Methodological factors influencing inhalation bioaccessibility of metal(loid)s in $PM_{2.5}$ using simulated lung fluid☆

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Abstract

In this study, methodological factors influencing the dissolution of metal(loid)s in simulated lung fluid (SLF) was assessed in order to develop a ~~conservative~~standardised method for the assessment of inhalation bioaccessibility in $PM_{2.5}$. To achieve this aim, the effects of solid to liquid (S/L) ratio (1:100 to 1:5000), agitation (magnetic agitation, occasional shaking, orbital and end-over-end rotation), composition of SLF (artificial lysosomal fluid: ALF; phagolysosomal simulant fluid: PSF) and extraction time (1–120 h) on metal(loid) bioaccessibility were investigated using $PM_{2.5}$ from three Australian mining/smelting impacted soils and a certified reference material. The results highlighted that SLF composition significantly ($p < 0.001$) influenced metal(loid) bioaccessibility and that when a S/L ratio of 1:5000 and end-over-end rotation was used, metal(loid) solubility plateaued after approximately 24 h. Additionally, in order to assess the exposure of metal(loid)s via incidental ingestion of surface dust, $PM_{2.5}$ was ~~then~~ subjected to simulated gastro-intestinal tract (GIT) solutions and the results were compared to extraction using SLF~~ alone~~. Although As bioaccessibility in SLF (24 h) was significantly lower than in simulated GIT solutions ($p < 0.05$), Pb bioaccessibility was equal to or significantly higher than that extracted using simulated GIT solutions ($p < 0.05$).

A S/L ratio of 1:5000, end-over-end rotation (45 rpm), 24-h extraction time using ALF is recommended as a standard~~s~~ inhalation bioaccessibility assay ~~from~~for $PM_{2.5}$.

Keywords: Bioaccessibility; $PM_{2.5}$; SLF; ALF; PSF; Inhalation

1 Introduction

Epidemiological studies have consistently linked chronic and short term exposure to fine particulate matter with an aerodynamic diameter of <2.5 µm ($PM_{2.5}$) to adverse health outcomes, e.g. cardiovascular and pulmonary morbidity, mortality, increased risk of chronic obstructive pulmonary disease (COPD), pneumonia and lung cancer mortality (Fajersztajn et al., 2017; Pinault et al., 2017; Pope and Dockery, 2006; Pun et al., 2017). The main constituents of airborne $PM_{2.5}$ ~~present in air particulate matter~~ include sulphate (20%), crustal materials (soil, sand, road and desert dust; 13.4%), equivalent black carbon (11.9%), NH_4NO_3 (4.7%), sea salt (2.3%), trace element oxides (1%), water (7.2%) and residual matter (40%) (Snider et al., 2016). A systemic review and meta-analysis of mortality and hospital admissions associated with daily $PM_{2.5}$ in 110 peer reviewed studies (until May 2011) found that an increase of

10 μg m^3 $PM_{2.5}$ was linked to an increased risk of hospital admissions and 1.04% rise in mortality (Atkinson et al., 2014). Furthermore, 1.51% of the rise in mortality risk was associated with respiratory illnesses (e.g. COPD, asthma, lower respiratory infections) and 0.84% rise in mortality risk associated with cardiovascular causes (e.g. heart failure, stroke, ischemic heart disease and dysrhythmia) (Atkinson et al., 2014). Recent research highlighted that metals in $PM_{2.5}$ (e.g. Cu, Fe, Mn and Ni) collected at traffic intersections may have considerable reactive oxygen species generation capabilities (Fujitani et al., 2017). Additionally, metals in aqueous extracts of $PM_{2.5}$ were demonstrated to cause lung inflammation and injury, oxidative stress, lipid and protein damage and cardiovascular injury in mouse and rat models (Gavett et al., 2003; Pardo et al., 2016; Shuster-Meiseles et al., 2016). Similarly, Fe, Cu, Ni, Co and Cr in $PM_{2.5}$ were also associated with inflammatory responses in mouse type II alveolar cells by increasing the expression of pro-inflammatory genes and proteins (He et al., 2017). Therefore, although metal oxides may comprise a small proportion of $PM_{2.5}$, it may represent substantial potential to cause human health injuries. Furthermore, because of its small mass, $PM_{2.5}$ may stay airborne for extended periods and travel long distances, impacting communities far from point sources.

Instead of using total meta(loid) concentration for human exposure assessment, it is more relevant to use the concentration of metal(loid)s in $PM_{2.5}$ that may potentially dissolve in lung fluid and be absorbed into the blood (Leclercq et al., 2017; Li et al., 2016; Pelfrêne and Douay, 2017). Although using metal(loid) bioavailability (i.e. metal(loid)s absorbed into the systemic circulation *in-vivo*) remains the most appropriate for exposure assessment, metal(loid) bioaccessibility (i.e. metal(loid)s extracted using simulated lung fluid (SLF) *in-vitro*) is often more desirable as a rapid and cost effective approach (Mukhtar and Limbeck, 2013a; Stopford et al., 2003). $PM_{2.5}$ may stimulate engulfment by lung macrophages in the respiratory system (d'Angelo et al., 2014) and metal(loid) dissolution may take place within the acidic environment of phagolysosomes (Kanapilly, 1977). Several SLFs have been developed to simulate phagolysosomal fluid, e.g. simulated intracellular fluid (Thelohan and De Meringo, 1994), artificial lysosomal fluid (ALF) (Midander et al., 2007; Stopford et al., 2003) and phagolysosomal simulant fluid (PSF) (Stefaniak et al., 2005), the latter two being more popular in bioaccessibility assays (Kastury et al., 2017). However, the extraction efficiencies of these SLFs with an acidic pH of 4.5 have not been compared. Additionally, significant knowledge gaps exist in methods currently used to determine metal(loid) bioaccessibility in $PM_{2.5}$ (Kastury et al., 2017; Wiseman, 2015). For example, the solid to liquid (S/L) ratio ranged from 1:100–1:1163 (Hamad et al., 2014; Potgieter-Vermaak et al., 2012; Wiseman and Zereini, 2014) or not reported because a part of the filter paper with which the particles were collected was used directly in assays (Mukhtar and Limbeck, 2013a; Schaider et al., 2007). Varying agitation frequencies and types have been used in the literature, for example, occasional (Wiseman and Zereini, 2014), continuous (Hamad et al., 2014; Potgieter-Vermaak et al., 2012) or ultrasonic (Mukhtar and Limbeck, 2013a). Large variability in extraction time is also observed in the literature, such as, 1 h in Mukhtar and Limbeck (2013a), 120 h in Schaider et al. (2007) or 30 days in Zereini et al. (2012). Furthermore, incidental ingestion of surface dust may be considered an important pathway for Pb exposure in children due to frequent hand to mouth activity (Scheckel et al., 2013). However, limited research has been conducted on the oral bioaccessibility of $PM_{2.5}$ from Pb mining/smelting impacted region.

