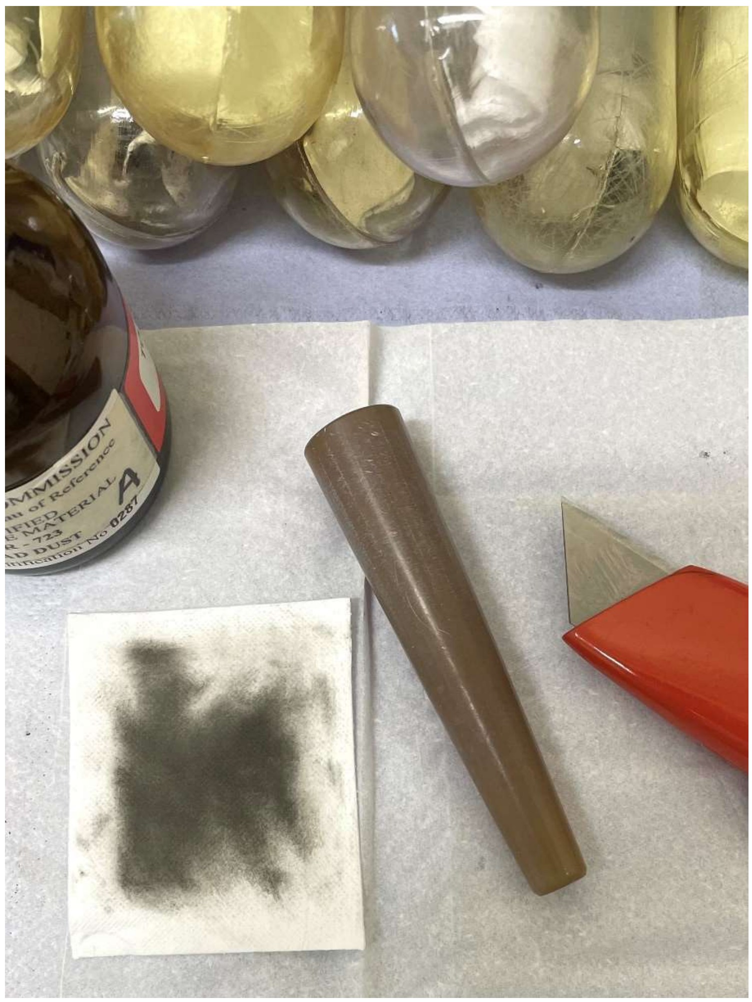
Evaluating in vitro inhalation bioaccessibility of potentially harmful elements in active and passive air sampling media

Environmental Change, Adaptation and Resilience

Internal Report OR/25/079



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Evaluating in vitro inhalation bioaccessibility of potentially harmful elements in active and passive air sampling media

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Foreword

This report showcases a study by the Inorganic Geochemistry Facility at the British Geological Survey. This study was conceived and designed by E.H. and M.J.W; and primarily managed by E.H. and M.J.W. Data output was supervised by E.H. and undertaken by M.T. Funding was gained by M.J.W. All authors contributed to the design, writing and/or review of the manuscript. The authors declare no conflict of interest.

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Elliott Hamilton now works for Agilent Technologies – elliott.hamilton@agilent.com

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Highlights

- Assessment of inhalation bioaccessibility in active and passive dust sampler media.
- There were significant differences ($p < 0.05$) between sampling media for some elements.
- Differences between sampling media are dependent on solid-to-liquid ratio.
- Active and passive sampling media suitable for inhalation bioaccessibility studies.

Summary

This study evaluated the use of Quartz Fibre (QF) and Polyurethane Foam (PUF) filter materials, commonly used in active and passive airborne particulate matter (APM) sampling respectively, in terms of their ability to capture statistically similar observations of *in vitro* inhalation bioaccessibility to better inform human health risk assessment from inhalation exposure to PHEs. The results indicated that both filter media can provide comparable inhalation bioaccessibility data at the lowest solid-to-liquid ratio (S/L 500) tested in this study, i.e. the largest amount of material deposited on the filter relative to the volume of extractant used. There was no statistical difference in the solid to liquid ratio, with the exception of Sr in the PUF and Cu for the QF. It is recommended that passive dust deposition samplers are either deployed for longer periods of time (in the order of months) to allow for sufficient sample material to be retained, or the extraction is scaled up to use larger filter portions. Further work is required to understand the impact of the increased porosity of the PUF on this recommendation, as the possibility of particle resuspension is greatly reduced compared to the densely woven surface of QF filters. This method of APM capture represents a cost-effective approach for application in the field with minimal risk of loss of equipment, no need for infrastructure (power and security) and their relatively low cost in the region of $200 per filter unit and $10 per filter. The lung bioaccessibility method provides a robust means of additional interpretation for human health risk assessment with context specific exposure.

