Review Article
High-performance liquid chromatographic determination of histamine in biological samples: The cerebrospinal fluid challenge – A review

Zhaopin Wang, Juanli Wu, Shihua Wu and Aimin Bao
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Review

Recent developments in assessment of bio-accessible trace metal fractions in airborne particulate matter: A review
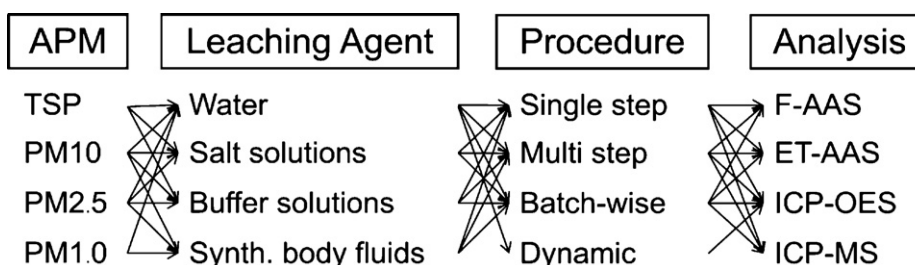
Azam Mukhtar, Andreas Limbeck*

Vienna University of Technology, Institute of Chemical Technologies and Analytics, Getreidemarkt 9/164, 1060 Vienna, Austria

HIGHLIGHTS

- ► Survey of leaching agents used for sample extraction.
- ► Multi-step fractionation schemes.
- ► Dynamic extraction procedures.
- ► Analytical approaches for element specific measurement.
- ► Detailed compilation of published results.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

In the last years a great deal of research has been focused on the determination of harmful trace metals such as Cd, Co, Cr, Cu, Ni or Pb in airborne particulate matter (APM). However, the commonly applied determination of total element concentrations in APM provides only an upper-end estimate of potential metal toxicity. For improved risk assessment it is important to determine bio-accessible concentrations instead of total metal contents. The present review gives an overview of analytical procedures reported for measurement of bio-accessible trace metal fractions in APM. The different approaches developed for extraction of soluble trace metals in APM are summarized. Furthermore the analytical techniques applied for accurate determination of dissolved trace metals in the presence of complex sample matrix are presented. Finally a compilation of published results for bio-accessible trace metals in APM is included.

Contents

* Corresponding author. Tel.: +43 1 58801 15180; fax: +43 1 58801 15199.
  E-mail address: A.Limbeck@tuwien.ac.at (A. Limbeck).

Azam Mukhtar completed his PhD at the Vienna University of Technology (Austria) at the Institute of Chemical Technologies and Analytics. His research work is dedicated to the field of environmental analytical chemistry with special emphasis on the measurement of bio-accessible trace element fractions in airborne particulate matter. Application of slurry procedures for the analysis of total element contents in aerosol samples is another important topic.

Andreas Limbeck is Privatdozent at the Vienna University of Technology (Austria), currently he is head of the research division of Instrumental Analytical Chemistry at the Institute of Chemical Technologies and Analytics. His research interests are focused on the application of ICP-MS, ICP-OES and ET-AAS for analysis of trace elements in environmental samples including the development of improved sample pre-treatment procedures. A further research field is the use of Laser-Ablation and Electro-Thermal-Vaporization for the direct analysis of solid samples.

1. Introduction

Airborne particulate matter (APM) is contributed through various natural as well as anthropogenic sources [1]. Primary particles are directly emitted as liquid or solids from sources such as biomass burning, incomplete combustion of fossil fuels, volcanic eruption, and wind-driven or traffic related suspension of road, soil, and mineral dust, sea salt and biological materials (plant fragments, micro-organisms, pollens, etc.). Secondary particles, on the other hand, are formed by gas to particle conversion in the atmosphere [2–5]. The suspended airborne particles undergo various physical and chemical interactions and transformations (atmospheric aging), that is, changes of particle size, structure, and composition (e.g. coagulation, restructuring, gas uptake, and chemical reactions). Therefore the concentration, composition, and size distribution of atmospheric aerosol particles are temporally and spatially highly variable. The components of APM can generally be classified as carbonaceous fractions including organic carbon, elemental carbon, carbonate carbon and inorganic components consisting of crustal elements, trace metals and ionic species. Each of these components typically contributes about 10–30% of the overall mass load. Depending on aerosol properties and meteorological conditions, the characteristic residence times (life times) of aerosol particles in the atmosphere range from hours to weeks [6,7]. The particles with diameter from some nm to tens of μm can remain buoyant in the atmosphere for days and thus can be transported over a long distance from the original source resulting in an enhanced level of ambient PM concentrations even at rural or background sites.

A particular APM component that is known to exert toxic effects on human being is metallic fraction [8]. Many elements, like Cd, Cr, Cu, Mn, Ni, Pb, V and Zn are widely distributed in PM and, hence, are suspected to be an important source of PM toxicity [9]. Therefore, trace element analysis of APM is highly demanded for assessing air quality and health risks [10]. Many epidemiological studies have demonstrated that exposure to such metals can cause adverse human health effects at concentrations commonly found in urban areas around the world [11,12]. Additionally, it has also been demonstrated in several recent studies conducted in vitro simulating the respiratory environment, or in vivo by animal models, that metal components of APM are correlated with the observed pulmonary toxicity [13–17]. Therefore, a great deal of research has been focused on the metal composition of atmospheric suspended particulate matter. However, most of the studies dealing with determination of trace metals in APM focus on the determination of total metal concentration without distinguishing the various species that are present [18]. But for risk assessment of metal toxicity, it is important to determine bio-accessible concentrations instead of total metal contents. Therefore, it is necessary to study the impact of easily released metallic components in APM on human health. This includes health research involving both epidemiology and toxicology, which continue to implicate metals as a possible cause of the observed adverse effects on human health.

The bio-accessibility of a metal indicates the upper level estimate of risk assessment and is defined as the value representing the availability of metal for absorption when dissolved in synthetic body fluid or juices in vitro, whereas bioavailability is the amount that is actually taken across the cell membrane [19,20]. There is misconception about the use of terms "bio-accessibility" and "bioavailability" in literature where determination of merely bio-accessibility is considered as bio-availability. But in fact, bio-accessibility measurement of trace metals in various fluids provides only the data about metallic fractions which could be available to living organisms for absorption in their physiological systems. Therefore, in lieu of obtaining bioavailability data from samples of body fluids like urine, blood, or other tissues, the measurement of metal "bio-accessibility" can be used as an in vitro substitute for measuring potential metal bioavailability [20,21]. The potential health effects from the trace metal fractions present in APM depend upon the bonding and hence solubility of metallic components in addition to particle size, shape, total human exposure and the health status of the population [22], whereas the solubility of an aerosol-borne trace metal is dependent on its source of origin (chemical form), the size of aerosol particle that bears it and the pH of the sample [23,24]. In general, metallic components of APM with high solubility can be more readily bio-activated and thus may be potentially more harmful for humans [25].
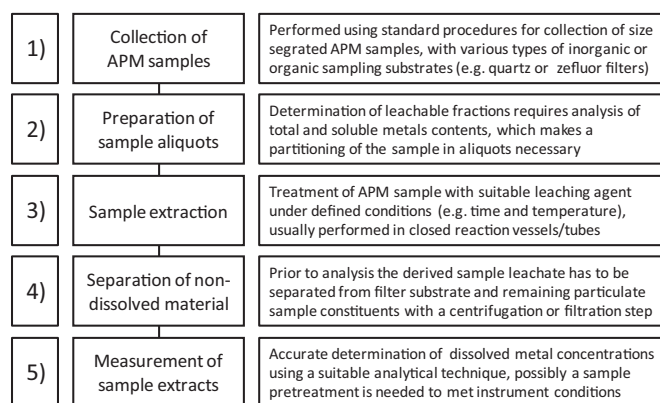
The health risk associated with metals in APM arises from the inhalation of these particles during breathing, followed by deposition of the particles in the human respiratory system. For example, after inhalation, particles in the 2.5–10 μm size fraction are in most cases deposited in the pharyngeal and tracheal region, 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 [19]. On the other hand, ultrafine particles less than 1 μm can travel deeper into alveolar region of lungs where they interact with the lung fluid [26–28]. After interaction with body fluid, bio-accessible trace metal fractions are released where they can be absorbed into human physiological systems thereby exerting adverse toxic effect.

For determination of bio-accessible trace metal fractions present within APM, use of various leaching agents, such as high purity water, weak acids, physiological sodium chloride solution and single chelating agents (EDTA, citrates, histidine, glycine, etc.), buffer solutions and synthetic body fluids have been reported in literature [13,14,29–35]. Therefore, there is lack of consistency for the use of appropriate solvents in order to determine the bio-accessible trace metal fractions, and hence a question arises for the estimation of true bio-accessible metal contents, since the procedure to extract bio-accessible trace metal fractions from APM has not been standardized so far. However, after appropriate extraction of APM samples the leached fractions have to be analyzed, which is rather difficult since the available metal amounts range between some hundred nanograms and few micrograms dissolved in the applied volume of leaching agent [31]. Therefore, highly sensitive and precise analytical procedures are required for the accurate quantification of bio-accessible trace metal fractions distributed in APM.

In this review, for the first time, a comprehensive survey of available approaches for determination of bio-accessible trace metal fractions in APM is reported, including a description of commonly used leaching procedures as well as the measurement techniques applied for analysis of derived sample extracts. Furthermore a compilation of the results published in literature so far is presented.