This study aimed to investigate how assay parameters affect metal(loid) bioaccessibility outcomes using $PM_{2.5}$ in order to recommend a standardised method. In addition, oral bioaccessibility of $PM_{2.5}$ metal(loid)s was also investigated to compare inhalation exposure to incidental ingestion of surface dust. To achieve this aim, the effect of solid to liquid (S/L) ratio, agitation, SLF composition and extraction time on metal(loid) bioaccessibility in $PM_{2.5}$ was assessed and compared to bioaccessibility results using simulated GIT solutions.

2 Materials and methods

2.1 Collection of $PM_{2.5}$

Surface soils (0–20 cm) from three Australian mining/smelting impacted sites were collected: historic non-ferrous slag impacted soil from York Peninsula (SH15), smelting impacted soil from Port Pirie (PP) and calcinated mine waste (CMW) from the golden triangle region of Victoria. Soil (<2 mm) was dried at 40 °C and sieved to recover the <53 μm particle size fraction using an Endecotts Octagon digital shaker. To extract the fine dust fraction ($PM_{2.5}$), 2–5 g of the <53 μm particle size fraction was applied to a hydrocyclone connected to a three speed vacuum cleaner (Pullman). Upon entering the system, the fine dust fraction travelled towards the top of the cyclone and was collected onto a glass microfiber filter paper (Whatman, grade GF/A, 1.2 μm pore size). The remaining >2.5 μm particle size fraction was captured in a plastic vessel below the cyclone and discarded. Fine particulate matter (stock $PM_{2.5}$) depositing on the filter paper was collected, pooled, homogenised (end-over-end rotation for 24 h) and stored at 20 °C.

2.2 Physicochemical characterisation of $PM_{2.5}$

Particle size distribution was analysed by dispersing 5 mg of $PM_{2.5}$ in 0.1 M NaCl overnight (end-over-end rotation), followed by analysis using a Particle Sizer 380 ZLS (NICOPM). The BET surface area in samples was determined using ASAP 2420 (Micrometritics) after thermal degassing at 200 °C overnight. Total metal(loid) concentration was determined using ICP-MS (ASX-500 series), following aqua-regia digestion (1:3–70% HNO_3: 36.5% HCl) (MARS-6 microwave (CEM) and USEPA method 3051 (USEPA, 1998)). X-ray absorption spectroscopy (XAS) was utilised to determine As and Pb speciation (MRCAT beamlines 10-BM (As and Fe) and 10-ID (Pb)), Sector 10, at the Advanced Photon Source of the Argonne National Laboratory, U.S) following methods described in (Kropf et al., 2010; Segre et al., 2000).

2.3 Formulation of simulated lung fluids

Artificial lysosomal fluid (ALF) (Midander et al., 2007) and phagolysosomal simulant fluid (PSF) (Stefaniak et al., 2005) were freshly made by dissolving constituents detailed in Table 1 in 1 L of MilliQ water and adjusting the pH to 4.5.

Table 1 Composition of simulated lung fluids.

alt-text: Table 1

References	Midander et al., 2007	Stefaniak et al., 2005
Constituents (g/L)	Artificial lysosomal fluid (ALF)	Simulated phagolysosomal fluid (PSF)
Sodium chloride	3.21	6.65
Sodium hydroxide	6.00	
Citric acid	20.8	
Calcium chloride (dihydrate)	0.128	0.029
Sodium hydrogen phosphate dibasic (anhydrous)	0.071	0.142
Sodium sulphate	0.039	0.071
Magnesium chloride (anhydrous)	0.05	
Glycine	0.059	0.45
Sodium citrate (dihydrate)	0.077	
Sodium tartrate (dihydrate)	0.09	
Sodium lactate	0.085	
Sodium pyruvate	0.086	
Potassium hydrogen phthalate		4.085
Benzalkonium chloride	0.05	0.05

2.4 Assessment of metal(loid) bioaccessibility using SLF

2.4.1 The effect of solid to liquid (S/L) ratio on metal(loid) bioaccessibility

Artificial lysosomal fluid (ALF) (Midander et al., 2007) was utilised in this assay as it is the most commonly used SLF to assess the bioaccessibility of metal(loid)s in $PM_{2.5}$. The solid (g) to liquid (mL) ratios (S/L) tested in this assay were 1:100, 1:500, 1:1000 and 1:5000. When necessary, the pH was re-adjusted to 4.5 ± 0.1 at the start of the assay using 1 M NaOH, which was performed at 37 °C for up to 120 h using end-over-end rotation at 45 rpm. Samples were collected at 1, 8, 24, 48, 72, 96 and 120 h and centrifuged at 13,000 rpm (18 g) for 3 min to separate the solid from liquid. The supernatant was diluted with 0.1 M HNO_3 and stored at 4 °C until analysis using ICP-MS (ASX-500 series).

2.4.2 The effect of agitation on metal(loid) bioaccessibility

Using a S/L ratio of 1:5000, metal(loid) bioaccessibility in $PM_{2.5}$ was assessed using ALF with magnetic stirring, occasional agitation (15 min of orbital rotation at 150 rpm, once a day), orbital rotation (150 rpm) and end-over-end rotation (45 rpm). Assays were conducted for up to 120 h at 37 °C, with sample collection, separation and metal(loid) analysis performed according to section 2.4.1.

2.4.3 The effect of SLF composition on metal(loid) bioaccessibility

The extraction efficiencies of ALF and PSF were investigated in this assay as they are the two most widely used SLFs. Using a S/L ratio of 1:5000 and end-over-end rotation (45 rpm), $PM_{2.5}$ was assessed for up to 120 h at 37 °C. The starting pH was readjusted to 4.5 ± 0.1 in ALF and PSF using 1 M NaOH and KOH respectively. Sample collection and separation was performed according to section 2.4.1. Metal(loid)s were analysed by ICP-MS using matrix matched calibration to avoid matrix bias.

2.5 Oral bioaccessibility assessment

Metal(loid) extraction using simulated GIT solution was conducted using the SBRC method (Kelley et al., 2002) with modification in (Juhasz et al., 2009a). Simulated gastric solution (40 mL, 0.4 M glycine, pH adjusted to 1.5 ± 0.05 using concentrated HCl) was added to PM$_{2.5}$ (8 mg) and rotated end-over-end (45 rpm). After 1 h, gastric phase samples (4 mL) were collected, syringe filtered (0.45 µm), acidified with 0.1 M HNO$_3$ and stored at 4 °C until analysis. Bovine bile (70 mg) and porcine pancreatin (20 mg), dissolved in 3 mL MilliQ water was then added to each tube to simulate the conditions in the small intestines, while the pH was adjusted to 7.0 ± 0.1 (using 50% and 5% NaOH; ~1 ml). Samples were rotated end-over-end for 4 h, after which, intestinal phase samples (10 mL) were collected by syringe filtration (0.45 µm), acidified with 0.1 M HNO$_3$ and stored at 4 °C until analysis. Metal(loid) analysis was conducted using ICP-MS and matrix matched calibration.