1 Introduction

The airborne transport of potentially harmful elements (PHEs), through both natural and anthropogenic activities, may cause adverse health effects to humans (Boisa et al., 2014; Sang et al. 2022; Schiavo et al. 2023). Inhalation of airborne particulate matter (APM), alongside ingestion of deposited material and dermal absorption, is one of the main routes of exposure for PHEs, particularly for individuals living near mining operations or in urban environments (Petavratzi et al., 2005, Okorie et al., 2012; Morai et al. 2019).

The sampling of APM can be undertaken using both active and passive methods (Bogdal et al., 2013). Active APM samplers draw in specific volumes of air through an installed filter over a defined time period, allowing for the determination of the concentration of an element in the sampled APM per volume of air sampled (typically µg/m^3 or ng/m^3 depending on local regulatory requirements) (Noble et al., 2017). Passive APM sampling methods are comparatively small, lightweight and less expensive to use, with their main drawbacks being the inability to alter the uptake rate of the sampling unit, and the lack of particle size fractionation during sampling (Markovic et al., 2015). Passive samplers are inherently better suited to long-term monitoring campaigns capturing seasonal variation (Seethapathy et al., 2008), allowing for the assessment of relationships between climate, and the likelihood of dust generation and subsequent human exposure (Zhang et al., 2019).

Approaches to human health risk assessment (HHRA) in APM typically evaluate potential risks based on the total concentrations of PHEs (Mehta et al., 2020). However, the use of total elemental concentrations alone has been shown to overestimate impacts on human health (Chi et al., 2020), as it makes the assumption that PHEs are in a form that is completely bioavailable without considering changes in absorption properties depending on the geochemical fractionation of the PHEs in the sample of interest (Yu and Yang, 2019). The use of oral bioaccessibility methods, as a proxy for more costly and complicated bioavailability assessments, have become commonplace in studies assessing human health risks from exposure to PHEs in a range of settings, including environmental consultancy (Pelfrêne et al., 2012), industrial (Marin Villegas and Zagury 2023), urban (Ma et al. 2021) and mining lifecycle monitoring (Mehta et al., 2019; Canovas et al. 2023).

Inhalation bioaccessibility methods, whereby the conditions of the respiratory system are simulated during sample extraction, are further behind in their development and deployment (Kastury et al., 2017). Whilst fluid composition generally utilises Alveolar Lung Fluid (ALF) or a modified Gambles Solution (GS) there is a lack of consensus surrounding fluid composition (Soares et al. 2025), particle size and fundamental test conditions (solid/liquid ratio, extraction time). Very few studies have applied inhalation bioaccessibility methods directly to sampling materials used to collect APM (e.g. polytetrafluoroethylene (PTFE) filters), which could overcome common issues in the recovery and analysis of APM such as contamination, and insufficient sample mass due to sample adhesion and loss of material (Tang et al., 2019).

This work aimed to assess the inhalation bioaccessibility of reference materials (BCR-723, NIST 2710a) deposited onto different APM collection substrates (quartz fibre, polyurethane foam), to understand potential artefacts and biases introduced by the different filter media. Evaluating the performance of these filters for inhalation bioaccessibility will diversify their useability and provide cost-effective, site-specific information for better refinement of human health risk assessments where inhalation of APM has been identified as a significant exposure route.

