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Chapter 31

Arsenic speciation in soils: An analytical challenge for understanding arsenic biogeochemistry

Guangliang Liu and Yong Cai

Abstract

The mobility, fate, bioavailability, and overall biogeochemical cycling of arsenic in soil depend on the individual form in which arsenic exists as well as physical and chemical characteristics of the soil. Speciation analysis of arsenic poses a great challenge and is the key to understand the biogeochemistry of arsenic in soils and sediments. The speciation of arsenic in soil may be defined functionally (e.g., bioavailable fraction), operationally, or chemical species-specifically. The extraction of arsenic species from solid matrices generally constitutes a critical step for speciation analysis of arsenic in soils and sediments. Various sequential extraction procedures have been developed to extract operationally defined arsenic species such as water soluble, exchangeable, Fe/Mn oxides associated, organic matter bound, and residual arsenic fractions. Several extractants have been used to leach chemically specific arsenic species, mainly arsenite, arsenate, monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) from a solid matrix. Efforts have been made to preserve the integrity of arsenic species during extraction. In this paper, extraction procedures with emphasis on the selection of extractants for chemical-species specifically and operationally defined arsenic species were reviewed. In addition, extraction method for evaluating bioavailable arsenic and spectroscopic technique for directly measuring arsenic species in soils were briefly discussed.

31.1. Introduction

31.1.1. Arsenic in the environment

Arsenic is the 20th most abundant element found in the Earth's crust and is ubiquitously distributed in soils, rocks, water, air, plants, and animals

(Cullen and Reimer, 1989; Cotton et al., 1999). It exists in inorganic and organic forms and in different oxidation states (−3, 0, +3, +5) (Frankenberger, 2002; Meng et al., 2003; Watt and Le, 2003). In recent years, there has been a renewed interest in the biogeochemistry of arsenic because of the frequently reported health problems associated with exposure to some toxic arsenic compounds worldwide.

The sources of arsenic in the environment include both natural and anthropogenic. More than 200 different arsenic mineral forms exist in nature, among them about 60% are arsenates, 20% sulfides and sulfosalts, and the remaining 20% include arsenides, arsenites, oxides, silicates, and elemental arsenic (As) (Onishi, 1969). The total arsenic amount in the earth's crust is estimated to be 4.01×10^{16} kg, and the natural arsenic liberation from the lithosphere into the exogenic cycle is about 1.715×10^{7} kg/year, mainly from volcanic exhalations and eruptions. About 4.87×10^{6} kg/year is added due to the submarine volcanism (Matschullat, 2002). Other natural activities that can release arsenic into the environment include erosion of rocks and forest fires (Smedley and Kinniburgh, 2002).

Arsenic compounds have been used in such fields as agriculture, metallurgy, and industry (Zhang et al. 1996; Mandal and Suzuki, 2002). These anthropogenic arsenic sources include insecticides, herbicides, desiccants, wood preservatives, high-temperature combustion (oil and coal burning power plants or waste incineration or cement works), mining process of metals, wastes from intense husbandry (disinfectants), glass ware production (discoloring agent), and electronics industries (admixture in semiconductor production, arsenide as laser material to convert electrical energy into coherent light) (Matschullat, 2002; Smedley and Kinniburgh, 2002). It was estimated that anthropogenic levels of arsenic exceeded natural levels in the environment at a ratio of 3 to 1 (Woolson, 1983).

31.1.2. Arsenic speciation and biogeochemistry

Arsenic is present in environmental and biological systems in a number of forms and species. A number of physiochemical and biological factors can affect arsenic speciation in the environment. It has been found that nearly two dozen arsenic species including organic and inorganic forms are present in the environmental and biological systems (Gong et al., 2002) despite the fact that arsenic is mostly present in its inorganic forms of arsenite (As^{III}) and arsenate (As^{V}) as a component in minerals. The two inorganic arsenic oxyanions, arsenite and arsenate, are widely distributed in the environment and are the major arsenic species found in

most water, soil, and sediment samples. Monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine, arsenocholine, and arsenosugars have been found as common organic arsenic compounds in environmental and biological samples. In many biological systems, arsenic is present mainly in organic forms such as arsenobetaine and arsenocholine. For instance, arsenobetaine exists widely in higher organisms including both invertebrates and vertebrates (Phillips, 1994; Edmonds and Francesconi, 2003; Kuehnelt and Goessler, 2003; 2002) and is by far the major form of arsenic in marine animals, often representing 80% of the extractable arsenic with concentrations ranging from 1–300 $\mu g\, g^{-1}$ dry mass (Francesconi and Edmonds, 1997; Francesconi and Kuehnelt, 2002).

In soils and sediments, arsenic is mainly present in inorganic forms, namely arsenite and arsenate. Although arsenate is the thermodynamically favored form under normal environmental conditions, transformation between arsenite and arsenate can easily occur in the presence of certain microorganisms under both aerobic and anaerobic conditions. As a result, it is likely inaccurate to predict the predominant form of arsenic in soils only based on redox potential in the specific environment. MMA and DMA are major organic arsenic compounds in soils and sediments, but they are generally present in much lower concentrations compared to arsenite and arsenate (Demesmay and Olle, 1997; Kuehnelt and Goessler, 2003). These methylated species are probably produced through microorganisms-mediated oxidation–reduction reactions and can also be demethylated to inorganic arsenic species under specific conditions (Garcia-Manyes et al., 2002; Craig et al., 2003; Kuehnelt and Goessler, 2003). These two organic species, as well as arsenite and arsenate, play important roles in the biogeochemical cycling of arsenic in soil and sediment environment.