2.6 Quality assuarance, quality control and statistical analysis

Trace grade nitric and hydrochloric acids were used during digestion and analysis. The accuracy of the aqua-regia digestion method was confirmed by including duplicate analysis, check values, spiked samples and a quantitative average As and Pb recovery from a standard reference material National Institute of Standards and Technology (NIST) SRM NIST 2710a (1540 ± 100 mg As kg^{-1} and 5522 ± 30 mg Pb kg^{-1}). Limit of detection (LOD) for As and Pb were 1 and 0.5 ppb respectively. Reagent blank (n = 2) values were below the LOD. The average deviation between duplicate samples (n = 3) was <4.8%, while the average recovery of As and Pb from spiked samples (n = 3) ranged from 93.8% to 107.8% respectively. The average check value recovery (30 ppb) (n = 3) for As and Pb was 99.8% and 104.3% respectively.

Statistical significance of differences among metal(loid) bioaccessibility determined in sections 2.4.1, 2.4.2 and 2.4.3 were conducted using Two-way ANOVA (α = 0.05), treating each time point independently. Statistical significance in section 2.5 for metal(loid) bioaccessibility was conducted using One-way ANOVA (α = 0.05).

3 Results and discussion

The complex nature of PM$_{2.5}$ composition, deposition and clearance has contributed to variability in *in-vitro* methods used for bioaccessibility assessment. This study focused on investigating the effects of methodological factors that influence metal(loid) bioaccessibility using SLF in order to standardise a conservative method that is biologically relevant to a human inhalation scenario. Although PM$_{2.5}$ may contain fine (0.1–2.5 µm) and ultrafine (≤0.1 µm) particles, those with aerodynamic sizes between 1 and 2 µm stimulate macrophage engulfment most efficiently (d'Angelo et al., 2014). Moreover, <1 µm particles may be exhaled or be absorbed into the pulmonary interstitium and exert additional toxicity, such as the generation of reactive oxygen species (Oberdörster, 2000). Because metal(loid) dissolution alone is assessed in a bioaccessibility assay, additional toxicity caused by fine and ultrafine particles was considered to be out of scope for this study. In discussing the results, particular focus was given to As and Pb as these two metal(loid)s have been identified by the Agency for Toxic Substances and Disease Registry as the number 1 and 2 contaminants in the Priority List of Hazardous Substances (2017).

3.1 Physico-chemical characteristics of mining/smelting impacted PM$_{2.5}$

The total concentrations of nine trace elements (As, Cd, Cr, Co, Cu, Mn, Ni, Pb and Zn) and six major elements (Al, Ca, Fe, K, Mg and P) in PM$_{2.5}$ ~~is~~are given in Table 2. The concentration of As was the highest in CMW (15240 ± 190 mg/kg), followed by SH15 (2010 ± 17.2 mg/kg) and PP (160 ± 0.8 mg/kg). Similar to CMW, elevated As concentrations in soil samples from gold mining regions have been reported previously (Meunier et al., 2010) and may be associated with the sulphidic phases in gold ores (Ollson et al., 2016). The results of speciation analysis demonstrated that the majority of the As present in all three samples was sorbed species (70–90%) with the remainder present as scorodite and beudantite (Table 3). The concentration of Pb was the highest in PP (7450 ± 490 mg/kg) as a result of historic Pb smelting activity with Pb present predominantly as sorbed/bound phases (93%; Table 3). Similar to PP, Pb in CMW (1800 ± 10 mg/kg) was also present as sorbed/bound phases, however, tertiary Pb phosphate, hydroxypyromorphite and litharge were observed in SH15 (1330 ± 16 mg/kg).

Table 2 Total metal(loid) concentration (mean ± SEM), particle size distribution (mean ± SEM) and BET surface area in PM$_{2.5}$ from mining and smelting impacted materials.

alt-text: Table 2

	Minor element concentration (mg/kg) (Because this table appeared to be too long and got cut off at the right hand side in the proof, I have uploaded another version which I believe will fit better in the manuscript without wasting an entire page. Please use this version of the table if possible.)									Major element concentration (mg/kg)				
	As	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn	Al	Ca	Fe	K	Mg

SH15	2011 ± 17.2	45.5 ± 0.4	16.9 ± 0.15	46.9 ± 0.19	175 ± 0.66	1319 ± 14.7	26.3 ± 0.34	1330 ± 16.2	17578 ± 245	26747 ± 187	113848 ± 743	40486 ± 415	6832 ± 18.6	23330 ± 209	3
PP	156 ± 0.8	37.2 ± 0.2	17.6 ± 0.7	46.1 ± 1.5	350 ± 21.5	1180 ± 85.7	27.1 ± 0.99	7454 ± 485	9873 ± 377	36726 ± 2353	57612 ± 2532	35044 ± 913	10616 ± 556	15777 ± 567	1
CMW	15242 ± 192	5.02 ± 0.07	225 ± 2.66	70 ± 0.59	551 ± 5.89	763 ± 9.01	533 ± 4.59	1803 ± 10.0	1592 ± 17.4	14178 ± 573	9913 ± 188	384934 ± 5659	4136 ± 226	8307 ± 59	5

Table 3 Arsenic and lead speciation in SH15, PP and CMW $PM_{2.5}$.

alt-text: Table 3

Sample	As Speciation Weighted Percentage					Pb Speciation Weighted Percentage					
	Mineral Sorbed As(V)	Mineral Sorbed As(III)	Scorodite	Jarosite – As (V)	Beudantite	Mineral Sorbed Pb	Organic Bound Pb	Ter. Pb Phosphate	Hydroxypyromorphite	Litherage	Plumbojarosite
SH15	73		17		9	66		17	11	6	
PP	70	5	17		7	65	28				7
CMW	80		5	15		42	58				

The concentration of As and Pb in particles with less than 10 µm in aerodynamic diameter (PM_{10}) in SH15, PP and CMW has been reported elsewhere (Kastury et al., 2018). There was no significant difference ($p > 0.05$) in As concentration between PM_{10} and $PM_{2.5}$, indicating that As was not enriched in the smaller particle size fraction in these samples. However, compared to $PM_{10,}$ Pb in $PM_{2.5}$ was enriched by up to 1.7 fold. A similar enrichment in the concentrations of other trace elements (e.g. Co, Cr, Cu, Mn, Ni and Zn) in $PM_{2.5}$ suggests that this fraction may act as a sink for toxic metals. Although the mean particle diameter was similar in $PM_{2.5}$ across the three samples (1.2 ± 0.05 µm in SH15, 2.17 ± 0.65 µm in PP and 1.6 ± 0.05 µm in CMW), noteworthy differences in BET surface area were observed between PP (32.1 m^2/g), CMW (18.7 m^2/g) and SH15 (4.4 m^2/g). The high BET surface area observed in PP $PM_{2.5}$ may be attributed to the emission of fine particles during smelting which may have contributed to the enrichment of Pb in the small particle size fraction.

