On- and off-line analysis by ICP-MS to measure the bioaccessible concentration of elements in PM_{10} using dynamic versions of the simplified bioaccessibility extraction test

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Abstract

Two dynamic versions of the simplified bioaccessibility extraction test (SBET) were developed – an off-line procedure and an on-line procedure coupled directly to ICP-MS. Batch, on-line and off-line procedures were applied to simulated PM_{10} samples prepared by loading NIST SRM 2711A Montana II Soil and BGS RM 102 Ironstone Soil onto 45 mm TX40 filters widely used in air quality monitoring. Three real PM_{10} samples were also extracted. A polycarbonate filter holder was used as an extraction unit for the dynamic procedures. Arsenic, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were determined in the extracts using an Agilent 7700x ICP-MS instrument. The residual simulated PM_{10} samples following application of the SBET were subjected to microwave-assisted *aqua regia* digestion and a 'mass balance' calculation performed with respect to digestion of a separate test portion of the SRM. Leachates were collected as subfractions for the off-line analysis or continuously introduced to the nebuliser of the ICP-MS for the on-line analysis.

The mass balance was generally acceptable for all versions of the SBET. Recoveries obtained with the dynamic methods were closer to pseudototal values than those obtained in batch mode. Off-line analysis performed better than on-line analysis, except for Pb. Recoveries of bioaccessible Pb relative to the certified value in NIST SRM 2711A Montana II Soil (1110 ± 49 mg kg^{-1}) were 99, 106, and 105% for the batch, off-line and on-line methods, respectively. The study demonstrates that dynamic SBET can be used to measure bioaccessibility of PTE in PM_{10} samples.

Keywords: Bioaccessibility; Inhaled particulate matter; Potentially toxic elements; dynamic extraction

1. Introduction

Since only the fraction that can be dissolved and is then available for absorption should be considered when human health effects caused by potentially toxic elements (PTE) in different substrates need to be assessed [1], many methods for measuring this bioaccessible fraction have been created, both *in vitro* and *in vivo* methods. However, the advantages of the *in vitro* methods – such as the ability to carry them out rapidly in a laboratory, low costs, and lack of ethical problems – make them preferred for assessing risks of PTE to human [2, 3].

In general, *in vitro* methods to assess oral bioaccessibility or bioaccessibility following inhalation are batch (static) models, with only limited dynamic models available, such as the TNO nutrition dynamic computer-controlled gastrointestinal model (TIM) [4-6]. Even these have their disadvantages. They are laborious, technologically complicated, high cost, and time consuming. Further, they are considered as equilibrium models, the same as batch models [7-9]. This is because only data at certain time points can be obtained.

Reactions that occur between substrates ingested or undergoing mucociliary transport and acids (as well as other constituents of gastrointestinal fluids) are non-equilibrium processes because PTE permeate across membranes once released [10]. Therefore, non-equilibrium dynamic versions (i.e. continuous on-line leaching) of the *in vitro* bioaccessibility methods more accurately represent the real conditions that substrates are subjected to in the body. In addition, when those models are applied, the maximum bioaccessible fraction that can be expected is obtained, as recommended by the International Organization for Standardization [10].

Many non-equilibrium dynamic models of extraction methods have been developed, the majority for sequential extraction. Some of these studies used a stirred-flow chamber as an extraction unit for fractionation or speciation of PTE either in soil [11-13], or in corrosion products from natural gas pipelines [14] and in solid biofuels [15]. Others dynamic sequential extraction models used a rotating coiled column for fractionation of PTE in soils, sludge, and sediments [16-22]. In addition, a column machined out of two polyoxymethylene end-caps was used as an extraction unit for fractionation of PTE either in soils and sludge [23-25] or in environmental and bio-shielding concrete samples [26]. Dynamic models of some single extraction method were also investigated. Mobility of trace elements in soil and sediments was dynamically studied by using the rotating coiled column coupled with inductively coupled plasma mass spectrometer (ICP-MS) [27]. Bioavailable Cr in soil was also

investigated using a dynamic model used a bi-conical micro column as an extraction unit [28, 29]. A recent study conducted by *Fedotov et al*., concluded that a dynamic extraction model based on the rotating coiled column can be used to measure the water-soluble fraction of As, Cd, Cu, Ni, Pb, S, Sb, and Zn in dust samples that were atmospherically deposited on window sills of a building near a copper smelter in Chelyabinsk region, Russia [30].

Dynamic leaching has not only been applied to single and sequential extraction methods. Versions of bioaccessibility extraction methods have also been developed and applied to various substrates, mainly foodstuffs but also a few environmental samples, as shown in Table 1. The overriding conclusions of the studies, both those conducted for single and sequential extraction methods and those for bioaccessibility extraction methods, was that non-equilibrium dynamic models offer advantages compared with batch models. They are simple and easy to apply, a good source for data on real-time element mobilisation, and less susceptible to potential contamination. In addition, they can offer short procedure times, represent best simulators of the gastrointestinal or environmental conditions, involve less probability of the occurrence of re-adsorption or redistribution of elements during extraction, and have less likelihood of analyte losses. A few disadvantages were also highlighted, such as the long time required for analysis and dilution effects [12].

It must be noted, however, that some of the advantages of dynamic models – such as shortened extraction time – are not the most important factors when gastrointestinal or inhalation bioaccessibility methods are considered, because the aim is to create the best simulator of the body tracts. The conditions of these tracts, such as the residence time of a substrate and volume of fluids present, should be maintained at physiologically relevant levels. For the gastrointestinal tract, one hour is typically adopted by several bioaccessibility methods as the residence time of a substrate in the stomach. Different volumes of fluids, and solid to fluid ratios, have been adopted by different authors. However, a review conducted in 2013 stated that, in the fasted state, the volume of gastric fluid in the human body must be near 50 mL [9]. Therefore, reduction of procedure time should not be the overriding aim when a new dynamic model for a bioaccessibility method is developed.

Extracts obtained by applying dynamic extraction models can be analysed by collecting subfractions with a defined volume or by interfacing a dynamic extraction system to an atomic spectrometer. These modes of detection are called off-line and on-line analysis, respectively. The relative advantages and disadvantages of off-line analysis and on-line analysis were reviewed in Ref 31 [31]. In off-line

analysis, the amount of leachate required for PTE quantification is small, therefore measurement of other parameters such as pH is possible. Secondly, leachates can be pre-treated before detection. Finally, they are generally simpler to use because complex interfaces between the extraction system and the spectrometer(s) are not required compared. However, on-line analysis provides real time extraction with insignificant contamination risks. As a result, extraction time can be reduced. It is also suitable when the purpose of a dynamic method is to monitor the leaching profile of elements that are extracted [31].

Since up to 99% of the total of the >2.5 μm fraction of inhaled PM_{10} is transported to the gastrointestinal tract by mucociliary clearance, oral bioaccessibility methods can be used to assess the bioaccessible fraction of the PTE in PM_{10}. The simplified bioaccessibility extraction test (SBET) [32], a simple oral bioaccessibility test, has been employed for determining the bioaccessible fraction of PTE in PM in a few studies [33-37]. Our previous study [37] revealed that it was possible to use the SBET with minor procedural modifications, in batch mode, to measure the bioaccessible PTE fraction in PM_{10} loaded on filter dynamic measurement system (FDMS) filters, as used in real air quality monitoring. Here, we report an extension of this work that, for the first time, demonstrates dynamic versions of the SBET procedure, which are applicable to PM on filters.
The specific aims of this study were:

1. Use of a (non-equilibrium)-based single-pass (SP) dynamic extraction model with fraction collection (FC) for the SBET (SPFC-SBET) to measure the bioaccessible concentration of PTE in PM_{10} using off-line analysis by ICP-MS.

2. On-line determination of the bioaccessible concentration of PTE in airborne particulate matter using the SP model of the SBET with direct coupling (DC) to ICP-MS (SPDC-SBET).

2. Experimental

2.1. Apparatus and Reagents

Blank Pallflex TX40 FDMS filters were supplied by Air Monitors (Gloucestershire, UK). This filter is borosilicate microfibers reinforced with woven glass cloth and bonded with polytetrafluoroethylene, and its typical weight and diameter is 5 mg cm^{-2} and 47 mm respectively. The pH of solutions was measured by using a Mettler-Teledo (SevenGo™) pH meter. Suspensions were shaken and incubated by using an end-over-end rotator placed inside an incubator (Stuart® SI500 shaking incubator)

manufactured by Barloworld Scientific Ltd., Staffordshire, UK. A multichannel peristaltic pump, REGLO ICC, 0.51 mm 3-stop cartridge tubing Tygon® LMT-55, 0.51 mm extension tubing Tygon® LMT-55, connector tube plastic for id 0.51 mm internal diameter tubing, and 47 mm in-line polycarbonate filter holder purchased from VWR International, Lutterworth, UK. All glassware and plastic ware were soaked overnight in 10% HNO_3 then rinsed three times with deionized water before use. All chemicals were of analytical grade. Hydrochloric acid (HCl) (36.5-38%) and nitric acid (HNO_3) (≥ 69 % Trace SELECT® for trace analysis) were obtained from Sigma Aldrich (Gillingham, Dorset, UK). Glycine was purchased from Fisher Scientific (Loughborough, UK). Multi-element standard stock solution (10 mg L^{-1} of As, Cd, Cr, Cu, Mn, Ni, Pb, and Zn) and Fe standard stock solution (1003 mg L^{-1}) were obtained from Qmx Laboratories, Essex, UK.

2.2. Simulation of PM_{10} samples

Samples of PM_{10} were simulated by smearing blank FDMS filters using a plastic spatula with 100 mg of NIST SRM 2711A Montana II Soil or BGS RM 102 Ironstone Soil. These were selected as test substrates (i) due to their relatively small particle size (<40 μm and <70 μm, where the size fraction of airborne PM collected on FDMS filters is <10 μm), (ii) because soil particles typically constitute a major component of airborne PM_{10}, and (iii) because NIST2711A is considered as a control material for the standard batch version of the SBET procedure [32]. Carrying out additional fractionation of the RMs to obtain particles <10 μm in diameter for study was considered but found not to be practicable (also the concentrations of PTE may differ between particle size fractions, rendering the certified value inapplicable). However, it is probable that use of larger particles will produce a more robust method since real PM_{10} samples will have larger specific surface area and so are likely to release PTE more readily than the test substrates.

2.3. Analytical procedures (off-line)

The SPFC flow through system is schematically illustrated in Figure 1. The device consists of the multichannel peristaltic pump, the 47 mm in-line polycarbonate filter holder (see Figure 2), extractant tube (50 mL centrifuge tube), water bath, pH meter, plastic rack, and thermometer. The four-channel peristaltic pump was chosen because it has three channels that can be individually controlled either from its keypad or from PC; as a result, throughput of the method is increased. The polycarbonate filter holder was chosen as the extraction cell because it has an area that is suitable to hold the FDMS filter and it can be vented. The caps of the 50 mL centrifuge tubes were holed. The wider central hole was used for inserting a pH electrode and for introducing reagents, and the two small holes were used to

insert the tubing. The wider hole was covered by laboratory Para-film during the extraction to prevent contamination.

A simulated PM_{10} sample was placed into the pre-cleaned filter holder and the holder was then tightly closed by hand. The filter holder and the extractant tube were fixed on the plastic rack using elastic bands. The 0.51 mm extension tubing was used to connect the inlet and outlet of the holder filter to the outlet of the pre-calibrated peristaltic pump (50 cm long) and the leachate tube (75 cm long), respectively as well as the inlet of the pump to the extractant tube (58 cm long).