2. Approaches for extraction of bio-accessible trace metal fractions

Trace metals associated with APM consists of fractions with different mobility that may have different bio-accessibility and potential risk to human beings. Thus, simple measurement of the total concentration of a metal, as commonly done in monitoring programs, may not be representative of its potential to participate in processes deleterious to health. Therefore, the ability to determine leachable fractions of metals in APM is becoming increasingly important. For example, the labile fraction of the particle-bound metals is considered to be more readily available to environmental receptors and hence possesses greater environmental risk than the resistant fraction [36]. Therefore different extraction and fractionation procedures have been proposed in literature for determining the distribution of soluble and insoluble trace metals in APM. In contrast to speciation procedures, which are aimed at the identification and/or measurement of the quantities of one or more individual chemical species in a sample, is the main purpose of fractionation procedures to classify analytes or group of analytes from a certain sample according to their physical (e.g. size, solubility) or chemical (e.g. bonding, reactivity) properties [37]. Application of such extraction or fractionation procedures should provide the information required for assessment of the relative biohazard of trace metals associated with airborne particulates. Procedures based on batch-wise extraction are mostly reported in

1)	Collection of APM samples	Performed using standard procedures for collection of size segrated APM samples, with various types of inorganic or organic sampling substrates (e.g. quartz or zefluor filters)
2)	Preparation of sample aliquots	Determination of leachable fractions requires analysis of total and soluble metals contents, which makes a partitioning of the sample in aliquots necessary
3)	Sample extraction	Treatment of APM sample with suitable leaching agent under defined conditions (e.g. time and temperature), usually performed in closed reaction vessels/tubes
4)	Separation of non-dissolved material	Prior to analysis the derived sample leachate has to be separated from filter substrate and remaining particulate sample constituents with a centrifugation or filtration step
5)	Measurement of sample extracts	Accurate determination of dissolved metal concentrations using a suitable analytical technique, possibly a sample pretreatment is needed to met instrument conditions

Batch-wise extraction procedures:
all sample manipulation steps were conducted manually, sample extraction is performed under equilibrium conditions, prepared APM leachates are measured in a separate step (off-line analysis)

Dynamic approaches:
extraction of APM samples is performed with a continuous flow of leaching agent directed through the sample, composition of derived eluate is determined on-line with suitable detection units, alternatively fractions of the eluate could be collected and subsequently measured off-line

Fig. 1. Schematic work flow of batch-wise and dynamic extraction procedures.

literature, involving treatment of APM with some leaching agent for defined time period and temperature under some agitation or ultrasonic treatment. These batch-wise extraction procedures are either consist of one single or some multiple steps, the most frequently applied leaching agents are compiled in Table 1. After extraction, the derived solution is subjected to centrifugation for the separation of remaining insoluble parts of the sample. Finally, the supernatant particle free solution containing the dissolved metal fractions is measured using a suitable analytical technique. The individual steps involved in the assessment of bio-accessible trace metal fractions in APM are summarized in Fig. 1.

The following section presents the various methods used for batch-wise extraction of bio-accessible trace metal fractions in APM. In addition to these bath-wise procedures, where extraction is performed under equilibrium conditions, some dynamic approaches were also reported in literature. These more complex techniques will be discussed in this article within Section 3 (analytical procedures), since these approaches are usually directly coupled with the detection process. Results derived from the application of the individual approaches are summarized in Section 4, which presents for the first time a detailed comparison of published data for bio-accessible trace metals in APM.

2.1. Single step extraction procedures

Batch wise extraction of APM has been performed using various leaching agents for the dissolution of leachable metal fractions. The simplest procedure involves the treatment of aerosol samples with water as the leaching agent. Improved approaches applied diluted acids and buffer solutions for sample extraction, which possess higher acidity and thus dissolution power than water. With the use of synthetic body fluids such as gastric juice or artificial lung fluids (Gamble solution and lysosomal fluid) more realistic estimates of leachable metal fractions in APM could be achieved, since the composition of these leaching agents are very similar to human body fluids. For example, synthetic gastric juice contains pepsin in addition to salts and HCl, which can increase metal's solubility. An outcome which indicates that dilute HCl is not a suitable model for the chemical conditions in the gastric intestinal juice (GIT) [34].

Table 1
Principle composition of various leaching agents used for the extraction of bio-accessible trace metal fractions in APM.

Leaching agents	Composition	References
Water	Neutral water (pH ~ 7)	[38–45]
Salt solutions/buffers and diluted acids	Physiological NaCl solution	[29]
	1 mM citrate solution	[30]
	EDTA, citrate, histidine, glycine	[49]
	0.01 M ammonium acetate (pH 7)	[27]
	0.01 M acetate buffer (pH 4.5)	[50]
	0.1 M HCl[a]	[39]
	0.1 M HNO$_3$ [a]	[42]
	0.1 N HCl[a]	[51]
	0.1 M HCl[b]	[53]
Tessier's scheme	1 mol L^{-1} MgCl$_2$ (pH 7) for exchangeable fractions 1 mol^{-1} NaAc (pH 5) with HAc solution for metallic fractions bound to carbonates0.04 mol L^{-1} NH$_2$OH·HCl in 25% (v/v) HAc (pH ~ 2) solutions for fractions bound to Fe and Mn oxides 0.02 mol L^{-1} HNO$_3$ + 30% H$_2$O$_2$ (pH 2 with HNO$_3$) 3.2 mol L^{-1} NH$_4$Ac in 20% (v/v) HNO$_3$ solution for fractions bound to organic matter HF + HClO$_4$ mixture for residual metals	[59,61–64]
Chester's scheme	1 mol L^{-1} NH$_4$Ac (pH 7) solution for mobile metallic fractions 1 mol L^{-1} NH$_2$OH·HCl + 25% (v/v) HAc mixture for fractions bound to carbonates, and oxides HNO$_3$ and HF (10:2) mixture for refractory associated metals	[65–69]
Zakta's scheme	0.1 mol L^{-1} (NH$_4$)$_2$-citrate solution (pH 4.4) for soluble metals 0.1 mol L^{-1} (NH$_4$)$_2$-citrate + 30% H$_2$O$_2$ (2:1, v/v) for sulfidic anhydrous methanol containing 0.2 mL of bromine for metallic fractions *Dust sample*: mixture of HNO$_3$ and HClO$_4$ *Sample on quartz filter*: HF + HNO$_3$/HClO$_4$ mixture for oxidic (insoluble) metals	[71–74]
BCR protocol	0.11 mol L^{-1} HOAc solution for acid soluble metals 0.1 mol L^{-1} NH$_2$OH·HCl (pH 2 with HNO$_3$) solution for reducible metallic fractions 30% (w/v) H$_2$O$_2$ + 1 mol L^{-1} NH$_4$OAc solution for oxidizable	[76,77]
Simulating serum	0.145 M Na$^+$, 0.0002 M Ca^{2+}, 0.010 M NH$_4^+$, 0.0005 M SO$_4^{2-}$, 0.126 M Cl$^-$, 0.027 M HCO$_3^-$, 0.0012 M PO$_4^{3-}$, 0.0002 M citrate, 0.005 M glycine and 0.001 M cysteine (pH 7.4)	[51]
Synthetic gastric juice	2 g L^{-1} NaCl, 7 mL of conc. HCl, 3.2 g L^{-1} pepsin (pH 2) 1.25 g L^{-1} pepsin, 0.5 g L^{-1} of sodium citrate, 0.5 g L^{-1} maleic acid, 0.5 g L^{-1} acetic acid, 420 μL L^{-1} lactic acid and HCl to adjust pH 2	[31,34] [78,79]
Artificial lysosomal fluid (ALF)	Sodium chloride (3.210 g L^{-1}), sodium hydroxide (6.000 g L^{-1}), citric acid (20.800 g L^{-1}), calcium chloride (0.097 g L^{-1}), sodium phosphate heptahydrate (0.179 g L^{-1}), sodium sulfate (0.039 g L^{-1}), magnesium chloride hexahydrate (0.106 g L^{-1}), glycerine (0.059 g L^{-1}), sodium citrate dihydrate (0.077 g L^{-1}), sodium tartrate dihydrate (0.090 g L^{-1}), sodium lactate (0.085 g L^{-1}), sodium pyruvate (0.086 g L^{-1}), formaldehyde (1.000 mL L^{-1}) (pH 4.5–5.0)	[54]
Gamble solution	Magnesium chloride (0.095 g L^{-1}), sodium chloride (6.019 g L^{-1}), potassium chloride (0.298 g L^{-1}), disodium hydrogen phosphate (0.126 g L^{-1}), Sodium sulphate (0.063 g L^{-1}), calcium chloride dihydrate (0.368 g L^{-1}), sodium acetate (0.574 g L^{-1}), sodium hydrogen carbonate (2.604 g L^{-1}), sodium citrate dihydrate (0.097 g L^{-1})	[54,32]

Note: Table indicates principle composition of leaching agents which could vary from study to study.
[a] For extraction of labile metallic fractions (water soluble plus dilute acid soluble fractions).
[b] Representative of gut solution, NaAc, NH$_4$Ac, and HAc (sodium acetate, ammonium acetate and acetic acid respectively).

Ultimately, the precise bio-accessibility of a metal in the human body fluids depends on the mineralogical, biogenic, and artificial phases that the metal exists in, and the kinetics of release under such acidic and enzymatic conditions and in the presence of potential inorganic and organic complexants [35]. The following sections present a compilation of the leaching agents applied so far in single step extraction procedures.

2.1.1. Water

Several studies report the use of water as leaching agent for estimation of bio-accessible trace metal fractions in APM [38–45]. According to this school of thought, the easily released water-soluble metal fractions are assumed to be easily mobilized when interacting with human body fluid and therefore, have been regarded as bio-accessible trace metal fractions. The other reason is that most toxicological studies have implicated the water-soluble metal as a possible harmful component of PM [13,43,46,47]. They have found that water-soluble metal fraction is capable of stimulating the toxic effects in human beings. The most important evidences originated from water-soluble metals that have been shown to generate reactive oxygen species (ROS), which switch on cellular pro-inflammatory response pathways in vitro and in

vivo. For example, Costa and Dreher [13] concluded that the dose of bioavailable (which these authors defined as water-extractable) transition metal, rather than PM mass, was the primary determinant of the acute inflammatory response for ambient PM, oil and coal fly ash instilled into rats. Using samples of PM10 from the Utah valley, Frampton et al. [48] showed the importance of soluble metal content for toxicity and pro-inflammatory potential in a range of human, animal and in vitro studies, whilst water-soluble concentration of Zn has been reported as the toxic factor in the mouse lung response to an atmospheric dust sample [46]. However, although frequently used, this approach does not provide realistic data about the true bio-accessible trace metal fractions in APM since water does not mimic the conditions of human body fluids – thus improved approaches are necessary resembling the properties of these reagents.

