Journal Pre-proof

Understanding how methodological aspects affect the release of trace metal(loid)s from urban dust in inhalation bioaccessibility tests

A. Expósito, B. Markiv, L. Ruiz-Azcona, M. Santibáñez, I. Fernández-Olmo

PII: S0045-6535(20)33378-6

DOI: https://doi.org/10.1016/j.chemosphere.2020.129181

Reference: CHEM 129181

To appear in: *ECSN*

Received Date: 29 July 2020

Revised Date: 18 November 2020

Accepted Date: 1 December 2020

Please cite this article as: Expósito, A., Markiv, B., Ruiz-Azcona, L., Santibáñez, M., Fernández-Olmo, I., Understanding how methodological aspects affect the release of trace metal(loid)s from urban dust in inhalation bioaccessibility tests, *Chemosphere*, https://doi.org/10.1016/j.chemosphere.2020.129181.

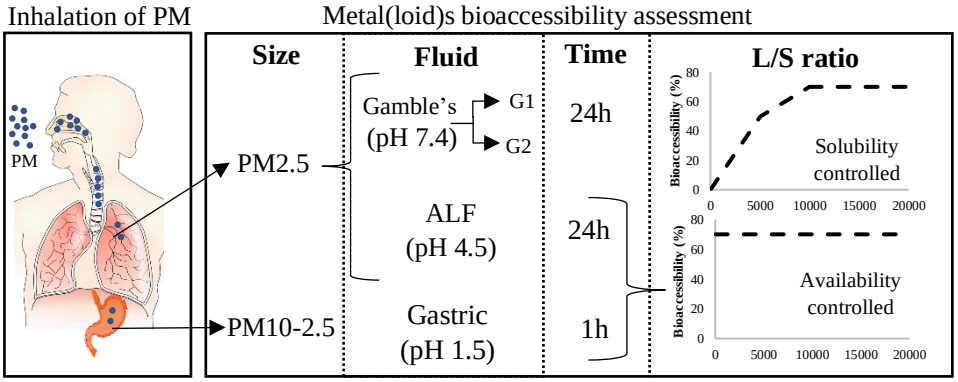
A. Expósito: Investigation, Writing- Original draft preparation, Formal analysis; B. Markiv: Investigation, Resources; L. Ruiz-Azcona: Investigation, Resources; M. Santibáñez: Supervision, Funding acquisition; I. Fernández-Olmo: Conceptualization, Methodology, Reviewing and Editing, Supervision, Funding acquisition

Understanding how methodological aspects affect the release of trace metal(loid)s from urban dust in inhalation bioaccessibility tests

A. Expósito[1,*], B. Markiv[1], L. Ruiz-Azcona[2], M. Santibáñez[2], I. Fernández-Olmo[1]
[1]Department of Chemical and Biomolecular Engineering, Universidad de Cantabria, Spain
[2]Department of Nursing. Global Health Research Group, Universidad de Cantabria, Spain.

[*]Corresponding author. Tel: +34 942 201579, E-mail: andrea.exposito@unican.es

GRAPHICAL ABSTRACT



Understanding how methodological aspects affect the release of trace metal(loid)s from urban dust in inhalation bioaccessibility tests

A. Expósito[1,*], B. Markiv[1], L. Ruiz-Azcona[2], M. Santibáñez[2], I. Fernández-Olmo[1]
[1]Department of Chemical and Biomolecular Engineering, Universidad de Cantabria, Spain
[2]Department of Nursing. Global Health Research Group, Universidad de Cantabria, Spain.

[*]Corresponding author. Tel: +34 942 201579, E-mail: andrea.exposito@unican.es

Abstract

The bioaccessibility of metal(loid)s in ambient particulate matter (PM) has been recently used to represent the risk of inhalation exposure. Nevertheless, different methodological factors affect the bioaccessibility values; among these, the type and composition of surrogate biological fluids and the liquid to solid ratio have been revealed to be the most important. To better understand how these methodological aspects affect the bioaccessibility, a reference material corresponding to urban dust (SRM 1648a) was contacted with synthetic biological fluids commonly used in the literature representing surrogate fluids that may interact with fine (Gamble's solutions, artificial lysosomal fluid (ALF)) and coarse particles (gastric fluid), for liquid to solid (L/S) ratios ranging from 500 to 20,000. Visual MINTEQ 3.1. was used to enhance the discussion on how the solubility of metals in the leaching solution depends on the composition of the simulated fluids and the speciation of metals. The results obtained indicate that a small change in the composition of Gamble's solution (the presence of glycine) may increase significantly the bioaccessibility at a L/S ratio of 5,000. The highest bioaccessibility of most of the studied metal(loid)s at a L/S ratio of 5,000 was found for ALF fluid. The study of the effect of the L/S ratio showed that metal(loid)s bioaccessibility in Gamble's fluid increased logarithmically with increasing L/S ratio, while it remained practically constant in ALF and gastric fluid. This different behavior is explained assuming that the leaching of metal(loid)s in Gamble's solution is solubility-controlled, while in ALF and gastric fluid is availability-controlled.

Keywords: inhalation bioaccessibility, trace metal(loid)s, synthetic body fluids, urban dust

1. Introduction

Inhalation of ambient particulate matter (PM) has been mainly associated with respiratory and cardiovascular diseases (Campen et al., 2001; Wallenborn et al., 2007; Hoek et al., 2013; Cesaroni et al, 2014). The major components of PM are sulfate, ammonium, nitrate, sodium, chloride, sea salt, carbonaceous material and crustal element, while the minor ones are trace metal(loid)s, in addition to persistent organic compounds (Seinfeld and Pandis, 2016). Trace metal(loid)s are potentially toxic because they may induce the formation of reactive oxygen species (ROS) that can damage DNA an initiate a catalytic cycle of cell membrane lipid peroxidation (See et al., 2007; Charrier et al., 2014; Bates et. al. 2015).

Recent toxicological research highlighted that the soluble forms of metal(loid)s participate in redox reactions, involving ROS production (Wallenborn et al., 2007; Charrier and Anastasio, 2015; Calas et al., 2017, Bates et al., 2019). Therefore, the potential health effects from the metal(loid)s present in PM depend upon the solubility of elements in human body. This solubility is influenced by the chemical speciation of these metal(loid)s and by the size and shape of particles (Kelly and Fussell, 2012). Different approaches to assess the toxicity of metal(loid)s derived from the inhalation exposure to ambient PM have been reported in the literature. In-vitro methods using surrogate biological fluids are considered a potential alternative to measure toxicity from PM since they are simple and unexpensive (Kastury et. al, 2017). Bioavailability is the fraction of a metal(loid) in exposure media absorbed by the organism, typically determined by in-vivo animal models, whereas bioaccessibility is the fraction of the total metal(loid) concentration released from the environmental matrix (e.g. PM, dust, soil, food, water) into a synthetic biological fluid and becomes available for absorption (Manjón et al., 2020).

Air quality regulation for metal(loid)s only consider the total metal(loid) content in the PM10 fraction (see e.g. EU Directives 2004/107/EC and 2008/50/EC); and total element contents are measured using different standards (see e.g. the European standard method “EN-UNE 14902-2006”). Accordingly, conventional inhalation risk assessment studies to metal exposure only

consider the total concentration of some trace metals in PM. However, the soluble concentration of PM-bound trace metal(loid)s instead of total content may better represent the exposure risk of such pollutants in humans (Mbengue et al., 2015; Hernández-Pellón et al., 2018; Weggeberg et al., 2019). The in-vitro analytical procedure to determine the bioaccessibility of elements consists of contacting PM filters with leaching agents that simulate body fluids. However, there is no unified protocol for the assessment of inhalation bioaccessibility, which, due to the high variability between samples and procedures found in the literature (Mukhtar and Limbeck, 2013; Wiseman, 2015; Kastury et. al, 2017, 2018a, 2018b), makes comparisons between studies difficult. Factors influencing element bioaccessibility can be classified as external and internal. External factors mainly include the composition of simulated lung fluid and the conditions for in-vitro methods, including extraction time, liquid to solid (L/S) ratio, agitation (Mukhtar and Limbeck, 2013; Kastury et al., 2018b) and the solid–liquid separation method (Laird et al., 2015). The most important internal factors are physiochemical characteristics of samples such as metal(loid)s speciation and types and sizes of particles (Ren et al., 2020).