The procedure for the SPFC-SBET was: 50 mL of 0.4 M glycine (37 °C, pH 1.5 ± 0.05) was transferred to the extractant tube by means of a micropipette. The pump flow rate was set at 1.5 mL min^{-1} for 5 min. The vent cap of the filter holder was then opened. To fill the filter holder, the pump was then run for 5 min, and the vent cap was then closed. The pump flow rate was then set at 1 mL min^{-1} and run for 5 min to pass 5 mL of extractant through the loaded filter. This was repeated 12 times, with a 15-second pause between delivery of each extractant volume, allowing for subfraction collection tubes (extract tubes) to be changed after each cycle. The plastic rack containing the filter holder and the extractant tube was then placed into the pre-thermostated water bath at 37 °C. The pump was then run for 1 hour, and the last 10 mL of the 0.4 M glycine was added to the extractant tube during the extraction. The extracts were collected as subfractions every 5 min with a 5 mL volume. The extracts obtained was checked to ensure that they were within pH 1.5 ± 0.5 (i.e. ≤ pH 2.0) since the SBET protocol requires than any extractions that do not meet this criterion are repeated. Results obtained were in the range pH 1.48 – 1.57 and so no repeats were necessary. The extracts were stored in polyethylene bottles at 4 °C prior to analysis by ICP-MS as described in Section 2.8.

Three simulated PM_{10} samples prepared using BGS RM 102 Ironstone Soil, three simulated PM_{10} samples prepared using NIST SRM 2711A Montana II Soil, and three blank FDMS filter, were used. In addition, a method blank was obtained by running the extractant only through the complete procedure. A blank spike was also studied for quality control purposes. This was obtained by running the extractant, spiked at 10020 μg L^{-1} for Fe and 250 μg L^{-1} for the other PTE, through the complete procedure.

A washing process was performed between samples when the system was used repeatedly. This was conducted after removing the extracted simulated PM_{10} samples from the filter holder. It involved

pumping 10 mL of 5% HNO_3, then 15 mL deionised water, then 5 mL of the extractant used, sequentially, at flow rate 1.5 mL min^{-1}, through the system The filter holder was then air-dried.

2.4. Analytical procedures (on-line)

A diagram of the system used for the SPDC, is shown in Figure 3. In addition to the constituents of the system described in Section 2.3, two further 50 mL centrifuge tubes were added, one for the internal standard and one for the residual extract accumulated inside the chamber of the ICP-MS. The four channels of the peristaltic pump were used. The first channel was used to deliver the internal standard solution from the internal standard tube to the T-connector. The second channel was used to deliver the extractant from the extractant tube to the filter holder. The third channel was used to remove the residual extracts from the chamber of the ICP-MS. The fourth channel was used to deliver the rinse solution (2% HNO_3) to the T-connector when washing process was conducted. The outlet of the holder filter was connected to the T-connector, which was connected to the nebulizer of the ICP-MS. The length of 0.51 mm extension tubing used for connecting the inlet and outlet of the pump's channels to the different parts of system were: 58 and 120; 58 and 50; 58 and 120; 58 and 120 cm for the first, second, third, and fourth channel, respectively.

The preparation of the holder filters containing simulated PM_{10} samples was as described in Section 2.3. A 50 mL aliquot of the 0.4 M glycine (37 °C, pH 1.5 ± 0.05) was transferred to the extractant tube by means of a micropipette. The flow rate of the second channel (extractant channel) was set at 1.5 mL min^{-1} for 5 min and other channels were on disabled mode. The vent cap of the filter holder was opened. The pump was then run for 5 min, and the vent cap was then closed. The flow rate of the extractant channel was then changed to 1 mL min^{-1}. The plastic rack contained the filter holder and the extractant tube was then placed into the pre-thermostated water bath at 37 °C.

The flow rate of the first channel (internal standard channel) was then set at 0.1 mL min^{-1}, with the other three channels on disabled mode, and the pump was run until the internal standard entered the T-connector. The flow rate of the third channel (extracts residual channel) was then set at 1.1 mL min^{-1}. The pump was then run (except the fourth channel, that was set on disabled mode) until the solution of the extract mixed with the internal standard reached the nebuliser. The pump was then run for 1 hour, simultaneously with the method of analysis described in Section 2.8. The last 10 mL of the 0.4 M glycine was added to the extractant tube during the extraction.

Three simulated PM_{10} samples prepared using NIST SRM 2711A Montana II Soil, and three blank FDMS filter were used. In addition, a method blank was obtained by running the extractant only through the complete procedure, and a blank spike (as described above for the off-line method). The washing process was performed by pumping 2% HNO_3 at flow rate 1 mL min^{-1} through the apparatus for 3 min using the fourth channel, whilst the third channel continued to remove residual solution from the ICP-MS, and the other two channels were on disabled mode.

2.5. Batch methods

The SBET was also carried out using the batch conventional model, as described in Ref [37]. To assess the efficiency of the methods developed, results obtained from the standard batch model were compared to results achieved from the new dynamic models. Extracts obtained from the batch model were analysed as described in Section 2.8.

2.6. Digestion of residues and mass balance

Mass balance of the batch and dynamic models of the SBET was checked by comparing the pseudototal PTE content (i.e. the *aqua-regia*-leachable PTEs) with the sum of the bioaccessible PTE concentration and the PTE remaining following extraction of samples. *Aqua regia* digestion was preferred to the determination of true total PTE content since it avoids the use of HF and is widely used in the investigation of anthropogenic pollution because it solubilises all but the refractory silicate-bound PTE fraction. Determination of the PTE remaining following extraction was performed by digestion of the residues remaining after sample leaching in 5 mL of freshly prepared *aqua regia*.

To determine the pseudototal PTE content, each batch of digestion involved: three simulated PM_{10} samples prepared using BGS 102, three simulated PM_{10} samples prepared using NIST 2711A, and three blank FDMS filters as well as a blank method. To assess the accuracy of sample digestion and PTE measurement, three samples of BCR CRM 143R Sewage Sludge Amended Soil were also digested. Digests obtained were analysed as described in Section 2.8.

2.7. Real PM_{10} samples

Three real PM_{10} samples were obtained from air quality monitoring stations operated by Glasgow City Council, Scotland, UK. These had been collected on FDMS filters, using TEOM FDMS instruments, as part of the City's routine air monitoring campaign. The stations were located at Byres Road, Broomhill Road and Burgher Street. The samples from Byres Road (exposed from 1st October to 15th October 2015) and Broomhill Road (no exposure dates available) were extracted using the

SPFC-SBET, while the sample from Burgher Street (no exposure dates available) was extracted using the SPDC-SBET.

2.8. Analyte quantification

Extracts and digests obtained from the batch and the SPFC model, and microwave digestion were analysed by ICP-MS (Model 7700x, Agilent Technologies, Cheshire, UK) using a spectrum analysis (multi tune) mode. Isotopes chosen and their internal standard for As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were ^{75}As and ^{72}Ge, ^{111}Cd and ^{115}In, ^{52}Cr and ^{45}Sc, ^{63}Cu and ^{45}Sc, ^{56}Fe and ^{45}Sc, ^{55}Mn and ^{45}Sc, ^{60}Ni and ^{45}Sc, ^{208}Pb and ^{209}Bi, and ^{66}Zn and ^{72}Ge respectively. Reagent-matched calibration standards were used. Those standards were: 0, 10, 100, 500, and 1000 **µg**/L for all PTE except Fe, where they were: 0, 300.9, 3009, 15045, and 30090 **µg**/L. All standards were prepared by pipetting out the volumes required from the standard stock solutions into 10 mL volumetric flasks, which were then made up to the mark with the same reagents used for preparing samples. For quality control, one of the calibration standards was re-run every 10 analysis and at the end of the sample run to check for instrumental drift.

Time resolved analysis mode (TRA) was used when the SPDC model was conducted. The parameters of TRA mode and the operation condition for the ICP-MS were as shown in Table 2. For the on-line analysis (i.e. TRA mode), the ICP-MS was calibrated using the same mode. The parameters of the TRA mode were similar to these described in Table 2, except the total acquisition time of analysis was 120 sec. The system (see Fig. 3) and the procedure described in Section 2.4, were used for calibration the ICP-MS with minor modifications. These modifications involved: removing the filter holder from the system; connecting the outlet of the second channel (extractant channel) of the pump directly to the T-connector; and connecting the inlet of the second channel to the standard solution tubes. Before analyzing standard solutions and the calibration blank, as well as between the analyses, the system was washed with 2% HNO_3 for 2 min using the fourth channel of the pump. The T-connector was then connected to the standard solutions or calibration blank using the second channel for 2 min, and then the method was run for 2 min.

For quality control, two of the calibration standards were re-analysed, one between the analyses and one at the end of the sample run to check for instrumental drift. This was conducted similarly to the procedure used for analyzing the standard solutions. The raw data obtained from the instrument in TRA mode was handled using Microsoft excel 2011.

2.9. Quality control and reference material

No certified reference material is currently available for bioaccessible PTE in airborne PM. Analytical performance was therefore assessed by processing triplicate samples and by use of spike recovery tests. Extractants were spiked to known concentrations of analytes (10020 μg L^{-1} for Fe and 250 μg L^{-1} for other PTE) and taken through the complete extraction procedure. The percentage spike recovery was calculated using equation 1.

$$\%\ \boldsymbol{spike\ recovery} = \left(\frac{|\boldsymbol{measured\ conc.\ of\ PTE\ in\ spiked\ reagent - measured\ conc.\ of\ PTE\ in\ unspiked\ reagent}|}{\boldsymbol{known\ conc\ of\ PTE\ in\ spiked\ reagent}}\right) \times \mathbf{100}$$

Equation 1

3. Results and discussion

3.1. Effect of loaded FDMS filters on the flow rate of extractant

As the porosity of a filter can affect the flow rate of an extractant flowed through it by a peristaltic pump[11], and since the porosity of FDMS filters is not known[38], the effect of introducing a loaded FDMS filters on the stability of flow rate of extractant was investigated. Three flow rates were tested: 1.0, 1.5, and 2.0 mL min^{-1}. Three loaded FDMS filters prepared using BGS102 soil were used for each flow rate. The analytical procedure for the SBET as described in Section 2.3 was followed. The volume of subfractions was measured using a 10 mL measuring cylinder. The theoretical flow rate was calculated by dividing the volume collected by the subfraction collection time (i.e. 5 min). Results obtained demonstrated that the loaded FDMS filters did not affect the extractant flow rate. The values obtained were 1.00 ± 0.02, 1.50 ± 0.01, and 2.00 ± 0.02 mL min^{-1}, corresponding well to the theoretical values set using the pump controls. To avoid leakage and reduce any flow resistance, 1.0 mL min^{-1} was chosen for subsequent work. This value meant that results obtained with the SPFC-SBET could be meaningfully compared with those achieved with the SPDC-SBET, as 1.0 mL min^{-1} is also a suitable flow rate for sample introduction into ICP-MS.

3.2. PTE in blank FDMS filters

The two dynamic models of the SBET were applied to blank FDMS filters. Three blank FDMS filters were used for each model. Bioaccessible PTE concentration in blank FDMS filters extracted using the dynamic models of the SBET as well as their residual fractions and pseudototal content of non-extracted filters are shown in Tables 3 and 4. As expected, the bioaccessible fraction was very low or below the IDLs for all PTE tested, except for Zn. The relatively high Zn values arise from the fact that

Zn is used as a binder for FDMS filters. The mass balance generally was acceptable according to the Student's t-test (see Tables 3 and 4).

3.3. Single-pass dynamic model of the SBET with fraction collection (SPFC-SBET) (off-line)

The extractograms of PTE in the simulated PM_{10} samples prepared using BGS 102 and NIST 2711A, extracted by the SPFC-SBET are presented in Figures 4 and 5. The largest release of bioaccessible species was observed in the first 5 mL leached (subfraction 1) for all PTE, except for Cr in BGS 102, where it was in subfraction 4. As NIST 2711A was prepared from a contaminated soil, this rapid mobilisation is expected since PTEs added from anthropogenic sources are likely to be adsorbed on surfaces of soil components and not strongly bound. However, this seems also to be the case for BGS 102, a soil naturally elevated in PTE. For Cr in BGS 102, the slow mobilisation may be because of the presence of Cr species such as CrO_4^{2-} and CrO_3^{3-}. These ions tend to be adsorbed on the anion exchange sites of soil minerals such as iron oxides, which are likely to be in greater abundance in the BGS 102 as it is a ferritic brown earth soil. This interpretation is supported by the fact that the Cr leaching profile was similar to some extent to that of Fe, which might suggest that Cr is mainly associated with Fe oxides in this soil.