2.1.2. Buffer, salt and chelating solutions

In contrast to water as the simplest leaching solvent, some authors used salt solutions, buffers and different chelating agents for the extraction of bio-accessible trace metal fractions. For example, Artelt et al. [29] used physiological NaCl solution for the extraction of soluble platinum from automobile exhaust. Smith

et al. [30] used a 1 mM citrate solution for the mobilization of Fe from coal fly ash and result showed enhanced solubility of Fe with the use of citrate solution. Harris and Silberman [49] used chelating agents like EDTA, citrate, histidine, and glycine for time dependent leaching of coal fly ash. Canepari et al. [50] applied a set of four different leaching agents for extraction of PM10 samples collected from an urban site in Rome, Italy. They compared the extraction efficiencies of deionized water (pH 6.44), acetate buffer (10 mM, pH 4.3), nitric acid (0.200 M, pH 0.78) and EDTA (1 mM, pH 4.77) in order to evaluate the soluble metal fraction and residual one. In the case of real PM10 samples, it was not possible to compare the absolute extraction efficiencies of these leaching agents with respect to each other. Therefore, they have taken acetate buffer as the reference leaching agent and then compared extraction efficiencies of other solutions relative to it. It has been found that almost comparable extractable metallic fractions were obtained with the use of water and acetate buffer. Similar results were obtained when EDTA was compared with acetate buffer with the exception of Pb where larger fractions were obtained with the use of EDTA. On the other hand, just nitric acid yielded greater percentage of metallic fractions in contrast to acetate buffer. Finally, they recommended the use of acetate buffer in order to determine the soluble metallic fractions in PM10 samples since it established the best compromise between method selectivity and robustness. Voutsa and Samara [51] investigated the bio-accessible and labile trace metal fractions in PM7.5 samples collected from urban and industrial sites in Thessaloniki, Greece. For extraction of potential bio-accessible trace metal fractions, aerosol samples were treated with synthetic serum which simulates realistic biological conditions as compared to simple water. According to authors, the labile metal fraction represents total amounts of easily soluble elements (water-soluble fraction plus metal fraction soluble in mild acidic conditions). Therefore they used a dilute acid solution (0.1 N HCl) for extraction of labile metallic components. The study revealed that in general, the bio-accessible fractions of all metals were lower than the corresponding labile fractions indicating that the bio-accessible fractions are loosely bounded to APM. These approaches discussed above were based on the concepts that for the release of loosely bound labile metallic species in APM, it is more appropriate to use extractants which possess higher ionic and chelating strength than water in order to better assess the bio-accessible trace metal fractions.

2.1.3. Synthetic body fluids

With the growing interest and progress in studies regarding the estimation of bio-accessible trace metal fraction in APM, some workers have realized that it is more realistic to use leaching agents which mimic conditions of human body fluid in order to better ascertain the bio-accessible trace metal fraction. For this purpose, in vitro physiologically based extraction tests (PBET) with synthetic agents analogous to human fluids were developed. Despite the fact that these artificial body fluids were similar to true body fluids in terms of pH, ionic strength, and major specific-ion interaction, this approach has inherent limitations because artificial fluids cannot contain all the constituents of human fluids (e.g. proteins, enzymes, etc.). Moreover, there are many other mechanisms, except dissolution, that control the bio-accessibility of elements [52]. Nevertheless, in vitro leaching methods, after validation, could be used as inexpensive alternatives to costly and labor-intensive animal models. Additionally, in lieu of obtaining bioavailability data from samples of urine, blood, or other tissues, these studies measured solubility of compounds in various artificial fluids as a surrogate for bioavailability.

In some studies, a synthetic gastric juice prepared according to US Pharmacopeia methodology has been used for one-step extraction of bio-accessible trace metal fractions in APM which follow the gastric intestine tract and thereby interact with synthetic gastric juice [31,34]. In this case, pepsin was used in acidic conditions to mimic conditions of human gastric juice. Twining et al. [53] investigated the risk ranking of bio-accessible trace metal fractions from fly ash dissolved in simulated gut and lung fluid. For this purpose they used 0.1 M HCl as representative of the gut fluid, whereas the simulated lung fluid was composed of various inorganic salts, citric acid, and albumin. Benzalkonium chloride was used as preservative for the simulated lung fluid. The study revealed that the proportion of bio-accessible to total metal contents ranged in coal fuel-derived fly ash from 0 (V) to 80% (Zn) when using simulated lung fluid as leaching agent, whereas with simulated gut fluid values between 2 (Th) and 100% (Cu) were found, indicating that the investigated elements exhibit differential solubility's in those media.

In addition to use of synthetic gastric juice for extraction of bio-accessible trace metal fractions, there are also some studies which have simulated the lung environment for assessment of bio-accessible fractions using synthetic lung fluids. The commonly used artificial lung fluids are Gamble solution and artificial lysosomal fluid (ALF). The main differences between Gamble's solution and ALF are the acidity (the former has pH 7.4 whereas the latter has pH 4.5) and the organic content (ALF has much higher organic content than Gamble's solution). Gamble's solution is representative of the interstitial fluid of the deep lung whereas ALF represents the more acidic environment within the lung. Colombo et al. [54] have investigated the risk associated with the inhalation of platinum group element (PGE) emissions from vehicle exhaust catalysts (VECs) by extracting road dust and milled auto catalyst with simulated lung fluids, i.e. Gamble solution and ALF. In a very recent study by Julien et al. [32], efforts have been made to improve the current knowledge on easily released metals from atmospheric particles. They investigated the release of bio-accessible trace metal fractions (Ba, Cd, Ce, Co, Cu, La, Mn, Mo, Ni, Pb, Rb, Sb and Zn) from four different reference materials representative of different particle sources. The bio-accessibility of metals was tested by using two extractants i.e. water and Gamble solution. Through this study, the influence of different parameters such as extraction-time, composition of the leaching solution and solid-to-liquid (S L^{-1}) ratios on the release of labile metal fractions were investigated. They clearly demonstrate that bio-accessibility is speciation and element dependent and the higher bio-accessibility of metals is obtained with the Gamble solution as compared to simple solvent water.

2.2. Sequential extraction procedures

The bio-accessibility of toxic metals depends on their characteristic surfaces, on the strength of their bonds, and on the properties of solutions in contact with them, therefore many research groups have designed and investigated multi-step extraction schemes aimed at the sequential solubilization of metals in order to investigate potential toxicity of trace metals bound to APM [55]. These sequential chemical extraction schemes are usually based on the same principle of successive extraction of metals bound in various chemical forms from the most mobile to those strongly bound to the particulate matter. The main objective of aerosol fractionation studies is the classification of metals according to particle size and chemical bonding in order to characterize metal mobility. The extractant converts the metal bound in solid phase into a soluble form. The fraction of individual element in each phase depends on the chemical reagents used as the extractant and the chemical and physical operating parameters involved, such as pH, reagent type and concentration, time of contact, particle size, stirring system temperature, and so on. In general terms, the fractions considered are exchangeable, weakly absorbed, hydrous-oxide bound, organically linked, and the lattice material component [33]. Applied fractionation schemes were basically developed for studying the

fractionation of metals in sediment samples and later on, adopted for the investigation of metallic fractions in APM. Although this classification provides useful information about how each element is partitioned among various components in APM, only the easily mobile metallic fraction could be considered as potentially bio-accessible. Thus in the case of sequential extraction procedures the concentrations derived from analysis of the first fraction constitutes the most relevant data about bio-accessible elements.

2.2.1. Two-step extraction procedures

In a very simple two-step extraction procedure, Kyotani and Iwatsuki [56] determined water-soluble fractions in atmospheric dust in the first step. In the second extraction step 0.1 M HCl was used for the dissolution of sulphate and carbonate bounded metallic components (e.g. Gypsum and calcite). Later on, Kyotani and Iwatsuki used this procedure to study the distribution of water and acid soluble trace metal fractions in PM2.5 and PM10 samples collected from Kofu, Japan [57]. In an another study reported by Sato et al. [58], a two-step extraction procedure based on water and 0.1 M HNO$_3$ for dissolution of acid soluble fractions was applied for investigating the distribution of major and trace metallic fractions in PM2.5 aerosol samples collected from Tokyo, Japan.

2.2.2. Multi-step procedures based on Tessier's and BCR scheme

Smichowski et al. published a review about survey schemes for chemically fractionation of metals and metalloids in airborne particulate matter [33]; thus in this work these approaches were just briefly summarized, furthermore some additional and new literature has been added.