The use of reference materials is suitable to study the methodological aspects (external factors). To take into account the influence of the particle size fraction, different surrogate body fluids should be used according to the fate of such particles in the human body. Only particles smaller than 10μm (PM10) have the potential to deposit in the tracheobronchial and alveolar region. Coarse particles in the 2.5-10 μm size fraction (PM2.5-10) are in most cases deposited in the pharyngeal and tracheal region, from where they are transported and swallowed toward the digestive system, where these coarse particles come into contact with gastric juice (Mukhtar and Limbeck, 2013). Besides, fine particles less than 2.5 μm (PM2.5) can be transported to the alveolar region. Accordingly, the use of gastric and pulmonary fluids as leaching agents with coarse and fine particles, respectively, seems to be appropriate. For this purpose, several surrogate synthetic fluids are applied in the literature. Gastric fluid has been widely used to assess the ingestion risk by measuring the metal bioaccessibility in soils applying standardization protocols such as U.S.EPA (2007), European Pharmacopoeia (2010), and Unified BARGE Method (Denys et al., 2012), but rarely used to assess the inhalation

bioaccessibility in the coarse fraction of PM (Mukhtar and Limbeck, 2013; Kastury et al., 2018a). Different Simulated Lung Fluids (SLFs) are used in the literature to assess the bioaccessibility in the fine fraction of PM: Gamble's solution and Artificial Lysosomal Fluid (ALF) are the most SLFs used to represent neutral and acidic conditions respectively. On one hand, Gamble's solution mimics the interstitial lung fluid, and on the other hand, ALF represents the acidic fluid (pH 4.5) resulting from the macrophages attack to the particles reaching the alveoli. Although, the composition of ALF is similar in most research studies (Colombo et al., 2008; Wiseman and Zereini, 2014; Kastury et al., 2018b, Meza-Figueroa et al., 2020), different compositions of Gamble's solutions are found in the literature, as observed in Table S1 of the Supplementary Material. These Gamble's solutions can be classified into two main groups; the first one was based on the composition used by Moss (1979) (Colombo et. al., 2008; Boisa et. al., 2014; Hernández-Pellón et al., 2018; Kastury et al., 2018a; Weggeberg et. al., 2019) and the second one by Eidson and Mewhinney (1983) (Gray et. al., 2010; Caboche et. al., 2011; Wragg and Klinck, 2007; Pelfrêne et. al., 2017). The main difference is that the group of Moss (1979) added sodium acetate and the group of Eidson and Mewhinney (1983) added glycine. Besides, some authors added other organic reagents, such as dipalmitoyl phosphatidyl choline (DPPC) (Caboche et al., 2011; Marques et al., 2011; Mbengue et al., 2015; Pelfrêne et al., 2017) to simulate better the interstitial fluid. Although Kastury et al. (2018a) compared the extraction efficiencies of different neutral lung lining fluids, the bioaccessibility of the two groups of Gamble's fluids has not been compared yet.

The use of the same reference material by different researchers allows the comparison between the bioaccessibility of some metal(loid)s contacted with different Gamble's solutions. Figure 1 shows the bioaccessibility of some metals (manganese, copper, zinc and lead) when a reference material of urban dust (SRM1648a) is contacted with different Gamble's solutions (Caboche et. al 2011; Pelfrêne et al., 2017; Weggeberg et al., 2019). The composition of the Gamble's solutions used by Caboche et al. (2011) and Pelfrêne et. al (2017) was based on that of Eidson and Mewhinney (1983), but with some small differences (see Table S1 of the Supplementary Material). However, Weggeberg et. al (2019) used a Gamble's solution similar than that used by

Moss (1979). In addition, different L/S ratios were used. The results shown in Figure 1 clearly indicate that Weggeberg et al. (2019) obtained much lower values of bioaccessibility and that the L/S ratio affects the solubility of metals in Gamble's solutions. However, the rationale of such differences in metals bioaccessibility is lack in the literature.

Therefore, the aim of this study is to understand how some external methodological factors affect the metals inhalation bioaccessibility from PM. To this end, the bioaccessibility of a reference material corresponding to urban dust (SRM1648a), using synthetic biological fluids commonly used in the literature (Gamble's, ALF and gastric) and water, was studied. Two Gamble's compositions (called G1 and G2, the latter containing glycine) were used. Since the liquid to solid (L/S) ratio seems to be one of the key factors governing the leaching of trace elements from solid particles, mainly under non-acidic conditions, L/S ratios ranging from 500 to 20,000 were used, considering the following metal(loid)s: V, Mn, Fe, Ni, Cu, Zn, As, Cd, Sb and Pb.

2. Materials and methods

2.1. Reference material

The standard reference material 1648a (SRM1648a) was selected to analyze the effect of the L/S ratio and the type and composition of leaching agents on the bioaccessibility of metal(loid)s. SRM1648a is a reference material of atmospheric PM collected in an urban area (St. Louise, MO, USA) with a particle size less than 100 μm and a median value of 5.85 μm. The certified mass fraction values for the studied elements are shown in Table S2 of the Supplementary Material. For each metal(loid), the bioaccessibility is calculated using the following formula: (Guney et al., 2017):

$$\%Bioaccessibility = \frac{C_{bio} \cdot V_{Fluid}}{C_{total} \cdot m} \cdot 100 \quad (1)$$

Where the C_{bio} is the concentration of an element in the surrogate fluid (mg/L), V_{Fluid} is the volume of fluid (mL), C_{total} is the total certified concentration of an element (mg/Kg) and m is the mass of reference material (g).

2.2. Inhalation bioaccessibility in vitro method

In-vitro bioaccessibility tests were performed by introducing 2.5 mg of SRM1648a accurately weighted into a 50 ml polypropylene vessel and adding each selected leaching agent. According to Hernández-Pellón et al. (2018), the daily mass of PM10 filters collected by a low sampler device (2.3 m^3/h) is typically between 0.6 and 3.1 mg. Then, the vessels were capped and placed in an end-over-end rotation incubator system (MRHX-04/SBS) at 30 rpm and at 37ºC, simulating the body temperature. The extraction time was 24 h for SLFs (Midander et al, 2007; Colombo et al., 2008; Caboche et al., 2011; Kastury et al., 2018a; Luo et al., 2019) as well as ultrapure water (Caboche et al., 2011) and 1 h for gastric fluid (Oomen et al., 2002; U.S.EPA, 2007; Drexler and Brattin, 2007; Deshommes et al., 2012; Kastury et al., 2018b). After the extraction test, the samples were centrifuged (Mistasel-BL/SELECTA) at 4,200 rpm for 10 min and the supernatants filtered through a 0.45 μm polypropylene syringe filter. The samples were stored until analysis at 4ºC and maximum storage time was 48h for Gamble's solution and ALF.

2.3. Selection and formulation of leaching solutions

To simulate the biological body conditions synthetic body fluids were selected as leaching agents. The selection of these fluids should be done based on the fate of PM in the body, which depends on the aerodynamic diameter of the particles. Thus, Gamble's solution and ALF were selected as lung fluids that can be contacted with PM2.5: Gamble's solution, as a surrogate of the interstitial lung fluid, and ALF, as a surrogate of the acidic conditions resulting from the macrophage attack to particles in alveoli. Gastric fluid was selected to represent the body fluid that can be contacted with coarse particles (PM 2.5-10), since these particles are swallowed from the upper airways to the digestive system. According to the discussion of the different composition of Gamble's solutions shown in the Introduction section, two Gamble's solutions

called as G1 and G2 were chosen; the composition of G1, G2 and ALF, and the order of addition of reagents are presented in Table 1.

Overall, five solutions were used to determine and compare the bioaccessibility of metal(loid)s in SRM1648a: ultrapure water (pH = 6.8±0.1), Gamble's (G1) based on Moss (1979) (pH = 7.4±0.1), Gamble's (G2) based on Eidson and Mewhinney (1983) (pH = 7.4±0.1), ALF (pH = 4.5±0.1) (Marques et al., 2011) and gastric (pH = 1.5±0.1) (U.S.EPA, 2007). The reagents used were of analytical grade or higher purity provided by Merck and Sigma Aldrich. HNO_3 and NaOH was used for pH adjustment. With respect to the gastric fluid, although complex formulations including different amino acids, enzymes and metabolic acids have been used in oral bioaccessibility tests (Ruby et al., 1993; Medlin, 1997; European Pharmacopoeia, 2010; Nie et al., 2018; Gao et al., 2018), a simpler formulation including glycine and HCl has been proved to provide similar results in bioaccessibility tests (U.S.EPA, 2007). Therefore, gastric fluid was prepared using 0.4M of glycine and adjusting the pH with HCl (37%) (U.S.EPA, 2007; Drexler and Brattin, 2007). The pH of the synthetic fluids was measured immediately before the beginning of the bioaccessibility test.

2.4. Influence of the L/S ratio on bioaccessibility

Different tests were conducted to analyze the influence of the L/S ratio on the bioaccessibility values in SRM1648a when applying the following leaching agents: Gamble's solution (G2), ALF and gastric fluid. The values of L/S ratio (expressed as mL/g) were 500, 1,000, 5,000 and 20,000. A further discussion about the selection of the range of L/S ratio was included in section 3.3. Besides, additional tests using Gamble's (G1) at L/S ratios of 500 and 5,000 and ultrapure water at L/S of 500 were conducted. In each case, 2.5 mg of SRM1648a was weighted and then between 1.25 and 50 ml of leaching agent was added.

2.5. Metal(loid) analysis

The concentration of V, Mn, Fe, Ni, Cu, Zn, As, Cd, Sb and Pb in the extracts of SRM1648a was measured by inductively coupled plasma mass spectrometry (ICP/MS, Agilent 7500 CE). Blanks were measured to check for the potential contamination from vessels and reagents.

Internal standards (^{89}Y, ^{103}Rh and ^{185}Re) were added to correct for instrumental drifts, and a collision cell with a helium flow rate of 4.8 ml/min was used to minimize spectral interferences. Since SLFs and gastric fluid contain various dissolved salts, which can cause spectral as well as non-spectral interferences (matrix effects) during ICP-MS analysis, the determination of the concentration of studied metal(loid)s in these leaching agents was performed by adding these fluids to the Multielement Standard Solution used to calibrate the instrument, leading to worse detection limits for the studied elements with respect to acidified ultrapure water; anyway, the concentrations of the studied elements were always above the detection limits. Seven calibration points between 0 and 25 ppb were used and samples were diluted between 1:1 to 1:100 when necessary. After calibration and at the end of each analytical run, quality control standards covering the concentration range of interest were measured to check for the accuracy of the measurements. The instrument was re-calibrated after not more than 20 samples.