3.2 The effect of S/L ratio on $PM_{2.5}$ metal(loid) bioaccessibility

Fig. 1 shows the influence of S/L ratio on As and Pb bioaccessibility in $PM_{2.5}$. For both As and Pb, dissolution plateaued within 24 h. Although As concentration varied significantly between samples, percentage As bioaccessibility after 24 h was similar across the four matrices (PP: 70.5%; SH15: 69.1%: CMW: 64.7%; SRM 2710a: 60.1%). The similarity in As bioaccessibility between the environmental samples stems from the similarity in As speciation (e.g. 70–90% of the As was present as sorbed species). In contrast, Pb bioaccessibility varied among the matrices, with values ranging from 50.6% (SRM 2710a) to 81.2% (PP). The high As and Pb bioaccessibility in PP can be attributed to the predominance of sorbed species and the high BET surface area (32.1 m^2/g) which will both influence metal(loid) dissolution.

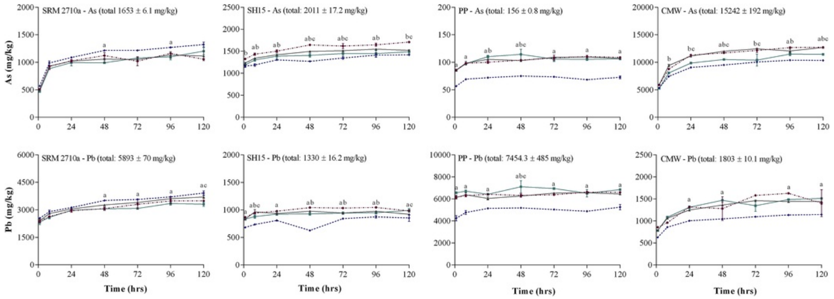

Fig. 1 (Is it possible to make the figures appear larger in the print? There is a lot of white space around the figures to be able to do that. also, there is also a lot of information on the figure that will be easier to read if the figures were a little larger)**Effect of solid to liquid ratio (1:100, ● 1:500, ▲ 1:1000 & ■ 1:5000) on As and Pb bioaccessibility (mg/kg) (37°C, ALF, end-over-end rotation) (mean ± SEM, n = 3).** SRM2710a = Standard reference material from the National Institute of Standards and Technology, SH15 = non-ferrous slag impacted $PM_{2.5}$, PP = smelter impacted $PM_{2.5}$ and CMW = calcinated mine waste impacted $PM_{2.5}$. Significant difference (ANOVA, α = 0.05) between 1:5000 & 1:100 = a, 1:5000 & 1: 500 = b, 1:5000 & 1:1000 = c.

alt-text: Fig. 1

S/L ratio did not significantly affect As and Pb bioaccessibility in SRM 2710a after 24 h ($p > 0.05$). This result is similar to a recent study conducted by Pelfrêne et al. (2017) who reported no significant difference in Cd, Pb and Zn bioaccessibility in three certified reference materials (BCR-723, SRM NIST 2710a and SRM NIST 1648a) using ALF, a S/L ratio of 1:1000–1:10,000 and a 24 h assessment period. In the present study, As and Pb bioaccessibility at 24 h was significantly higher ($p < 0.05$) in PP, SH15 and CMW when a S/L ratio of 1:5000 was used compared to 1:100. However, As bioaccessibility decreased by 8% in SH15 and between 12 and 14% in CMW when S/L ratio of 1:5000 was used compared to 1:500 and 1:1000 ($p < 0.05$). A similar decrease in the bioaccessibility of other elements (Al, Fe, Mn and Zn) was also observed in these samples (Fig. S1). Schaider et al. (2007) suggested that phosphate may form insoluble $Zn_3(PO_4)_2$ during SLF extractions at pH 4.5, which may account for lower of Zn bioaccessibility with increasing S/L ratio. Arsenic and Pb have also been shown to form complexes with inorganic and organic constituents in SLF (Marschner et al., 2006), decreasing the fraction that remains in solution over time.

Although the concentration of $PM_{2.5}$ in air may vary significantly depending on location (1–217 µg/m^3) (WHO, 2016), the acceptable concentration of $PM_{2.5}$ is 10 µg/m^3 (Apte et al., 2015) (with a 2015 world average $PM_{2.5}$ of 44 µg/m^3 (Bank, 2017)). Using an air intake value of 20 m^3/day (Caboche et al., 2011), the mass of $PM_{2.5}$ that may be inhaled using $PM_{2.5}$ concentrations of 10-217 µg/m^3 falls between 0.2 and 4.34 mg. With a total lung fluid volume of 20 mL (Caboche et al., 2011), the biologically relevant S/L ratio may be estimated to be 1:4650–1:100,000, although, it may be assumed that the total volume of phagolysosomal liquid within the macrophages would be lower than lung lining fluid. The lowest biologically relevant particle loading used in this study was 1:5000, because concentrations of several metals were below the level of quantification when a lower S/L ratio was used. Also, at lower particle loading, scaling up becomes impracticable as it would require large volumes of SLF. As a consequence, a S/L ratio of 1:5000 was utilised as a biologically relevant particle loading for further studies assessing the influence of other operational parameters on inhalation bioaccessibility.

3.3 The effect of agitation on $PM_{2.5}$ metal(loid) bioaccessibility

It has been suggested that certain agitation methods may result in particle agglomeration and reduce the available surface area for metal-chelator interaction *in-vitro* (Caboche et al., 2011). Therefore, the effect of four different agitation methods was assessed for their influence on metal(loid) bioaccessibility: magnetic stirring, occasional stirring, orbital rotation and end-over-end rotation. Fig. 2 demonstrates that with the exception of magnetic stirring, As and Pb bioaccessibility appears to be unaffected by the choice of agitation, plateauing after 24 h. This result agrees with findings of ~~(~~Kastury et al., 2018) (Please change this from (Kastury et al., 2018) to Kastury et al. (2018)), where using a S/L ratio of 1:5000 and Gamble's solution (pH 7.4), there was no significant difference in the bioaccessibility of metal(loid)s as a result of agitation types other than magnetic stirring. As strong mechanical mixing is not congruent to the mixing processes in the lung, magnetic stirring was concluded as not being biologically relevant. Particles were visually the most dispersed using end-over-end shaking and for this reason, end-over-end shaking was selected as the mixing approach for sample agitation ~~(see Fig. 3)~~ (Please delete the words highlighted here.).