When the cumulative bioaccessible concentrations achieved using the dynamic model were compared with those obtained using the standard batch extraction, results for a few elements – As in BGS 102 and Cu in both soils – were higher. However, for the majority of elements, concentrations were lower than those obtained in batch mode This was not expected as the sample was exposed to 60 mL of fresh SBET reagent over the course of one hour when the SPFC-SBET was applied, compared with only 10 mL in the batch model. Even where the bioaccessible concentrations achieved using the SPFC-SBET were as expected (i.e. higher than that obtained by the batch model). the volume required to obtain the batch-equivalent concentrations was greater than 10 mL (the volume of the SBET reagent used in batch model), e.g. 25 mL for As in BGS 102. This was probably due to the effect of contact time between the reagent and samples, where it was one hour for 10 mL for the batch model, while for the SPFC-SBET, it was only 10 min for 10 mL.

3.4. Single-pass dynamic model of the SBET with direct coupling to ICP-MS (SPDC-SBET) (on-line)

The leaching profiles for PTE in simulated PM_{10} samples prepared using NIST 2711A, extracted by the SPDC-SBET, are depicted in Figure 7. Although a result was measured every 5 seconds by the ICP-MS instrument, the cumulative bioaccessible concentration obtained at every 60^{th} measurement is

presented in order to create a plot that can be readily compared with the results obtained at 5-minute intervals using the off-line SPFC-SBET model. The maximum release of bioaccessible PTE occurred in the first 10 second of the 3600 s leaching time, with the leaching rate then gradually decreasing. The exception was Cr, where concentrations were below the IDL throughout (hence no extractograms is presented for this element in Figure 7). The leaching trend was in agreement with the same dynamic model with off-line analysis, where the maximum PTE concentrations were released in the first 300s (i.e. found in the first subfraction). As shown in Figure 7, and according to the Student's t-test at 95% confidence level (see Table 5), there was no significant difference between the cumulative bioaccessible PTE concentration obtained by extraction the simulated PM_{10} samples (NIST 2711A on FDMS filter) using the SPFC-SBET and the SPDC-SBET.

3.5. Mass balance

Tables 6-8 show the bioaccessible and residual PTE fractions, as well as the sum of fractions and mass balance with respect to the pseudototal PTE content, for simulated PM_{10} samples prepared using BGS 102 and NIST 2711A, extracted by the batch model, the SPFC-SBET, and SPDC-SBET. Values of Z-score shown in Tables 6-8 were calculated using Equation 2, where the predicted standard deviation(s) was calculated using Equation 3. According to these values, mass balances agree with the measured pseudototal PTE concentration in all cases, either when batch or the dynamic model was applied. For the batch model of the SBET, 73% of Z-scores were acceptable, 20% satisfactory, and 7% not satisfactory. For the SPFC-SBET, 100% of Z-scores were acceptable, while for the SPDC-SBET, 86% of Z-scores were acceptable and 14% were satisfactory. For Zn, Z-score was not calculated, as the pseudototal content of the blank FDMS filters was variable (see Tables 3 and 4).

$$Z - score = \frac{\overline{x}_A - \overline{x}_B}{s/\sqrt{n}}$$

Equation 2

Where, $\overline{x}_A$, $\overline{x}_B$, and n are a test mean (certified or measured mean), and a number of replicates of the test sample, respectively.

$$s = \frac{C}{10}$$

Equation 3

Where, C, is the concentration (a certified or measured mean).

3.6. Quality control

The RSD was used to ascertain the precision of the models of the SBET. All of the RSD values were less than 10% for the batch models of the SBET, while for the SPFC-SBET, 83% of RSD's values were less than 10% (see Tables 6 and 7). However, the remaining 17% of RSD's values ranged from only 13.4 to 16.0%. For the SPDC-SBET, the repeatability (RSD) was not as good as the off-line model, where it ranged from 13.2% for Pb to 41.7% for Zn (see Table 8).

In addition to the validation of mass balance as described in Section 2.6, spike recovery tests were also performed to ascertain the trueness of the models. For the batch model of the SBET, spike recoveries were 100 ± 4% (except for Zn, where it was 87%), whilst for the dynamic models, they were 100 ± 2% for the SPFC-SBET and 100 ± 9% for the SPDC-SBET (see Tables 6-8).

Although the certified value of bioaccessible Pb in NIST 2711A (1110 ± 49 $mg.kg^{-1}$) was established for the batch model of the SBET, it was still considered suitable to verify the accuracy of the two dynamic SBET models developed in the current study. For off-line analysis (SPFC-SBET), the recovery was 106 ± 6 % while for the on-line model (SPDC-SBET) it was 105 ± 15%. Accuracy of *aqua regia* microwave digestion followed by PTE quantification by ICP-MS was ascertained using CRM BCR143R. The results obtained were within 100±10% of certified values, as illustrated in Table 9, indicating the method is under control.

3.7. Analysis of real samples

To verify the applicability of the dynamic models to determine the bioaccessible concentration of PTE in real samples collected during air quality monitoring, three PM_{10} samples from the City of Glasgow were analysed (two for the SPFC-SBET and one for the SPDC-SBET). This was done purely as proof-of-concept, since the aim of this work was not to assess air pollution levels or sources. The SPFC-SBET was conducted by continuously pumping 60 mL of the extractants at 1.0 mL min^{-1} flow rate through the real samples of PM_{10}. The leachate was then collected in one fraction (60 mL) instead of subfractions. The extracts were stored in polyethylene bottles at 4 °C prior to analysis by ICP-MS as described in Section 2.8. Table 10 shows the bioaccessible PTE concentration in real PM_{10} samples extracted by the SPFC-SBET. The results showed high bioaccessible concentration for Cu, Fe, Pb, and Zn. This was in agreement with results obtained by a study[36] conducted in an industrial city (Nanjing) in China, where the SBET was used to measure the bioaccessible concentration of PTE in aerosol (total suspended particulates and $PM_{2.5}$) collected on filters. For a real PM_{10} sample (obtained from Burgher Street), the SPDC-SBET was conducted as described in Section 2.4. Table 10 and Figure 8 show the

bioaccessible PTE concentration measured in the real PM_{10} sample extracted by the SPDC-SBET. Higher bioaccessible concentration was observed for Cu, Fe, Pb, and Zn, whereas the bioaccessible concentration for the rest of the PTE measured was low. Similar to the NIST 2711A, the highest amount of PTE in the real PM_{10} sample was extracted in the first 5 min.

4. Conclusion

In this work, two dynamic models for the SBET were successfully developed and applied to measure the bioaccessible PTE concentration in simulated PM_{10} samples prepared by smearing blank FDMS filters with 100 mg BGS 102 soil and NIST 2711A, as well as in real PM_{10} samples. Extracts obtained from samples by applying these dynamic models were analysed off-line and on-line by ICP-MS.

Bioaccessible PTE concentration obtained from these dynamic models and those achieved using modified versions of the batch models of the SBET were compared. Results indicated that some 67% of bioaccessible PTE values obtained using the batch models were higher than those achieved by applying the dynamic model of the SBET with off-line analysis (SPFC-SBET). This is unexpected since dynamic extraction should give the higher results, as fresh reagent is continuously pumped through the sample, and may reflect potential contamination during batch mode samples preparation. On-line determination of PTE using the tandem system of the dynamic model of the SBET and the ICP-MS (SPDC-SBET) was shown to be applicable to both simulated and real PM_{10} samples. Statistical analysis indicated that there was no significance different between the bioaccessible PTE concentration obtained using the SPFC-SBET and those obtained by the same model but with on-line analysis (SPDC-SBET).

The quality of these dynamic models was controlled using mass balance, spike recovery, replicates of samples, and recoveries of the available certified or guidance values. Mass balance values were in most cases in agreement with measured pseudototal PTE values.

Although low sample throughput and long analysis time for each sample were their disadvantages, the dynamic models of the SBET were successfully applied to measure the bioaccessible PTE concentration in simulated and real PM_{10} samples, with less contamination than can potentially occur in conventional batch mode. Their further investigation and application are therefore recommended.

Acknowledgement

JA acknowledges financial support from the Iraqi Ministry of Higher Education and Scientific Research and the Iraqi Ministry of Education. Authors also acknowledge Alex Clunie for assistance with the ICP-MS.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Legends for Figures

Figure 1. Schematic diagram of the single-pass dynamic extraction model with fraction collection device

Figure 2. Constituents of 47 mm in-line polycarbonate filter holder

Figure 3. Schematic diagram of the single-pass dynamic extraction device with direct coupling to ICP-MS

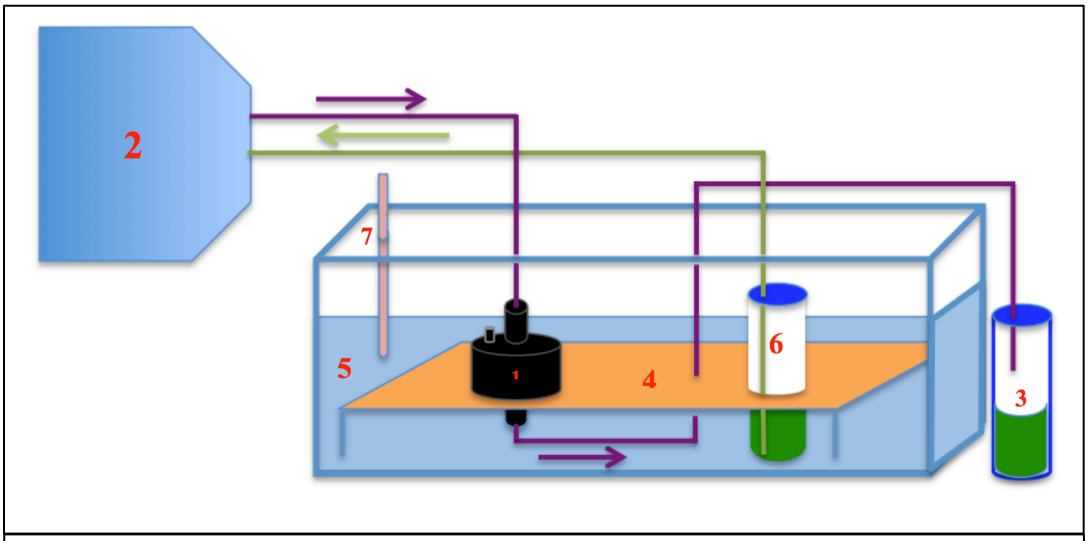
Figure 4. Bioaccessible concentration of PTE in subfractions obtained by extraction of the simulated PM_{10} samples (BGS RM 102 Ironstone Soil on FDMS filter) using the single-pass dynamic model of the simplified bioaccessibility extraction test (SBET) with fraction collection (SPFC-SBET)

Figure 5. Bioaccessible concentration of PTE in subfractions obtained by extraction of the simulated PM_{10} samples (NIST SRM 2711A Montana II Soil on FDMS filter) using the single-pass dynamic model of the simplified bioaccessibility extraction test (SBET) with fraction collection (SPFC-SBET)