Tessier's scheme [59] has been initially developed for the investigation of sediment samples and comprises of four steps and is one of the most thoroughly investigated and analytically well-documented schemes. The fractions obtained and reagents used in this scheme are: exchangeable (1.0 mol L^{-1} magnesium chloride), bound to carbonate (1.0 mol L^{-1} sodium acetate), bound to Fe and Mn oxides (0.04 mol L^{-1} hydroxylamine chloride in 25% acetic acid), bound to organic matter (0.02 mol L^{-1} nitric acid + 30% hydrogen peroxide + 3.2 mol L^{-1} ammonium acetate in 20% nitric acid) and residual (hydrofluoric and perchloric acids). Obiols et al. [60] modified Tessier's scheme [59] for the investigation of metal solubility in APM samples from an urban area of Barcelona. The developed four step fractionation procedure allowed the partitioning of exchangeable metals into four fractions: (1) soluble and exchangeable, (2) bound to carbonate and Fe–Mn oxides, (3) bound to organic matter, and (4) residual. Differences to Tessier's fractionation scheme are the replacement of magnesium chloride with sodium chloride, and metals bound to carbonates and oxides were extracted in the same step. In the PM samples analyzed by flame atomic absorption spectrometry (FAAS), only Cd was predominant in the soluble and exchangeable fraction whereas other investigated metals were largely associated with carbonate and Mn oxides. Later the group of Fernandez carried out series of extraction experiments based on Tessier's scheme with several modifications with the aim to study the distribution of different chemical forms of trace metals in APM [61–63]. The scheme differentiated four fractions which define the distribution and partitioning of key metals: (1) soluble and exchangeable metals, (2) carbonate, oxides and reducible metals, (3) bound to organic matter, oxidizable and sulfidic metals, and (4) residual metals. One of the important modifications to Tessier's scheme adopted by Fernandez was the replacement of magnesium chloride by water in order to investigate soluble and exchangeable trace metal fractions which could be regarded as the bio-accessible trace metal fraction. The scheme was applied for the extraction of urban particulate matter collected from Seville (Spain). Fujiwara et al. [64] used a very similar four-step chemical sequential leaching procedure to that developed by the group of Fernandez for

fractionation of atmospheric aerosols. The distribution of eleven elements amongst soluble, bound to carbonates, oxides (reducible elements), bound to organic matter (oxidizable and sulfidic elements) and environmentally immobile fractions was investigated in PM10 samples collected from an industrial city in Argentina.

Chester et al. [65] introduced a fractionation scheme especially designed for aerosol samples. The procedure consists of a three-stage sequential leaching procedure i.e. (1) loosely held environmentally mobile fractions, (2) bound to carbonates and oxides, and (3) organic and refractory associated fractions. The extractants used for fractionation of these fractions were: (1) 1.0 mol L^{-1} ammonium acetate, (2) 1.0 mol L^{-1} hydroxylamine and 25% acetic acid, and (3) nitric and hydrofluoric acid respectively. In this scheme the environmentally mobile fraction could be considered as the bio-accessible trace metal fraction. This three-step fractionation scheme has been applied to urban and marine aerosols. Derived results indicated that Cu, Pb and Zn were mostly found in loosely bound bio-accessible fractions, whereas Al and Fe were generally present in the refractory aerosol fraction. In Chester's scheme use of acetate salt for determining the environmentally mobile fraction could enhance the extraction of trace metals, since acetate ions have better chelating capabilities in contrast to water and simple inorganic salt solutions. Many comprehensive fractionation studies based on Chester's scheme [66–68] were conducted by group of Hlavay to investigate various elemental fractions (mobile, carbonate and oxide associated, and bound to silicates) in aerosol samples collected from Hungary. Mustafa et al. [69] studied the distribution of various fractions of Al, Fe, Cu, Pb, Cd, Zn and Mn in aerosol samples collected from the Turkish Eastern Mediterranean coastline. The Chester scheme has also been applied for analysis of the standard reference material 1648a (urban particulate matter) from NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) [70]. The distribution of the selected metals in the three fractions indicated that Zn was the most environmentally mobile element whereas Fe was mostly associated in refractory fractions.

Zatka and Maskery [71] introduced a four-step fractionation scheme for determining the distribution of Ni in airborne dust samples. The four metallic fractions were classified as (1) soluble, (2) sulfidic, (3) metallic, and (4) oxidic (insoluble). In this scheme, 0.1 mol L^{-1} NH$_4$-citrate solution (pH 4.4) was used for fractionation of soluble metallic fractions. Later on, the procedure was adopted by other authors [72,73] to study distribution of various metallic fractions in aerosol samples. Füchtjohann et al. [74] modified Zatka's scheme by replacing ammonium acetate by EDTA for leaching of soluble trace metal fractions. The procedure was applied to study the speciation of Ni in aerosol samples.

The original BCR scheme *elaborated in 1993 by the European Community's Bureau of References* [75] was developed for chemical speciation analysis of heavy metals in soils and sediments which distinguishes the metallic fractions into the acid soluble, reducible and oxidizable species. For extraction of these three fractions, 0.11 mol L^{-1} solution of acetic acid, 0.1 mol L^{-1} NH$_2$OH·HCl solution (pH 2), and a mixture of H$_2$O$_2$ and 1 mol L^{-1} ammonium acetate are applied respectively. Hlavay et al. [76] used BCR scheme to monitor the chemical fractionation of aerosols, and later on a capillary electrophoresis method combined with BCR sequential extraction procedure for determining distribution of Fe, Zn, Cu, Mn, and Cd in APM was developed [77].

2.2.3. Multi-step procedures using synthetic body fluids

In addition to the above mentioned procedures there exist also methods which use synthetic body fluids instead of simple salt solutions. For example, Colombo et al. [78] used a gastric solution for the PBET of vehicle exhaust and road dust in order to estimate bio-accessibility of platinum group elements (PGE). The gastric juice was prepared by adding 2.50 g of pepsin (activity of

800–2500 units mg^{-1}), 1.00 g of sodium citrate, 1.00 g of malic acid, 840 µL of lactic acid and 1 mL of acetic acid to deionized water (2 L) followed by addition of HCl to adjust pH of the solution to 2. After extraction with synthetic gastric juice, the residual substrate was subjected to further leaching by using a simulated small intestinal juice. For this purpose, they used bile salt and pancreatin at neutral pH. In one more study [79], the bio-accessibilities of PGE and the catalyzator poison (Pb) have been determined in particles derived from milled automotive catalytic converters using a PBET that simulates, sequentially, the chemical conditions encountered in the human stomach and intestine. They employed the PBET recommended by the British Geological Survey (BGS) for the rapid screening of contaminated soils. The approach simulates the chemical conditions of the pediatric gastro-intestinal tract, and is based on a simplified version of an earlier protocol described by Ruby et al. [80]. For extraction of PGEs and Pb, the autocatalytic sample was treated with synthetic gastric solution for 1 h at 37 °C with constant lateral agitation to mimic stomach mixing via peristalsis. After 1 h, a sample aliquot representing the stomach phase was taken and preserved for analysis. The rest of the sample mixture was subjected to simulated intestinal conditions by neutralizing the solution to pH 7 with saturated sodium bicarbonate solution and adding pancreatin and bile salts. The extraction was continued as in the case of stomach phase and sample aliquots were collected after 2 h and 4 h which represented intestinal phase I and II respectively.

3. Techniques and procedures for measurement of bio-accessible trace metals

In contrast to other solid environmental samples such as soil, sediments, dust and fly ash the amount of APM available for analysis is usually restricted – typically the sample mass deposited on the filter substrate used for sample collection is in the order of few milligrams (mg). Moreover it has to be considered that the sample has to be shared if determination of bio-accessible fractions is intended (requires analysis of leachable and total contents), which causes a further reduction of the available sample mass. The use of organic sampling substrates such as cellulose ester filters

creates another problem; they often produce electro-static charges which make sample handling much more complicated. Thus the application of conventional leaching procedures is often not simple or straightforward, since the sensitivity required for measurement is not achievable. Analysis of APM samples is further hampered by sometimes very low analyte concentrations which have to be determined in the presence of complex matrix with varying composition. Typically the whole trace metals fraction (cumulated mass of all trace metals) is less than 1% of the total APM mass. Furthermore it has to be considered that the various fractions of metals bound to APM as discussed above in section 2 are merely a small part of the total concentrations. Crustal elements like Al, Ca, Mg, Fe, and Si are usually present in order of some µg m^{-3} whereas trace metals such as As, Ba, Cd, Cr, Co, Cu, Mn, Ni, Pb, Sb, Sn, Sr, Ti, V and Zn are available from few ng m^{-3} to some hundred ng m^{-3} [81]. When considering typical sample volumes collected with low volume systems (50–60 m^{-3}), this translates to a total APM mass of 2–4 mg per sample and a cumulated mass of some µg trace metals (target analytes) only. Therefore, for accurate determination of such small sample amounts, highly sensitive but also selective analytical procedures are required. Fig. 2 presents an overview of the most frequently applied techniques, including a comparison of their analytical characteristics. The procedures described in literature so far are summarized in the following sections.

3.1. Batch-wise extraction procedures

For assessment of bio-accessible trace metal fractions, typical batch-wise extraction procedures are used. The collected aerosol samples are treated with leaching agents such as water, salt solutions, buffers or synthetic body fluids using simple shaking or ultrasonic agitation for a defined period of time at definite temperature. Dissolved species are separated from remaining particles by centrifugation of the derived suspension. Finally the supernatant solution containing the soluble species of the investigated aerosol sample is removed; subsequent analysis is usually accomplished using analytical techniques such as FAAS, electro thermal atomic absorption spectrometry (ET-AAS), inductively coupled plasma

	ET-AAS	ICP-OES	ICP-MS
Capabilities	single element	multi element	multi element
Sensitivity	0.1 to 10 µg l^{-1}	0.1 to 10 µg l^{-1}	< 1 to 100 ng l^{-1}
Dynamic working range	up to some hundred µg l^{-1}	up to some hundred mg l^{-1}	up to some mg l^{-1}
Matrix effects - spectral interferences -non spectral Interferences	- very few - thermal behavior of analytes could be changed	- some - variations in the sample introduction efficiency - emission of spectral underground	- many - variations in the sample introduction efficiency - signal suppression - formation of isobaric interferences
Correction methods	- use of modifiers - Zeeman background correction	- separation procedures - selection of different wavelengths - inter element corrections	- separation procedures - mathematical corrections - collision/reaction cells - high resolution instruments

Fig. 2. Analytical techniques applied for determination of trace metals in APM sample extracts.

Table 2
Techniques used for the assessment of bio-accessible trace metal fractions in APM.