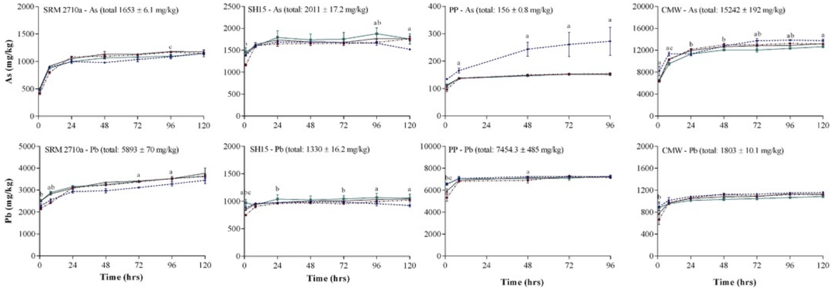

Fig. 2 **(Is it possible to make the figures appear larger in the print? There is a lot of white space around the figures to be able to do that. also, there is also a lot of information on the figure that will be easier to read if the figures were a little larger)** **Effect of agitation (magnetic stirring◆, orbital rotation●, occasional stirring▲, end-over-end rotation■) on As and Pb bioaccessibility (mg/kg) (37°C, ALF, S/L ratio of 1:5000) (mean ± SEM, n = 3).** SRM2710a = Standard reference material from the National Institute of Standards and Technology, SH15 = non-ferrous slag impacted $PM_{2.5}$, PP = smelter impacted $PM_{2.5}$ and CMW = calcinated mine waste impacted $PM_{2.5}$. Significant difference between end over end vs magnetic stirring = a, end over end vs occasional stirring = b and end over end vs orbital rotation = c).

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3.4 The effect of extraction time and SLF composition on $PM_{2.5}$ metal(loid) bioaccessibility

A recent review by Kastury et al. (2017) identified four fluid compositions simulating the environment inside a phagolysosome: Simulated intracellular fluid (Thelohan and De Meringo, 1994), two versions of artificial lysosomal fluid (ALF) (Midander et al., 2007; Stopford et al., 2003) and phagolysosomal simulant fluid (PSF) (Stefaniak et al., 2005). Simulated intracellular fluid has not been used in recent metal(loid) bioaccessibility studies (Kastury et al., 2017), while preliminary experiments demonstrated that there was no significant difference in metal(loid) extraction efficiencies between the two versions of ALFs (data not shown). Due to their extensive use, metal(loid) bioaccessibility using ALF (Midander et al., 2007) and PSF was assessed in order to compare extraction efficacies (Fig. 3 and Fig. S3). As with previous assessments, As and Pb solubility plateaued at 24 h. As the majority of metal(loid) solubilisation was observed to take place in SLF within 24 h, it may be presumed that dissolved metal(loid)s would potentially be absorbed into the systemic circulation via the pulmonary interstitium (Kanapilly, 1977), indicating that this timeframe may be adequate for the assessment of metal(loid) bioaccessibility in $PM_{2.5}$.

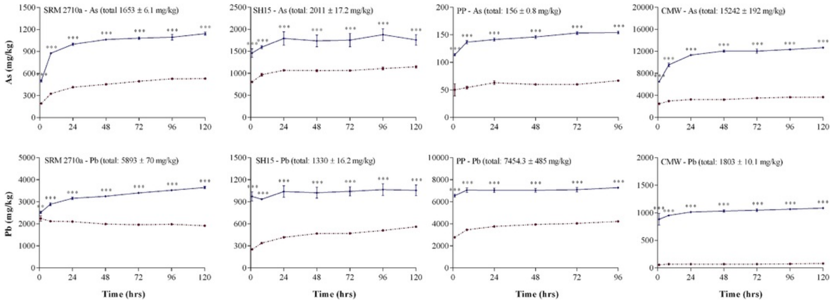

Fig. 3 **(Is it possible to make the figures appear larger in the print? There is a lot of white space around the figures to be able to do that. also, there is also a lot of information on the figure that will be easier to read if the figures were a little larger)** **Effect of SLF composition (ALF◆, PSF●) on As and Pb bioaccessibility (mg/kg) (37°C, S/L ratio of 1:5000 and end-over-end rotation: 45 rpm) (mean ± SEM, n = 3).** SRM2710a = Standard reference material from the National Institute of Standards and Technology, SH15 = nor ferrous slag impacted $PM_{2.5}$, PP = smelter impacted $PM_{2.5}$ and CMW = calcinated mine waste impacted $PM_{2.5}$. Significant differences (two-way ANOVA, α = 0.05) in bioaccessibility between the two simulated lung fluids are indicated as * = P < 0.05, ** = p < 0.01 and *** = p < 0.001.

alt-text: Fig. 3

When the 24 h time point was assessed, both As and Pb bioaccessibility was significantly higher ($p < 0.001$) when assessed using ALF compared to PSF. Arsenic bioaccessibility was 1.6 (SH15) to 3.4 fold higher (CMW) while Pb bioaccessibility was 1.5 (SRM 2710a) to 3.9 fold greater (CMW) using ALF compared to PSF. The bioaccessibility of other elements (Al, Cd, Fe, Mn and Zn; Fig. S4) was also higher in ALF, with values plateauing within 24 h. Although both ALF and PSF methodologies are conducted under the same pH conditions, there are important differences in fluid composition with respect to metal chelation capacity, e.g., amino acids and organic molecules. Glycine was the only metal chelator present in PSF while ALF contains a mixture of glycine, citrate, tartrate, lactate and pyruvate, which may contribute to the higher metal(loid) extraction capacity. Consequently, for a conservative estimate of metal(loid) inhalation bioaccessibility, ALF should be utilised as the *in-vitro* fluid.

3.5 Oral bioaccessibility of metal(loid)s in $PM_{2.5}$

Fine particulate matter may be transported over a long distance and deposit in areas far from point sources (Mukhtar and Limbeck, 2013b). This may be particularly hazardous for residential areas surrounding current or historic mining/smelting region because metal(loid) (Cd and Pb) impacted dust (0.3–5 μm) have been isolated from the sidewalks of residential areas ten years after the closedown of a smelter in northern France (Pelfrêne and Douay, 2017). Incidental ingestion of soil and surface dust represents a major pathway for toxic metals (e.g. Pb) and may contribute to childhood Pb exposure (Boreland et al., 2006; Li et al., 2014; Scheckel et al., 2013). Due to hand to mouth activity of children, surface dust may be swallowed, metal(loid)s may solubilise in the acidic environment of the stomach and be absorbed in the small intestines (Scheckel et al., 2013). Consequently, in addition to inhalation exposure of $PM_{2.5}$, the oral bioaccessibility in simulated gastric [G] and intestinal tract solutions [G + I] were also assessed in this study (Fig. 4). Arsenic and Pb bioaccessibility in SH15, PP and CMW ranged between 53.4 to 78.1% and 44.9–78.1% respectively. When As was assessed, bioaccessibility was 16.6–29.4% lower following G + I assessment compared to gastric phase alone. Similar findings have been reported for other contaminated materials (Juhasz et al., 2009b; Li et al., 2015) using the SBRC assay (used in this study), physiologically based extraction test (PBET), in vitro gastro-intestinal method (IVG) and Deutches Institut fur Normung (DIN). Similarly, compared to gastric phase alone, Pb bioaccessibility following G + I extraction decreased significantly (18.8–61.9%), which occurs as a result of the increase in pH from 1.5 to 7 (Smith et al., 2011). The mechanisms responsible for the decrease in As and Pb bioaccessibility under G + I conditions include co-precipitation with amorphous Fe or re-adsorption onto the sample matrix as a result of the increase in pH (Martínez and McBride, 2001; O'Reilly and Hochella, 2003; Ruby et al., 1996).