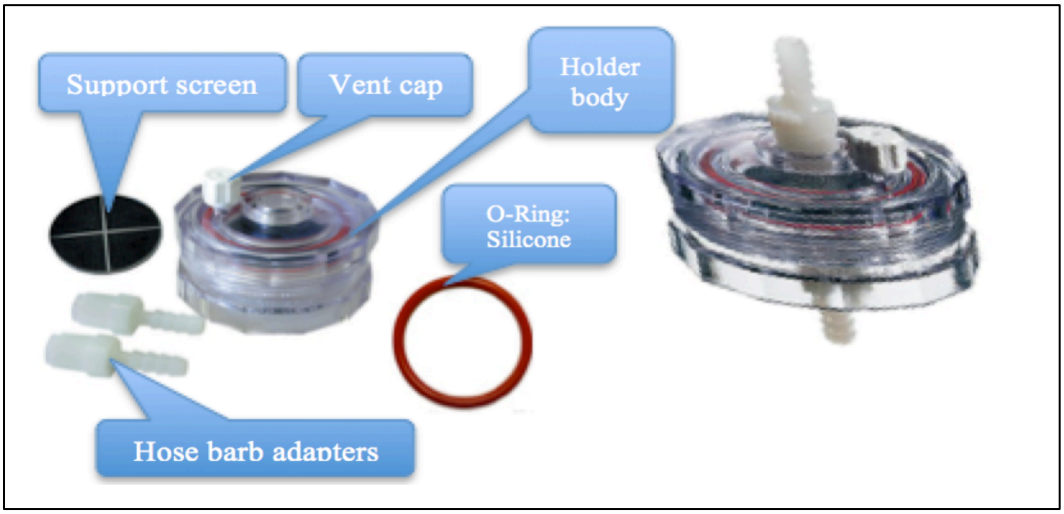
Figure 6. Values of pH for each subfraction obtained by applying the the single-pass dynamic model of the simplified bioaccessibility extraction test (SBET) with fraction collection (SPFC-SBET) using BGS RM 102 Ironstone Soil and NIST SRM 2711A Montana II Soil

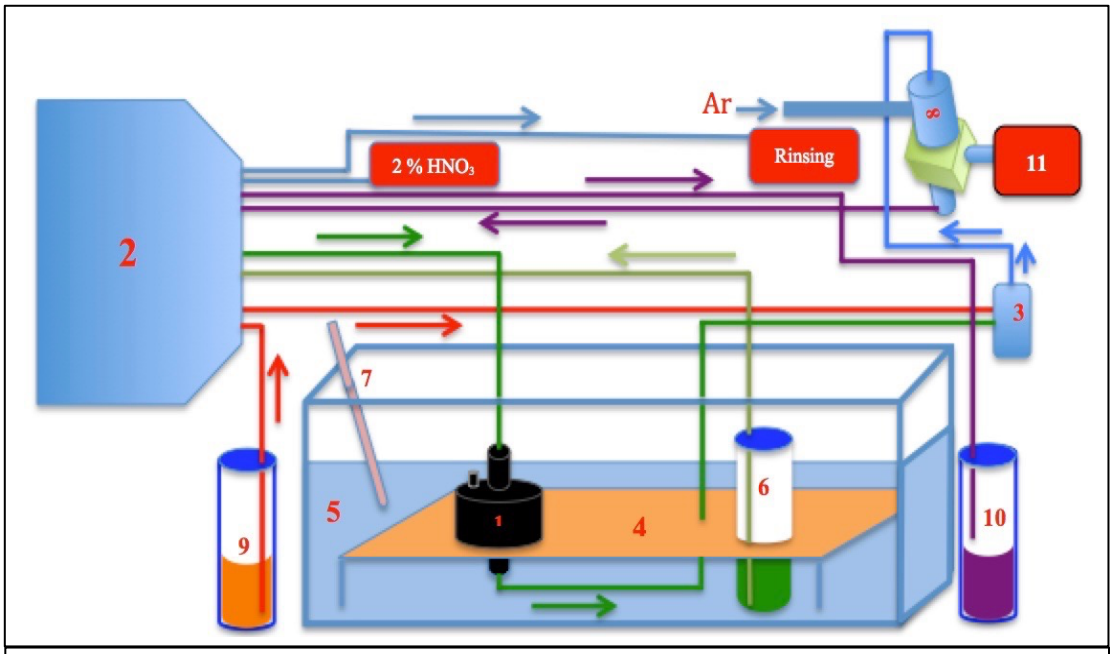
Figure 7. Cumulative bioaccessible concentration of PTE obtained by extraction of the simulated PM_{10} samples (NIST SRM 2711A Montana II Soil on FDMS filter) using the single-pass dynamic model of the simplified bioaccessibility extraction test (SBET) with fraction collection (SPFC-SBET) and with direct coupling to ICP-MS (SPDC-SBET); error bars represent one standard deviations (n=3)

Figure 8. Bioaccessible concentration of potentially toxic elements (PTE) in a real PM_{10} sample obtained by applying the single-pass dynamic model of the simplified bioaccessibility extraction test (SBET) with direct coupling to ICP-MS (SPDC-SBET)

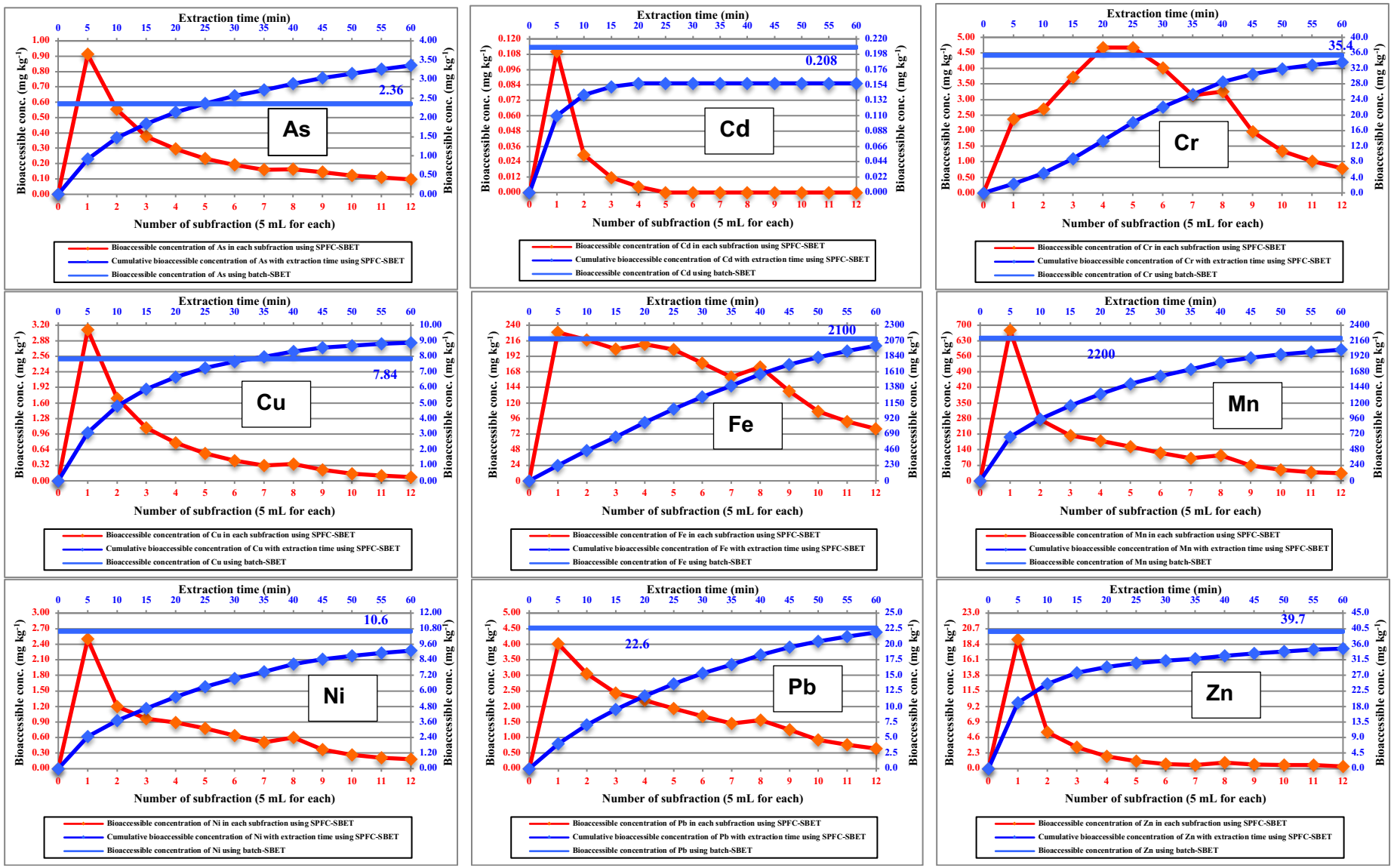


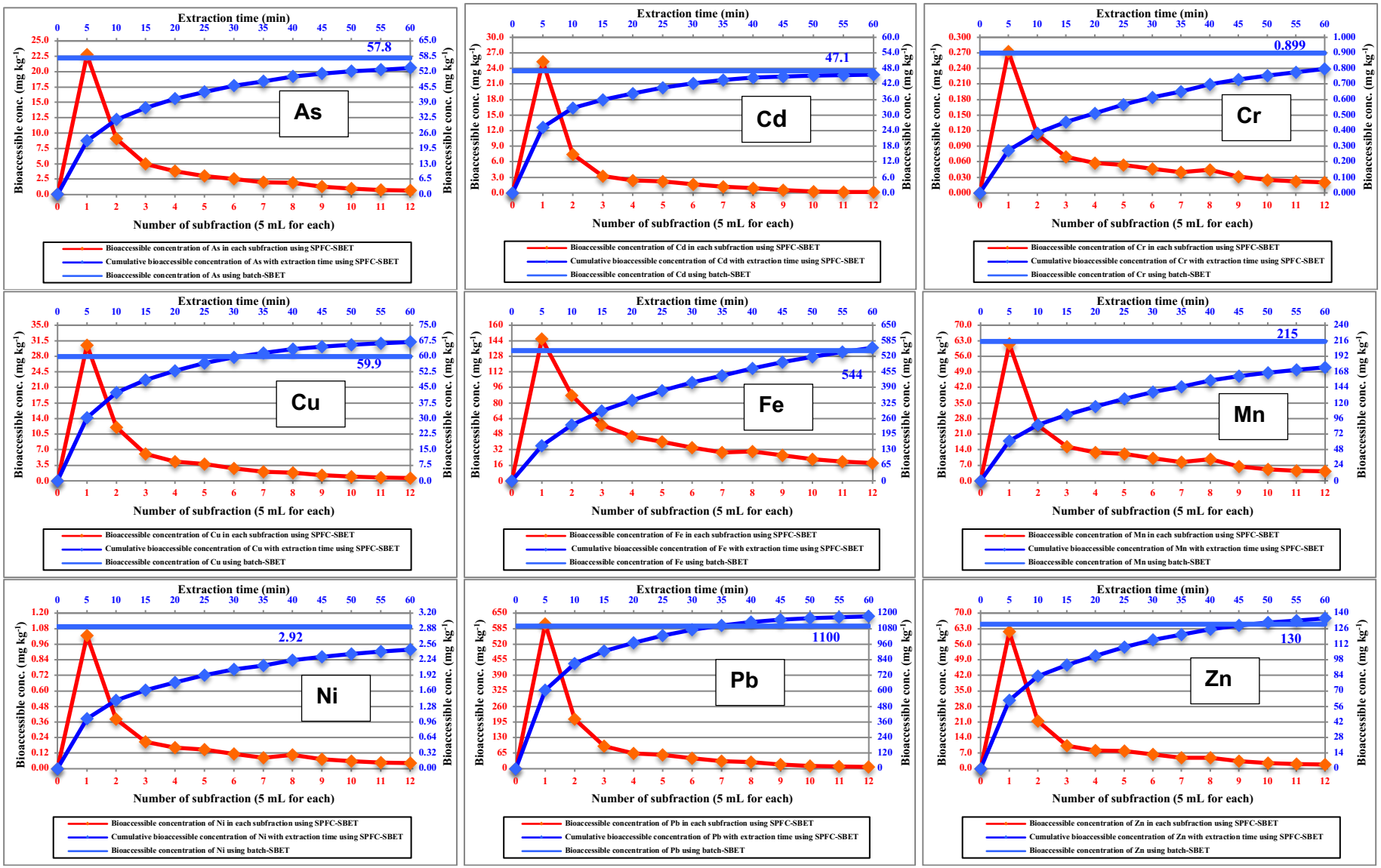
(1) Polycarbonate filter holder (2) Peristaltic pump (3) Leachate tube (4) Plastic rack (5) Water bath (6) Extractant tube (7) Thermometer