The environmental fate, bioavailability, and toxicity of arsenic vary dramatically with the chemical forms of arsenic. The two inorganic forms of arsenic were previously known to be much more toxic than their organic forms and arsenite has higher toxicity than arsenate (Jain and Ali, 2000). Recent studies, however, suggested that monomethylarsinous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}) were at least as toxic as, if not so more than, arsenite (Lin et al., 2001; Mass et al., 2001; Petrick et al., 2001; Le et al., 2000, 2004). Dimethylarsinic acid (DMA^{V}), once viewed simply as a detoxification product of inorganic arsenic, has been proven to be a complete carcinogen in the rat urinary bladder (Wei et al., 1999; Kenyon and Hughes, 2001). It is believed that arsenite is more mobile than arsenate in most environmental conditions, suggesting that arsenite in soils is subject to leaching into soil solution and thus is able to

move longer distances both in horizontal and vertical directions compared with arsenate. The knowledge of the chemical forms of arsenic is critical for the understanding of arsenic mobilization to the aqueous phase in equilibrium with the soils or sediments and for the assessment of environmental impacts of arsenic. Frequently the lack of the speciation information is the major limitation to our understanding of the biogeochemical cycling of arsenic. Therefore, it has become very important and challenging to accurately quantify the amount of each form of arsenic that is present in the environment through speciation analysis.

31.1.3. Arsenic speciation analysis

Speciation analysis of an element has been defined by Florence (1982) as the determination of the concentrations of the individual physico-chemical forms of the element in a sample that together, constitute its total concentration. Speciation analysis involves a complex scheme of operations. Numerous publications concerned with speciation analysis of an element have appeared. Depending on the research interests, this term has been used in a number of different ways in such fields as environmental chemistry, biogeochemistry, biochemistry, and environmental toxicology. In a recent attempt to end the present confusion regarding the usage of the term speciation (analysis), IUPAC has defined speciation analysis as analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a particular system (Templeton et al., 2000). This definition confines speciation to chemical species that refer to isotopic composition, electronic or oxidation state, and/or complex or molecular structure. Such activities as classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties are defined as fractionation. The fractionation was often called speciation and regarded as a type of speciation analysis (e.g., Van Herreweghe et al., 2003). The following discussion adopted this approach and defined fractionation as a part of speciation analysis.

Arsenic speciation analysis of soils has been carried out in three different ways which can be defined as: chemical species-specifically, operationally, and functionally. The chemical species-specifically defined speciation analysis involves various levels of structure in the IUPAC definition including electronic or oxidation state, inorganic compounds and complexes, organoarsenic compounds, and organic and macromolecular complexes. This is the most common type of arsenic speciation. Various inorganic and organic arsenic compounds may be further combined with inorganic (e.g., Fe and Mn oxides) or organic ligands

(e.g., thiol and humic substances) to produce coordination complexes with different degrees of thermodynamic stability and lability (Templeton et al., 2000; Gong et al., 2002). The operationally defined speciation analysis differentiates these arsenic species according to the extractants used. For example, arsenic species can be categorized into water soluble, exchangeable, Fe/Mn oxides associated, organic matter bound, and residual fractions or mobile and mobilizable fractions. The functionally defined speciation analysis has recently been used to extract and evaluate the bioaccessible arsenic fraction, i.e., the fraction that dissolves in the gastrointestinal tract and is available to be absorbed into the blood (bioavailable). From the point of view of biogeochemistry, the most important structure levels of arsenic speciation are arsenic compounds with different oxidation states and chemical bonds, those physically distinguished forms present with different molecular sizes (particulate, colloidal, and dissolved phases), and those with different operationally defined forms (Tessier et al., 1979; Cai, 2003).

31.2. Arsenic speciation analysis in soil

The operationally defined as well as chemical species-specifically defined speciation analyses of arsenic in soil have been extensively studied whereas few studies have dealt with functionally defined arsenic species. Different sequential extraction procedures (SEP) have been developed for the operationally defined speciation analysis of arsenic for the purpose of assessing mobility of arsenic. Meanwhile, several sets of extraction and analysis procedures for chemical specifically defined arsenic species in soil, mainly arsenite, arsenate, MMA and DMA, have also been studied. All these studies, however, had limited success. For operationally defined speciation analysis, the specificity and the reproducibility of SEP were usually questionable. For chemical specifically defined speciation analysis, analytical protocols are not available to date to achieve satisfactory recoveries as well as avoidance of species transformation during extraction steps.

Accurate measurement of arsenic species in soil is determined by a number of factors (Loeppert et al., 2003). Most important of these is the selection of suitable extraction procedures that can selectively extract/release target arsenic species (different phase association forms or oxidation states) from the solid matrix while at the same time protecting the original integrity of arsenic species. There is a large number of suitable methods for the identification of the dissolved arsenic compounds; these include: hydride generation (HG) atomic fluorescence or absorbance

spectrometry (AFS or AAS) and high performance liquid chromatography (HPLC) coupled with inductively coupled plasma mass spectrometry (ICPMS) or AFS. Combinations of other chromatographic separation with spectrometric detection have also been utilized to arsenic speciation analysis although to a lesser extent. Details in instrumentation for arsenic speciation analysis were previously reviewed (Guerin et al., 1999; Vilanó et al., 2000; Gong et al., 2002) and therefore are not discussed further in this paper.

