Comparison of the extraction efficiencies of different leaching agents for reliable assessment of bio-accessible trace metal fractions in airborne particulate matter

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Abstract. In present study, an in-vitro physiologically based extraction test has been applied for extraction of bio-accessible trace metal fractions in airborne particulate matter (APM) samples collected from different urban sites in Austria and Pakistan using the leaching agents H2O, sodium chloride, ammonium acetate, ammonium citrate, synthetic gastric juice and artificial lung fluids. Obtained extracts were then measured using an ETV-ICP-OES procedure which allowed highly sensitive measurement of dissolved analytes even in the presence of leaching agents. Derived results indicated that the investigated leaching agents extract different amounts of trace metals. In general, leaching agents with organic nature generate comparatively greater extractable and thus bio-accessible trace metal fractions to that of simple solvents like H2O or aqueous NaCl solution. With water, only 26.3±4.0% of Cd was found to be bio-accessible whereas 88.4±24.8 of Cd was obtained as bio-accessible fraction with the use of synthetic gastric juice. The concentrations of bio-accessible metal fractions varied from 0.4 ng m⁻³ (Cd) to 714 ng m⁻³ (Zn) and 0.3 ng m⁻³ (Cd) to 190 ng m⁻³ (Zn) for PM10 samples collected from Karachi (Pakistan) and Graz (Austria) respectively.

Key words: Bio-accessibility; trace metals; airborne particulate matter; synthetic body fluids

Introduction

In recent years, many toxicological studies have implicated metal contents as a possible harmful component of APM (Prieditsis and Adamson, 2002) since they can be absorbed into human tissues during breathing especially particles with an aerodynamic diameter less than 10 microns (PM10). The toxicity of metal depends upon its species that are present. Thus, for risk assessment of metal toxicity, it is important to determine bio-accessible concentrations instead of total metal contents (Michelozzi et al., 1998).

Particles in the 2.5- to 10-µm size fraction are in most cases deposited in the tracheal and the bronchial region after inhalation, from where they are transported within hours by the so-called mucociliary clearance adoral and are mainly swallowed. This fraction reaches the gastrointestinal tract (GIT), where it comes into contact with gastric juice (Hamel et al., 1998). On the other hand particles less than 1 µm can enter into the alveolar region of lungs where they interact with the lung fluid (Jianjum et al., 2010 and Song et al., 2011). For risk assessment of metal toxicity the determination of bio-accessible fractions is recommended. For this purpose extraction procedures with different leaching agents such as water, buffer solutions or synthetic body fluids were reported in literature. Since the chemical composition of these leaching agents is different it is expected that inconsistent amount of bio-accessible metal fractions is obtained via the use of applied leaching agents. Therefore, it is highly needed to evaluate the extraction efficiencies of various commonly used leaching agents for true estimation of bio-accessible trace metal fractions in APM.

In the present study, an in-vitro physiologically based extraction test (PBET) was employed for extraction of APM samples from Graz (Austria) and Karachi (Pakistan) with the leaching agents water, sodium chloride, ammonium acetate, ammonium citrate, synthetic gastric juice, artificial lysosomal fluid and Gamble solution, followed by measurement of resulting extracts using electrothermal vaporization.
inductively-coupled plasma atomic emission spectrometry (ETV-ICP-OES). Derived results were discussed in order to gain more detailed information about the extraction behavior of the investigated leaching agents.

**Materials and Methods**

**Reagents and materials**

High purity water was obtained by purifying de-ionized water (reverse osmosis/ion exchange combination Euro 20 plus, SG Water Systems, Germany) with an Easypure 2 system (Thermo Barnstead, USA). All used chemicals and reagents were of analytical reagent grade and were procured from Merck (Darmstadt, Germany). Pepsin from porcine stomach mucosa (456 units mg⁻¹ solid) was purchased from Sigma Aldrich (Chemie GmbH, Steinheim, Germany). Certified multielement standard solutions of In, As, Ba, Cd, Co, Cu, Mn, Ni, Pb and Zn (1000 mg/L) were purchased from Merck (Darmstadt, Germany) and diluted with 1% HNO₃ (v/v) in order to prepare various working standard solutions. A 1 mol l⁻¹ solution of ammonium acetate was prepared by dissolving 77.0825 g weight in one litre of bi-distilled water and pH of the solution was maintained at 7. Similarly, 0.1 mol l⁻¹ ammonium citrate solution was prepared by dissolving 22.619 g in one litre of high purity water and pH of the solution was maintained at 4.4 with the use of HCl. The synthetic gastric juice was prepared according to a US Pharmacopeia methodology by dissolving 2 g NaCl in 50 ml of high purity water followed by addition of 7 ml of conc. HCl. The derived extracts were centrifuged (Hettich, Zentrifugen-EBA 20) at 5000 rpm for 10 min followed by addition of 700 mg of leaching agent. From each sample three replicates have been prepared with each type of leaching agent. Closed tubes were treated in an ultrasonic bath (Sonorex TK30, Bandelin, Germany) at 37°C for 1 h in order to extract soluble trace metal fractions. After cooling down the sample solutions to room temperature, the derived extracts were centrifuged (Hettich, Zentrifugen-EB A 20) at 5000 rpm for 10 min for separation of undissolved material and remaining filter substrate. The supernatant clear sample solutions were transferred to new 3 ml polypropylene tubes and stored until further analysis.