This reference material was previously used by the research group to check the validity of the total digestion method (Hernández-Pellón et al., 2018); however, there is not a reference material/method that certifies inhalation bioaccessibility values of trace metal(loid)s. Therefore, a comparison was made between bioaccessibility data obtained in the literature using the same reference material, and the same leaching agents and conditions when possible (Pelfrêne et al., 2017), obtaining a reasonable match for most of the studied elements (this is discussed further below). In addition, all bioaccessibility tests were performed in triplicate (n=3). The average deviation between replicate samples was < 3.3 % for ALF, < 7.4 for gastric and <7.7 % for Gamble's solution.

2.6. Simulation of metal(loid)s solubility by Visual MINTEQ

Visual MINTEQ 3.1. (Gustafsson, 2014) was used to simulate the contact between the leaching agent and the reference material, in order to understand how the solubility of metals in the leaching solution changes depending on the composition of simulated fluid and the speciation of metals. The composition of fluids was introduced as cations and anions, besides, pH, L/S ratio and temperature were set. The metal(loid)s speciation in SRM1648a is unknown, therefore,

different metal(loid) species were used to simulate the leaching process. In each simulation, the solubility fraction of metal(loid)s and species distribution were obtained.

3. Results and discussion

3.1. Influence of the composition of interstitial lung fluids on the bioaccessibility

Gamble's G1 and G2 solutions were used to analyze the influence of the composition of neutral lung lining fluids on the bioaccessibility of metal(loids) in SRM1648a. As Figure 2 shows, analyzed metal(loid)s had low bioaccessibility in Gamble's G1 using L/S ratios of 500 and 5,000. The results show a stronger impact of L/S ratio on the bioaccessibility of metal(loid)s in Gamble's G2: the bioaccessibility using a L/S ratio of 5,000 was much higher than using a L/S ratio of 500, for example, the mean value of Mn changed from 1.5% at a L/S ratio of 500 to 31.7% at a L/S ratio of 5,000. Besides, the bioaccessibility of some metal(loid)s was similar using a L/S ratio of 500 in Gamble's G1 and G2, with the exception of Ni (16.3% G1 and 21.59% G2) and Cu (12.5% G1 and 31.6% G2) that had higher bioaccessibility in Gamble's G2.

The great difference between bioaccessibility of metal(loid)s in Gamble's G1 and G2 at a L/S ratio of 5,000 suggests that some components of Gamble's G2 increase the solubility of metal(loid)s. The ionic composition of both fluids is presented in Table S3 of the Supplementary Material; the main difference is that Gamble's G1 contents acetate and Gamble's G2 glycine.

Since the speciation of metal(loid)s in SRM1648a is unknown, Visual MINTEQ 3.1. was used to simulate the leaching of some target metal species in Gamble's G1 and G2 (pH=7.4). For example, the leaching of Cu, one of the metals showing the greatest difference in solubility between G1 and G2 fluids, was simulated using $Cu_3(PO_4)_2$ (0.001M) as target compound. Visual MINTEQ 3.1. results indicated that the solubility of Cu^{+2} in Gamble's G1 and G2 was 3% and 82%, respectively; the different solubility can be explained in Figure 3, where the distribution of soluble species for Cu^{+2} in both solutions is shown. With Gamble's G1, soluble Cu is mainly associated with $CuCO_3$(aq) and Cu-Citrate$^-$ species (Figure 3a). However, when

Gamble's G2 was used, the speciation changed significantly with more than 90% of soluble Cu in the form of Cu-$(Glycine)_2$ (aq) (Figure 3b). Although this should not be interpreted in a quantitative way, Visual MINTEQ results confirmed that the presence of glycine in Gamble's G2 allowed the formation of soluble metal complexes increasing the bioaccessibility of such metals, as discussed by Kastury et al. (2017).

It is well known that the presence of proteins in SLFs increases the solubility of metal(loid)s. For example, albumin may play a role in higher metal(loid) extraction observed for Hatch's solution (Kastury et al., 2018a), because it has the capacity to bind metals through sequestration (Peters and Blumenstock, 1967). However, amino acids are usually used instead of proteins for simplicity, and glycine is the most employed, although it was not clear why glycine was chosen to represent protein among all amino acids (Kastury et al., 2017). In addition, the chelating efficiency of amino acids is different (Harris and Silberman, 1983) and this may lead to different bioaccessibility values of selected metal(loid)s (Kastury et al., 2017). Therefore, in the formulation of Gamble's solution, it is important to use always the same amino acid (glycine) at the same concentration.

3.2. Influence of the type of leaching agent on bioaccessibility

A detailed protocol to account for the bioaccessibility of metal(loid)s from inhaled PM should consider different synthetic body fluids depending on the fate of particles on the human body, which also depends on the particle size of PM. The pH and chemical composition of these fluids will affect the solubility of metal(loid)s. For this reason, ultrapure water and different simulated body fluids (Gamble's G2 representing interstitial lung fluid, ALF as a surrogate of the acidic conditions resulting from the macrophage attack to fine particles in alveoli, and gastric fluid to represent the body fluid in which the coarse particles will be) were used for assessing the bioaccessibility of potentially toxic metal(loid)s from SRM1648a. Figure 4 shows the mean and standard deviation of the bioaccessibility of metal(loid)s in the studied fluids at a L/S ratio of 5,000. This L/S ratio was selected for comparison purposes because this value can be achieved in humans when inhalating 50 $\mu g/m^3$ of PM10, and considering a total alveolar fluid volume of

267 5 mL and a daily air uptake of 20 m^3, and assuming that 100 % of the inhaled particles reach the
268 pulmonary alveoli. Results shown in Figure 4 confirm that the bioaccessibility of metal(loid)s
269 depends on the type of leaching agents, which differ in pH and chemical composition. The
270 soluble fraction of metal(loid)s was lower in ultrapure water than in synthetic biological fluids,
271 with the exception of Ni, which showed similar bioaccessibility values in all the studied
272 leaching agents.
273 When the studied neutral fluids were compared (i.e., Gamble's G2 and water), most metal(loids)
274 showed higher bioaccessibility in Gamble's G2, which was attributed to the presence of organic
275 and inorganic reagents in this SLF that can form soluble metal(loid) complexes, as discussed in
276 Section 3.1. In accordance with this, Caboche et al. (2011) indicated that the bioaccessibility of
277 metals in Gamble's solution was higher than in water.
278 The bioaccessibility of most metal(loid)s was higher in ALF than in Gamble's solution, as
279 reported in the literature (Wiseman and Zereini, 2014; Mukhtar et al., 2015; Guney et al., 2017;
280 Hernández-Pellón et al., 2018; Weggeberg et al., 2019; Gosselin et al., 2020; Meza-Figueroa et
281 al., 2020). Iron and Pb were the metals with the highest difference as shown in Figure 4 (Fe: 1.1
282 vs 20.0 %; Pb: 1.9 vs 77.3 % in G2 and ALF respectively). This behavior was not observed for
283 Ni, V and Cu; Pelfrêne et al. (2017) also obtained a similar bioaccessibility in Gamble's
284 solution and ALF at a L/S ratio of 5,000.
285 Moreover, the highest bioaccessibility of Mn, Fe, Zn, As, Cd, Sb and Pb was found in ALF
286 (pH=4.5), even higher than in gastric fluid (pH=1.5), supporting the idea that pH is not the only
287 factor that affects the solubility of metal(loid)s. This agrees with the results found by Kastury et
288 al. (2018b), which showed that the bioaccessibility of Pb, Fe, As and Mn from a reference
289 material of soil (SRM2710a) was also higher in ALF than in gastric fluid.
290 Finally, the differences between the bioaccessibility obtained in Gamble's solution and gastric
291 fluid at a L/S ratio of 5,000 were not relevant, with the exception of Pb (2 % in Gamble's (G2)
292 vs 55 % in gastric), Fe (1 % in Gamble's (G2) vs 11 % in gastric) and Cd (42 % in Gamble's
293 (G2) vs 53 % in gastric). This may be due to how the different composition and pH of both
294 fluids affect the solubility of the studied metal(loid)s. Visual MINTEQ 3.1. was used to explain

295 semi-quantitatively why a few metal(loid)s showed different bioaccessibility in gastric and
296 Gamble's fluids, whereas the other metal(loid)s obtained similar bioaccessibility values. In
297 particular, Pb and Mn were selected as target metals, because Pb showed the greatest difference
298 in bioaccessibility while Mn solubility was similar in both fluids. As Pb speciation in
299 SRM1648a was unknown, four Pb solid species were considered; the concentration of Pb
300 species was calculated in mol/kg from the total certified content of Pb in SRM1648a. The
301 simulation results using Visual MINTEQ 3.1. are shown in Table S4 of the Supplementary
302 Material: the soluble fraction of Pb was 100% in gastric fluid in all hypotheses, whereas the
303 soluble fraction of Pb in Gamble's solution varies with metal speciation from 5 to 100 %. Also,
304 a simulation with the four checked Pb solid species was carried out, showing that the soluble
305 fraction of Pb^{+2} was higher in gastric fluid (Gamble's solution 1% vs Gastric fluid 100%). With
306 respect to Mn, Visual MINTEQ results showed that the soluble fraction of Mn^{+2} was similar in
307 the two fluids and for all the studied Mn species (results not shown here). These results explain,
308 at least qualitatively, the different behavior of Pb and Mn when they are released from the
309 reference material in Gamble's and gastric fluids. In addition, this analysis also indicated that
310 bioaccessibility of some metals, such as Pb, not only may depend on the composition and pH of
311 synthetic fluid (external factor), but also on their speciation (internal factor). Therefore, the high
312 variability in metal(loid) bioaccessibility typically found when working with real PM samples
313 using the same bioaccessibility method with the same extraction fluid may be explained by
314 changes in the speciation of these metal(loid)s due to the different contribution of sources over
315 the course of the sampling campaigns.