Techniques	Elements determined	Interference correction	Ref.
F-AAS	Cd, Cr, Cu, Fe, Pb, Mn	–	[60]
	Al, Cu, Fe, Mn, Pb, Zn	–	[65]
	Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn		[68]
	Al, As, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, V	–	[67]
	Cd, Cu, Mn, Ni, Pb, Zn	MMC	[53]
	Co, Cu, Fe, Ni	–	[72]
ET-AAS	Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn	–	[66]
	Cd, Cu, Mn, Ni, Pb, Zn	MMC	[51]
	Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn	–	[76]
	Ni	–	[74]
	Cr, Cu, Fe, Mn, Ni, Pb, Ti, V, Zn,	–	[45]
ICP-OES	Al, Br, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, S, Si, Ti, V, Zn	IS (Y)	[58]
	Al, As, Ba, Ca, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Ti, V, Zn	–	[39]
	Al, Ba, Ca, Cr, Cu, Fe, Mg, Mn, Pb, Se, Ti, Zn	–	[41]
	Al, Ca, Fe, K, Mg, Na		[58]
	Al, As, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, S, Sb, Se, Si, Ti, V, Zn	–	[50]
	Ca, Cd, Co, Cu, Fe, Mg, Mn, Ni, Pb, Ti, V	–	[61]
	Al, As, Cr, Cu, Fe, Mn, Ni, Pb, Ti, V, Zn	–	[64]
ICP-MS	As, Be, Ba, Cd, Co, Cr, Cu, Li, Mn, Mo, Ni, Sb, Se, Pb, Ti, V, Zn	IS (In and Re)	[58]
	As, Cd, Cu, Cr, Fe, Mn, Ni, Pb, Ti, V, Zn	Various isotopes	[43]
	Ag, Al, As, Ba, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, K, La, Li, Mg, Mn, Mo, Na, Ni, Nb, P, Pb, Pd, Rb, Rh, S, Sb, Sc, Se, Sr, Sn, Ti, Tl, U, V, Y, Zn	–	[44]
	Ag, Al, Ba, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Pt, Se, Sn, Ti, Zn	Drift control using standard solutions	[38]
	Ag, Al, As, Ba, Br, Cd, Ce, Co, Cr, Cu, Eu, La, Mg, Mn, Mo, Nd, Ni, Pb, Rb, Sb, Se, Sm, Sr, Ti, V, Zn		[40]
	Al, As, Ca, Cd, Co, Cr, Fe, Mn, Mg, Ni, Pb, Se, Sb, V, Zn		[25]
	Ba, Cd, Cu, Fe, Mn, Ni, Mo, Pb, Sb, Se, Sn, Sr, V, Zn		[27]
	As, Al, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn	IS (In, Bi, Sc, and Ge)	[36]
	Ag, Cd, Co, Cu, Ti, Mn, Mo, Pb, Sb, Sn, Tl, Zn	IS (In)	[34]
	Cr, Ni	DRC	[34]
	Pd, Pt and Rh Pd and Pt	Math. correction IDMS	[78,82]
ETV-ICP-OES	Ba, Co, Cu, Mn, Ni and Zn	IS (In)	[31]

	Elements	Techniques	Ref.
Dynamic	Al, As, B, Ba, Cd, Cu, Fe, Mn, Pb, Se, Zn	ICP-OES	[84]
Extraction	Zn	FI-FAAS	[85]
Procedures	Ba, Cr, Cu, Fe, Mn, Ni	FI-ICP-OES	[86]

MMC, matrix matched calibration; IS, internal standard; DRC, dynamic reaction cell.

optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Table 2 presents a compilation of the instrumental approaches reported so far.

Batch-wise extraction procedures are well established and are commonly applied; their main advantages are simplicity and the absence of cost intensive equipment. However, batch-wise extraction procedures are accompanied by lengthy and laborious sample handling steps e.g. shaking the sample with the leaching agent, centrifugation of the resulting heterogeneous solution and the separation of supernatant solution. A further problem of such procedures is that the extracted soluble analyte species may adsorb on the remaining insoluble part of sample, which can lead to under estimation of target analytes.

3.2. Improved analytical approaches in combination with batch-wise extraction procedures

The presence of soluble components such as alkali elements or water soluble ions and organic compounds as well as matrix introduced from the complex chemical components of leaching agents (buffers, salts, enzymes, etc.) can cause interferences during analysis. The main problems are non-spectroscopic as well as spectroscopic interferences impeding reliable measurement of target analytes. A common way to overcome these problems is simple dilution of the extracts in order to enable sample analysis. But in the case of aerosol samples, where only limited sample amounts

are available, simple dilution may lead to analyte signals insufficient for accurate quantification of bio-accessible metal fractions. Thus, some improved procedures have also been reported in literature in order to overcome problems related with sample matrix, signal drift and interferences during measurement. With the use of such techniques, a more accurate and reliable estimation of bio-accessible trace metal fractions could be ensured.

3.2.1. Single element analysis using ET-AAS

In the case of ET-AAS technique, volatile matrix constituents can be removed prior to sample atomization by optimizing the steps of the temperature furnace program [68]. However, accurate analysis of PM extracts could be hampered by losses of volatile analyte species during the pyrolysis step, since many elements form in the presence of organic compounds volatile organo-metal species which could lead to removal of analytes prior to the atomization step, thus accurate measurement of bio-accessible trace metals becomes difficult. A common way to overcome such problems is the use of special modifiers or matrix matched standard solutions for signal quantification. With the use of modifiers the existing analyte species could be converted into less volatile forms, thus the temperature of the pyrolytic step could be increased, which helps to remove volatile sample constituents such as organic compounds prior to the final analysis step without losses of analyte. Thereby the influence of these interferences could be minimized (e.g. absorption caused from non-dissociated molecules), furthermore a

quantitative measurement of the analyte could be obtained. Application of matrix matched standards for signal evaluation allows compensation of possible differences in the behavior of sample extracts and calibration standards during pyrolysis and atomization.

One procedure which involves the use of matrix matched calibration has been reported by Voutsa and Samara [51]. They determined bio-accessible, labile and total concentrations of heavy metals in PM7.2 samples collected from various urban and industrial sites in the Greater Thessaloniki area, Greece. The total metal concentrations were ultrasonically extracted from aerosol samples by treatment with a mixture of 2.6 M HNO$_3$ and 0.9 M HCl for 60 min and labile metals were extracted with the use of 0.1 N HCl. A neutral simulating serum solution (pH 7.4) consisting of 0.145 M Na$^+$, 0.0002 M Ca^{2+}, 0.010 M NH$_4$$^+$, 0.0005 M SO$_4$$^{2-}$, 0.126 M Cl$^-$, 0.027 M HCO$_3$$^-$, 0.0012 M PO$_4$$^{3-}$, 0.0002 M citrate, 0.005 M glycine and 0.001 M cysteine was used for the investigation of bio-accessible trace metal fractions. The obtained extracts were measured using F-AAS and ET-AAS techniques and quantification of derived signals was performed by matrix matched calibration using the three leaching agents.

3.2.2. Multi-element analysis with ICP-OES or ICP-MS

3.2.2.1. Correction of non-spectroscopic interferences.
In the case of ICP-OES or ICP-MS analysis the presence of complex sample matrix may result in non-spectroscopic interferences during measurement which can disable accurate analysis of target analytes. Organic constituents or enhanced salt concentrations present in the leaching agents may lead to differences in sample introduction efficiency as compared to conventional aqueous standard solutions, since such solutions may possess different viscosity, surface tension and density. Furthermore, complex matrix may influence the atomization, excitation and ionization of target analytes causing signal suppression when compared to standard solutions. Approaches to overcome such non-spectroscopic interferences include the use of internal standards and/or the use of matrix matched standard solutions for signal evaluation. The use of an internal standard helps to correct instrumental drifts, and with the use matrix matched standards the effect of matrix on sample introduction efficiency and analyte excitation/ionization in the plasma can be compensated. Kyotani and Iwatsuki [57] have used yttrium (Y) as internal standard for ICP-OES measurement of 14 elements in water and weak acid extracts of PM10 and PM2.5 samples collected from an urban site Kofu (central Japan). In another study carried out by Sato et al. [58], major elements (Na, Mg, Al, K, Ca and Fe) were determined by ICP-OES whereas for determination of trace elements (As, Ba, Be, Cd, Co, Cr, Cu, Li, Mn, Mo, Ni, Pb, Sb, Se, Ti, V and Zn), an ICP-MS method was applied using In and Re as internal standards for analysis of calibration standards and sample solutions (water and weak acid extracts of PM2.5). Recently, chemical fractionation of trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn) in fine particle (PM2.5) collected in Guangzhou (China) has been investigated using Fernandez extraction scheme and ^{45}Sc, ^{72}Ge, ^{115}In, and ^{209}Bi as internal standards for ICP-MS analysis [36]. Julien et al. [32] used Rh as internal standard in all calibration standards and sample solutions in order to monitor the drift of the ICP-MS signal for measuring Ba, Cd, Ce, Co, Cu, La, Mn, Mo, Ni, Pb, Rb, Sb and Zn in four reference materials of urban atmospheric particles, urban dust and fly ash. However, it was not possible to measure As, Cr, Fe and Ti accurately in these reference materials due to spectroscopic interferences introduced from the plasma gas Ar, and chlorides, which were part of the extraction solvent. In one more study, Turner and Lp [35] have also used In and Ir as internal standard to correct for detector drift and variations in plasma conditions for analysis of Ag, Cd, Co, Ni, Cu, Pb, Sn and U in dust samples from indoor environment.

3.2.2.2. Correction of spectroscopic interferences.
In addition to non-spectroscopic interferences the presence of organic and inorganic sample or leaching agent constituents may result also in so called spectroscopic interferences. In the case of ICP-OES organic matrix can cause alterations in the background signal hampering accurate signal quantification. Major inorganic sample constituents may results in emission signals overlapping with the analytical lines of the target elements. In theory these problems could be solved with improvements in the optical resolution of the used instruments, in reality the operator has no possibility to influence the resolution of a given instrument since the respective parameters (e.g. optical path length) are predetermined by the manufacturer. Thus strategies to overcome this kind of interferences are limited to the selection of undisturbed emission lines – which is sometimes difficult since this might be related with a loss in sensitivity; inter element corrections and use of matrix adjusted standards for signal evaluation. Nevertheless most studies report the use of more than one spectroscopic line per element to improve quality of derived results.