2 Materials and methods

2.1 REFERENCE MATERIALS

The performance of the inhalation bioaccessibility method was assessed using reference material BCR-723 (road dust, <90 µm particle size, European Commission Institute for Reference Materials and Measurements). This material was chosen due to its representative particle size, and its use in previously published inhalation bioaccessibility method development (Pelfrêne et al., 2017, Wiseman et al., 2018). In addition, road dust is frequently used in human health risk studies as a proxy for atmospheric contamination (Dietrich et al., 2022).

2.2 FILTER MEDIA

Two airborne particulate matter collection substrates were employed in this study: quartz fibre (QF) and polyurethane foam (PUF). Quartz fibre filters are commonly used in epidemiological and environmental monitoring studies due to their high thermal stability and low metal(loid) background concentration (Ogrizek et al., 2021). Polyurethane foam substrates, in conjunction with passive dry deposition (PAS-DD) sampling units, are becoming an attractive alternative to active sampling methods due to their relatively low cost and ease of use (Cleaver et al., 2022, Mastin et al., 2023). Each material is representative of the typical sampling configuration that is used in active and passive air sampling, respectively (Bidleman and Tysklind, 2018).

Quartz fibre filters (type AQFA, Merck Millipore, UK) were used as received. Prior to use, each PUF disk (Tisch Environmental, USA) was cleaned according to previously published methods (Gaga et al., 2019). Briefly, each disk was triple rinsed with deionised water (DIW, 18.2 MΩ·cm at 25°C, Merck Millipore, UK) before ultrasonication in 1% v/v HNO_3 (Romil, UK) for 1.5 hours. After ultrasonication, the disks were triple rinsed with DIW to remove acid residue and dried in a laminar flow cabinet.

2.3 INHALATION BIOACCESSIBILITY

The most widely used synthetic lung fluids are Gamble's solution (GS) and artificial lysosomal fluid (ALF) (Wiseman, 2015). Artificial lysosomal fluid typically yields higher, and therefore more conservative, bioaccessibility values than GS, due to the lower pH of the fluid (Marin Villegas and Zagury, 2023). In addition, bioaccessibility values have been shown to be dependent on the solid-to-liquid (S/L) ratio when using other lung fluid compositions such as GS (Guney et al., 2017); ALF was subsequently chosen for this work to reduce the impact of extraction artefacts on the assessment of the filter media performance.

Aliquots of the reference material were spiked onto quartered segments of each filter media (QF or PUF) and transferred to precleaned (20% v/v HNO_3) polycarbonate centrifuge tubes, before the addition of 50 mL of ALF solution (pH 4.5 ± 0.1, 37°C). The centrifuge tubes were then placed into a temperature-controlled water bath and subjected to end over end rotation at 37°C for 24 h. Following this, the tubes were centrifuged at 4,500 × g for 15 minutes, before an aliquot of the supernatant was collected and preserved to 1% v/v HNO_3 (Romil, UK). Each reference material was extracted in triplicate and at three different S/L ratios (1/500, 1/1000, 1/5000) to assess the possibility of S/L ratio bioaccessibility dependence as highlighted above.

2.4 ELEMENTAL ANALYSIS

Major and trace element concentrations in ALF extracts were determined using an Agilent Technologies 8900 series inductively coupled plasma-tandem mass spectrometer (ICP-MS/MS), using previously reported operating conditions (Ondayo et al., 2024). Internal standards Sc, Ge, Rh, In, Te, Ir were deployed for signal drift correction. The full dataset along with performance data for reproducibility is presented in Table S1 for Quartz Fibre filters (QF) and Table S2 for Polyurethane Foam filters (PUF) of the Supplementary Materials. Within these tables, each element is accompanied by a denotion for the collision cell gas [e.g. He, O_2] and the method detection limits for each solid:liquid ratio is provided accounting for the analytical limits of detection for each element and method dilutions. For the purposes of comparison with previously published inhalation bioaccessibility data, only a subset of elements (Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, Sr, and Zn) will be presented and discussed in this article.

2.5 DATA ANALYSIS

Statistical description was undertaken using Microsoft Excel. One–way analysis of variance (ANOVA) was used to determine whether S/L ratio caused statistically significant differences in the bioaccessible elemental concentrations. Independent sample *t*-test was used to determine significant differences between QF and PUF filters; a p value < 0.05 was taken to reveal statistical significance. Means denoted by an asterix (*) in the displayed figures indicate significant differences ($p < 0.05$).