316 **3.3. Influence of the L/S ratio on the bioaccessibility of metal(loid)s**

317 According to Macklin (1955), an average alveolar fluid depth of 0.2 μm covering a surface area
318 of 100 m^2 would lead to a total alveolar fluid volume of 20 mL. Considering conditions of
319 inhalation exposure for 24 h under a large range of particle concentrations from 20 to 500
320 $\mu g/m^3$, and assuming that 100% of the inhaled particles reach the pulmonary alveoli with a daily
321 air uptake between and 10 and 20 m^3 and a total alveolar fluid volume ranging from 5 to 20 mL,

the corresponding L/S ratio could vary between 100,000 and 500 mL/g (Caboche et al., 2011; Pelfrêne et al., 2017; Kastury et al., 2018a). This has resulted in a wide range of L/S ratios used in the literature for bioaccessibility tests. For example, Caboche et al. (2011) used L/S ratios between 30 and 50,000 to obtain the bioaccessibility of four reference materials representing different types of particles (NIST 1648a, BC 038, NIES 8 and NIST 2548) in Gamble's solution. These authors suggest that L/S ratios below 500 present risk of saturation of solution or competition between the soluble elements. Pelfrêne et al. (2017) studied the bioaccessibility of three reference materials (BCR-723, NIST2710a and NIST1648a) using four ratios from 100 to 10,000 in Gamble's solution and ALF; these authors observed an impact of the L/S ratio on the bioaccessibility of some metal(loid)s in Gamble's solution. In the same way, Kastury et al. (2018a; 2018b) reported an increased metal(loid)s bioaccessibility at higher L/S ratios when using Gamble's solution, but not when using ALF.

Besides, the standardization protocol developed by U.S.EPA to measure the metal bioaccessibility in soils recommended a L/S ratio of 100 to reduce the effect of metal dissolution (U.S.EPA, 2007). However, although this ratio can be applied with reference materials, higher L/S ratios are needed with ambient PM samples because of the low sample weight typically collected with low volume samplers. As a summary, the variation of bioaccessibility of metal(loid)s in Gamble's solution with the L/S ratio is uncertain in the literature and in gastric fluid is unknown for urban dust.

Taking into account the results found in the literature and the experimental constraints, four L/S ratios ranging from 500 to 20,000 were used to analyze the influence of L/S ratio on bioaccessibility in Gamble's solution, ALF and gastric fluid. As Figure 5 shows, the bioaccessibility of V, Mn, Fe, Ni, Cu, Zn, As, Cd, Sb and Pb in Gamble's G2 increased logarithmically with the L/S ratio, while it remained almost constant in gastric and ALF fluids. The effect of the L/S ratio on the bioaccessibility in Gamble's solution is similar to that found in a study conducted by Pelfrêne et al. (2017) for Cd, Mn and Zn. However, the study of Pelfrêne et al. (2017) showed that the behavior of Cu and Ni was different: the bioaccessibility of Cu was constant while that of nickel decreased with the L/S ratio; the later can be due to the worse

detection limit of the ICP-OES used by Pelfrêne et al. (2017) with respect to that of ICP-MS used in this work, which may difficult the determination of some metals at high L/S ratios. Kastury et al. (2018a) also found that As and Pb bioaccessibility in SRM2710a and some PM10 filters prepared from soil at 120 h using a L/S ratio of 5,000 was significantly higher than that using L/S ratios of 100, 500 and 1,000. A similar trend was also found by these authors for Al, Cd, Fe, Mn and Zn, with the highest metal bioaccessibility at L/S of 5,000. Sysalová et al. (2014) also found a similar behavior for As, Cd, Cr, Mn, Ni, Pb and Zn with real PM samples, but using the Hatch's solution in a range of L/S between 100 and 1,000. With respect to ALF, Pelfrêne et al. (2017) and Kastury et al.(2018b) reported that metal(loid)s bioaccessibility is not significantly affected by the L/S ratio in accordance with the results shown in Figure 5.

However, the results showing the effect of the L/S ratio on the bioaccessibility of many metal(loid)s have not been explained yet in the literature. First, we have to be aware that some physiochemical parameters can affect the release of pollutants from solids, such as contact, particle size, temperature, pH and composition of leaching agents, kinetics, leaching mechanisms, etc. A first hypothesis may be that when using Gamble's solution, the concentration of solubilized metal(loid)s at the end of the extraction period may not be in equilibrium due to kinetic limitations of the release of such metal(loid)s. However, according to the literature, a contact time of 24 hours should be enough to reach the equilibrium conditions; thus, Caboche et al. (2011) reported that the results obtained for four SRMs suggest that the bioaccessibility is maximized after a 24 h extraction time for L/S ratios ranging from 500 to 50,000. Similarly, Kastury et al. (2018b) reported that for As and Pb dissolution in ALF plateaued within 24 h. In addition, bioaccessibility in gastric fluid is practically constant using a contact time of only one hour.

To xplain the different behavior of bioaccessibility with the L/S ratio in Gamble's, ALF and gastric fluids, it was assumed that different leaching controlling mechanisms may occur when using Gamble's solution with respect to ALF and gastric fluid according to their different metal(loid)s solubilities. Solubility control occurs when the solution in contact with a solid is saturated with respect to the constituent species of interest, this means that at a given L/S ratio

the soluble concentration is the saturation concentration. When the L/S ratio is increased, the release of a given metal increases at higher L/S ratios to reach the equilibrium concentration. This means that the soluble fraction (bioaccessibility) will increase with L/S ratio until the maximum solubility is reached (see Figure 6a); this is the behavior observed for Gamble's solution. Availability is defined as the maximum amount of a component that can be released from a solid into solution under aggressive leaching conditions. Under availability-controlled conditions, the resulting metal concentration in solution will decrease with L/S ratio because the same amount of metal will be released, leading to a metal dilution because of the larger leachate volume. However, the soluble fraction will remain constant with the L/S ratio (see Figure 6b), this value being the availability (Kosson et al., 1996). This behavior agrees well with that found in ALF and gastric fluid. These leaching mechanisms have been used previously to explain the different leaching behavior of some elements from solid wastes when changing the L/S ratio (Van der Sloot et al, 1997). According with these hypotheses, the leaching of metal(loid)s in Gamble's solution is assumed to be solubility-controlled, while in more acidic fluids (i.e. when using ALF and gastric fluid), is assumed to be availability-controlled.

3.4. Recommendations for a unified inhalation bioaccessibility protocol

In view of these results, the composition of the simulated interstitial fluid, the type of leaching agent and the L/S ratio are factors that influence the assessment of inhalation bioaccessibility. The following recommendations are derived from the present study: (i) The assessment of the bioaccessibility of PM-bound metal(loid)s by the inhalation route should consider both the use of synthetic lung fluids for fine particles (PM2.5) and gastric fluid for coarse particles (PM10-2.5).

(ii) The use of two types of lung fluids (neutral, such as Gamble's solution, and acid, such as ALF) allows to account for two scenarios in which fine particles may be found in the lung. (iii) Since the composition of neutral lung lining fluids can dramatically affect the solubility of some metal(loid)s, a unique composition that closely simulates the interstitial pulmonary fluid should be used; Gamble's G2 solution is recommended for this purpose. (iv) Since the bioaccessibility

in ALF and gastric fluid is almost independent of the L/S ratio, a relatively low L/S ratio (e.g. 500 to 1,000) is recommended, because this will allow to determine the bioaccessibility of elements present in very low concentrations in PM and even when small amounts of PM are collected in filters (e.g. PM personal samplers). However, it is important to avoid working at L/S ratios less than 5,000 when using Gamble's (G2) fluid, since bioaccessibility increases logarithmically with the L/S ratio, mainly in the range between 500 and 5,000. Ideally, this test should be conducted at the highest possible L/S ratio that allows the metal concentration to be quantified in the Gamble's bioaccessibility assay.