The extraction of arsenic species constitutes a critical step for speciation analysis of arsenic in soils and sediments. This is true for chemical species-specifically defined as well as operationally defined speciation analyses. There are some exceptions in cases where direct spectroscopic techniques have been applied to determine arsenic speciation. Sequential extraction procedures are usually adopted to differentiate operationally defined arsenic species while simple extraction using a selective extractant for arsenite or arsenate is applied to distinguish arsenic species with different oxidation states. Both extraction procedures are generally done by mechanical shaking. Recently, these extraction procedures have been simplified by introducing ultrasonic or microwave-assisted extraction techniques (Thomas et al., 1997). In the following text, direct spectroscopic speciation techniques are briefly introduced and then extraction procedures with the emphasis on the selection of extractants for chemical species-specifically and operationally defined arsenic species are reviewed in detail.

31.2.1. Direct arsenic speciation in soil using spectroscopic techniques

The identification of arsenic-bearing phases in solid samples, especially in minerals or mineral waste, has been attempted using spectroscopic techniques without sample extraction. The commonly used instruments include extended X-ray absorption fine-structure spectroscopy (EXAFS) (Foster et al., 1998; Savage et al., 2000), scanning electron microscope-energy dispersive spectrometer (SEM-EDS), X-ray powder diffraction (XRD) (Jones et al., 1997; Juillot et al., 1999), and electron microprobe analysis (Davis et al., 1996). These direct spectroscopic techniques, however, are limited by their sensitivity for many real environmental samples.

By using EXAFS for arsenic analysis in mine tailings, Foster et al. (1998) observed the formation of scorodite ($FeAsO_4 \cdot 2H_2O$) in mining wastes and also suggested that arsenates could be substituted by sulfates in the crystalline structure of jarosite ($KFe_3(SO_4)_2(OH)_6$). Savage et al. (2000) recently confirmed this substitution by using the same technique. By using electron microprobe analysis, Davis et al. (1996) observed the

formation of metal arsenic oxides, iron–arsenic oxide similar to scorodite ($FeAsO_4 \cdot 2H_2O$) and arsenic phosphates such as $FeAlPbAs(PO_4)(SO_4)$ in smelter-impacted soils.

In addition to studying arsenic-bearing phase in minerals, direct spectroscopic techniques have also been used to determine arsenic oxidation state and coordination environment of arsenic-metal oxides complex in soils. A shift in the X-ray absorption near edge structure (XANES) spectra to a higher energy (e.g., from ~11,871 to ~11,874 eV) can be an indication of oxidation of As^{III} to As^{V} (Manning et al., 2003; Smith et al., 2005). EXAFS is another method to differentiate As^{V} from As^{III}. This can be done by comparing the coordination number (N) and interatomic distance (R_{As-O}) of the As–O shell, a shell of oxygen atoms surrounding the As atom which is formed when As is associated with metal oxides in soils. In theory, there are three O atoms surrounding As for As^{III} ($N = 3$) while four O atoms for As^{V} ($N = 4$). It was known by EXAFS that R_{As-O} values for As^{III} and As^{V} were approximately 1.79 Å and 1.69 Å, respectively (Fendorf et al., 1997; Foster et al., 1998; Manning et al., 1998). By fitting adjustable parameters of the theoretical EXAFS function to the experimental data, Manning et al. (2003) obtained an N value of 3.54 at a distance (R_{As-O}) of 1.71–1.72 Å for Aiken clay and Wyo loam soil samples fortified with 20 ml of 67 μM As^{III} to 2 g soil. The obtained N and R_{As-O} values provided evidences that As^{III} initially spiked has been partially oxidized to As^{V} so that a mixture of As^{III} and As^{V} was observed in these soils. Also in this study, a second shell of Fe atoms (As–Fe shell) further away from the As–O shell was observed, indicating that arsenic was predominantly combined to Fe oxide surface. In addition to X-ray absorption spectroscopy (XANES and EXAFS), the energy dispersive X-ray microanalysis (EDXMA) was also used to investigate association forms of arsenic in solid phase. Using EDXMA technique combined with SEP, Lombi et al. (2000) confirmed that 0.2 M NH_4-oxalate and 0.2 M NH_4-oxalate buffer + ascorbic acid could extract amorphous and poorly crystalline hydrous oxides of Fe and Al associated and well-crystallized hydrous oxides of Fe and Al bound arsenic fractions from five Cambisol soils in Austria, respectively.

31.2.2. Sequential extraction

The sequential extraction technique used for studying solid-phase association of elements, originally proposed by Tessier et al. (1979) and modified by many authors (e.g., Lum and Edgar, 1983; Voigt et al., 1996; Roussel et al., 2000), has been extensively applied to metals and metalloids. This procedure involves sequential chemical extractions separating

trace metals into five fractions: exchangeable, bound to carbonates, bound to Fe–Mn oxides, bound to organic matter, and residual. Simplified sequential extraction methods have also been developed for practical applications (Sahuquillo et al., 1999, 2003; Quevauviller, 2002). For example, the three-level evaluation system, which separates metals into mobile, mobilizable, and pseudo total metal fractions, seems to be attractive because of its simplicity and potential application in risk assessment and risk management (Gupta, 1996; Cai et al., 2002).