3 Results and Discussions

3.1 IMPACT OF S/L RATIO ON INHALATION BIOACCESSIBILITY MEASUREMENTS BETWEEN QUARTZ FIBRE AND POLYURETHANE FOAM

The amount of APM collected on either QF or PUF filter media will vary significantly depending on source strength and weather patterns (Omokungbe et al., 2020). Figure 1 and 2 show that there is no statistical difference in the solid to liquid ratio with the exception of Cu for the quartz fibre filter and Sr in the polyurethane foam filter. This may be due to the mineralogy of the dust within the CRM and/or physical properties of the filter media. Cleaver et al. (2022) also reported similar observations in that the PUF retained dust to a greater extent than conventional filter media.

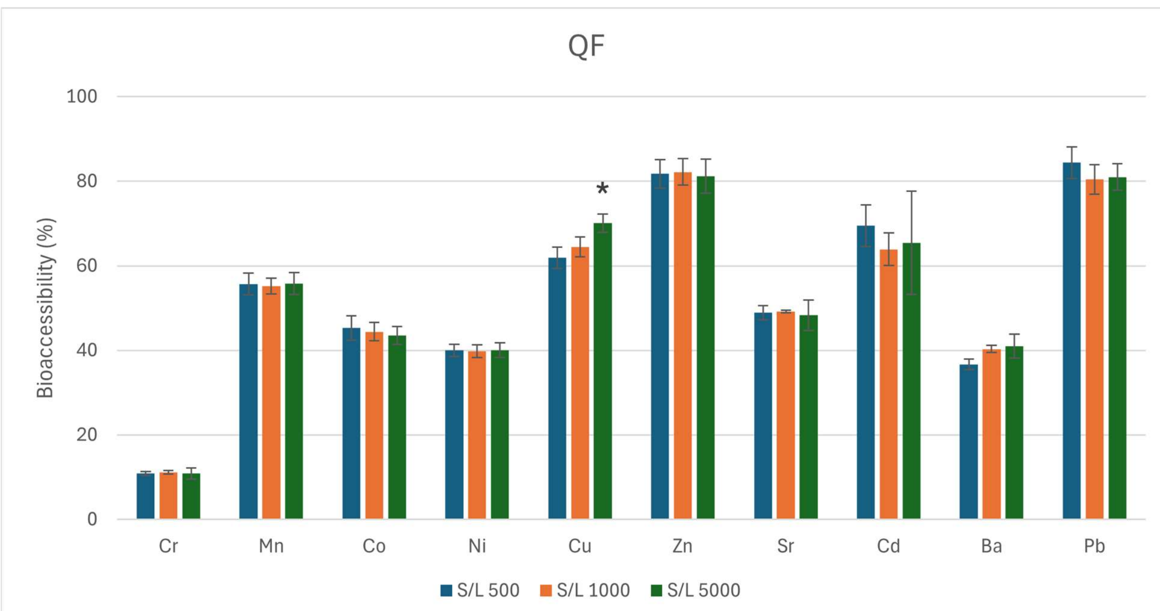

Figure 1 Bioaccessibility values (%; mean and SD; n = 3) in BCR 723 added to QF filter media at three S/L ratios. The asterisk (*) denotes statistically significant differences ($p < 0.05$) between the S/L ratios.