The remaining eight aerosol filter punches (diameter 12 mm) were used for determination of total trace metal contents. For sample digestion the filter punches were transferred into pre-cleaned Bernas type Teflon lined bombs followed by addition of 1 ml conc. HNO₃, 1 ml HCl and 50 µl of HClO₄. The Teflon lined bombs were then placed in indigenously developed refractory oven and treated at 130 °C for 1 h in order to dissolve total metal contents. Finally, the temperature of the refractory oven was increased to 150 °C and maintained for other 30 min for evaporation of excessive amount of HNO₃ and HCl. After cooling the insoluble filter material including the small droplet of HClO₄ remaining in the Teflon lined bombs were transferred in new PP tubes and . diluted to a final mass of approximately 2 g with 1% (v/v) HCl. Simultaneously a defined amount of In as internal standard was added. After centrifugation the supernatant solutions were removed and stored in new PP tube at 4°C until ananalysis.

**ETV-ICP-OES Analysis of standard solutions and PM10 samples**

Measurement of standard solution and prepared PM10 extracts was carried out according to Mukhtar and Limbeck, 2011. Briefly, 40 µl of the prepared
extracts/digests were pipetted into precleaned graphite boats and dried using an IR-lamp. For analysis, the graphite boats were inserted into the graphite furnace tube of the ETV system and the furnace program was started by the ICP-OES software and the emission spectra of the vapor introduced into plasma was measured.

**Results and Discussion**

Total metal concentrations in PM10 samples from Karachi ranged from few ng m\(^{-3}\) to some hundred ng m\(^{-3}\). The highest concentrations among the measured elements were observed for Zn ranging from 361 ng m\(^{-3}\) to 918 ng m\(^{-3}\) whereas lowest concentrations were observed for trace element Cd varying from 1.9 ng m\(^{-3}\) to 4.2 ng m\(^{-3}\). As and Co revealed results below their detection limits i.e., less than 0.5 ng m\(^{-3}\). These results were found in accordance to literature findings as reported by Venkataraman et al., 2002, and Salam et al., 2003 for mega south Asian cities Mumbai and Dhaka respectively. Similar observations were found for PM 10 samples collected from Graz, with concentrations of Zn varying from 71 ng m\(^{-3}\) to 300 ng m\(^{-3}\) and Cd concentrations ranging from 0.9 to 1.5 ng m\(^{-3}\). These findings were in agreement with the results reported in literature from various sites in central Europe. For example, Limbeck et al., 2009 have reported concentrations of Cd and Zn in the order of 0.1 ng m\(^{-3}\) and 200 ng m\(^{-3}\) respectively in PM10 samples collected from various urban sites in Vienna. The results indicated clearly that atmosphere of Karachi is significantly more contaminated with toxic trace elements as compared to Graz.

Bio-accessible fractions were found to be lower than the corresponding total metal concentrations, indicating that only a fraction of metal is soluble in various leaching agents. Since bio-accessibility test has been performed with different leaching agents, variable amounts of bio-accessible trace metal fractions have been released which reflect differences in their ionic strength and composition. Therefore a question arises about the trueness of bio-accessible fractions. It was found that lowest bio-accessible trace metal fractions were obtained with the use of water and NaCl as compared to leaching agents with organic composition like synthetic gastric juice and artificial lysosomal fluid (Figure 1a and 1b). However, the Gamble solution released comparable quantities of bio-accessible trace metal fractions to that of water and NaCl. Furthermore, the low leaching ability of Gamble solution as compared to synthetic gastric juice and artificial lysosomal fluid could be explained on the basis that it is neutral (pH-7), thus the interaction of Gamble solution with PM10 metal particles is not so aggressive. In contrast, synthetic gastric juice and artificial lysosomal fluid being having complex organic nature and acidic pH causes the metals to release easily, thereby posing serious health risks when trace fractions become part of body fluid. It can also be deduced from above figure that the metal extracting behavior of applied leaching agents is quite similar for PM10 samples collected from two entirely different sites i.e., Graz (Central Europe) and Karachi (South Asia), indicating that bio-accessibility is element as well as leaching agent dependent.

Using synthetic gastric juice for assessment of bio-accessible trace metal fractions concentrations in PM10 ranging from 0.4 ng m\(^{-3}\) (Cd) to 714 ng m\(^{-3}\) (Zn) were found in aerosol samples collected in Karachi, whereas bio-accessible trace metal concentrations in PM10 samples from Graz varied from 0.3 ng m\(^{-3}\) (Cd) to 190 ng m\(^{-3}\) (Zn). Comparison of bio-accessible fraction in PM10 samples reported in present study with literature data is not possible, since current study is carried out for the first time where an attempt has been made to provide a guideline for estimation of actual bio-accessible trace metal fractions in APM.

**Conclusion**

Fig. 1. Trace metal fractions extractable with different leaching agents (%), results are average of twenty investigated PM10 samples.
In this study an attempt has been made for the first time in order to propose a model for the estimation of true bio-accessible trace metal fractions in APM which could be used as a guideline for future studies. In present study, an in-vitro PBET was applied for extraction of bio-accessible metal fractions present in APM using various leaching agents followed by subsequent measurement of gastric extracts using a recently developed ETV-ICP-OES procedure. The obtained results indicated severe differences in the extraction efficiencies of the investigated leaching agents. Highest bio-accessible trace metal fractions are obtained with the use of synthetic body fluids, lowest results were observed for water and sodium chloride solution. Generally it was found that the presence of organic complexing agent as well as acidic conditions improve the solubility of trace metals significantly. Therefore, for future studies it is highly recommended to use synthetic body fluids for estimation of bio-accessible trace metal fractions in APM, since they enable a more reliable assessment of bio-accessible trace metal fractions in PM10 than pure inorganic solutions.

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References