Sequential extraction procedures provide operational definition of the metal phases one is trying to measure. Strictly speaking, the measurements performed by sequential extractions correspond to fractionation studies and not to real speciation measurements. Sequential extraction was developed to evaluate the lability and availability of trace metal in real environment, but the physicochemical conditions used during extraction procedures (strong reagents and short extraction time) did not exactly coincide the naturally occurring processes (weak reagents and slow kinetics). Additionally, the extractants used in each extraction step are not specifically selective for the target phase of trace metal. It has been observed that same extractants could extract different phase of metal species when applied to different samples. The validation of sequential extraction protocols are hard to achieve, though several attempts have been made to do so (Rendell et al., 1980; Tipping et al., 1985; Kheboian and Bauer, 1987; Martin et al., 1987). The specificity and reproducibility of the method greatly depend upon the chemical properties of the element and the chemical composition of the samples. Accordingly, these methods provide, at best, a gradient for the physicochemical association strength between trace elements and solid particles rather than their actual speciation (Nirel and Morel, 1990).

Despite these inherent limitations, sequential extraction procedures have been extensively applied to assess the mobility, environmental fate and behavior, and environmental risk of trace metals in soils and sediments. Some useful information has been obtained in the fields of environmental chemistry and geochemistry through extensive individual validation and consideration of particular conditions. Most of the sequential extraction procedures are developed for metal cations (Martin et al., 1987; Hirner, 1992; Shan and Chen, 1993; Ngiam and Lim, 2001; Gleyzes et al., 2002), but recently speciation studies dealing with oxyanions such as arsenate (Gruebel et al., 1988; Keon et al., 2001; Fedotov et al., 2005) have been considerably extended. Table 31.1 summarizes some commonly used sequential procedures for arsenic speciation in soils and sediments.

Table 31.1. Sequential extraction protocols used for speciation analysis of arsenic in soil

Extractants	As fraction	Reference
1 M $MgCl_2$	Ionically bound As	Keon et al. (2001)
1 M NaH_2PO_4	Strongly adsorbed As	
1 N HCl	As coprecipitated with AVS, carbonates, Mn oxides, and very amorphous Fe oxyhydroxides	
0.2 M ammonium oxalate/ oxalic acid	As coprecipitated with amorphous Fe oxyhydroxides	
0.05 M Ti^{III}-citrate-EDTA-bicarbonate	As coprecipitated with crystalline Fe oxyhydroxides	
10 M HF	As oxides and As coprecipitated with silicates	
16 N HNO_3	As coprecipitated with pyrite and amorphous As_2S_3	
16 N HNO_3 + 30% H_2O_2	Orpiment and remaining recalcitrant As minerals	
$(NH_4)_2SO_4$ (0.05 M)	Non-specifically sorbed	Wenzel, et al. (2001); Fedotov, et al. (2005)
$(NH_4)H_2PO_4$ (0.05 M)	Specifically sorbed	
NH_4-oxalate buffer (0.2 M)	Amorphous Fe and Al oxides bound	
NH_4-oxalate buffer (0.2 M) + ascorbic acid (0.1 M)	Crystalline Fe and Al oxides bound	
HNO_3/H_2O_2	Residual	
NH_4Cl (1 M)	Soluble	Jones et al. (1997)
NaOH (0.1 M)	Non-occluded Fe/Al oxides	
Citrate-bicarbonate (0.3 M)	Amorphous Fe	
Citrate-dithionite-bicarbonate (0.3 M)	Reducible Fe	
HCl (1 M)	Ca-associated	
30% H_2O_2 + 0.8 M NH_4OAC	Organic matter	
Acids	Residual	
Anion-exchange resin	Exchangeable	McLaren et al. (1998)
$NaHCO_3$ (0.5 M)	Easily labile	
NaOH (0.1 M)	Adsorbed on Al/Fe	
HCl (1 M)	Ca-associated	
Aqua regia	Recalcitrant As	
Ultrapure water	Water soluble	Shiowatana et al. (2001)
$NaHCO_3$ (0.5 M)	Surface adsorbed	
NaOH (0.1 M)	Fe- and Al-associated	
HCl (1 M)	Carbonate-bound	
HNO_3-HF (1:1)	Residual	
Water	Water soluble	Van Herreweghe et al. (2003)
Anion exchange membrane strips	Exchangeable anions	

Table 31.1. (*Continued*)

Extractants	As fraction	Reference
NH_4F (0.5 M)	NH_4F-extractable	
NaOH (0.1 M)	NaOH-extractable	
Sodium citrate (0.5 M) + $NaHCO_3$ (1 M) + $Na_2S_2O_4$	Reducible	
HNO_3/H_2O_2	Oxidizable	
$HCl + HNO_3 + HF$	Residual	
HCH_3COO (0.1 M)	Exchangeable	Sahuquillo et al. (1999, 2003); Quevauviller (2002)
$NH_2OH \cdot HCl$ (0.5 M)	Reducible	
NH_4CH_3COO/ H_2O_2	Oxidizable	
$NaNO_3$ (0.1 M)	Mobile	Cai et al. (2002)
KH_2PO_4 (0.1 M)	Mobilizable	
HNO_3/H_2O_2	Residual	

