Casegas) and 30.7 (UV) times the maximum reference value range (RVR_{As} = 2 to 20 mg kg^{-1}) and the acceptable risk value (ARV_{As} = 20 mg kg^{-1}). Enrichment Factor calculations confirms this statement, in particular for As, with a maximum value in SFA greatly above the EF classification for extremely high enrichment of 40. In addition, SEM-EDS analysis identified particles that reveal weathered agglomerates rich in As, Ca, Fe, Cu, Al, Ti.

Gastric (G) and gastrointestinal (GI) phases bioaccessible fractions for As, Cd, Co, Cr, Cu, Fe and Pb, determined by the UBM assay reveals, as a main conclusion, that bioaccessibility extraction vary depending on the phase extraction and sample location. The comparison between the two phases show that G phase has higher median concentrations than GI phase, except for As in the areas considered not impacted by the mining activities. When compared the pseudototal (PT) and bioaccessible G and GI fractions, risk assessment for arsenic, results points out that Risk represented by the pseudototal fraction is overestimated, nevertheless, total G and GI Risk is above the carcinogenic target risk (1.00E-06) in most of the samples. Carcinogenic Risk of the BAF reveal that As represents the higher risk of developing cancer over a lifetime and the ingestion route is the one that represents higher risk.

Regarding the link between the increase of gastric and gastrointestinal bioaccessible extracted concentrations and the decrease of particles grain size, further tests with other particle sizes will be performed in order to assess the bioaccessible fraction in different granulometric classes and understand which represents a higher risk to humans.

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