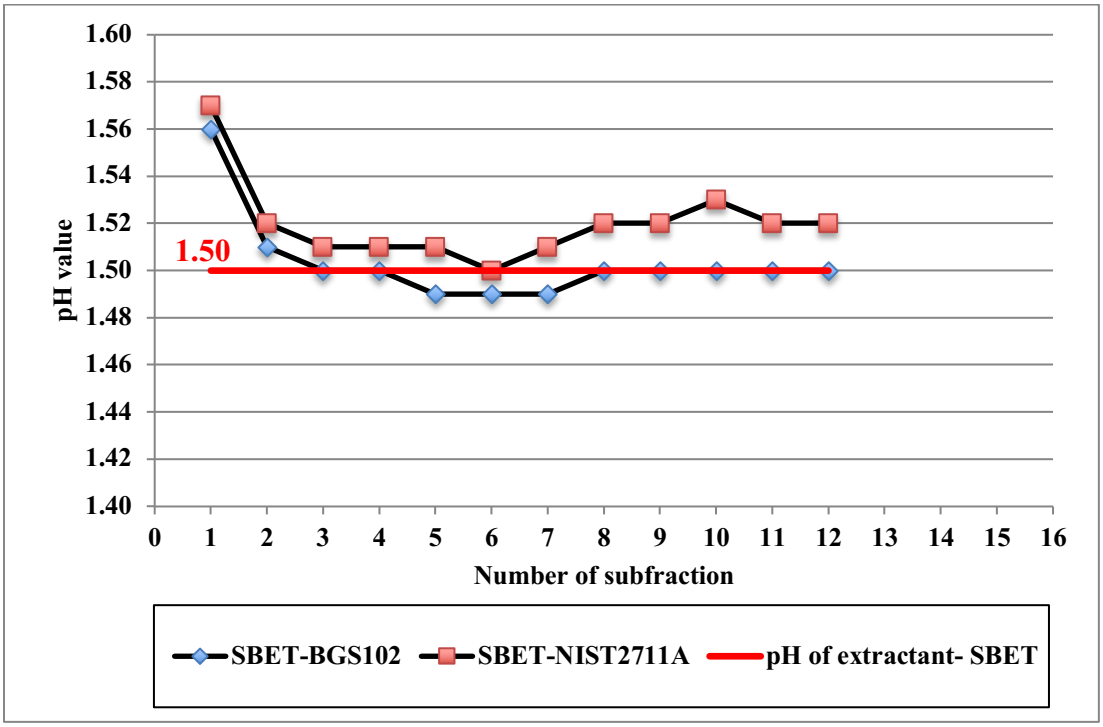

Support screen
Vent cap
Holder body
O-Ring: Silicone
Hose barb adapters

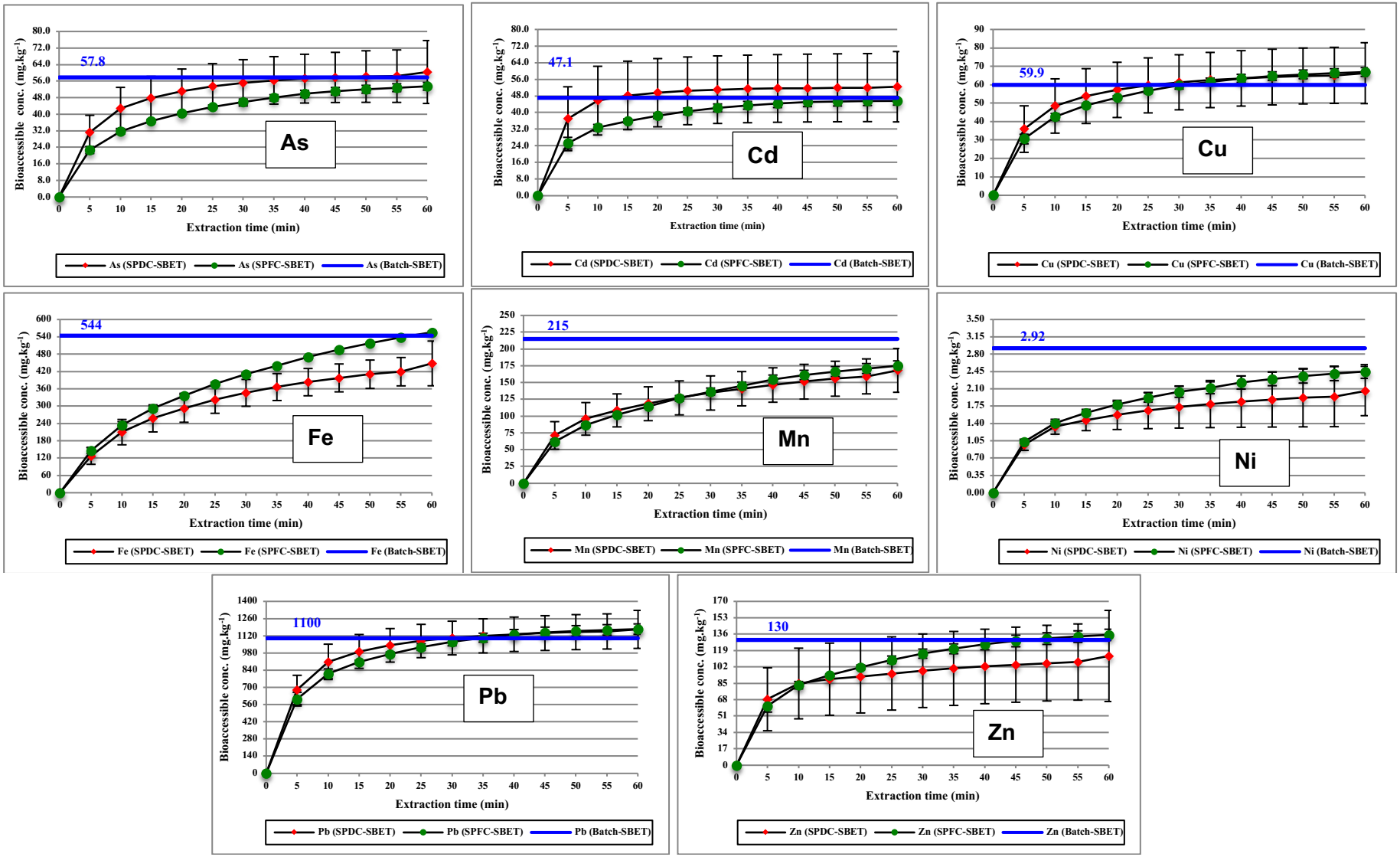


(1) Polycarbonate filter holder (2) Peristaltic pump (3) T-connecter (4) Plastic rack (5) Water bath (6) Extractant tube (7) Thermometer (8) Nebuliser (9) Internal standard tube (10) residual tube (11) Torch-ICP-MS


Extraction time (min)
Bioaccessible conc. (mg kg-1)
Number of subfraction (5 mL for each)
As
2.36
Bioaccessible concentration of As in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of As with extraction time using SPFC-SBET
Bioaccessible concentration of As using batch-SBET
Cd
0.208
Bioaccessible concentration of Cd in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Cd with extraction time using SPFC-SBET
Bioaccessible concentration of Cd using batch-SBET
Cr
35.4
Bioaccessible concentration of Cr in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Cr with extraction time using SPFC-SBET
Bioaccessible concentration of Cr using batch-SBET
Cu
7.84
Bioaccessible concentration of Cu in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Cu with extraction time using SPFC-SBET
Bioaccessible concentration of Cu using batch-SBET
Fe
2100
Bioaccessible concentration of Fe in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Fe with extraction time using SPFC-SBET
Bioaccessible concentration of Fe using batch-SBET
Mn
2200
Bioaccessible concentration of Mn in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Mn with extraction time using SPFC-SBET
Bioaccessible concentration of Mn using batch-SBET
Ni
10.6
Bioaccessible concentration of Ni in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Ni with extraction time using SPFC-SBET
Bioaccessible concentration of Ni using batch-SBET
Pb
22.6
Bioaccessible concentration of Pb in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Pb with extraction time using SPFC-SBET
Bioaccessible concentration of Pb using batch-SBET
Zn
39.7
Bioaccessible concentration of Zn in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Zn with extraction time using SPFC-SBET
Bioaccessible concentration of Zn using batch-SBET


Extraction time (min)
Bioaccessible conc. (mg kg-1)
Number of subfraction (5 mL for each)
As
57.8
Bioaccessible concentration of As in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of As with extraction time using SPFC-SBET
Bioaccessible concentration of As using batch-SBET
Cd
47.1
Bioaccessible concentration of Cd in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Cd with extraction time using SPFC-SBET
Bioaccessible concentration of Cd using batch-SBET
Cr
0.899
Bioaccessible concentration of Cr in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Cr with extraction time using SPFC-SBET
Bioaccessible concentration of Cr using batch-SBET
Cu
59.9
Bioaccessible concentration of Cu in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Cu with extraction time using SPFC-SBET
Bioaccessible concentration of Cu using batch-SBET
Fe
544
Bioaccessible concentration of Fe in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Fe with extraction time using SPFC-SBET
Bioaccessible concentration of Fe using batch-SBET
Mn
215
Bioaccessible concentration of Mn in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Mn with extraction time using SPFC-SBET
Bioaccessible concentration of Mn using batch-SBET
Ni
2.92
Bioaccessible concentration of Ni in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Ni with extraction time using SPFC-SBET
Bioaccessible concentration of Ni using batch-SBET
Pb
1100
Bioaccessible concentration of Pb in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Pb with extraction time using SPFC-SBET
Bioaccessible concentration of Pb using batch-SBET
Zn
130
Bioaccessible concentration of Zn in each subfraction using SPFC-SBET
Cumulative bioaccessible concentration of Zn with extraction time using SPFC-SBET
Bioaccessible concentration of Zn using batch-SBET


1.60
1.58
1.56
1.54
1.52
1.50
1.48
1.46
1.44
1.42
1.40
pH value
1.50
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Number of subfraction
SBET-BGS102
SBET-NIST2711A
pH of extractant- SBET