The sequential extraction procedures listed in Table 31.1 can be divided into three categories: traditional phase-association scheme, BCR (the Community Bureau of Reference) scheme, and three-level differential system (mobile, mobilizable, and residual). Traditional phase-association sequential extraction scheme differentiates arsenic species based on the classification system of Tessier et al. (1979) by choosing extraction reagents commonly used for metals, Se or P. For instance, based on the chemical similarity of P and As, modified versions of the sequential procedures for P (Chang and Jackson, 1957) have been adopted for As (Woolson et al., 1973). The extraction reagents in this scheme include NH_4Cl, NH_4F, NaOH, and H_2SO_4, corresponding to easily exchangeable, and Al-, Fe-, and Ca-associated As. However, the adaptability of extraction steps from SEP for P and Se is questionable when they are used for As fractionation. Some extractants that have been used in SEPs for P and Se, including NH_4NO_3, NaOAc, $NH_2OH \cdot HCl$, EDTA, NH_4OH, and NH_4F, were shown to either have only low extraction efficiency for As, or to be insufficiently selective or specific for the phases targeted. This was possibly caused by re-adsorption on other mineral phases during reductive and oxidative dissolution of As from a certain mineral phase as well as subsequent desorption of As in the next extraction step (Gruebel et al., 1988).

In order to overcome these limitations, Wenzel et al. (2001) integrated the Chang and Jackson (1957) procedure for P, the Saeki and Matsumoto (1994) procedure for Se, and the Han and Banin (1995) approach to

extract carbonates-associated metal fractions into a five-step SEP for As. In this scheme, 0.05 M $(NH_4)_2SO_4$, 0.05 M $NH_4H_2PO_4$, 0.2 M NH_4-oxalate, 0.2 M NH_4-oxalate buffer + ascorbic acid, and HNO_3/H_2O_2, were sequentially used to extract non-specifically sorbed, specifically sorbed, amorphous and poorly crystalline hydrous oxides of Fe and Al associated, well-crystallized hydrous oxides of Fe and Al bound, and residual arsenic fractions. The results of repeatability and recovery tests suggested that this SEP was dependable. The application of this SEP in 20 soils showed a partitioning pattern of As among these five fractions as follows: amorphous and poorly crystalline hydrous oxides of Fe and Al associated (42.3%) > well-crystallized hydrous oxides of Fe and Al bound (29.2%) > residual (17.5%) > specifically sorbed (9.5%) > non-specifically sorbed (0.24%) (Wenzel et al, 2001).

The lack of uniformity in a number of SEP protocols resulted in little comparability amongst the data obtained with different schemes. In order to achieve comparability among various studies and among different laboratories, the European Commission attempted to establish well-defined and standardized sequential extraction schemes for assessing lability of trace metals, including arsenic in soil (Quevauviller, 1998a, 1998b, 2002). A three-step extraction scheme (acetic acid—step 1; hydroxylammonium chloride—step 2; and, hydrogen peroxide/ammonium acetate—step 3) has been developed and improved by the addition of a fixed volume of dilute nitric acid into hydroxylamine hydrochloride to adjust pH to 1.5 in Step 2. Corresponding with these three steps, exchangeable (associated mainly with carbonates), reducible (associated with Fe/Mn oxides), and oxidizable (associated with organic matter) metal fractions were extracted, respectively. Speciation of these three metal fractions was known to be a good estimation of extractable metal in soils (Sahuquillo et al., 2003). This three-step sequential extraction protocol has been used for developing the sediment certified reference material (CRM 601) (Quevauviller et al., 1997; Sahuquillo et al., 1999). The so-called BCR scheme now appears to be internationally recognized as a reference method for soil and sediment studies (Davidson et al., 1998; Martin et al., 1998; Gómez Ariza et al., 2000; Tokalioglu et al., 2000; Pérez Cid et al., 2001), and this is supported by the increasing number of scientific papers referring to this protocol.

In order to simplify the sequential extraction method and make it easy to use for practical application, the protocol for separating metals into mobile, mobilizable, and pseudo total metal fractions, has also been widely used (Gupta et al., 1996). In this scheme, the mobile fraction was generally extracted by neutral unbuffered salt solutions, such as $NaNO_3$, $CaCl_2$, and NH_4NO_3, whereas buffered and unbuffered complexing and

chelating reagents like EDTA, DTPA + $CaCl_2$, and acetic acid have been proposed as extraction media for the mobilizable fraction. This simple sequential extraction procedure has been adopted to evaluate the mobility of arsenic in South Florida golf course soils by extracting mobile arsenic fraction with 0.1 M $NaNO_3$ and mobilizable arsenic with 0.1 M KH_2PO_4, respectively (Cai et al., 2002).

31.2.3. Extraction of arsenate and arsenite

Similar to arsenic speciation using SEP, difficulties in chemical specifically defined speciation analysis of arsenic in soils lie in the choice of the extraction media. A desirable extraction method has to quantitatively extract all arsenic species and to preserve the identity of the native species in the samples during extraction process. Moreover, the arsenic species in the soil extracts should be stable until analysis and the solvent used to extract samples should not interfere with the species analysis. The stability of arsenic species depends on a number of factors, such as the pH value and chemical composition of the extraction media, and soil properties (Wang et al., 1995). For example, Pantsar-Kallio and Manninen (1997) investigated the stability of dissolved As^{III} and As^{V} in soil extracts obtained using $NaNO_3$, K_2SO_4, $NaHCO_3$, and Na_2CO_3, in the pH range of 1 to 12. It was found that As^{V} was stable in the whole pH range whereas As^{III} was converted to As^{V} at a pH value of 1 and 12.