4. Conclusions

The bioaccessibility of metal(loid)s in urban dust (SRM 1648a) has been investigated using different surrogate fluids (Gamble's G1 and G2, ALF, gastric and ultrapure water), and L/S ratios in order to provide new insights into the development of a unified protocol for the assessment of inhalation bioaccessibility. The results first showed the high influence of the composition of the neutral lung fluid (Gamble's solution) on the bioaccessibility of metal(loid)s; at a L/S ratio of 5,000 it was one order of magnitude lower in Gamble's G1 than in G2, due to the presence of glycine in Gamble's G2 leading to the formation of soluble metal complexes. As expected, the type of leaching agent (ultrapure water, Gamble's G2, ALF and gastric) influenced the soluble fraction of metal(loid)s: most the analyzed metal(loid)s at a L/S ratio of 5,000 achieved the highest bioaccessibility in pulmonary ALF and the lowest in ultrapure water, with the exception of Ni and V, which showed similar bioaccessibility values in all the studied leaching agents. When gastric and Gamble's G2 fluids were compared at a L/S ratio of 5,000, similar bioaccessibility values were found for most of the studied metal(loid)s, with the exception of Pb and Fe (2 %Pb in Gamble's G2 vs 55 %Pb in gastric; 1 %Fe in Gamble's G2 vs 11 %Fe in gastric). Visual MINTEQ 3.1. was used to explain semi-quantitatively why Pb showed such different bioaccessibility in gastric and Gamble's fluids; Visual MINTEQ results also indicated that metal(loid)s bioaccessibility in PM samples could depend not only on the composition and pH of the synthetic fluid, but also on their speciation. Finally, the

bioaccessibility of metal(loid)s for L/S ratios ranging from 500 to 20,000 in ALF and gastric fluid was almost constant with the L/S ratio; however, it increased logarithmically with the L/S ratio in Gamble's G2. This different behavior was attributed to different leaching mechanisms of metal(loid)s from the studied reference material: in Gamble's solution it was assumed to be solubility-controlled, while in ALF and gastric fluid availability-controlled.

Acknowledgments

This work was funded by the Spanish Ministry of Science, Innovation and Universities through the CTM2017-82636-R Project. Bohdana Markiv also thanks this Ministry for the PhD grant awarded, PRE2018-085152.

References

Bates, J. T., Fang, T., Verma, V., Zeng, L., Weber, R. J., Tolbert, P. E., Russell, A. G. (2019). Review of Acellular Assays of Ambient Particulate Matter Oxidative Potential: Methods and Relationships with Composition, Sources, and Health Effects. *Environmental Science and Technology*, *53*(8), 4003–4019. https://doi.org/10.1021/acs.est.8b03430

Bates, J. T., Weber, R. J., Abrams, J., Verma, V., Fang, T., Klein, M., Russell, A. G. (2015). Reactive Oxygen Species Generation Linked to Sources of Atmospheric Particulate Matter and Cardiorespiratory Effects. *Environmental Science and Technology*, *49*(22), 13605–13612. https://doi.org/10.1021/acs.est.5b02967

Boisa, N., Elom, N., Dean, J. R., Deary, M. E., Bird, G., Entwistle, J. A. (2014). Development and application of an inhalation bioaccessibility method (IBM) for lead in the PM10 size fraction of soil. *Environment International*, *70*, 132–142. https://doi.org/10.1016/j.envint.2014.05.021

Caboche, J., Perdrix, E., Malet, B., Alleman, L. Y. (2011). Development of an in vitro method to estimate lung bioaccessibility of metals from atmospheric particles. *Journal of Environmental Monitoring*, *13*(3), 621–630. https://doi.org/10.1039/c0em00439a

457 Calas, A., Uzu, G., Martins, J. M. F., Voisin, Di., Spadini, L., Lacroix, T., Jaffrezo, J. L. (2017).
458 The importance of simulated lung fluid (SLF) extractions for a more relevant evaluation of
459 the oxidative potential of particulate matter. *Scientific Reports*, *7*(1), 1–12.
460 https://doi.org/10.1038/s41598-017-11979-3

461 Campen, M. J., Nolan, J. P., Schladweiler, M. C. J., Kodavanti, U. P., Evansky, P. A., Costa, D.
462 L., & Watkinson, W. P. (2001). Cardiovascular and thermoregulatory effects of inhaled
463 PM-associated transition metals: A potential interaction between nickel and vanadium
464 sulfate. *Toxicological Sciences*, *64*(2), 243–252. https://doi.org/10.1093/toxsci/64.2.243

465 Cesaroni, G., Forastiere, F., Stafoggia, M., Andersen, Z. J., Badaloni, C., Beelen, R., Peters, A.
466 (2014). Long term exposure to ambient air pollution and incidence of acute coronary
467 events: Prospective cohort study and meta-analysis in 11 european cohorts from the escape
468 project. *BMJ (Online)*, *348*(January), 1–16. https://doi.org/10.1136/bmj.f7412

469 Charrier, J. G., Anastasio, C. (2015). Rates of Hydroxyl Radical Production from Transition
470 Metals and Quinones in a Surrogate Lung Fluid. *Environmental Science and Technology*,
471 *49*(15), 9317–9325. https://doi.org/10.1021/acs.est.5b01606

472 Charrier, J. G., McFall, A. S., Richards-Henderson, N. K., Anastasio, C. (2014). Hydrogen
473 peroxide formation in a surrogate lung fluid by transition metals and quinones present in
474 particulate matter. *Environmental Science and Technology*, *48*(12), 7010–7017.
475 https://doi.org/10.1021/es501011w

476 Colombo, C., Monhemius, A. J., Plant, J. A. (2008). Platinum, palladium and rhodium release
477 from vehicle exhaust catalysts and road dust exposed to simulated lung fluids.
478 *Ecotoxicology and Environmental Safety*, *71*(3), 722–730.
479 https://doi.org/10.1016/j.ecoenv.2007.11.011

480 Denys, S., Caboche, J., Tack, K., Rychen, G., Wragg, J., Cave, M., Feidt, C. (2012). In vivo
481 validation of the unified BARGE method to assess the bioaccessibility of arsenic,
482 antimony, cadmium, and lead in soils. *Environmental Science and Technology*, *46*(11),

6252–6260. https://doi.org/10.1021/es3006942

Deshommes, E., Tardif, R., Edwards, M., Sauvé, S., Prévost, M. (2012). Experimental determination of the oral bioavailability and bioaccessibility of lead particles. *Chemistry Central Journal*, *6*(1). https://doi.org/10.1186/1752-153X-6-138

Drexler, J. W., Brattin, W. J. (2007). An in vitro procedure for estimation of lead relative bioavailability: With validation. *Human and Ecological Risk Assessment*, *13*(2), 383–401. https://doi.org/10.1080/10807030701226350

Eidson, A. F., Mewhinney, J. A. (1983). In vitro dissolution of respirable aerosols of industrial uranium and plutonium mixed-oxide nuclear fuels. *Health Physics*, *45*(6), 1023–1037. https://doi.org/10.1097/00004032-198312000-00001

European Pharmacopoeia. (2010). Recommendarions on methods for dosage forms testing. *European Pharmacopoeia*, (1), 727–729.

Gao, P., Guo, H., Zhang, Z., Ou, C., Hang, J., Fan, Q., Xing, B. (2018). Bioaccessibility and exposure assessment of trace metals from urban airborne particulate matter (PM10 and PM2.5) in simulated digestive fluid. *Environmental Pollution*, *242*, 1669–1677. https://doi.org/10.1016/j.envpol.2018.07.109

Gosselin, M., Zagury, G. J. (2020). Metal(loid)s inhalation bioaccessibility and oxidative potential of particulate matter from chromated copper arsenate (CCA)-contaminated soils. *Chemosphere*, *238*, 124557. https://doi.org/10.1016/j.chemosphere.2019.124557

Gray, J. E., Plumlee, G. S., Morman, S. A., Higueras, P. L., Crock, J. G., Lowers, H. A., Witten, M. L. (2010). In vitro studies evaluating leaching of mercury from mine waste calcine using simulated human body fluids. *Environmental Science and Technology*, *44*(12), 4782–4788. https://doi.org/10.1021/es1001133

Guney, M., Bourges, C. M. J., Chapuis, R. P., Zagury, G. J. (2017). Lung bioaccessibility of As, Cu, Fe, Mn, Ni, Pb, and Zn in fine fraction (< 20 μm) from contaminated soils and mine

508 tailings. *Science of the Total Environment*, *579*, 378–386.
509 https://doi.org/10.1016/j.scitotenv.2016.11.086

510 Gustafsson, J.P. 2014. Visual MINTEQ ver. 3.1. [Accesed 20 December 2019]. Available from:
511 https://vminteq.lwr.kth.se/

512 Harris, W. R., Sllberman, D. (1983). Time-Dependent Leaching of Coal Fly Ash by Chelating
513 Agents. *Environmental Science and Technology*, *17*(3), 139–145.
514 https://doi.org/10.1021/es00109a004

515 Hernández-Pellón, A., Nischkauer, W., Limbeck, A., Fernández-Olmo, I. (2018). Metal(loid)
516 bioaccessibility and inhalation risk assessment: A comparison between an urban and an
517 industrial area. *Environmental Research*, *165*, 140–149.
518 https://doi.org/10.1016/j.envres.2018.04.014

519 Hoek, G., Krishnan, R. M., Beelen, R., Peters, A., Ostro, B., Brunekreef, B., Kaufman, J. D.
520 (2013). Long-term air pollution exposure and cardio-respiratory mortality: A review.
521 *Environmental Health: A Global Access Science Source*, *12*(1).
522 https://doi.org/10.1186/1476-069X-12-43