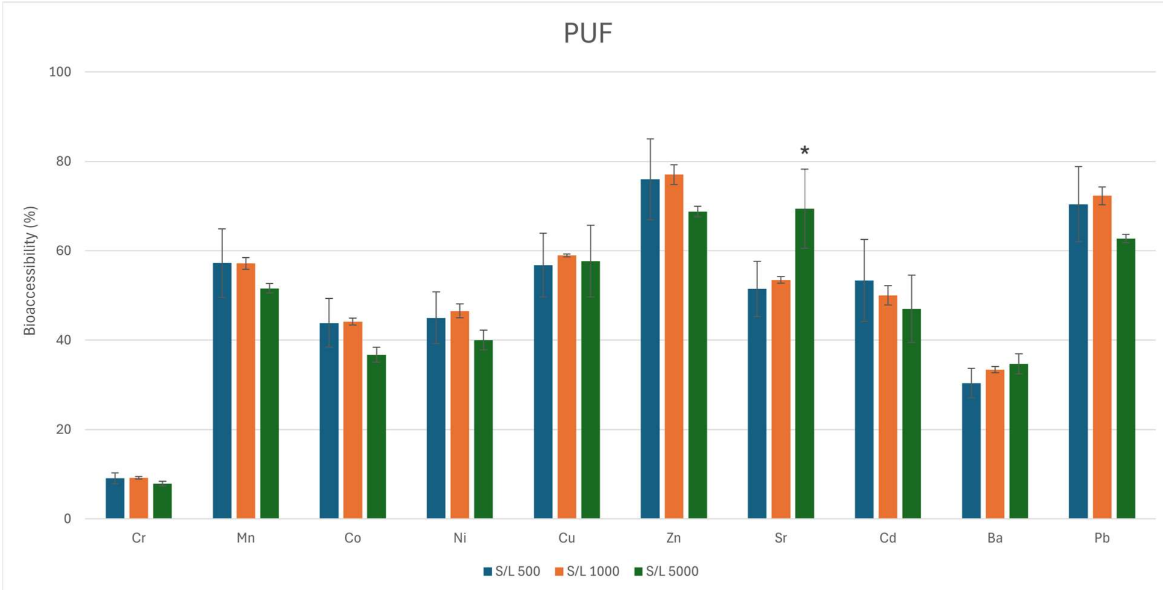

Figure 2 Bioaccessibility values (%; mean and SD; n = 3) in BCR 723 added to PUF filter media at three S/L ratios. The asterisk (*) denotes statistically significant differences ($p < 0.05$) between the S/L ratios.

3.2 EFFECTS OF QUARTZ FIBRE (QF) AND POLYURETHANE FOAM FILTERS (PUF) ON INHALATION BIOACCESSIBILITY MEASUREMENTS

Comparison of the QF and PUF filters suggests that higher S/L (1/1000, 1/5000) ratios result in some statistically significant differences between the measured inhalation bioaccessibility values for each of the filter types as denoted by an asterisk (*) in Table 1. In the case of the lowest S/L ratio (1/500), only Cd was significantly different (lower in the PUF compared to the QF). This is possibly an analytical artefact resulting from the relatively high dilution (20-fold) employed on the extracts due to the elevated sodium (Na) concentrations in the ALF solution to reduce the impact on the plasma efficiency of the ICP-MS. However, the precision for the 1/500 S/L ratio PUF measurements were generally poor at 12-13% (see Supplementary Tables) across all elements, suggesting a 1/500 S/L ratio is not appropriate for PUF, whereas for the QF precision of measurements were generally 4-5% across all elements, suggesting a preference for the 1/500 S/L for QFs compared to other S/L ratios. The precision of ratios for the 1/1000 S/L ratio were preferable across all elements for the QF filters, whilst showing a significant difference between QF and PUFs in results for S/L ratios of 1/1000 and 1/5000 across four of the ten elements. Overall, the 1/1000 S/L ratio is preferable for PUF and 1/500 for QF providing good reproducibility, for which the lower dilution may enable reliable analyses for less sensitive ICP-MS instruments than the triple quadrupole configuration, particularly for low abundance elements.

Table 1 Inhalation bioaccessibility values (mg kg^{-1} ± SD, n = 3) of BCR-723 spiked onto QF and PUF filter media and extracted at S/L ratios of 1/500, 1/1000 and 1/5000. Average values with an asterix (*) differ significantly by t-test ($p < 0.05$).