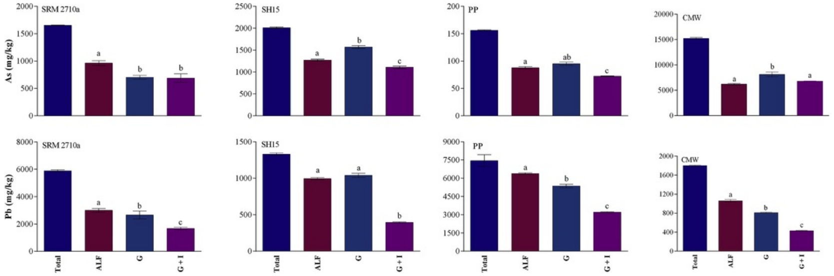

Fig. 4 (Is it possible to make the figures appear larger in the print? There is a lot of white space around the figures to be able to do that. also, there is also a lot of information on the figure that will be easier to read if the figures were a little larger)**Comparison of As and Pb bioaccessibility when assessed using ALF and simulated GIT solutions (gastric [G] or gastric and intestinal [G + I]) (mean ± SEM, n = 3).** Total metal(loid) concentration (■), ALF (■), gastric solution (■), gastric + intestinal solution (■). SRM2710a = Standard referenc material from the National Institute of Standards and Technology, SH15 = non-ferrous slag impacted $PM_{2.5}$, PP = smelter impacted $PM_{2.5}$ and CMW = calcinated mine waste impacted $PM_{2.5}$. $PM_{2.5}$ was assessed in ALF for 24 h, followed by gastric (G) and intestinal (I) solutions (SBRC method). Additionally, $PM_{2.5}$ was assessed in gastric (G) and intestinal (I) solutions only to determine the difference between inhalation + ingestion and ingestion only pathways. Statistically significant differences (ANOVA, α = 0.05) between metal(loid) bioaccessibility is indicated by dissimilar letters.

alt-text: Fig. 4

Arsenic bioaccessibility was higher when assessed using gastric phase conditions (G) compared to ALF for PP (1.08 fold; $p > 0.05$), SH15 (1.2 fold; $p < 0.05$) and CMW (1.3 fold; $p < 0.05$). Higher As bioaccessibility when assessed using simulated gastric solution was expected as the extent of As (V) solubility, which is the principle form of As present in $PM_{2.5}$ samples, is pH dependent (Gersztyn et al., 2013). In contrast, Pb bioaccessibility in ALF was 1.12, 1.2 and 1.3 fold higher ($P < 0.05$) in SRM 2710a, PP and CMW respectively compared to the gastric phase alone; while no significant difference ($p > 0.05$) was observed in SH15. It is likely that the higher Pb bioaccessibility in ALF was a combination of increased contact time of the ALF assay (24 h) compared to the SBRC assay (1 h gastric phase + 4 h small intestinal tract phase) and the concentration of metal chelators in ALF (e.g. citrate, tartrate, lactate, pyruvate). Similarly Pb, Fe and Mn bioaccessibility (Fig. S4) was also higher in ALF, compared to simulated GIT solutions. In a similar study by Pelfrêne and Douay (2017), Pb bioaccessibility in 0.3–5 μm fraction dust was 81.4% in gastric solution, 36.4% in intestinal solution and 69% in ALF. The results of this study suggest that Pb exposure from fine particulate matter may be high when compared to oral bioaccessibility as a result of macrophage mediated digestion of small particulate matter.

4 Conclusion

The results of this study demonstrate that S/L ratio, fluid composition, as well as extraction time significantly influences metal(loid) bioaccessibility in $PM_{2.5}$. A S/L ratio of 1:5000, end-over-end rotation (45 rpm), 24-h extraction time using ALF is recommended for inhalation bioaccessibility assay to simulate exposure from fine particulate matter via macrophage engulfment. Furthermore, in addition to inhalation, incidental ingestion of $PM_{2.5}$ with elevated toxic metal(loid)s may be a potential hazard to human health.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.05.094.

References

Apte J.S., Marshall J.D., Cohen A.J. and Brauer M., Addressing global mortality from ambient $PM_{2.5}$, *Environ. Sci. Technol.* **49**, 2015, 8057–8066.

Agency for Toxic Substances and Disease Registry (ATSDR), Priority List of Hazardous Substances, 2017.

Atkinson R., Kang S., Anderson H., Mills I. and Walton H., Epidemiological ~~T~~time ~~S~~series ~~S~~studies of $PM_{2.5}$ and ~~D~~daily ~~M~~mortality and ~~H~~hospital ~~A~~admissions: a ~~S~~systematic ~~R~~review and ~~M~~meta-analysis. Thorax, Thoraxjnl-2013–204492, 2014.

Bank T.W., $PM_{2.5}$ air pollution, mean annual exposure (micrograms per cubic meter), $PM_{2.5}$ air pollution, 2017.

Boreland F., Lyle D.M., Wlodarczyk J. and Balding W.A., Lead dust in Broken Hill homes: relationship between house dust and children's blood lead levels, *Environ. Health* **6**, 2006, 15.

Caboche J., Esperanza P., Bruno M. and Alleman L.Y., Development of an in vitro method to estimate lung bioaccessibility of metals from atmospheric particles, *J. Environ. Monit.* **13**, 2011, 621–630.

d'Angelo I., Conte C., La Rotonda M.I., Miro A., Quaglia F. and Ungaro F., Improving the efficacy of inhaled drugs in cystic fibrosis: challenges and emerging drug delivery strategies, *Adv. Drug Deliv. Rev.* **75**, 2014, 92–111.

Fajersztajn L., Saldiva P., Pereira L.A.A., Leite V.F. and Buehler A.M., Short-term effects of fine particulate matter pollution on daily health events in Latin America: a systematic review and meta-analysis, *Int. J. Publ. Health* **62**, 2017, 729–738.

Fujitani Y., Furuyama A., Tanabe K. and Hirano S., Comparison of oxidative abilities of PM$_{2.5}$ collected at traffic and residential sites in Japan. Contribution of transition metals and primary and secondary aerosols, *Aerosol. Air Qual. Res.* **17**, 2017, 574–587.

Gavett S.H., Haykal-Coates N., Copeland L.B., Heinrich J. and Gilmour M.I., Metal composition of ambient PM$_{2.5}$ influences severity of allergic airways disease in mice, *Environ. Health Perspect.* **111**, 2003, 1471.