A number of studies have investigated the arsenic speciation involving As^{III} and As^{V} in soil and sediment samples using a variety of solvent extraction techniques (Chappell et al., 1995; Manning and Martens, 1997; Thomas et al., 1997; Pongratz, 1998; Ellwood and Maher, 2003; Pizarro et al., 2003). However, the results do not always reflect the original arsenic speciation status from the samples studied due to possible conversion between arsenic species, especially oxidation of As^{III} to As^{V} during the extraction process (Wang et al., 1995; Pantsar-Kallio and Manninen, 1997; Ellwood and Maher, 2003; Georgiadis, 2004). A few studies have shown limited success in preserving the original arsenic redox integrity during extraction of different arsenic species. Commonly used extraction methods for speciation analysis of As^{III}, As^{V}, MMA, and DMA are illustrated in Table 31.2 and some representative procedures were taken as examples and further discussed in detail in the following text.

It is worthy noting that no suitable CRMs of the soils and sediments are nowadays available for validating As speciation in such materials (Pongratz, 1998). Reference materials certified for total As content were therefore used for speciation analysis to validate extraction methods and to intercompare the results with other laboratories. The validation and

Table 31.2. Extractants used for speciation analysis of arsenic in soil

Extractants	Extracting method	Sample type	Arsenic species targeted	Recovery for total arsenic (%)	Reference
Hydroxylammonium hydrochloride	Occasional shaking at 95°C for 8 h	Estuarine sediments	As^{V}, As^{III}	98.2[a]	Gómez-Ariza et al. (1998)
		CRM 320	As^{V}, As^{III}	67.0[b]	
Orthophosphoric acid	Microwave irradiation at 40 W for 20 min	CRM 320	As^{V}, As^{III},	93.6–96.5[b]	Gallardo et al. (2001)
		SRM 2709	As^{V}, As^{III},	37.1–61.6[b]	
		CRM 007-040	As^{V}, As^{III}, MMAA, DMAA	92.2–99.1[b]	
		Indonesian soil/ sediment	As^{V}, As^{III}, MMAA[c], DMAA[c]	72.5–96.6[a]	
		CRM 320	As^{V}, As^{III},	81.9[b]	Thomas et al. (1997)
		CRM 141	As^{V}	50.5[b]	
		IAEA Soil-7	As^{V}	86.6[b]	
		In-house soil	As^{V}	81.0[a]	
		In-house sediment	As^{V}, As^{III}	99.8[a]	
	Heated at 150 °C for 3 h	Contaminated soil	As^{V}, As^{III}, MMA, DMA	82	Pizarro et al., 2003
Phosphoric acid and ascorbic acid	Microwave irradiation at 20–60 W for 10 min	GBW07405	As^{V}, As^{III}, MMA	81.7[b]	Garcia-Manyes et al. (2002)
		GBW07311	As^{V}, As^{III},	101[b]	
		BCR 320	As^{V}, As^{III},	95.8[b]	
		Soil	As^{V}, As^{III}, MMA[c], DMA[c]	56.5–101[a]	

Table 31.2. (*Continued*)

Extractants	Extracting method	Sample type	Arsenic species targeted	Recovery for total arsenic (%)	Reference
Phosphoric acid and hydroxylamine hydrochloride	Agitated on a mixing wheel for 1 h	BCSS-1 NIST 1646 PACS-1 NIST 1941	As^{V}, As^{III},	32–72	Ellwood and Maher (2003)
KH_2PO_4 and K_2HPO_4	Shaken on a reciprocating shaker for 2 h	MESS-1 PACS-1 NIST 1646 NIST 1633b	As^{V}, As^{III},	<5	Manning and Martens (1997)
Ammonium oxalate	Agitating at room temperature for 1 h	Contaminated soil	As^{V}	0.04–20[a]	Bissen and Frimmel (2000)
Sodium carbonate			As^{V}	0.04–20[a]	
Sodium bicarbonate			As^{V}	0.04–20[a]	
hydrochloric acid—chloroform water	Shaken vigorously for 30 min	Soil	As^{V}, As^{III}, organic As	85	Chappell et al. (1995)
10 mM phosphate + 0.5% NaDDC	Shaken at 300 rpm on a orbital shaker for 1–24 h	PACS-2 Everglades soil	As^{V}, As^{III}	7	Georgiadis (2004); Georgiadis et al. (2006)

[a]Recoveries are calculated against total arsenic concentration obtained through strong acid (e.g., nitric acid) digestion procedure.
[b]Recoveries are calculated against certified value of total arsenic concentration.
[c]MMA or DMA were detected only in few cases for real soil/sediment samples.

intercomparisons are questionable, at least in some cases, because the speciation of As in reference materials may have been modified by the rather energetic treatments to which these samples have been submitted during their preparation (Gallardo et al., 2001).