Similar problems were reported for measurements using ICP-MS for element specific analysis, in this technique matrix elements can contribute to the formation of polyatomic ions which disables correct measurement of the elements of interest. A descriptive example is the measurement of Cu in a sample extract with high sodium contents, which leads to the formation of an ArNa-ion with the same nominal mass to charge ratio of 63 as the most abundant Cu isotope. To overcome the interference of such polyatomic ions two or more possible isotopes of target elements can be monitored in order to cross check against potential interferences by other species of the same, or closely overlapping, m/z value. This analytical strategy has been adopted by Heal et al. [43] for the investigation of bio-accessible (water-soluble) fractions of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Ti, V and Zn in PM10 and PM2.5 samples collected from an urban background site Edinburgh, UK. An alternative approach to overcome this problem is to correct interferences mathematically by estimating the contribution of interfering species to the analyte signal through the analysis of single element standard solutions of interferents. Colombo et al. [78] have utilized this technique for correction of spectroscopic interference from monoatomic and polyatomic ions produced in the argon plasma by the matrix constituents: elements such as Cu,, Hf, Pb, Rb, Sr and Y which were usually present in road dust and catalyst samples and hamper therefore the accurate quantification of the investigated platinum group elements.

With the use of a collision cell or a dynamic reaction cell it is possible to separate such overlapping polyatomic ions prior to introduction into MS system. Falta et al. [34] applied an instrumental approach for the determination of bio-accessible trace metal fractions in PM10 and PM2.5 samples collected from downtown Vienna. The aerosol samples were subjected to batch-wise extraction procedure with the use of synthetic gastric juice as leaching agent and concentrations of 14 elements (Ag, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sn, Ti, Tl and Zn) were determined in the prepared extracts using ICP-MS. In was used as internal standard for correction of non-spectroscopic interferences. Moreover, isotopes of ^{52}Cr and ^{60}Ni were measured using dynamic reaction cell (DRC) with NH$_3$ as the reaction gas. With the use of DRC, polyatomic ions like ArC$^+$ (m/z = 52) and ^{44}Ca^{16}O$^+$ or ^{23}Na^{37}Cl$^+$ (both mass with m/z = 60) were removed, which can overlap with the target ions ^{52}Cr and ^{60}Ni respectively. With the use of this procedure, excellent limit of detection values (LOD) were obtained even in the presence of complex organic matrix originating from the used synthetic gastric juice, which enabled quantification of bio-accessible Cr and Ni fractions up to concentration levels of some pico gram per cubic meter (pg m^{-3}) only.

Another possibility to distinguish between polyatomic interferences and target ions is the use of high resolution ICP-SFMS (sector field mass spectrometry). Depending on the used mass resolution with this technique a separation of interferences is possible, but improvements in selectivity are always associated with losses in sensitivity. Puls et al. [82] applied this approach successfully for the determination of bio-accessible Pt and Pd concentrations in APM samples, analysis was performed using the high resolution mode of an Element 2 sector field ICP-MS equipped with a membrane desolvatization system for reduction of oxide interferences. Quantification of derived signals was performed via isotope dilution analysis.

In addition to these instrumental approaches the strategy of interference elimination also exists prior to analysis with ICP-OES or ICP-MS. For this purpose all kind of pre-concentration and/or matrix separation techniques could be used, including well established procedures such as co-precipitation, liquid–liquid extraction and solid phase extraction. An example for the preliminary separation of possible interferences prior to measurement is reported by Puls et al. [82], who applied a cation exchange procedure for reduction of interfering element concentrations in APM extracts derived via sample leaching with synthetic gastric juice, followed by ICP-MS analysis of the target analytes. Recently an alternative approach for separation of organic matrix present in synthetic gastric juice extracts of APM samples has been presented, which removes the more volatile organic matrix prior to element specific analysis [31]. The method is based on the preliminary extraction of trace metals with synthetic gastric juice using a physiologically based extraction test (PBET) and the subsequent measurement of gastric extracts using electro-thermal vaporization (ETV) combined with ICP-OES detection. Application of PBET improves risk assessment but hampers analysis [34], since the organic matrix used for sample extraction cause spectroscopic and non-spectroscopic interferences during ICP-OES measurement. With the use of an ETV as sample introduction unit a quantitative separation of solvent and organic matrix was possible, this enabled after careful optimization of the ETV temperature program an interference free measurement of the target analytes. Furthermore, there was no need to digest or dilute sample extracts containing pepsin, thereby saving time and reducing the risk of sample contamination. Differences in the sample evaporation process and the sample introduction efficiency of APM extracts and standard solutions were corrected with the use of In as internal standard. Quantification of derived signals was performed using external calibration with aqueous standards, leading to detection limits of some hundred $pg\,m^{-3}$ for bio-accessible trace elements in APM.

3.2.3. Dynamic extraction procedures with online detection

Dynamic extractions procedures provide a continuous flow of the leaching agent through the sample, thereby soluble species are dissolved, followed by subsequent on-line or off-line measurement of the derived extracts using a suitable detection unit. In contrast to their batch-wise counterpart where extraction is achieved under equilibrium conditions, dynamic extraction procedures provide information about the kinetics of the leaching process. Furthermore, the risk of sample losses as well as contamination is reduced since troublesome and time consuming sample handling steps such as sample shaking, filtration or centrifugation could be avoided. Moreover, application of dynamic procedures with on-line detection allows an increase in sample throughput and sensitivity as compared to batch-wise counterparts [83]. But most important, with the use of flow injection systems (FI) a complete automation of on-line dynamic extraction procedures is also possible, which further enhances sensitivity and precision of analysis.

In literature, dynamic extraction procedures based on the concepts of stirring chambers, rotating coiled columns and packed

micro-cartridges are routinely used for the analysis of samples like fly ash, rock, soil and sediments [83]. Stirred flow cells are furnished with closed extraction chambers and used for sequential extraction of solid substrates (soil, sediments and fly ash) capitalizing on continuous flow analysis. The extractants are replaced manually and propelled forward into the extractor by either negative or positive pressure using a peristaltic pump. After extraction of the substrate, the eluents are directed to the detection unit. On the other hand, rotating coiled columns procedures are based on the technique of retaining one phase (stationary) in a column without solid support under the action of centrifugal forces, while the other liquid phase (mobile) is continuously pumped through. These methods have been frequently used in liquid–solid systems for continuous leaching of trace metals from contaminated soils and sediments. The third dynamic extraction technique involves the use of packed microcolumns. In this case the cylindrical microcolumns are filled with a defined amount of sample through which different extracting agents are pumped sequentially for fast assessment of metal distribution among different phases. The microcolumns can either be connected to the conduits of flow-injection set up or to the second generation of flow injection analysis known as sequential injection analysis (SIA). These dynamic extraction procedures described above have been successfully applied to soil, sediments and fly ash, but never applied to APM in order to investigate the extraction behavior of different metallic fractions present in aerosol samples. The reason is that, unlike soil, sediments and fly ash, APM is only available with very limited sample amounts. As mentioned earlier is APM collected with the use of filters and the material deposited is usually homogenously distributed on the whole filter surface. Therefore, the application of traditional approaches is difficult, where the powdered sample is filled into a chamber, column or coil before analysis. However, despite of this limitation, some dynamic extraction procedures have been reported for the assessment of leachable metal fractions in APM. To overcome problems associated with this special kind of sample, a different approach has been introduced. In this case, instead of whole sample only aliquots of the sample in the form of either circular parts or punches of the sampling substrate were used, which enabled loading of APM sample into the extraction units. So far, only two groups have reported results for dynamic extraction procedures for aerosol filter samples which are discussed below.

Morales-Riffo and Richter [84] introduced a procedure for dynamic extraction of inorganic elements in APM samples collected from Santiago, Chile. In this method, aerosol samples were divided into circular parts of $9.2\,cm^2$ and loaded into an extraction cell made of poly ether ketone (PEEK). The extraction cell was placed into an aluminum chamber-oven extractor and the oven was coupled to a heating device electronically controlled by means of a thermocouple in order to maintain a temperature of $150\,^\circ C$. Acidified water was then pumped through the system to extract the analytes from the particulate matter at a flow rate of $2\,mL\,min^{-1}$. Throughout the dynamic and static acidified subcritical-water extraction, aliquots of the derived extract have been collected, followed by off-line ICP-OES determination of the elements present in the sample aliquots. Although this approach offers distinct improvements when compared to batch-wise counter parts, the collection of leachate aliquots with subsequent off-line analysis is still rather disadvantageous.

Recently, at the Vienna University of Technology a dynamic procedure for the extraction of water-soluble trace metal fractions in APM with on-line F-AAS or ICP-OES detection has been developed [85,86]. For this purpose, the filter punches were packed into sample cartridges, which were inserted into a flow-injection (FI) manifold and continuously extracted with leaching agent, thereby dissolving soluble trace metal fractions followed by on-line measurement of dissolved metallic fractions. In the first study

[84], the APM sample inserted into the cartridge was preliminary extracted with water under dynamic conditions and the derived eluate was then directed to a F-AAS as element specific detection unit, where the obtained transient elution profile was recorded. Therefore, time-resolved information on the ongoing leaching process was obtained. The authors found that, in contrast to conventional batch-wise extraction procedure, the developed FI-procedure led to an improved sensitivity, since this online approach enabled measurement of the undiluted extract, whereas the off-line procedure determines only average species concentrations in the collected eluate. For validation of the developed on-line procedure a set of PM10 samples was processed using the proposed method and a conventional batch-wise extraction procedure, revealing an excellent agreement between the results derived for water-soluble zinc concentrations in PM10. Applicability of developed procedure was demonstrated by analysis of PM10 samples collected from three different urban sites in Austria and the reported results were found to be in accordance to literature findings.