Gersztyn L., Karczewska A. and Gałka B., Influence of pH on the solubility of arsenic in heavily contaminated soils/Wpływ pH na rozpuszczalność arsenu w glebach silnie zanieczyszczonych, *Ochrona Srodowiska Zasobów Nat.* **24**, 2013, 7–11.

Hamad S.H., Schauer J.J., Shafer M.M., Al-Rheem E.A., Skaar P.S., Heo J. and Tejedor-Tejedor I., Risk assessment of total and bioavailable potentially toxic elements (PTEs) in urban soils of Baghdad–Iraq, *Sci. Total Environ.* **494**, 2014, 39–48.

He M., Ichinose T., Yoshida S., Ito T., He C., Yoshida Y., Arashidani K., Takano H., Sun G. and Shibamoto T., PM$_{2.5}$-induced lung inflammation in mice: differences of inflammatory response in macrophages and type II alveolar cells, *J. Appl. Toxicol.* 2017.

Juhasz A.L., Weber J., Smith E., Naidu R., Marschner B., Rees M., Rofe A., Kuchel T. and Sansom L., Evaluation of SBRC-gastric and SBRC-intestinal methods for the prediction of in vivo relative lead bioavailability in contaminated soils, *Environ. Sci. Technol.* **43**, 2009a, 4503–4509.

Juhasz A.L., Weber J., Smith E., Naidu R., Rees M., Rofe A., Kuchel T. and Sansom L., Assessment of four commonly employed in vitro arsenic bioaccessibility assays for predicting in vivo relative arsenic bioavailability in contaminated soils, *Environ. Sci. Technol.* **43**, 2009b, 9487–9494.

Kanapilly G., Alveolar microenvironment and its relationship to the retention and transport into blood of aerosols deposited in the alveoli, *Health Phys.* **32**, 1977, 89–100.

Kastury F., Smith E. and Juhasz A.L., A critical review of approaches and limitations of inhalation bioavailability and bioaccessibility of metal (loid)s from ambient particulate matter or dust, *Sci. Total Environ.* **574**, 2017, 1054–1074.

Kastury F., Smith E., Karna R.R., Scheckel K.G. and Juhasz A., An inhalation-ingestion bioaccessibility assay (IIBA) for the assessment of exposure to metal (loid)s in PM$_{10}$, *Sci. Total Environ.* **631**, 2018, 92–104.

Kelley M.E., Brauning S., Schoof R. and Ruby M., Assessing ~~O~~oral ~~B~~bioavailability of ~~M~~metals in ~~S~~soil, 2002, Battelle Press.

Kropf A., Katsoudas J., Chattopadhyay S., Shibata T., Lang E., Zyryanov V., Ravel B., McIvor K., Kemner K. and Scheckel K., The new MRCAT (Sector 10) bending magnet beamline at the advanced photon source, In: *AIP Conference Proceedings. AIP,* 2010, 299–302.

Leclercq B., Alleman L.Y., Perdrix E., Riffault V., Happillon M., Strecker A., Lo-Guidice J.-M., Garçon G. and Coddeville P., Particulate metal bioaccessibility in physiological fluids and cell culture media: toxicological perspectives, *Environ. Res.* **156**, 2017, 148–157.

Li H.-B., Cui X.-Y., Li K., Li J., Juhasz A.L. and Ma L.Q., Assessment of in vitro lead bioaccessibility in house dust and its relationship to in vivo lead relative bioavailability, *Environ. Sci. Technol.* **48**, 2014, 8548–8555.

Li H.-B., Li J., Zhu Y.-G., Juhasz A.L. and Ma L.Q., Comparison of arsenic bioaccessibility in housedust and contaminated soils based on four in vitro assays, *Sci. Total Environ.* **532**, 2015, 803–811.

Li S.-W., Li H.-B., Luo J., Li H.-M., Qian X., Liu M.-M., Bi J., Cui X.-Y. and Ma L.Q., Influence of pollution control on lead inhalation bioaccessibility in PM$_{2.5}$: a case study of 2014 Youth Olympic Games in Nanjing, *Environ. Int.* **94**, 2016, 69–75.

Marschner B., Welge P., Hack A., Wittsiepe J. and Wilhelm M., Comparison of soil Pb in vitro bioaccessibility and in vivo bioavailability with Pb pools from a sequential soil extraction, *Environ. Sci. Technol.* **40**, 2006, 2812–2818.

Martínez C.E. and McBride M.B., Cd, Cu, Pb, and Zn coprecipitates in Fe oxide formed at different pH: aging effects on metal solubility and extractability by citrate, *Environ. Toxicol. Chem.* **20**, 2001, 122–126.

Meunier L., Walker S.R., Wragg J., Parsons M.B., Koch I., Jamieson H.E. and Reimer K.J., Effects of soil composition and mineralogy on the bioaccessibility of arsenic from tailings and soil in gold mine districts of Nova Scotia *Environ. Sci. Technol.* **44**, 2010, 2667–2674.

Midander K., Pan J., Wallinder I.O. and Leygraf C., Metal release from stainless steel particles in vitro—influence of particle size, *J. Environ. Monit.* **9**, 2007, 74–81.

Mukhtar A. and Limbeck A., Comparison of the extraction efficiencies of different leaching agents for reliable assessment of bio-accessible trace metal fractions in airborne particulate matter, In: *E3S Web of Conferences. EDP Sciences,* 2013a.

Mukhtar A. and Limbeck A., Recent developments in assessment of bio-accessible trace metal fractions in airborne particulate matter: a review, *Anal. Chim. Acta* **774**, 2013b, 11–25.

O'Reilly S.E. and Hochella M.F., Lead sorption efficiencies of natural and synthetic Mn and Fe-oxides, *Geochem. Cosmochim. Acta* **67**, 2003, 4471–4487.

Oberdörster G., Pulmonary effects of inhaled ultrafine particles, *Int. Arch. Occup. Environ. Health* **74**, 2000, 1–8.

Ollson C.J., Smith E., Scheckel K.G., Betts A.R. and Juhasz A.L., Assessment of arsenic speciation and bioaccessibility in mine-impacted materials, *J. Hazard Mater.* **313**, 2016, 130–137.

Pardo M., Porat Z., Rudich A., Schauer J.J. and Rudich Y., Repeated exposures to roadside particulate matter extracts suppresses pulmonary defense mechanisms, resulting in lipid and protein oxidative damage, *Environ. Pollut.* **210**, 2016, 227–237.

Pelfrêne A., Cave M., Wragg J. and Douay F., In v~~V~~itro investigations of human bioaccessibility from reference materials using simulated lung fluids, *Int. J. Environ. Res. Publ. Health* **14**, 2017, 112.

Pelfrêne A. and Douay F., Assessment of oral and lung bioaccessibility of Cd and Pb from smelter-impacted dust, *Environ. Sci. Pollut. Control Ser.* 2017 (Please correct this is "***25***, 2018, 3718–3730.").