Hydrochloric acid was a reagent used for the extraction of As^{III} and As^{V} from soil in earlier studies for speciation (Chappell et al., 1995). The strong acidic condition used is probably too aggressive and is suspected to lead to the alternation of arsenic species (Gong et al., 2000; Gallardo et al., 2001). In order to avoid species changing, some weak extractants such as phosphate, acetate, citrate, and oxalate buffers have been proposed for the selective extraction of As^{III}, As^{V}, MMA, and DMA (Bissen and Frimmel, 2000; Shi et al., 2003; Georgiadis et al., 2006).

In a study of evaluating the mobility and differentiating the chemical species of arsenic, an extraction procedure at different pH values similar to the natural environment has been developed (Bissen and Frimmel, 2000). Stepwise extractions were performed with 0.3 M ammonium oxalate (pH = 3), milli-Q water (pH = 5.8), 0.3 M sodium carbonate (pH = 8), and 0.3 M sodium bicarbonate (pH = 11). As^{III}, As^{V}, MMA, and DMA were monitored by HPLC-ICPMS in each extract. As^{V} was the predominant form while neither MMA nor DMA was found in the extracts of two contaminated soils sampled at a former tannery site and a former paint production site (Bissen and Frimmel, 2000). The concentrations of arsenic species in different extracts varied remarkably and the overall extraction yields were fairly low (0.04% for tannery site and 20% for paint production site, respectively), indicating that the mobilization of arsenic depended on the pH value of the extraction solution and the kind of extracted soil (Bissen and Frimmel, 2000). Similarly, an analytical procedure for determination of As^{III} and As^{V} in soil extracts obtained by sequentially applying water, 0.6 M KH_2PO_4 solution, 1% (v/v) HCl solution and 1% (w/v) NaOH solution was developed (Shi et al., 2003).

Phosphate was another frequently used mild extractant for leachable As species in solid samples. The similarity of chemical properties between phosphate and arsenate, such as ion size and acid dissociation constants, made the ion-exchange reaction between phosphate and arsenate occur easily. Through this kind of ion-exchange reaction, phosphate is able to release extractable As fractions from soils. However, it was found that As^{III} could be oxidized to As^{V} during phosphate extraction process. Georgiadis et al. (2005) attempted to add complexing reagents such as ethylenediaminetetraacetic acid (EDTA) and sodium diethyldithiocarbamate trihydrate (NaDDC) or reductant hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) into phosphate to preserve As^{III} integrity. The results for a Florida Everglades peat soil with high content of organic matter and

low content of Fe and Mn and marine sediment CRM PACS-2 with high content of Fe and Mn suggested that the addition of EDTA in the phosphate solution did not prevent As^{III} from oxidation in the extracts despite the fact that EDTA was an effective preservative for As^{III} in water samples. When 1% $NH_2OH \cdot HCl$ was added, it was effective to preserve As^{III} in a limited time (<12 h). The addition of 0.5% NaDDC, however, minimized As^{III} oxidation and the recoveries of spiked As^{III} into these samples ranged from 80 to 120%. The tests on several other types of soil and sediment samples indicated that the combined phosphate and NaDDC solution seemed to be a promising extraction procedure for the speciation analysis of phosphate-extractable As^{III} and As^{V} (Georgiadis et al., 2006).

In addition to these mild extractants mentioned above, a simple extraction for arsenic species using certain particular reagent could be feasible for soils and sediments with specific characteristics. For instance, reducible reagents are likely efficient extraction media for solid samples with high content of Fe oxides because As species are predominantly associated with this phase. Gómez-Ariza et al. (1998) reported a procedure for the extraction and analysis of arsenic species (As^{III}, As^{V}, MMA, and DMA) from estuarine iron oxide rich sediments using $NH_2OH \cdot HCl$ solution as leaching agent. When samples were extracted with 40 ml of 0.4 M $NH_2OH \cdot HCl$ at 95°C for 8 h with occasional shaking, no changes in the arsenate oxidation state were observed despite the reducing character of the extractant. As^{V} was found to be the predictably predominant form while As^{III} was present as a minor constituent. A good recovery higher than 90% ($As^{III} + As^{V}$ versus total As concentration determined after *aqua regia* digestion) was obtained for this oxic estuary sediment sample after three repetitive extractions. However, low recovery (67.0%) was observed for CRM 320 river sediment, indicating the influence of the matrix composition on the extraction.

More recently, the use of phosphoric acid for the extraction of arsenic from soils has been studied. Several studies have investigated the influence of phosphoric acid concentration, extraction time, extraction method (shaking, heating, or microwave irradiation), and the addition of reducible agents such as $NH_2OH \cdot HCl$ and ascorbic acid on arsenic extraction efficiency and species integrity (Demesmay and Ollé, 1997; Thomas et al., 1997; Hegesen and Larsen, 1998; Martin et al., 2001; Garcia-Manyes et al., 2002; Ellwood and Maher, 2003; Pizarro et al., 2003). The efficiency of orthophosphoric acid extraction was shown to be related with acid concentration. High concentrations of phosphoric acid could extract more arsenic species from the soil, but the oxidation of As^{III} to As^{V} was also observed at high concentration (Hegesen and Larsen, 1998; Martin

et al., 2001). Ascorbic acid as well as $NH_2OH \cdot HCl$ was observed to enhance the protection of As^{III} from oxidation. The recovery of spiked As^{III} increased up to 98.9% with addition of ascorbic acid from 93.7 without the addition of ascorbic acid (Garcia-Manyes et al., 2002) while recoveries between 89 and 104% were observed for As^{III} spiked into four oxic sediment CRMs and an anoxic sediment when $NH_2OH \cdot HCl$ was used as a sacrificial oxidant (Ellwood et al., 2003). Regarding extraction method, microwave assisted extraction seemed to be more efficient compared with mechanical shaking (Thomas et al., 1997).