Although this approach offered several improvements, however with the use of F-AAS only single element detection was possible, therefore Limbeck et al. [86] developed a dynamic extraction procedure with on-line ICP-OES detection for fast and accurate multielement analysis of soluble trace metal fractions in APM. In this work further improvements in the previous FI procedure were performed which enabled the use of ICP-OES as element specific detection system for on-line multi-element analysis of Ba, Cr, Cu, Fe, Mn and Ni in aqueous PM10 extracts. Extraction cartridges were further miniaturized to reduce the inner volume thus more concentrated sample extracts were derived, resulting in an improved sensitivity for the investigated elements. An on-line acidification of the extract with simultaneous addition of La as internal standard was also implemented, to overcome the severe matrix effects caused by the co-elution of alkaline and earth alkaline elements as well as water-soluble organic sample constituents present in APM. The proposed dynamic procedure was found to be less labor intensive and time consuming than batch-wise counterparts, since sample manipulation steps such as filtration, centrifugation or dilution could be avoided. On-line coupling of the detection device enabled sensitive multi-element analysis. One more advantage of the proposed approach was that the automation of the FI-system delivered reproducible results for elution profiles. Demonstration of accuracy has been performed by calculating the mass balance of water soluble and residual fractions of trace elements in a series of PM10 samples and comparison with the total metal contents of the same sample set, the absence of significant differences confirmed the applicability of the developed procedure for fast and accurate determination of water soluble metal fractions in airborne particulate matter. The developed procedure was applied for the analysis of water soluble metal fractions in PM10 collected at two urban sites in Linz (Austria). Derived results indicated differences in the solubility between elements with predominantly anthropogenic origin such as Cu or Mn and the crustal element Fe. But more important, the proposed approach allowed the interpretation of the extraction profiles to differentiate between metal fractions which are easily soluble and such which require more time for quantitative dissolution. For risk assessment this information is quite important since the readily mobilizable metal fraction in APM is assumed to be more accessible for environmental processes and living organisms.

Table 3
Bio-accessible trace metal concentrations in APM determined using water as leaching agent.

Authors	Birmili et al.	Wang et al.		Gutierrez-Castillo et al.	Santos et al.		Karthikeyan et al.	
Reference	[38]	[39]		[45]	[41]		[42]	
Sampling site	Birmingham, UK	Nanjing, China		Mexico City, Mexico	Buenos Aires, Argentina		Singapore	
Leaching agent	Water	Water		Water	Water		Water	
Extraction time	20 min	30 min		15 min	60 min		5 min	
Temperature	RT	RT		RT	RT		Microwave	
Unit	$(ng\,m^{-3})$	$(ng\,m^{-3})$		$(ng\,m^{-3})$	$(ng\,m^{-3})$		$(ng\,m^{-3})$	
APM fraction	PM7.2	PM10	PM2.5	PM10	PM10	PM2.5	PM10	PM2.5
Ag	0.010 ± 0.012							
Al	13 ± 6.4	53.19	7.62		19.8	29.4		
As		11.99	3.44		5.96	1.49	1.4 ± 0.3	1.2 ± 0.2
Ba	1.3 ± 0.74	17.25	16.40					
Ca		11,634	676		608	402		
Cd	0.013 ± 0.010						0.12 ± 0.03	0.33 ± 0.30
Co	0.020 ± 0.012						0.12 ± 0.04	0.06 ± 0.01
Cr		5.35	5.41	0.8 ± 0.4	1.02	2.95	1.9 ± 0.6	7.3 ± 1.5
Cu	1.3 ± 0.74	38.83	113	41 ± 36	4.5	9	3.97 ± 0.5	4.8 ± 0.81
Fe	28 ± 16	45.18	8.42	3.3 ± 1.1	1.7	2	30.3 ± 5.3	23.5 ± 5.9
K		12,414	4507					
La								
Li		53.48	65.1					
Mg		583	535		148	85.3		
Mn	0.85 ± 0.46	81.94	91.2	5 ± 2	3.40	3.2	1.8 ± 0.3	1.5 ± 1.5
Mo								
Na		5044	4022					
Ni	0.083 ± 0.100	8.91	8.31	7 ± 7			2.9 ± 2.4	5.6 ± 2.1
Pb	0.75 ± 0.65	9.84	9.53	0.9 ± 1.3	7.8	8.7	3.9 ± 2.1	6.65 ± 1.7
Rb								
Sb								
Sc								
Se	0.017 ± 0.027				0.54	5.36		
Sn	0.16 ± 0.14							
Sr								
Ti	0.43 ± 0.28	2.89	2.58					
Tl								
V		5.31	5.27	29 ± 19			17.9 ± 12.2	20.1 ± 5.6
Zn	3.6 ± 3.6	607	666	210 ± 70	17.5	29.8	18 ± 6	34 ± 19

In studies where sampling for more than one conditions (e.g. location or seasons, substrates, and leachants) is done, results with higher bio-accessible contents are presented.

4. Application examples

For assessment of bio-accessible trace metal fractions, different approaches have been reported in literature which vary greatly in terms of use of leaching solution and extraction procedure including extraction time, temperature and volume of the leaching agent. Additionally, investigated APM size fraction, meteorological conditions and emission sources at sampling site vary from study to study. Therefore, comparison of literature results for bio-accessible trace metal fractions is difficult.

Interpreting the findings reported for water soluble concentrations it can be concluded, that results for various elements show distinct differences from study to study. For example varied the water-soluble concentration of Cu from 1.3 ± 0.7 ng m^{-3} in PM10 samples collected from Birmingham, UK [38] to 41 ± 36 ng m^{-3} in PM10 samples from Mexico city [45] indicating a more than 30 times higher level of labile Cu in urban area of Mexico city as compared to Birmingham. Comparable findings were observed for elements such as As, Ni or Pb, for details see Table 3 which presents a general overview of reported water soluble trace metal concentrations in APM.

However, comparison of water soluble concentrations only is rather difficult since overall pollution levels differ from site to site. To overcome this influence it has become usual to determine water soluble and total element concentrations in APM, which enables the calculation of water-soluble fractions – defined as the concentration ratio of extractable and total metal concentration in percent. Table 4 gives a compilation of the results published for water soluble trace metal fractions in APM. Interpreting these results

it becomes obvious that even water soluble fractions show great variations from site to site. E.g. the percentage fraction of water-soluble Ni reported in literature varied from 45% to $89 \pm 7\%$ in PM2.5 samples collected from Edinburgh, UK [43] and Towson, USA [40] respectively. This outcome indicates that the transformation of natural or anthropogenic metal emissions into water-soluble species is affected by regional parameters such as APM composition, climate conditions (maritime or continental climate) or period of sample collection (e.g. summer or winter).

When we compare the results of bio-accessible trace metal fractions obtained via the use of buffer solutions or synthetic body fluids (for details see Table 5) similar findings were observed. The bio-accessible fractions of Pb varied from 29% to 85% in PM10 samples collected from Ottawa, Canada [27] and Puszta, Hungary [67] respectively. However, a comparison of results for synthetic gastric juice as reported by Falta et al. [34] and Mukhtar and Limbeck [31] in PM10 samples collected from Vienna, Austria and Styria, Austria respectively indicated comparable bio-accessible fractions of Co, Cu, Mn and Pb although total metal concentrations were distinctly different in these two cities. An expected outcome since both sampling sites were situated in Mid Europe with continental climate conditions, thus the transformation of metal emissions into soluble species should be similar.

Comparing the results presented in Tables 3–5 it becomes obvious that with the use of different leaching agents inconsistent amounts of bio-accessible trace metals fractions are obtained, an outcome which could be only partially explained with differences between the investigated sampling sites. For example, the maximum bio-accessible fraction of Cu obtained with the use of water

Table 4
Bio-accessible trace metal fractions in APM determined using water as leaching agent.

Authors	Graney et al.	Kyotani et al.	Qureshi et al.	Heal et al.		Fernandez et al.	Schneidemesser et al.	
Reference	[40]	[56]	[25]	[43] [a]		[61] [b]	[44]	
Sampling site	Towson, USA	Tokyo, Japan	New York, USA	Edinburgh, UK		Seville, Spain	Lahore, Pakistan	
Leaching agent	Water	Water	Water	Water		Water	Water	
Extraction time	90 min	20 min	90 min	60 min		3 h	???	
Temperature	40 °C	RT	70 °C	RT		RT	RT	
Unit	(%)	(%)	(%)	(%)		(%)	(%)	
APM fraction	PM2.5	Dust sample	PM2.5	PM10	PM2.5	PM0.61	PM10	PM2.5
Ag							1.8 ± 3.9	1.1 ± 2.2
Al	83 ± 13	67	11 ± 8				0.23 ± 0.3	1.5 ± 1.0
As			84 ± 1.0	60	64		25 ± 17	36 ± 23
Ba	63 ± 20						11 ± 3.4	30 ± 9.6
Ca		93	47 ± 14			27.1	60 ± 23	76 ± 15
Cd	100 ± 53		12 ± 7	74	75	24.9	60 ± 21	73 ± 17
Co	35 ± 11		73 ± 1.2			35.1	14 ± 5.1	22 ± 5.7
Cr			5 ± 4	14	28		3.7 ± 2.7	9.6 ± 6.6
Cu	68 ± 15			46	50	26.5	13 ± 7.3	13 ± 8.4
Fe		35	62 ± 1.1	6	10	3.7	0.44 ± 0.6	1.8 ± 1.3
K		99					41 ± 13	67 ± 15
La	48 ± 22						0.76 ± 0.6	3 ± 1.7
Li							13 ± 5.8	26 ± 9.5
Mg	86 ± 6	94	88 ± 1.2			10.1	16 ± 6.4	22 ± 5.7
Mn	89 ± 7		84 ± 1.0	40	45	32.5	31 ± 10	37 ± 9.4
Mo							30 ± 6.7	37 ± 8.5
Na		83					62 ± 13	79 ± 10
Ni	89 ± 7		64 ± 0.9	10	45	39.9	15 ± 8.2	38 ± 19
Pb	70 ± 23	32	88 ± 1.0	40	50	3.8	6.4 ± 4.6	23 ± 16
Rb	100 ± 46						18 ± 9.3	50 ± 15
Sb	94 ± 57		71 ± 0.8				34 ± 17	36 ± 18
Sc							0.15 ± 0.4	
Se			70 ± 0.8				73 ± 19	70 ± 19
Sn							5.7 ± 6.3	5.7 ± 5.3
Sr	84 ± 8						52 ± 16	65 ± 13
Ti	77 ± 32			4	14	7.7	0.10 ± 0.2	0.3 ± 0.2
Tl							49 ± 12	64 ± 7.7
V			88 ± 0.8	66	80	50.4	6.9 ± 7.4	19 ± 15
Zn	91 ± 6	95	90 ± 1.3	38	75		58 ± 26	65 ± 22