57.8
As
Bioaccessible conc. (mg.kg-1)
Extraction time (min)
As (SPDC-SBET)
As (SPFC-SBET)
As (Batch-SBET)
47.1
Cd
Cd (SPDC-SBET)
Cd (SPFC-SBET)
Cd (Batch-SBET)
59.9
Cu
Cu (SPDC-SBET)
Cu (SPFC-SBET)
Cu (Batch-SBET)
544
Fe
Fe (SPDC-SBET)
Fe (SPFC-SBET)
Fe (Batch-SBET)
215
Mn
Mn (SPDC-SBET)
Mn (SPFC-SBET)
Mn (Batch-SBET)
2.92
Ni
Ni (SPDC-SBET)
Ni (SPFC-SBET)
Ni (Batch-SBET)
1100
Pb
Pb (SPDC-SBET)
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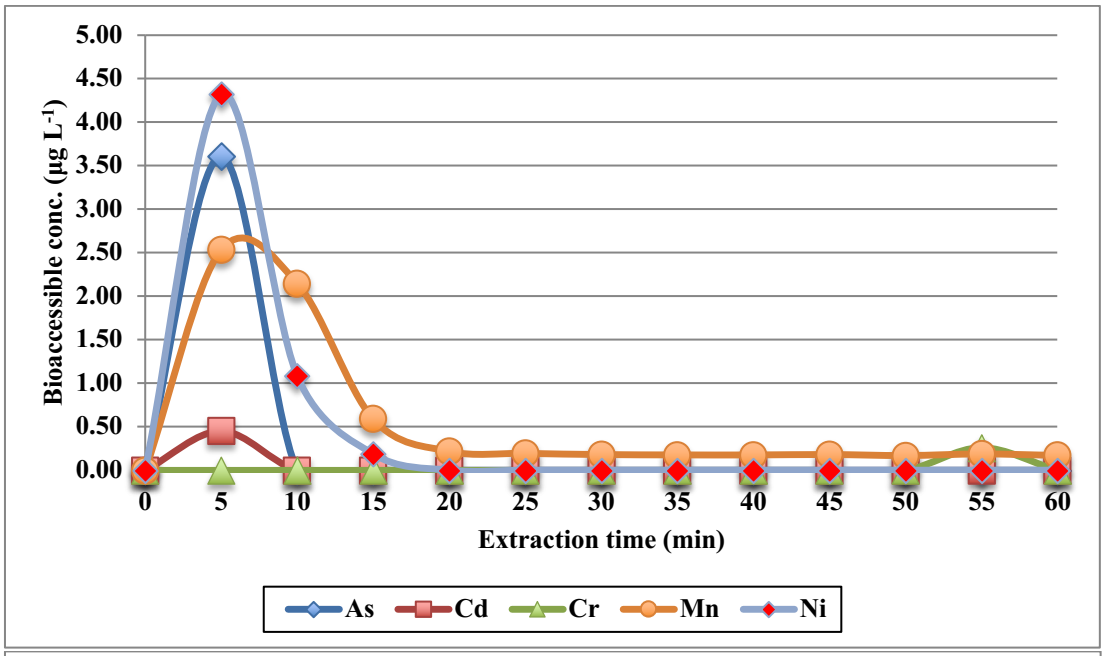
130
Zn
Zn (SPDC-SBET)
Zn (SPFC-SBET)
Zn (Batch-SBET)


Bioaccessible conc. (µg L-1)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0
5
10
15
20
25
30
35
40
45
50
55
60
Extraction time (min)
As
Cd
Cr
Mn
Ni

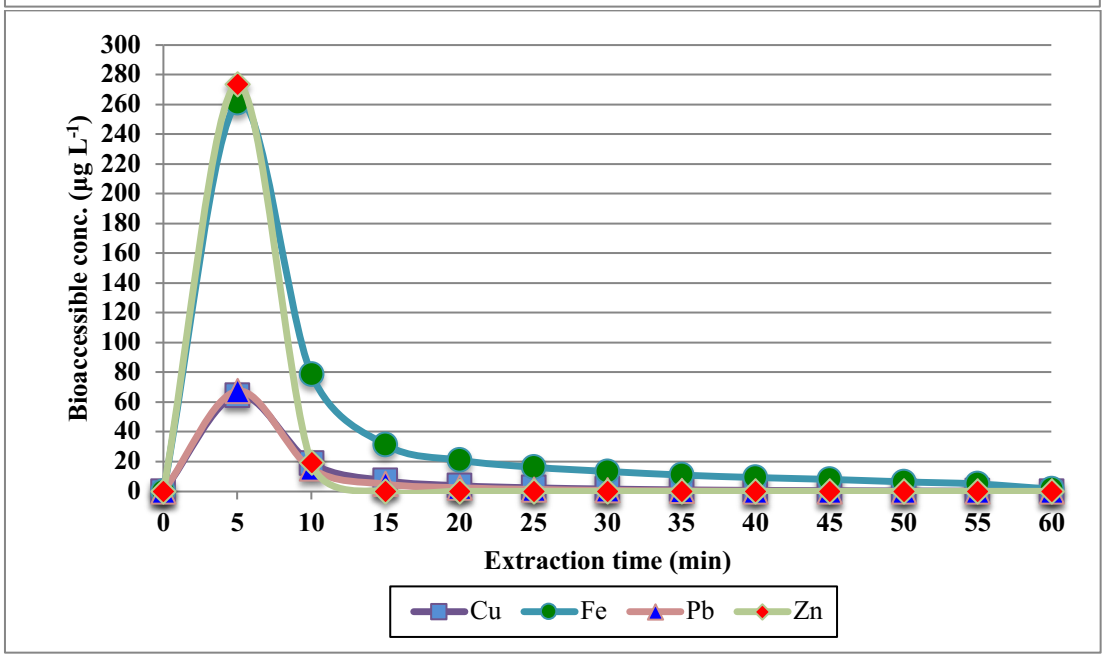

Bioaccessible conc. (µg L-1)
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
50
55
60
Extraction time (min)
Cu
Fe
Pb
Zn

Table 1
Studies on dynamic models of bioaccessibility extraction methods

Bioaccessibility method	Substrates	Analytes	Extraction unit (Sample container)	Analysis mode (sample uptake rate)	Digestive fluids used	Reagents driver	References
USP XXIII (without enzymes)	Food (NIST SRM-8433 corn bran)	Pb and Zn	Micro-column (4-cm long and 3.17 mm ID PTFE tube)	ICP-MS on-line (1.2 mL min^{-1})	Artificial saliva, gastric juice, and intestinal juice	Flow injection	Chu, M. Y. and Beauchemin, D., 2004[7]
USP XXIII	Seafood reference materials and real sample of seafood	As	Mini-column (8-cm long and 3.17 mm ID PTFE tube)	ICP-MS on-line (1.2 mL min^{-1})	Artificial saliva, gastric juice, and intestinal juice	Peristaltic pump	Dufailly *et al*., 2008[39]
USP XXIII	Rice reference material (SRM 1568a) and real sample of white rice	As	Mini-column (8-cm long and 3.17 mm ID PTFE tube)	ICP-MS on-line (0.8 mL min^{-1})	Artificial saliva, gastric juice, and intestinal juice	Peristaltic pump	Horner, N. S. and Beauchemin, D., 2012[40]
USP XXIII for preparing the gastric juice and intestinal juice; In vitro digestion (RIVM) method for artificial saliva	Seafood reference materials and real sample of seafood	As	Mini-column (5-cm stainless steel tube)	ICP-MS on-line (1 mL min^{-1})	Artificial saliva, gastric juice, and intestinal juice	High pressure liquid chromatography pump	Leufroy *et al*., 2012[4]

Table 1

Studies on dynamic models of bioaccessibility extraction methods continued …

Bioaccessibility method	Substrates	Analytes	Extraction unit (Sample container)	Analysis mode (sample uptake rate)	Digestive fluids used	Reagents driver	References
USP XXIII for preparing the gastric juice and intestinal juice; In vitro digestion (RIVM) method for artificial saliva	Seafood reference materials and real sample of seafood	Al, Cd, Cu, Hg, Mn, Pb, V, and Zn	Mini-column (5-cm stainless steel tube)	ICP-MS on-line, (1 mL min^{-1})	Artificial saliva, gastric juice, and intestinal juice	High pressure liquid chromatography pump	Leufroy *et al*., 2012[41]
UBM-like test	Forest and residential garden soils	Cr, Cu, Ni, Pb, and Zn	Stirred flow chamber	Hybrid flow ICP-AES off-line, (1.5 mL min^{-1})	Gastric juice alone without saliva	Bi-directional syringe pump	Rosende *et al*., 2014[10]
USP XXIII with no enzyme was added to artificial saliva	Bread	As, Cd, Cr, and Pb	A mini-column (10-cm long and 3.17 mm ID PTFE tube)	On-line by ICP-MS	Artificial saliva, gastric juice, and intestinal juice	Instrument's built-in peristaltic pump	Lamsal, R. P. and Beauchemin, D., 2015[42]
UBM	Soybean	Cu, Fe, and Mn	150 mL polypropylene bottle	On-line by ICP-OES	Sliva, gastric, duodenal, and bile fluid	Bi-directional syringe pump	M.A. Herrera *et al*., 2016 [43]

Table 1

Studies on dynamic models of bioaccessibility extraction methods continued …

Bioaccessibility method	Substrates	Analytes	Extraction unit (Sample container)	Analysis mode (Sample uptake rate)	Digestive fluids used	Reagents driver	References
Simulate Lung fluid	PM_{10} collected by an automated sampler on mixed cellulose ester filters 47 mm	Zn	A Chromafix® SPE column (12 mm diameter and 14 mm length)	On-line by Flame atomic absorption spectrometry (1.0 mL min^{-1})	High purity water	FI (a peristaltic pump + a six port injection valve)	Mukhtar A. and Limbeck A., 2010[44]
Simulated Lung fluid	PM_{10} collected by a digital high volume sampler on Pallflex Tissue Quartz 2500 QAT-UP filters	Ba, Cr, Cu, Fe, Mn, and Ni	A Chromafix® SPE column (12 mm diameter and 14 mm length)	On-line by ICP-AES (0.8 mL min^{-1})	High purity water	FI (a syringe pump + a six port injection valve)	Limbeck *et al*., 2012[45]
SBET	BGS102 Soil and NIST2711A reference material	As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn	Polycarbonate holder filter	ICP-MS off-line, 1 mL min^{-1}	Gastric fluid	Peristaltic pump	The current study
SBET	NIST2711A reference material	As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn	Polycarbonate holder filter	ICP-MS on-line, 1 mL min^{-1}	Gastric fluid	Peristaltic pump	The current study

Table 2
Operation conditions of the ICP-MS and parameters of the TRA

ICP-MS conditions	
Power (watt)	1550
Quadrupole bias (v)	-15
Octopole bias (v)	-18
Nebulizer gas flow (L min^{-1})	1.05
Plasma gas flow (L min^{-1})	15
Auxiliary gas flow (L min^{-1})	0.9
Collision cell gas (L min^{-1})	He (4.5) He (4.5) for all masses determined, except for ^{111}Cd and ^{208}Pb, no gas mode was chosen
Sample uptake rate (mL min^{-1})	1
TRA Parameters	
Number of peaks	1
Number of points per peak	1
Signals monitored for quantification	^{75}As, ^{111}Cd, ^{52}Cr, ^{63}Cu, ^{56}Fe, ^{55}Mn, ^{60}Ni, ^{208}Pb, and ^{66}Zn
Signals monitored for internal standard	^{209}Bi, ^{115}In, ^{72}Ge, ^{175}Lu, and ^{45}Sc
Integration time (sec)	(0.1) For all masses determined, except ^{75}As, ^{111}Cd, ^{52}Cr, and ^{60}Ni, was (1.0)
Sampling period (sec)	5.029
Number of repetitions for data acquisition	1
Acquisition time per sampling period (sec)	5.029
Total acquisition time of analysis (sec)	3600
Real time plot (time chart) (sec)	3600
Type of running a sample analysis	Running a sample manually using acquired data run

Table 3

Concentrations of potentially toxic elements (PTE) in the bioaccessible and residual fractions, together with pseudototal content and mass balance in blank FDMS filters using the single-pass dynamic model of the simplified bioaccessibility extraction test (SBET) with fraction collection (SPFC-SBET)