Applying microwave-assisted phosphoric acid extraction method to an arsenic-contaminated soil sample, Garcia-Manyes et al. (2002) evaluated the stability of As species in the soil extracts. The extraction was conducted using a solution containing 1 M phosphoric acid and 0.1 M ascorbic acid and 60 W microwave power during 10 min extraction. The extract was then analyzed for the concentration of As^{III}, As^{V}, DMA, and MMA at different periods of time (0, 3, 6, and 7 days after extraction). The results showed that As^{III} remained practically the same as the original, whereas both DMA and MMA had decreased more than 50% after the extract was kept for 6 days at 4°C under darkness. After 7 days, As^{III}, DMA, and MMA were not detectable in the soil extract. The authors suspected that the original species As^{III}, DMA, and MMA were probably transformed into As^{V} (Garcia-Manyes et al., 2002).

From these studies, it seems that phosphoric acid combined with appropriate reducible agents was an efficient extraction method for the speciation analysis of As species in soil. Meanwhile, it should be noted that the efficiency of phosphoric acid extraction significantly depended upon the nature of the material analyzed. For example, excellent (90–100%) recoveries of total As were obtained for the sediment and the sludge reference materials (CRM 320 and CRM 007-040) whereas yield did not exceed 62% for the soil reference materials (SRM 2709) (Gallardo et al., 2001).

It should be kept in mind that most extraction procedures for arsenite and arsenate discussed above were developed to evaluate arsenic mobility or leachability in soils, which suggests that the extraction of arsenic species is incomplete and only labile or selected solvent extractable arsenic fractions in soils are extracted. When using 1 mM phosphate buffer to extract As from several sediment CRMs, very low extraction yields (at most 2.2%) were obtained (Manning and Martens, 1997). Georgiadis et al. (2006) extracted 7% of total arsenic from PACS-2 using 10 mM phosphate combined with 0.5% NaDDC, whereas Bissen and Frimmel (2000) extracted 0.04–20% of arsenic from contaminated soils using four different extractants, namely 0.3 M ammonium oxalate, Milli-Q water,

0.3 M sodium carbonate, and 0.3 M sodium bicarbonate. These extractions with low yields could provide an estimate for labile arsenic pool. However, information provided by these procedures may not be sufficient for overall evaluation of arsenic biogeochemistry. With the assistance of microwave, orthophosphoric acid extraction seemed to be efficient to some kinds of soils and sediments (Demesmay and Ollé, 1997; Thomas et al., 1997).

31.2.4. Extraction of functionally defined arsenic species

The functionally defined speciation analysis of As in soil was generally used to evaluate the bioavailability of As. In this extraction scheme, synthetic leaching fluids closely analogous to those of the human stomach and small intestine was utilized to release bioavailable As fraction from mining waste or contaminated soil at pH, temperature, and duration which mimics human gastro-intestinal tract environment. For example, Ruby et al. (1996) developed a physiologically based extraction test (PBET) to assess in vitro bioavailability of metals. The simulated gastric fluid was prepared by adding 1.25 g pepsin (activity of 800–2500 units mg^{-1}), 0.5 g sodium citrate, 0.5 g malate (DL-Malic acid, disodium salt 97%), 420 µl lactic acid syrup (free acid 85%) and 500 µl acetic acid into 1 l of de-ionized water, and adjusting pH to 2.5 with HCl. This method has been used for different types of samples (Williams et al., 1998; Rodriguez et al., 1999; Reimer et al., 2003). Williams et al. (1998), using a modified version of PBET, evaluated the post-ingestion bioavailability of arsenic in alluvial soil and mineral beneficiation waste in Thailand. It was found that an average stomach absorption of 11.2% of total soil As could occur, which provided a direct basis for assessment of As bioavailability following juvenile hand–mouth ingestion. Reimer et al. (2003) applied this test to a mining area at Yellowknife, Canada and found that As in crushed rock samples ($<12\%$ of total As extracted) was less bioavailable than As in lawn soils ($>30\%$ of total As extracted). The PBET provides a potentially valuable mechanism for refining risk assessments of sites subject to natural or anthropogenic As contamination.

31.3. Concluding remarks

Speciation analysis of arsenic in soils and sediments can provide crucial information in our understanding the biogeochemical cycle of arsenic and meanwhile is a challenge in the field of environmental and analytical chemistry. The speciation of arsenic in soil may be defined functionally,

operationally, or chemical specifically. The extraction of arsenic species from solid matrices generally constitutes a critical step for speciation analysis of arsenic in soils and sediments. Sequential extraction procedures were used to extract operationally defined arsenic species (phase association forms) while simple extraction with selective extractants were applied to leach chemically specific arsenic species (As^{III}, As^{V}, MMA, and DMA) from a solid matrix. Simulated gastric fluid was usually used to release functionally defined As species (bioavailable As fraction). Additionally, direct spectroscopic technologies without an extraction step were also useful tools for speciation analysis of As, which could provide information for As phase association as well as As oxidation state. The combined application of different types of speciation analysis seems to be an encouraging area and will serve to give a better view of As biogeochemical cycling.

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