[a] Values taken for graphical presentation of results, therefore may be associated with some uncertainty, R.T. = room temperature, results for measurement with SRM as well as for some studies where it is not possible to interpret the bio-accessible contents are not presented.
[b] Only results derived for the first fraction of the applied fraction scheme are considered.

Table 5
Bio-accessible trace metal fractions in APM determined using salt solutions or synthetic body fluids as leaching agent.

Authors	Jianjun et al.	Mustafa et al.	Bikkes et al.		Falta et al.		Mukhtar et al.	Voutsa et al.
Reference	[27][a]	[69]	[66][a]		[34][a]		[31]	[51][a]
Sampling site	Ottawa, Canada	Erdemli, Turkey	K-Puszta, Hungary		Vienna, Austria		Styria, Austria	Thessaloniki, Greece
Leaching agent	0.01 M NH$_4$Ac	1 M NH$_4$Ac	1 M NH$_4$Ac		Synthetic gastric juice		Synthetic gastric juice	Simulating serum
Extraction time	2 h	15 min	15 min		2 h		60 min	60 min
Temperature	37 °C	RT	RT		37 °C		37 °C	RT
Unit	(%)	(%)	(%)		(%)		(%)	(%)
APM fraction	nm – μm PM	PM	PM10	PM1	PM10	PM2.5	PM10	PM7.2
Ag					60	58		
Al		3.9 ± 1.2	27	42				
As			25	13	–			
Ba							78 ± 23	
Ca								
Cd		91.7 ± 7.7	55	68	88	86		12
Co					44	44	38 ± 23	
Cr			30	27	82	18		
Cu	55	37.0 ± 4.4	44	14	74	78	80 ± 36	42
Fe	5	2.6 ± 1.1	3	11				
K								
La								
Li								
Mg								
Mn	60	52.9 ± 4.1	10	8	58	56	55 ± 21	52
Mo	26				38	44		
Na								
Ni			48	32	80	64	32 ± 14	45
Pb	29	84.9 ± 10.5	85	62	84	82	97 ± 36	5
Rb								
Sb					38	54		
Sc								
Se								
Sn	11				18	24		
Sr								
Ti					6	2		
Tl					78	82		
V	41							
Zn	82	65.6 ± 8.6	82	43	72	74		40

[a] Values taken for graphical presentation of results, therefore may be associated with some uncertainty, R.T. = room temperature, Results for measurement with SRM as well as for some studies where it is not possible to interpret the bio-accessible contents are not presented.

as leaching agent was found to be 68 ± 15% in PM10 samples collected from Towson, USA [40] whereas with the use of ammonium acetate buffer an average value of only 55% was observed in PM10 samples collected from Ottawa, Canada [27]. Highest results for bio-accessible fractions of Cu in PM10 have been reported for samples from Styria, Austria which yielded on average 80% with synthetic gastric juice as leaching agent. Although this outcome is also attributed to differences in the total APM concentrations and the climate conditions of the investigated sampling sites, it reflects also the nature of the applied leaching agents. Water is considered as simplest solvent, whereas synthetic gastric juice contains organic constituents and possesses more acidic pH as compared to water, thus higher yield of bio-accessible trace metal fractions is expected with the use of synthetic gastric juice. Due to differences in sample pH and salinity a similar effect could be expected for ammonium acetate buffer, but the findings reported for bio-accessible fractions are lower than for water as leaching agent, which hints to great differences in the composition and transformation of APM at the compared sampling sites. In the case of Mn, the obtained bio-accessible fractions were reported to be 84 ± 1.0% in PM2.5 samples collected from New York, USA with the use of water as leaching agent [25], whereas only 56% of Mn was obtained with the use of synthetic gastric juice in PM2.5 samples collected from Vienna, Austria [34]. In the case of PM10 samples comparable bio-accessible fractions of Mn were obtained with the use of acetate buffer (60%) [27] and synthetic gastric juice (58%) [34], whereas with water as leaching agent a reduced value of 40% was found [43]. Almost identical results were observed for lead, with the use of water as leaching agent a bio-accessible fraction of Pb

88% was reported for PM10 samples collected in New York, USA [25], application of acetate buffer delivered a result of 85% for PM10 samples from Puszta, Hungary [67]. Comparatively higher fractions of bio-accessible Pb (97%) were obtained with the use of synthetic gastric acid in PM10 samples collected from Styria, Austria [31]. Similar findings were obtained for Zn, with an average water soluble fraction of 84% in PM10 samples collected from various sites in Austria [82] and approximately 82% for samples collected in Hungary using acetate buffer as leaching agent [67]. Again with the use of synthetic gastric juice reduced values of 72% only were found for bio-accessible Zn in PM10 samples from Vienna, Austria [34]. In the case of PM2.5 samples comparable results were found, the bio-accessible Zn obtained with the use of water was found to be 91% [40], whereas with synthetic gastric juice only 74% of the Zn content from samples collected in Vienna, Austria were determined as bio-accessible [34].

5. Conclusion

Interpreting literature data it is obvious that the results reported for bio-accessible fractions of different metals vary greatly from study to study. Furthermore differences in the behavior of the investigated elements were observed, for some elements the use of various leaching agents revealed distinct differences in the final results for bio-accessible fractions, whereas for other elements more or less no influence was observed. This outcome indicates that the chemical forms of the elements present in APM play a fundamental role in the determination of the bio-accessible fraction, for species which could easily be dissolved the chemical properties

of the leaching agent are only of minor importance, whereas for less soluble species a dependence of leachability from sample pH, salinity, etc. could be expected. Thus noticeable deviations between individual studies could be explained with differences in the applied leaching agents, since their extraction efficiencies differ with variations in sample pH, salinity and presence of complexing agents. But variations in the main emission sources (e.g. traffic, industry, civil population, etc.) and thus composition of APM samples as well as differences in meteorology (e.g. climate conditions, date of sample collection e.g. winter versus summer) of the sampling sites must also be considered, since these parameters influence the presence of soluble metal species in APM. The effect of varying leaching agents is enforced by apparent variations in extraction time and temperature, parameters which were known to influence the leaching process. Thus the lack of uniformity in the determination of bio-accessible metal contents in APM disables inter-comparison of results from different sampling sites. For that reason, it is highly needed to propose a standard protocol for assessment of bio-accessible trace metal fractions in APM, which defines variable parameters such as composition of leaching agent or operating conditions like extraction time and temperature.

Furthermore, most of the studies dealing with estimation of bio-accessible metal in APM involve batch-wise extraction procedures, which were performed under equilibrium conditions and thus provide no information about the kinetics of the leaching process. Another drawback of this approach is the re-adsorption and redistribution of dissolved metals among remaining solid APM constituents, causing an underestimation of the bio-accessible metal fraction in APM. To overcome these disadvantages in the field of soil chemistry dynamic flow through approaches were used, which allow sample extraction with a continuous flow of leaching agent, and thus to ascertain better the bio-accessibility of trace metals in solid environmental matrices since natural processes occur normally under non steady-state conditions. However, for the investigation of APM samples until now only two groups reported such dynamic approaches. Thus research should be focused on the further improvement of these techniques, additionally similarities and discrepancies between batch-wise and kinetic leaching methods should be investigated thoroughly for APM samples.

Beside the problems associated with sample extraction the determination of bio-accessible trace metal fractions in APM is also an analytical challenge, since accurate analysis is hampered by small sample amounts and the presence of highly complex sample matrix mainly derived from the used leaching agents. These factors can cause matrix interferences and insufficient sensitivity if standardized routine procedures with ET-AAS, ICP-OES or ICP-MS detection were used for analysis. Thus the use of inadequate analytical procedures might also contribute to the observed variations for bio-accessible trace metal fractions in APM. To overcome the limitations of routine methods improved procedures were developed, applying special strategies for sample introduction, separation of interferences or signal quantification. The use of a dynamic reaction cell has recently been published as an example for an instrumental approach to overcome matrix based interferences in ICP-MS analysis. Electro-thermal-vaporization (ETV) has been shown to be a successful technique for the separation of organic sample matrix prior to ICP-OES measurement. Alternatively common sample procedures such as solid phase extraction could be applied to remove interfering sample constituents. However, there is still room for improvement in particular regarding accuracy of applied procedures. For this purpose a standard reference material with defined contents of bio-accessible trace metal fractions is needed, which could be used for validation of developed procedures. A first step in this direction could be the characterization of soluble metal fractions in NIST 1648a, which has been reported by some groups. Nevertheless ongoing research work should be focused

on the production of a certified reference material which could be used for validation of different procedures. Thus the quality of published results could be improved, which finally facilitates data comparison and interpretation, enabling the collection of new or additional knowledge in this research field.

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