523 Kastury, F., Smith, E., Juhasz, A. L. (2017). A critical review of approaches and limitations of
524 inhalation bioavailability and bioaccessibility of metal(loid)s from ambient particulate
525 matter or dust. *Science of the Total Environment*, *574*, 1054–1074.
526 https://doi.org/10.1016/j.scitotenv.2016.09.056

527 Kastury, F., Smith, E., Karna, R. R., Scheckel, K. G., Juhasz, A. L. (2018a). An inhalation-
528 ingestion bioaccessibility assay (IIBA) for the assessment of exposure to metal(loid)s in
529 PM10. *Science of the Total Environment*, *631–632*, 92–104.
530 https://doi.org/10.1016/j.scitotenv.2018.02.337

531 Kastury, F., Smith, E., Karna, R. R., Scheckel, K. G., Juhasz, A. L. (2018b). Methodological
532 factors influencing inhalation bioaccessibility of metal(loid)s in PM2.5 using simulated

lung fluid. *Environmental Pollution*, *241*, 930–937. https://doi.org/10.1016/j.envpol.2018.05.094

Kelly, F. J., & Fussell, J. C. (2012). Size, source and chemical composition as determinants of toxicity attributable to ambient particulate matter. *Atmospheric Environment*, *60*, 504–526. https://doi.org/10.1016/j.atmosenv.2012.06.039

Kosson, D. S., Van Der Sloot, H. A., Eighmy, T. T. (1996). *An approach for estimation of contaminant release during utilization and disposal of municipal waste combustion residues*. *Journal of Hazardous Materials* (Vol. 47). https://doi.org/10.1016/0304-3894(95)00109-3

Laird, B. D., Weiseth, B., Packull-McCormick, S. R., Peak, D., Dodd, M., Siciliano, S. D. (2015). Solid-liquid separation method governs the in vitro bioaccessibility of metals in contaminated soil-like test materials. *Chemosphere*, *134*, 544–549. https://doi.org/10.1016/j.chemosphere.2014.12.019

Luo, X., Zhao, Z., Xie, J., Luo, J., Chen, Y., Li, H., Jin, L. (2019). Pulmonary bioaccessibility of trace metals in PM2.5 from different megacities simulated by lung fluid extraction and DGT method. *Chemosphere*, *218*, 915–921. https://doi.org/10.1016/j.chemosphere.2018.11.079

Macklin, C. C. (1955). Pulmonary Sumps, Dust Accumulations, Alveolar Fluid And Lymph Vessels. *Cells Tissues Organs*, *23*(1), 1–33. https://doi.org/10.1159/000140979

Manjón, I., Ramírez-Andreotta, M. D., Sáez, A. E., Root, R. A., Hild, J., Janes, M. K., Alexander-Ozinskas, A. (2020). Ingestion and inhalation of metal(loid)s through preschool gardening: An exposure and risk assessment in legacy mining communities. *Science of the Total Environment*, *718*. https://doi.org/10.1016/j.scitotenv.2019.134639

Marques, M. R. C., Loebenberg, R., Almukainzi, M. (2011). Simulated biological fluids with possible application in dissolution testing. *Dissolution Technologies*, *18*(3), 15–28.

https://doi.org/10.14227/DT180311P15

Mbengue, S., Alleman, L. Y., Flament, P. (2015). Bioaccessibility of trace elements in fine and ultrafine atmospheric particles in an industrial environment. *Environmental Geochemistry and Health*, *37*(5), 875–889. https://doi.org/10.1007/s10653-015-9756-2

Medlin, E.A. (1997). An *in vitro* method for estimating the relative bioavailability of lead in humans. *Master's thesis*. Department of Geological Sciences, University of Colorado, Boulder.

Meza-Figueroa, D., Barboza-Flores, M., Romero, F. M., Acosta-Elias, M., Hernández-Mendiola, E., Maldonado-Escalante, F., Pedroza-Montero, M. (2020). Metal bioaccessibility, particle size distribution and polydispersity of playground dust in synthetic lysosomal fluids. *Science of the Total Environment*, *713*. https://doi.org/10.1016/j.scitotenv.2019.136481

Midander, K., Wallinder, I. O., Leygraf, C. (2007). In vitro studies of copper release from powder particles in synthetic biological media. *Environmental Pollution*, *145*(1), 51–59. https://doi.org/10.1016/j.envpol.2006.03.041

Moss, O. R. (1979). Simulants of lung interstitial fluid. *Health Physics*, *36*, 447–448.

Mukhtar, A., Limbeck, A. (2013). Recent developments in assessment of bio-accessible trace metal fractions in airborne particulate matter: A review. *Analytica Chimica Acta*, *774*, 11–25. https://doi.org/10.1016/j.aca.2013.02.008

Mukhtar, A., Mohr, V., Limbeck, A. (2015). The suitability of extraction solutions to assess bioaccessible trace metal fractions in airborne particulate matter: a comparison of common leaching agents. *Environmental Science and Pollution Research*, *22*(21), 16620–16630. https://doi.org/10.1007/s11356-015-4789-8

Nie, D., Wu, Y., Chen, M., Liu, H., Zhang, K., Ge, P., Ge, X. (2018). Bioaccessibility and health risk of trace elements in fine particulate matter in different simulated body fluids.

583 *Atmospheric Environment*, *186*(April), 1–8.
584 https://doi.org/10.1016/j.atmosenv.2018.05.024

585 Oomen, A. G., Hack, A., Minekus, M., Zeijdner, E., Cornelis, C., Schoeters, G., Van Wijnen, J.
586 H. (2002). Comparison of five in vitro digestion models to study the bioaccessibility of
587 soil contaminants. *Environmental Science and Technology*, *36*(15), 3326–3334.
588 https://doi.org/10.1021/es010204v

589 Pelfrêne, A., Cave, M. R., Wragg, J., Douay, F. (2017). In vitro investigations of human
590 bioaccessibility from reference materials using simulated lung fluids. *International*
591 *Journal of Environmental Research and Public Health*, *14*(2), 1–15.
592 https://doi.org/10.3390/ijerph14020112

593 Peters, J. T., Blumenstock, F. A. (1967). Copper-binding properties of bovine serum albumin
594 and its aminoterminal peptide fragment. *Biological Chemistry*, *242*(7), 1574–1578.

595 Ren, H., Yu, Y., An, T. (2020). Bioaccessibilities of metal(loid)s and organic contaminants in
596 particulates measured in simulated human lung fluids: A critical review. *Environmental*
597 *Pollution*, *265*, 115070. https://doi.org/10.1016/j.envpol.2020.115070

598 Ruby, Michael V., Davis, A., Link, T. E., Schoof, R., Chaney, R. L., Freeman, G. B.,
599 Bergstrom, P. (1993). Development of an in Vitro Screening Test to Evaluate the in Vivo
600 Bioaccessibility of Ingested Mine-Waste Lead. *Environmental Science and Technology*,
601 *27*(13), 2870–2877. https://doi.org/10.1021/es00049a030

602 See, S. W., Wang, Y. H., Balasubramanian, R. (2007). Contrasting reactive oxygen species and
603 transition metal concentrations in combustion aerosols. *Environmental Research*, *103*(3),
604 317–324. https://doi.org/10.1016/j.envres.2006.08.012

605 Seinfeld, J.H., Pandis, S.N. (2016). Atmospheric Chemistry and Physics: From Air Pollution to
606 Climate Change. John Wiley & Sons, Hoboken, 3 rd Edition.

607 Sysalová, J., Száková, J., Tremlová, J., Kašparovská, K., Kotlík, B., Tlustoš, P., Svoboda, P.

(2014). Methodological Aspects of In Vitro Assessment of Bio-accessible Risk Element Pool in Urban Particulate Matter. *Biological Trace Element Research*, *161*(2), 216–222. https://doi.org/10.1007/s12011-014-0101-x

U.S. Environmental Protection Agency. (2007). Estimation of relative bioavailability of lead in soil and soil-like materials using in vivo and in vitro methods.

Van Der Sloot, H. A., Heasman, L., Quevauviller, P. (1997). Harmonization of leaching/extraction tests. *Studies in Environmental Science, Elseiver*, *70*, 30–165.

Wallenborn, J. G., McGee, J. K., Schladweiler, M. C., Ledbetter, A. D., Kodavanti, U. P. (2007). Systemic translocation of particulate matter-associated metals following a single intratracheal instillation in rats. *Toxicological Sciences*, *98*(1), 231–239. https://doi.org/10.1093/toxsci/kfm088

Weggeberg, H., Benden, T. F., Lierhagen, S., Steinnes, E., Flaten, T. P. (2019). Characterization and bioaccessibility assessment of elements in urban aerosols by extraction with simulated lung fluids. *Environmental Chemistry and Ecotoxicology*, *1*, 49–60. https://doi.org/10.1016/j.enceco.2019.10.001

Wiseman, C. L. S. (2015). Analytical methods for assessing metal bioaccessibility in airborne particulate matter: A scoping review. *Analytica Chimica Acta*, *877*, 9–18. https://doi.org/10.1016/j.aca.2015.01.024

Wiseman, C. L. S., Zereini, F. (2014). Characterizing metal(loid) solubility in airborne PM10, PM2.5 and PM1 in Frankfurt, Germany using simulated lung fluids. *Atmospheric Environment*, *89*, 282–289. https://doi.org/10.1016/j.atmosenv.2014.02.055

Wragg, J., Klinck, B. (2007). The bioaccessibility of lead from Welsh mine waste using a respiratory uptake test. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, *42*(9), 1223–1231. https://doi.org/10.1080/10934520701436054

Table 1. Composition of simulated lung fluids: Gamble's (G1), Gamble's (G2) and ALF.