PTE	Models	Bioaccessible fraction (µg filter^{-1}) Mean (n=3) ± SD	Residual fraction (µg filter^{-1}) Mean (n=3) ± SD	Sum ± SD_C (µg filter^{-1})	Pseudototal (µg filter^{-1}) Mean (n=3) ± SD	% Mass balance ± SD_C	Student's t-test at 0.05 significance level	
							$t_{calculated}$	$t_{critical}$
As	**Batch**	< RB	0.318 ± 0.039	0.318 ± 0.039	0.251 ± 0.027	127 ± 21	2.44	2.78
	SPFC	< RB	0.330 ± 0.030	0.330 ± 0.030	0.281 ± 0.047	118 ± 22	1.54	2.78
Cd	**Batch**	< IDL	< RB	NC	< RB	NC	NC	NC
	SPFC	< IDL	< RB	NC	< RB	NC	NC	NC
Cr	**Batch**	0.001 ± 0.0003	2.80 ± 0.03	2.81 ± 0.03	2.72 ± 0.11	103 ± 4	1.05	3.18
	SPFC	0.002 ± 0.001	2.77 ± 0.16	2.77 ± 0.16	2.88 ± 0.25	96.4 ± 10.0	0.59	2.78
Cu	**Batch**	< IDL	< RB	NC	< RB	NC	NC	NC
	SPFC	0.003 ± 0.002	0.256 ± 0.081	0.259 ± 0.081	< RB	NC	NC	NC
Fe	**Batch**	0.422 ± 0.148	83.0 ± 6.1	83.4 ± 6.1	60.5 ± 2.3	138 ± 11	6.31	3.18
	SPFC	0.026 ± 0.003	67.0 ± 4.8	67.0 ± 4.8	59.6 ± 3.7	112 ± 11	2.11	2.78
Mn	**Batch**	0.076 ± 0.039	1.42 ± 0.44	1.49 ± 0.45	0.932 ± 0.114	160 ± 52	2.24	3.18
	SPFC	< RB	1.26 ± 0.12	1.26 ± 0.12	0.869 ± 0.139	145 ± 27	3.73	2.78
Ni	**Batch**	0.002 ± 0.0005	0.053 ± 0.018	0.054 ± 0.018	0.084 ± 0.007	64.5 ± 22.3	2.76	3.18
	SPFC	< RB	< IDL	NC	< IDL	NC	NC	NC
Pb	**Batch**	< RB	0.530 ± 0.048	0.530 ± 0.048	0.456 ± 0.027	116 ± 13	2.31	2.78
	SPFC	< IDL	0.543 ± 0.056	0.543 ± 0.056	0.471 ± 0.025	115 ± 13	2.04	2.78
Zn	**Batch**	2.07 ± 0.10	1010 ± 28	1010 ± 28	867 ± 154	117 ± 21	1.26	3.18
	SPFC	2.20 ± 0.47	939 ± 105	941 ± 105	604 ± 315	156 ± 83	1.76	2.78

n= number of replicates; SD_C: combined standard deviation; $\text{Sum} = (\text{Bioaccessible fraction} + \text{Residual fraction})$; $\%\ \text{Mass balance} = \frac{\text{Sum}}{\text{pseudototal}} \times 100$; < IDL: less than instrumental detection limit; < RB: less than reagent blank; NC: not calculated

Table 4

Concentrations of potentially toxic elements (PTE) in the bioaccessible and residual fractions, together with pseudototal content and mass balance in blank FDMS filters using the single-pass dynamic model of the simplified bioaccessibility extraction test (SBET) with direct coupling to ICP-MS (SPDC-SBET)

PTE	Models	Bioaccessible fraction (µg filter^{-1}) Mean (n=3) ± SD	Residual fraction (µg filter^{-1}) Mean (n=3) ± SD	Sum ± SD_C (µg filter^{-1})	Pseudototal (µg filter^{-1}) Mean (n=3) ± SD	% Mass balance ± SD_C	Student's t-test at 0.05 significance level	
							$t_{calculated}$	$t_{critical}$
As	**Batch**	< RB	0.318 ± 0.039	0.318 ± 0.039	0.251 ± 0.027	127 ± 21	2.44	2.78
	SPDC	< IDL	0.185 ± 0.029	0.185 ± 0.029	0.324 ± 0.034	57.1 ± 10.8	5.34	2.78
Cd	**Batch**	< IDL	< RB	NC	< RB	NC	NC	NC
	SPDC	< IDL	< RB	NC	< RB	NC	NC	NC
Cr	**Batch**	0.001 ± 0.0003	2.80 ± 0.03	2.81 ± 0.03	2.72 ± 0.11	103 ± 4	1.05	3.18
	SPDC	< IDL	2.10 ± 0.25	2.10 ± 0.25	2.63 ± 0.18	79.8 ± 10.9	3.01	2.78
Cu	**Batch**	< IDL	< RB	NC	< RB	NC	NC	NC
	SPDC	< IDL	< RB	< RB	< RB	NC	NC	NC
Fe	**Batch**	0.422 ± 0.148	83.0 ± 6.1	83.4 ± 6.1	60.5 ± 2.3	138 ± 11	6.31	3.18
	SPDC	< IDL	47.9 ± 33.4	47.9 ± 33.4	62.8 ± 4.6	76.3 ± 53.5	0.76	2.78
Mn	**Batch**	0.076 ± 0.039	1.42 ± 0.44	1.49 ± 0.45	0.932 ± 0.114	160 ± 52	2.24	3.18
	SPDC	< IDL	1.06 ± 0.19	1.06 ± 0.19	1.19 ± 0.15	89.1 ± 19.6	0.93	2.78
Ni	**Batch**	0.002 ± 0.0005	0.053 ± 0.018	0.054 ± 0.018	0.084 ± 0.007	64.5 ± 22.3	2.76	3.18
	SPDC	< IDL	0.178 ± 0.025	0.178 ± 0.025	0.280 ± 0.040	63.6 ± 12.8	3.73	2.78
Pb	**Batch**	< RB	0.530 ± 0.048	0.530 ± 0.048	0.456 ± 0.027	116 ± 13	2.31	2.78
	SPDC	< IDL	0.401 ± 0.064	0.401 ± 0.064	0.430 ± 0.038	93.3 ± 17	0.67	2.78
Zn	**Batch**	2.07 ± 0.10	1010 ± 28	1010 ± 28	867 ± 154	117 ± 21	1.26	3.18
	SPDC	3.35 ± 0.39	876 ± 128	879 ± 128	945 ± 108	93.0 ± 17.2	0.63	3.18

n= number of replicates; SD_C: combined standard deviation; $\text{Sum} = (\text{Bioaccessible fraction} + \text{Residual fraction})$; $\%\ \text{Mass balance} = \frac{\text{Sum}}{\text{pseudototal}} \times 100$; < IDL: less than instrumental detection limit; < RB: less than reagent blank; NC: not calculated

Table 5

T-test at 0.05 significance level between the mean (n=3) of the cumulative bioaccessible concentration (mg kg^{-1}) of potentially toxic elements obtained by extraction of the simulated PM_{10} samples (NIST SRM 2711A Montana II Soil on FDMS filter) using the single-pass dynamic model of the simplified bioaccessibility extraction test (SBET) with fraction collection (SPFC-SBET) and with direct coupling to ICP-MS (SPDC-SBET) (n=3)

Time (min)	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
5	1.74[a]	1.29[a]	< IDL	0.727[a]	1.02[a]	0.801[a]	0.904[a]	1.00[a]	0.359[a]
10	1.88	1.36	< IDL	0.686	0.897[a]	0.670	0.832[a]	1.11[a]	0.082
15	1.86	1.29	< IDL	0.601	1.22[a]	0.478	1.16[a]	1.09	0.192
20	1.72	1.18	< IDL	0.489	1.55[a]	0.274	1.18[a]	0.905	0.431
25	1.59	1.04	< IDL	0.340	1.99	0.045[a]	1.21[a]	0.618[a]	0.646
30	1.41	0.920	< IDL	0.206	2.38	0.142[a]	1.21[a]	0.360[a]	0.800
35	1.26	0.831	< IDL	0.109	2.71	0.273[a]	1.16[a]	0.178[a]	0.911
40	1.07	0.756	< IDL	0.001	3.18	0.545[a]	1.25[a]	0.013[a]	1.03
45	0.942	0.718	< IDL	0.063	3.52	0.643[a]	1.26[a]	0.074[a]	1.08
50	0.843	0.699	< IDL	0.100	3.79	0.687[a]	1.25[a]	0.116[a]	1.11
55	0.751	0.687	< IDL	0.138	4.17	0.747[a]	1.27[a]	0.146[a]	1.14
60	0.779	0.692	< IDL	0.064	2.41	0.360[a]	1.32[a]	0.028[a]	0.796

[a] means that F-test was passed, a degree of freedom (ν) = 4, and $t_{critical}$ = 2.78; < IDL: less instrumental detection limit; SD: standard deviation; ν = 2, $t_{critical}$ = 4.30, and F-test was failed unless otherwise indicated

Table 6

Concentrations of potentially toxic elements (PTE) in the bioaccessible and residual fractions, together with pseudototal content and mass balance in simulated PM_{10} samples (BGS RM 102 Ironstone Soil on FDMS filters) using the single-pass dynamic model of the simplified bioaccessibility extraction test (SBET) with fraction collection (SPFC-SBET)

PTE	Models	Bioaccessible fraction (mg kg^{-1}) Mean (n=3) ± U (% RSD)	Residual fraction (mg kg^{-1}) Mean (n=3) ± U	Sum ± U_C (mg kg^{-1})	Pseudototal (mg kg^{-1}) Mean (n=3) ± U	% Mass balance ± U_C	%Spike recovery	Z-Score
As	**Batch**	2.36 ± 0.27 **(0.314)**	82.9 ± 9.6	85.3 ± 9.6	98.1 ± 11.3	86.9 ± 14.0	100	-1.8
	SPFC	3.20 ± 0.37 **(14.5)**	90.6 ± 10.5	93.8 ± 10.5	100 ± 12	93.4 ± 15.0	102	-0.9
Cd	**Batch**	0.208 ± 0.024 **(1.27)**	< RB	NC	< RB	NC	100	NC
	SPFC	0.147 ± 0.017 **(13.4)**	0.155 ± 0.018	0.302 ± 0.025	0.304 ± 0.035	99.4 ± 14.1	102	-0.1
Cr	**Batch**	35.4 ± 4.1 **(2.74)**	140 ± 16	176 ± 17	188 ± 22	93.6 ± 14.0	101	-0.9
	SPFC	32.9 ± 3.8 **(7.82)**	143 ± 17	176 ± 17	196 ± 23	90.0 ± 13.5	100	-1.4
Cu	**Batch**	7.84 ± 0.91 **(0.514)**	12.8 ± 1.5	20.6 ± 1.7	27.5 ± 3.2	75.0 ± 10.7	104	-3.5
	SPFC	8.36 ± 0.97 **(16.0)**	16.7 ± 1.9	25.1 ± 2.2	23.8 ± 2.7	105 ± 15	101	0.8
Fe	**Batch**	2100 ± 242 **(0.073)**	115000 ± 13300	117000 ± 13300	135000 ± 15600	86.4 ± 14.0	103	-1.9
	SPFC	1930 ± 223 **(9.95)**	127000 ± 14700	129000 ± 14700	139000 ± 16000	92.9 ± 15.1	100	-1.0
Mn	**Batch**	2200 ± 254 **(1.45)**	3950 ± 456	6150 ± 522	6410 ± 740	96.0 ± 13.8	104	-0.6
	SPFC	1950 ± 225 **(6.71)**	4260 ± 492	6210 ± 541	6530 ± 755	95.0 ± 13.7	100	-0.7
Ni	**Batch**	10.6 ± 1.2 **(2.77)**	58.4 ± 6.7	69.0 ± 6.9	72.7 ± 8.4	95.0 ± 14.5	103	-0.7
	SPFC	8.75 ± 1.01 **(7.69)**	60.4 ± 7.0	69.1 ± 7.0	74.0 ± 8.5	93.5 ± 14.4	101	-0.9
Pb	**Batch**	22.6 ± 2.6 **(3.42)**	47.9 ± 5.5	70.5 ± 6.1	74.9 ± 8.6	94.2 ± 13.6	101	-0.8
	SPFC	21.0 ± 2.4 **(9.36)**	54.0 ± 6.2	75.1 ± 6.7	77.3 ± 8.9	97.1 ± 14.2	100	-0.4
Zn	**Batch**	39.7 ± 4.6 **(8.17)**	< FB	NC	< FB	NC	87	NC
	SPFC	32.2 ± 3.7 **(6.54)**	< FB	NC	< FB	NC	102	NC