Pinault L.L., Weichenthal S., Crouse D.L., Brauer M., Erickson A., Donkelaar A.v., Martin R.V., Hystad P., Chen H., Finès P., Brook J.R., Tjepkema M. and Burnett R.T., Associations between fine particulate matter and mortality in the 2001 canadian census health and environment cohort, *Environ. Res.* **159**, 2017, 406–415.

Pope C.A. and Dockery D.W., Health effects of fine particulate air pollution: lines that connect, *J. Air Waste Manag. Assoc.* **56**, 2006, 709–742.

Potgieter-Vermaak S., Rotondo G., Novakovic V., Rollins S. and Van Grieken R., Component-specific toxic concerns of the inhalable fraction of urban road dust, *Environ. Geochem. Health* **34**, 2012, 689–696.

Pun V.C., Kazemiparkouhi F., Manjourides J. and Suh H.H., L~~O~~ong-term PM$_{2.5}$ exposures and respiratory, cancer and cardiovascular mortality in american older adults, *Am. J. Epidemiol.* 2017.

Ruby M.V., Davis A., Schoof R., Eberle S. and Sellstone C.M., Estimation of lead and arsenic bioavailability using a physiologically based extraction test, *Environ. Sci. Technol.* **30**, 1996, 422–430.

Schaider L.A., Senn D.B., Brabander D.J., McCarthy K.D. and Shine J.P., Characterization of zinc, lead, and cadmium in mine waste: implications for transport, exposure, and bioavailability, *Environ. Sci. Technol.* **41**, 2007, 4164–4171.

Scheckel K.G., Diamond G.L., Burgess M.F., Klotzbach J.M., Maddaloni M., Miller B.W., Partridge C.R. and Serda S.M., Amending soils with phosphate as means to mitigate soil lead hazard: a critical review of the state of the science, *J. Toxicol. Environ. Health, Part B* **16**, 2013, 337–380.

Segre C., Leyarovska N., Chapman L., Lavender W., Plag P., King A., Kropf A., Bunker B., Kemner K. and Dutta P., The MRCAT insertion device beamline at the Advanced Photon Source, In: *AIP Conference Proceedings. AIP,* 2000 419–422.

Shuster-Meiseles T., Shafer M.M., Heo J., Pardo M., Antkiewicz D.S., Schauer J.J., Rudich A. and Rudich Y., ROS-generating/ARE-activating capacity of metals in roadway particulate matter deposited in urban environment, *Environ. Res.* **146**, 2016, 252–262.

Smith E., Weber J., Naidu R., McLaren R.G. and Juhasz A.L., Assessment of lead bioaccessibility in peri-urban contaminated soils, *J. Hazard Mater.* **186**, 2011, 300–305.

Snider G., Weagle C.L., Murdymootoo K.K., Ring A., Ritchie Y., Stone E., Walsh A., Akoshile C., Anh N.X., Balasubramanian R., Brook J., Qonitan F.D., Dong J., Griffith D., He K., Holben B.N., Kahn R., Lagrosas N., Lestari P., Ma Z., Misra A., Norford L.K., Quel E.J., Salam A., Schichtel B., Segev L., Tripathi S., Wang C., Yu C., Zhang Q., Zhang Y., Brauer M., Cohen A., Gibson M.D., Liu Y., Martins J.V., Rudich Y. and Martin R.V., Variation in global chemical composition of $PM_{2.5}$: emerging results from SPARTAN, *Atmos. Chem. Phys.* **16**, 2016, 9629–9653.

Stefaniak A., Guilmette R., Day G., Hoover M., Breysse P. and Scripsick R., Characterization of phagolysosomal simulant fluid for study of beryllium aerosol particle dissolution, *Toxicol. Vitro* **19**, 2005, 123–134.

Stopford W., Turner J., Cappellini D. and Brock T., Bioaccessibility testing of cobalt compounds, *J. Environ. Monit.* **5**, 2003, 675–680.

Thelohan S. and De Meringo A., In vitro dynamic solubility test: influence of various parameters, *Environ. Health Perspect.* **102**, 1994, 91.

USEPA, Microwave ~~A~~assissted ~~A~~acid ~~D~~digestion of ~~S~~sediments, ~~S~~sl~~i~~udges, ~~S~~soils, and ~~O~~oils, 1998.

WHO, WHO ~~G~~global ~~U~~urban ~~A~~ambient ~~A~~air ~~P~~pollution ~~D~~database, ~~P~~public ~~H~~health, ~~E~~environmental and ~~S~~social ~~D~~determinants of ~~H~~health (PHE), 2016.

Wiseman C.L., Analytical methods for assessing metal bioaccessibility in airborne particulate matter: a scoping review, *Anal. Chim. Acta* **877**, 2015, 9–18.

Wiseman C.L. and Zereini F., Characterizing metal (loid) solubility in airborne PM_{10}, $PM_{2.5}$ and PM_1 in Frankfurt, Germany using simulated lung fluids, *Atmos. Environ.* **89**, 2014, 282–289.

Zereini F., Wiseman C.L. and Püttmann W., In vitro investigations of platinum, palladium, and rhodium mobility in urban airborne particulate matter (PM_{10}, $PM_{2.5}$, and PM_1) using simulated lung fluids, *Environ. Sci. Technol.* **46**, 2012, 10326–10333.

Appendix A. Supplementary data

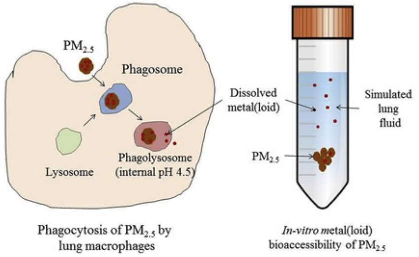
The following is the supplementary data related to this article:

Multimedia Component 1 (The graphs were incorrect. Please use the docx attached.)

Multimedia component 1

alt-text: Multimedia component 1

Graphical abstract



alt-text: Image 1

Highlights

- Fine air particulate matter ($PM_{2.5}$) may act as a sink for toxic metal(loid)s.
- Solid to liquid ratio may affect bioaccessibility of metal(loid)s using ALF.
- ALF results in significantly higher metal(loid) bioaccessibility than PSF ($p < 0.001$).
- $PM_{2.5}$ assessment using ALF and 1:5000 for 24 h is a conservative bioaccessibility approach.

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Answer: ok

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Answer: Zereini, F.,Wiseman, C.L.S., Püttmann,W., 2012. In vitro investigations of platinum, palladium,
and rhodium mobility in urban airborne particulate matter (PM_{10}, $PM_{2.5}$, and
PM_1) using simulated lung fluids. Environmental Science and Technology 46, 10326–10333.

Query: Please note that 'Fig.3' were not cited in the text. Please check that the citation(s) suggested by the copyeditor are in the appropriate place, and correct if necessary.
Answer: Fig 3 is cited in the next paragraph (8th line) as (Fig. 3 and Fig S3). Please do not write (see Fig. 3) at the end of the previous paragraph.

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