Reagents	Formula	Gamble G1(g/L) PH 7.4±0.1	Gamble G2(g/L) PH 7.4±0.1	ALF (g/L) 4.5±0.1
Magnesium chloride hexahydrate	$MgCl_2\,6{\cdot}H_2O$	0.095	-	0.050
Sodium chloride	NaCl	6.019	6.779	3.210
Potassium chloride	KCl	0.298	-	-
Disodium hydrogen phosphate	Na_2HPO_4	0.126	0.142	0.071
Sodium sulphate	Na_2SO_4	0.063	-	0.039
Calcium chloride dihydrate	$CaCl_2\,2{\cdot}H_2O$	0.368	0.026	0.128
Sodium acetate	$NaC_2H_3O_2$	0.574	-	-
Sodium hydrogen carbonate	$NaHCO_3$	2.604	2.268	-
Sodium citrate dihydrate	$C_6H_5Na_3O_7\,2{\cdot}H_2O$	0.097	0.055	0.077
Ammonium chloride	$NH_4\,Cl$	-	0.535	-
Sodium hydroxide	NaOH	-	-	6.000
Citric acid	$C_6H_8O_7$	-	-	20.800
Glycine	NH_2CH_2COOH	-	0.375	0.059
Sodium tartrate dihydrate	$C_4H_4O_6Na_2\,2{\cdot}H_2O$	-	-	0.090
Sodium lactate	$C_3H_5NaO_3$	-	-	0.085
Sodium pyruvate	$C_3H_3O_3Na$	-	-	0.086

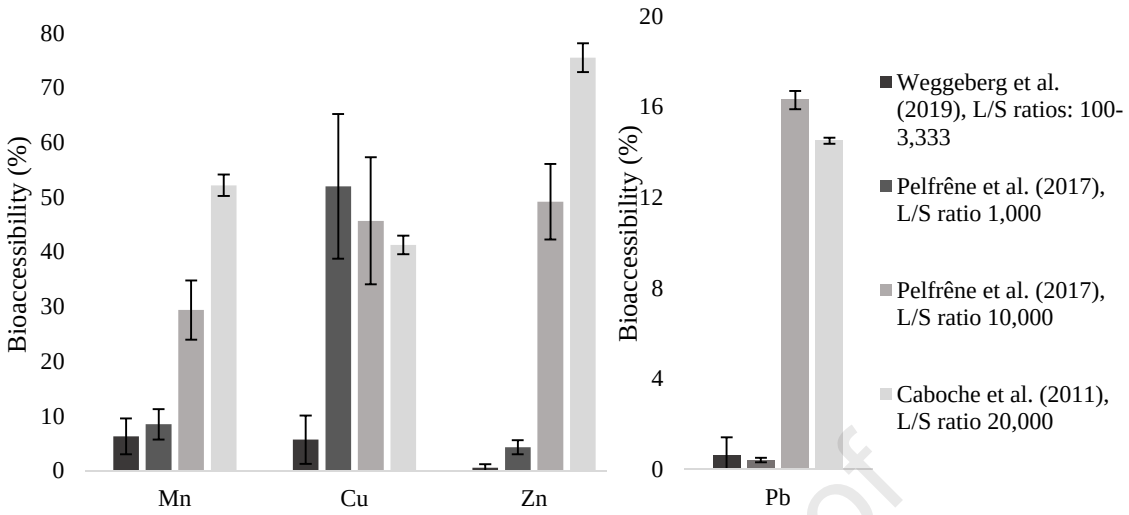


Figure 1. *Bibliographic comparison of the bioaccessibility values of Mn, Cu, Zn and Pb in SRM 1648a using Gamble's solutions.*

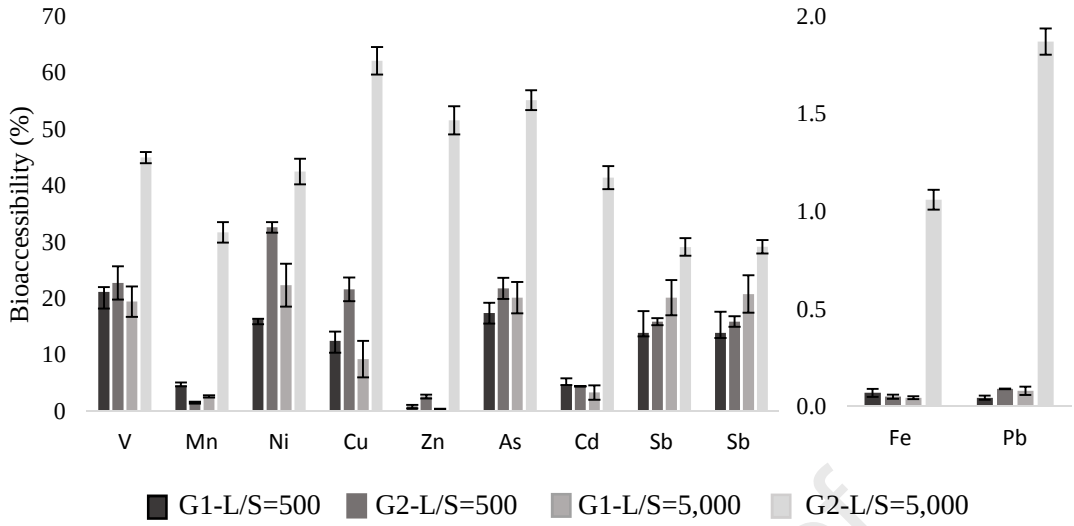
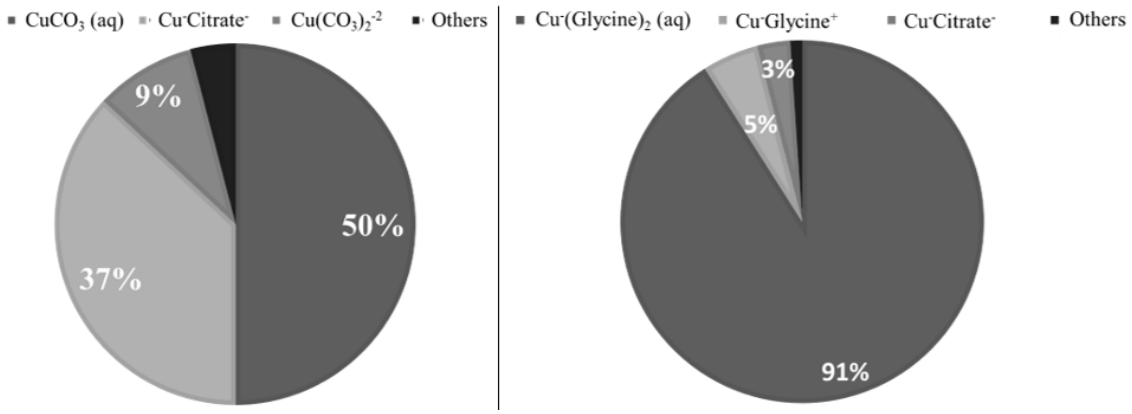


Figure 2. *Bioaccessibility of metal(loid)s in SRM1648a using two type of Gamble's fluid (G1 and G2) and two L/S ratios (500 and 5,000).*



(a) Gamble's G1 (b) Gamble's G2

Figure 3. *Visual MINTEQ 3.1. speciation modeling results for contacting Gamble's G1 and G2 with $Cu_3(PO_4)_2$ 0.001M.*

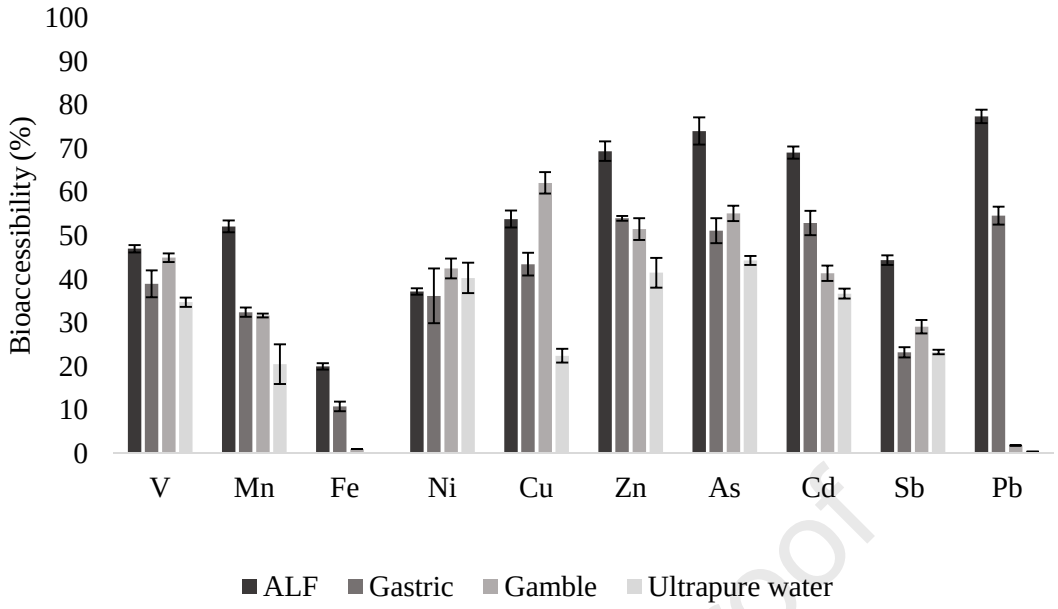


Figure 4. *Bioaccessibility (%) of SRM1648a in water, Gamble's solution (G2), gastric fluid and ALF at a L/S ratio of 5,000.*