S/L 500	QF	PUF	S/L 1000	QF	PUF	S/L 5000	QF	PUF
Cr	47.7 ± 2	39.9 ± 5.3	Cr	49.2 ± 1.8	40.5 ± 1.2	Cr	47.7 ± 5.8	34.7 ± 2.2
Mn	712 ± 33	733 ± 98	Mn	706 ± 24	732 ± 17	Mn	714 ± 33	661 ± 13
Co	13.5 ± 0.9	13.3 ± 1.6	Co	13.2 ± 0.6	13.5 ± 0.2	Co	13.0 ± 0.6	13.3 ± 0.5
Ni	68.4 ± 2.4	76.9 ± 9.9	Ni	68.1 ± 2.6	79.6 ± 2.7*	Ni	68.5 ± 2.9	68.4 ± 3.8
Cu	140 ± 6	128 ± 16	Cu	146 ± 5	133 ± 1	Cu	158 ± 5	130 ± 18
Zn	1358 ± 56	1261 ± 151	Zn	1364 ± 51	1279 ± 36*	Zn	1347 ± 67	1141 ± 20*
Sr	124 ± 4	131 ± 16	Sr	125 ± 1	136 ± 2*	Sr	123 ± 9	176 ± 22*
Cd	1.74 ± 0.12	1.33 ± 0.23*	Cd	1.60 ± 0.10	1.25 ± 0.05	Cd	1.64 ± 0.31	1.18 ± 0.19
Ba	169 ± 6	140 ± 15	Ba	185 ± 4	154 ± 3*	Ba	189 ± 13	160 ± 10*
Pb	731 ± 32	610 ± 73	Pb	697 ± 30	627 ± 17*	Pb	701 ± 27	548 ± 8*

The porosity of the PUF filters has been shown to minimise resuspension of trapped particles (Eng et al., 2014). In this work, the S/L ratio was varied by reducing the mass of the reference material spiked onto the filter; therefore, it is possible that the ALF solution was unable to efficiently and reproducibly extract the smaller masses during the procedure. Further work is required to understand the significance of this observation, as PUF filters have also been shown to accumulate more APM than QF filters and so the absolute amount of material available for extraction after any sampling interval will still be greater than that obtained from conventional sampling media (Strandberg et al., 2018). The amount of APM that can reach the alveolar region varies depending on the individual physiology of exposed populations, and the particle size and quantity of APM present in the environment (Rennard et al., 1986). In addition, large volumes of lung fluid may cause solution saturation, whilst large masses of APM can reduce the efficiency of the extraction as less effective particle surface area will be exposed to the synthetic lung fluid during the extraction procedure (Julien et al., 2011).

The PUF disks also offer the ability to sample both gas and particle phase chemicals, allowing for assessment of persistent organic pollutants (POPs) in APM without additional sampling equipment (Saini et al., 2020) and potentially microplastics, albeit with limitations on particle sizes (Zheng et al., 2024). They can be used for both passive and active air sampling, providing long-term average concentrations for pollutants in both gas and particle phases, although their efficiency for particle bound compounds can be variable and environmental factors (e.g. wind speed, temperature) can affect the sampling rate.

4 Conclusion

This study evaluated the use of QF and PUF filter materials, commonly used in active and passive APM sampling respectively, in terms of their ability to capture statistically similar observations of *in vitro* inhalation bioaccessibility to better inform human health risk assessment from inhalation exposure to PHEs.

There is no statistical difference in the solid to liquid ratio, with the exception of Sr in the PUF and Cu for the quartz fibre filter. Therefore, consideration of the mineralogy of the dust and/or physical properties of the filter media is critical when interpreting inhalation bioaccessibility data using such APM data capture methods.

The results indicated that both filter media can provide comparable inhalation bioaccessibility data at the lowest solid-to-liquid ratio (S/L 500) tested in this study, i.e. the largest amount of material deposited on the filter relative to the volume of extractant used, although for PUFs, a 1/1000 S/L ratio provided acceptable precision of measurements across all elements compared to the 1/500 S/L ratio. It is recommended that passive dust deposition samplers are either deployed for longer periods of time (in the order of months) to allow for sufficient sample material to be retained, or the extraction is scaled up to use larger filter portions. Further work is required to understand the impact of the increased porosity of the PUF on this recommendation, as the possibility of particle resuspension is greatly reduced compared to the densely woven surface of QF filters. This method of APM capture represents a cost-effective approach for application in the field with minimal risk of loss of equipment (e.g. expensive active samplers $1000's), no need for infrastructure (power and security) and their relatively low cost in the region of $200 per filter unit and $10 per filter. The lung bioaccessibility method provides a robust means of additional interpretation for human health risk assessment with context specific exposure.

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