$U = \frac{(K \times \text{Mean} \times \%RSD)}{100 \times \sqrt{n}}$, K = 2, % RSD = 10, n= number of replicates; $Z - \text{Score} = \frac{(\text{Sum} - \text{Pseudototal})}{SD_R/\sqrt{n}}$, $SD_R = \frac{\text{Mean of pseudototal} \times 10}{100}$, n= number of independent replicates; U_C: combined uncertainty; $\text{Sum} = (\text{Bioaccessible fraction} + \text{Residual fraction})$; $\%\ \text{Mass balance} = \frac{\text{Sum}}{\text{pseudototal}} \times 100$; < RB: less than reagent blank; < FB: less than filter blank; NC: not calculated

Table 7

Concentrations of potentially toxic elements (PTE) in the bioaccessible and residual fractions, together with pseudototal content and mass balance in simulated PM_{10} samples (NIST SRM 2711A Montana II Soil on FDMS filters) using the single-pass dynamic model of the simplified bioaccessibility extraction test (SBET) with fraction collection (SPFC-SBET)

PTE	Models	Bioaccessible fraction (mg kg^{-1}) Mean (n=3) ± U (% RSD)	Residual fraction (mg kg^{-1}) Mean (n=3) ± U	Sum ± U_C (mg kg^{-1})	Pseudototal (mg kg^{-1}) Mean (n=3) ± U	% Mass balance ± U_C	Z-Score
As	**Batch**	57.8 ± 6.7 **(1.26)**	37.7 ± 4.4	95.4 ± 8.0	94.7 ± 10.9	101 ± 14	0.1
	SPFC	54.1 ± 6.2 **(3.45)**	40.0 ± 4.6	94.1 ± 7.8	94.9 ± 11.0	99.1 ± 14.1	-0.1
Cd	**Batch**	47.1 ± 5.4 **(2.32)**	5.18 ± 0.60	52.2 ± 5.5	49.3 ± 5.7	106 ± 17	0.8
	SPFC	46.2 ± 5.3 **(4.33)**	5.16 ± 0.60	51.4 ± 5.4	50.2 ± 5.8	102 ± 16	0.3
Cr	**Batch**	0.899 ± 0.104 **(2.57)**	24.9 ± 2.9	25.8 ± 2.9	32.1 ± 3.7	80.3 ± 12.9	-2.8
	SPFC	0.803 ± 0.093 **(5.01)**	29.6 ± 3.4	30.4 ± 3.4	30.9 ± 3.6	98.6 ± 15.9	-0.2
Cu	**Batch**	59.9 ± 6.9 **(2.69)**	59.4 ± 6.9	119 ± 10	130 ± 15	91.7 ± 13.0	-1.2
	SPFC	67.4 ± 7.8 **(3.51)**	59.1 ± 6.8	127 ± 10	130 ± 15	97.4 ± 13.8	-0.4
Fe	**Batch**	544 ± 63 **(2.67)**	19400 ± 2240	19900 ± 2240	24500 ± 2830	81.3 ± 13.1	-2.6
	SPFC	554 ± 64 **(0.661)**	23400 ± 2700	24000 ± 2700	24300 ± 2810	98.7 ± 15.9	-0.2
Mn	**Batch**	215 ± 25 **(1.97)**	281 ± 32	496 ± 41	573 ± 66	86.7 ± 12.3	-1.9
	SPFC	176 ± 20 **(4.11)**	389 ± 45	565 ± 49	574 ± 66	98.4 ± 14.2	-0.2
Ni	**Batch**	2.92 ± 0.34 **(2.34)**	12.5 ± 1.4	15.4 ± 1.5	18.4 ± 2.1	83.9 ± 12.6	-2.3
	SPFC	2.48 ± 0.29 **(5.62)**	15.3 ± 1.8	17.8 ± 1.8	18.0 ± 2.1	98.9 ± 15.2	-0.2
Pb	**Batch**	1100 ± 127 **(2.75)**	265 ± 31	1360 ± 131	1310 ± 151	104 ± 16	0.5
	SPFC	1180 ± 136 **(3.56)**	164 ± 19	1340 ± 138	1320 ± 152	102 ± 16	0.2
Zn	**Batch**	130 ± 15 **(3.53)**	< FB	NC	< FB	NC	NC
	SPFC	137 ± 16 **(4.28)**	< FB	NC	< FB	NC	NC

$U = \frac{(K\times \text{Mean} \times \%RSD)}{100 \times \sqrt{n}}$, K = 2, % RSD = 10, n= number of replicates; $Z - \text{Score} = \frac{(\text{Sum}-\text{Pseudototal})}{SD_R/\sqrt{n}}$, $SD_R = \frac{\text{Mean of pseudototal} \times 10}{100}$, n= number of independent replicates; U_C: combined uncertainty; $\text{Sum} = (\text{Bioaccessible fraction} + \text{Residual fraction})$; $\%\ \text{Mass balance} = \frac{\text{Sum}}{\text{pseudototal}} \times 100$; < FB: less than filter blank; < RB: less than reagent blank; NC: not calculated

Table 8

Concentrations of potentially toxic elements (PTE) in the bioaccessible and residual fractions, together with pseudototal content and mass balance in simulated PM_{10} samples (NIST SRM 2711A Montana II Soil on FDMS filters) using the single-pass dynamic model of the simplified bioaccessibility extraction test (SBET) with direct coupling to ICP-MS (SPDC-SBET)

PTE	Models	Bioaccessible fraction (mg kg^{-1}) Mean (n=3) ± U (% RSD)	Residual fraction (mg kg^{-1}) Mean (n=3) ± U	Sum ± U_C (mg kg^{-1})	Pseudototal (mg kg^{-1}) Mean (n=3) ± U	% Mass balance ± U_C	%Spike recovery	Z-Score
As	**Batch**	57.8 ± 6.7 **(1.26)**	37.7 ± 4.4	95.4 ± 8.0	94.7 ± 10.9	101 ± 14	100	0.1
	SPDC	60.4 ± 7.0 **(25.3)**	29.8 ± 3.4	90.1 ± 7.8	96.1 ± 11.1	93.8 ± 13.5	105	-0.9
Cd	**Batch**	47.1 ± 5.4 **(2.32)**	5.18 ± 0.60	52.2 ± 5.5	49.3 ± 5.7	106 ± 17	100	0.8
	SPDC	52.4 ± 6.1 **(32.3)**	3.78 ± 0.44	56.2 ± 6.1	50.1 ± 5.8	112 ± 18	91	1.7
Cr	**Batch**	0.899 ± 0.104 **(2.57)**	24.9 ± 2.9	25.8 ± 2.9	32.1 ± 3.7	80.3 ± 12.9	101	-2.8
	SPDC	< IDL	28.4 ± 3.3	NC	29.7 ± 3.4	NC	100	NC
Cu	**Batch**	59.9 ± 6.9 **(2.69)**	59.4 ± 6.9	119 ± 10	130 ± 15	91.7 ± 13.0	104	-1.2
	SPDC	66.3 ± 7.7 **(25.0)**	52.1 ± 6.0	118 ± 10	127 ± 15	93.3 ± 13.2	101	-0.9
Fe	**Batch**	544 ± 63 **(2.67)**	19400 ± 2240	19900 ± 2240	24500 ± 2830	81.3 ± 13.1	103	-2.6
	SPDC	448 ± 52 **(17.3)**	19900 ± 2300	20400 ± 2300	24000 ± 2770	85.0 ± 13.7	102	-2.1
Mn	**Batch**	215 ± 25 **(1.97)**	281 ± 32	496 ± 41	573 ± 66	86.7 ± 12.3	104	-1.9
	SPDC	168 ± 19 **(19.4)**	352 ± 41	520 ± 45	571 ± 66	91.1 ± 13.1	101	-1.3
Ni	**Batch**	2.92 ± 0.34 **(2.34)**	12.5 ± 1.4	15.4 ± 1.5	18.4 ± 2.1	83.9 ± 12.6	103	-2.3
	SPDC	2.05 ± 0.24 **(24.3)**	13.8 ± 1.6	15.9 ± 1.6	18.1 ± 2.1	87.6 ± 13.5	107	-1.7
Pb	**Batch**	1100 ± 127 **(2.75)**	265 ± 31	1360 ± 131	1310 ± 151	104 ± 16	101	0.5
	SPDC	1170 ± 135 **(13.2)**	147 ± 17	1320 ± 136	1320 ± 152	100 ± 15	97	0.0
Zn	**Batch**	130 ± 15 **(3.53)**	< FB	NC	< FB	NC	87	NC
	SPDC	113 ± 13 **(41.7)**	< FB	NC	< FB	NC	108	NC

$U = \frac{(K \times \text{Mean} \times \%RSD)}{100 \times \sqrt{n}}$, K = 2, % RSD = 10, n= number of replicates; $Z - \text{Score} = \frac{(\text{Sum} - \text{Pseudototal})}{SD_R/\sqrt{n}}$, $SD_R = \frac{\text{Mean of pseudototal} \times 10}{100}$, n= number of independent replicates; U_C: combined uncertainty; $\text{Sum} = (\text{Bioaccessible fraction} + \text{Residual fraction})$; $\%\ \text{Mass balance} = \frac{\text{Sum}}{\text{pseudototal}} \times 100$; < IDL: less than instrumental detection limit; < FB: less than filter blank; NC: not calculated

Table 9

Comparison between found and certified values for BCR CRM 143R Sewage Sludge Amended Soil subjected to microwave assisted *aqua regia* digestion in parallel to residual material from the single-pass dynamic models of the simplified bioaccessibility extraction test (SBET) with fraction collection (SPFC-SBET) and with direct coupling to ICP-MS (SPDC-SBET)

PTE	Cd	Cr	Mn	Ni	Pb	Zn
Certified pseudototal values (Mean ± SD)	72.0 ± 1.8	426 ± 12	858 ± 11	296 ± 4	174 ± 5	1063 ± 16
	Measured pseudototal PTE content for the SPFC-SBET					
Measured values (Mean ± SD) (n=3)	70.0 ± 1.0	460 ± 5	892 ± 11	294 ± 8	173 ± 3	1030 ± 5
% Recovery	97.2 ± 2.8	108 ± 3	104 ± 2	99.4 ± 2.9	99.5 ± 3.4	96.8 ± 1.5
	Measured pseudototal PTE content for the SPDC-SBET					
Measured values (Mean ± SD) (n=3)	68.8 ± 1.1	454 ± 15	886 ± 16	294 ± 3	173 ± 5	1050 ± 11
% Recovery	95.5 ± 2.8	107 ± 5	103 ± 2	99.5 ± 1.7	99.3 ± 4.2	98.5 ± 1.8

SD: standard deviation, n= number of replicates

Table 10

Bioaccessible concentration of potentially toxic elements (PTE) in real PM_{10} samples obtained by applying the single-pass dynamic model with fraction collection of the simplified bioaccessibility extraction test (SPFC-SBET) and the SPDC-SBET

PTE	SPFC-SBET (µg filter^{-1})		SPFC-SBET (ng m^{-3})		Burgher Street	
	Byres Road	Broom Hill	Byres Road	Broom Hill	SPDC-SBET (µg filter^{-1})	SPDC-SBET (ng m^{-3})
As	0.037	0.038	0.617	**NA**	0.036	**NA**
Cd	0.013	0.009	0.213	**NA**	0.005	**NA**
Cr	0.010	0.015	0.158	**NA**	0.003	**NA**
Cu	0.673	0.828	11.1	**NA**	1.02	**NA**
Fe	1.14	1.891	18.8	**NA**	4.63	**NA**
Mn	0.182	0.203	3.01	**NA**	0.069	**NA**
Ni	0.056	0.047	0.929	**NA**	0.056	**NA**
Pb	0.763	0.606	12.6	**NA**	0.928	**NA**
Zn	3.46	0.876	57.3	**NA**	2.93	**NA**

NA: no exposure dates available