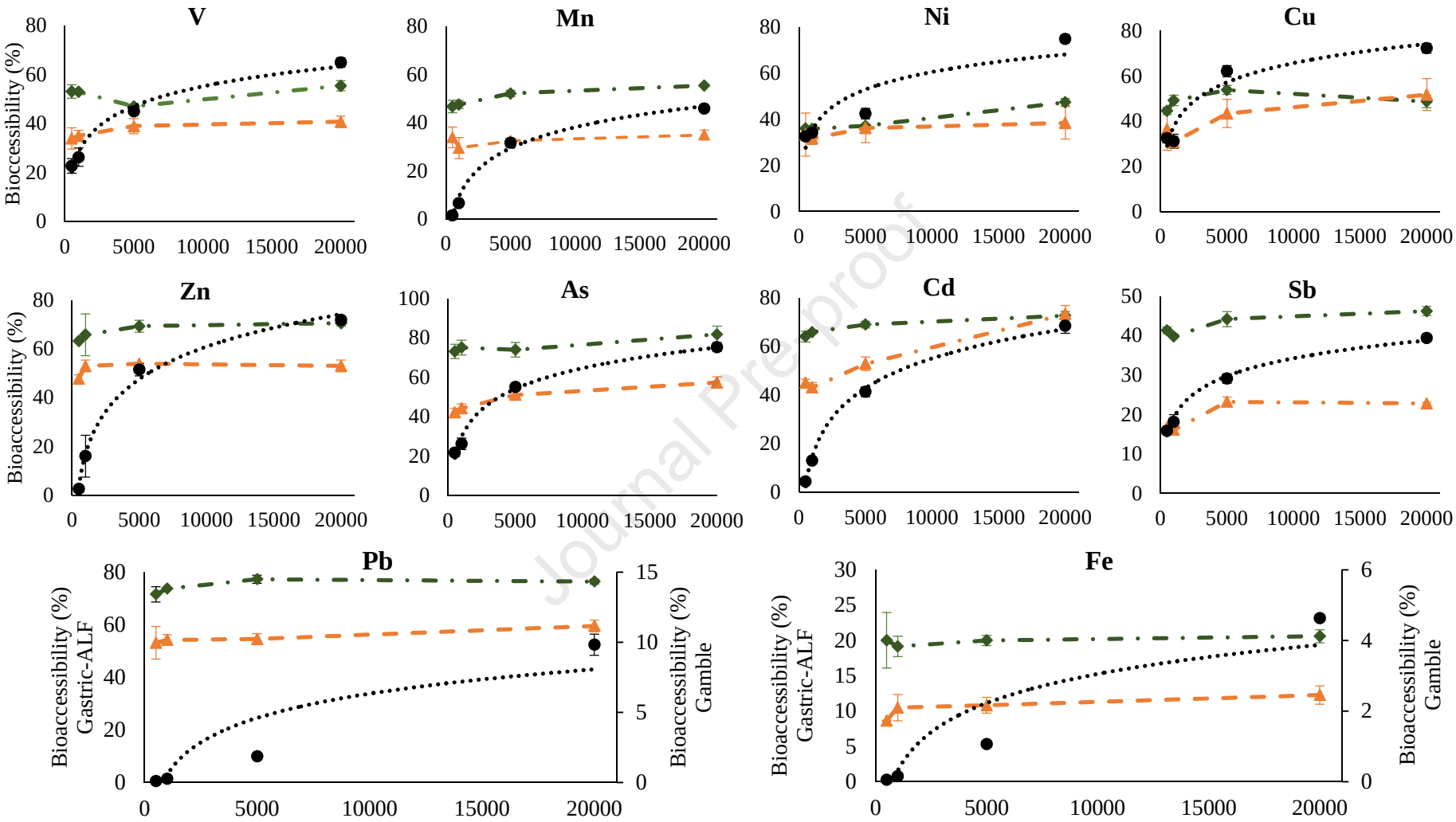


Figure 5. *Variation of bioaccessibility (%) of SRM1648a in Gamble's G2 (●), gastric fluid (▲) and ALF fluid (♦) with the L/S ratio for metal(loid)s.*

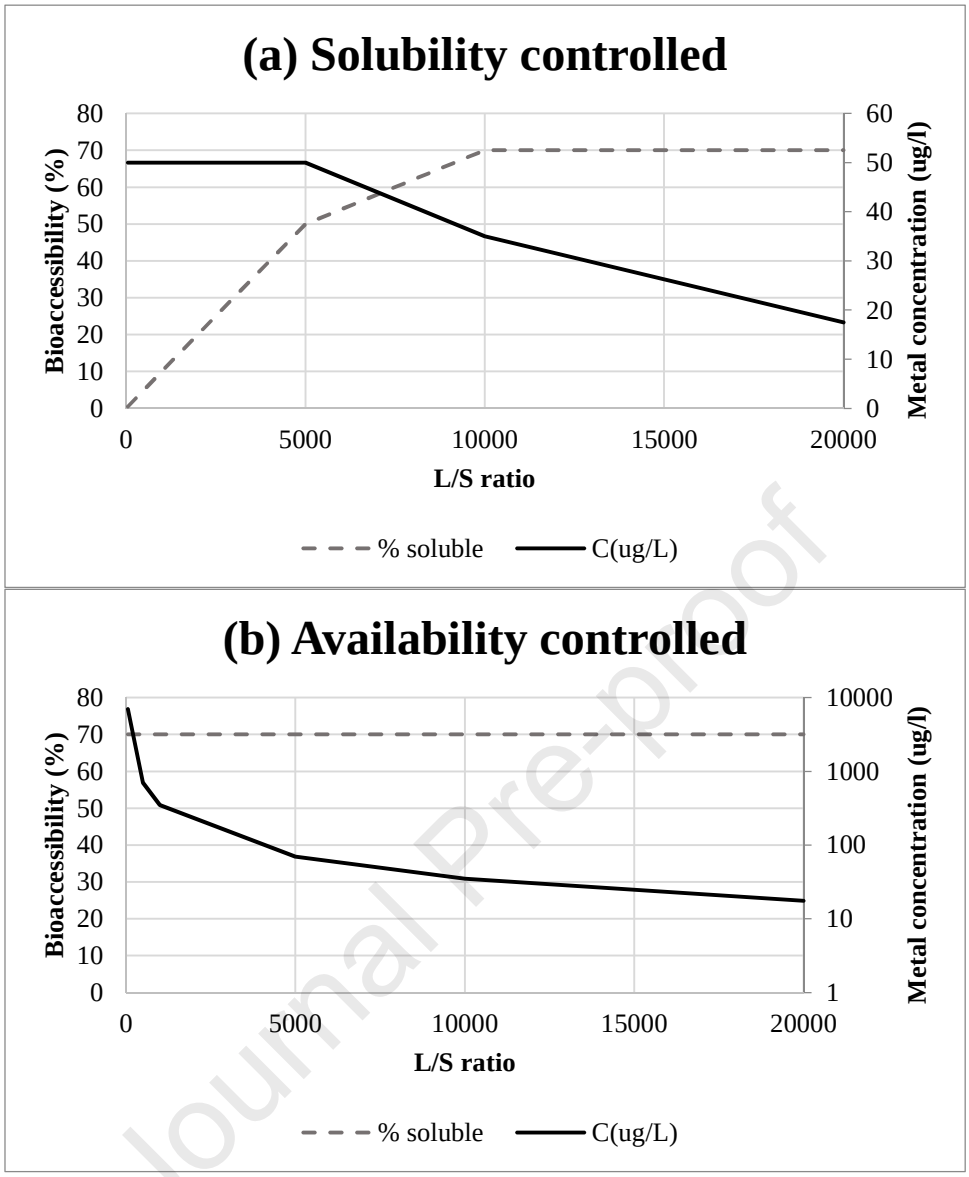


Figure 6. *Solubility vs availability controlled leaching mechanisms: (a) Solubility controlled: total metal concentration 500 mg/kg; metal availability 350 mg/kg; metal solubility 50 µg/L. (b) Availability controlled: total metal concentration 500 mg/kg; metal availability 350 mg/kg; metal solubility 7000 µg/L.*

HIGHLIGHTS

- The inhalation bioaccessibility of metal(loid)s was assessed in urban dust, SRM1648a
- Bioaccessibility depends on the surrogate biological fluid and metal(loid) speciation
- The composition of the interstitial pulmonary fluid affects the bioaccessibility
- Increased liquid-solid ratios lead to greater bioaccessibility in Gamble's solution

Declaration of Competing Interest

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.