Regional and Global Scale Challenges for Controlling Arsenic Contamination in Agricultural Soil, Water Supplies, Foods and Ayurvedic Medicines

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Arsenic is naturally present at high concentration levels in aquifers adversely affecting the life of some 200 million people in a number of countries on four continents. Human exposure to As from dietary sources such as marine fish, seafood, poultry, cereals is generally much lower compared to exposure through drinking contaminated water, using contaminated water in food preparation and irrigation of crops. Arsenic toxicity depends on its four valences [As(-III), As⁰, As(III) and As(V)] and chemical compounds. Thus, in seafood, As is mainly found in its less toxic organic forms. The qualitative and quantitative determinations of individual As species are crucial to understand the environmental fate and behavior of As. The aim of the present review is to give a brief overview on the main As speciation methods and to present how to control As contamination at local and global scales in several environmental (soil, waters) and biological (crops, basic and processed food) samples, as well as complementary and alternative medicinal products marketed as food supplements. In terms of chromatographic separation, emphasis is placed on separation by thin layer chromatography and solid phase extraction. Some approaches to address As contamination (e.g., stabilization in soil, provision of a safe water supply in affected communities) at global and regional scales are also presented.

Keywords: arsenate, arsenite, drinking water, organic arsenicals, speciation

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Abbreviations used in this Review

| Acronym | Meaning | Acronym | Meaning |
|---------|---------|---------|---------|
| AC      | activated charcoal | $K_a$  | acid dissociation constant |
| AsB     | arsenobetaine     | LA     | laser ablation           |
| AsC     | arsenocholine     | LC     | linear combination       |
| ASE     | accelerated solvent extraction | LD$_{50}$ | median lethal dose |
| ASHRAM  | Arsenic health risk assessment and molecular epidemiology | LMWOA | low molecular weight organic acid |
| ATR-FTIR| attenuated total-reflection Fourier—transformed infrared spectroscopy | LOD | limit of detection |
| BCR     | Bureau Communautaire de Référence or (European) Community Bureau of Reference | MMA(III) | monomethylarsonous acid |
| BMDL    | benchmark dose limit | MMA(V) | monomethylarsonic acid |
| BW      | body weight       | MOE    | margin of exposure       |
| CAM     | complementary and alternative medicine | MW     | microwave                |
| DMA(III)| dimethylarsinous acid | NIST   | National Institute for Standards and Technology |
| DMA(V)  | dimethylarsinic acid (cacadyllic acid) | OPLC   | overpressured layer chromatography |
| DSHEA   | Dietary Supplement Health and Education Act | PEI    | polyethylene imide       |
| DW      | deionized water   | PTFE   | polytetrafluoroethylene  |
| EC      | European Commission | Q-ICP-MS | quadrupole inductively coupled plasma mass spectrometry |
| EDTA    | ethylene diamine tetraacetic acid | $R_f$  | retention factor |
| EDXRF   | energy dispersive X-ray fluorescence | RSD   | relative standard deviation (%) |
| EXAFS   | extended X-ray absorption fine structure | SAM   | S-adenosyl methionine |
| FAO     | Food and Agriculture Organization of the United Nations | SAX   | strong anion exchanger |
| FIA     | flow injection analysis | SPE   | solid phase extraction |
| FRW     | fresh root weight | SRM   | standard reference material |
| FTIR    | Fourier-transformed infrared spectroscopy | SR-XANES | synchrotron radiation X-ray absorption near edge spectroscopy |

Continues on the next page.
Abbreviations used in this Review (Continuation)

| Acronym       | Meaning                                              | Acronym       | Meaning                        |
|---------------|------------------------------------------------------|---------------|--------------------------------|
| GFAAS         | graphite furnace atomic absorption spectrometry      | TETRA         | tetramethylarsine               |
| GSH           | reduced glutathione                                  | TMA           | trimethylarsine                 |
| HG-AAS        | hydride generation atomic absorption spectrometry    | TFA           | trifluoroacetic acid            |
| HPLC          | high performance liquid chromatography               | THGA          | transversely heated graphite atomizer |
| HR-CS-GFAAS   | high resolution continuum source graphite atomic absorption spectrometry | TMAO          | trimethylarsine oxide           |
| iAs(III)      | arsenite                                             | TXRF          | total-reflection X-ray fluorescence (spectrometry) |
| iAs(V)        | (hydrogen) arsenate ions                             | TLC           | thin layer chromatography       |
| ICP-(SF)-MS   | inductively coupled plasma (sector field) mass spectrometry | WHO           | World Health Organization       |
| IRMM          | Institute for Reference Materials and Measurements   | XANES         | X-ray absorption near edge spectroscopy |
| JECFA         | Joint FAO/WHO Expert Committee on Food Additives     | µ-XRF         | µ-X-ray fluorescence            |

RATIONALITY OF THIS REVIEW

In the 21st century, not only did the pace of scientific development accelerate tremendously, but thanks to infocommunications, our way of life has been completely transformed. We spend nearly 90% of our time indoors, and global warming has led to the creation of energy-efficient air-conditioned buildings. There is a growing demand for healthy diet. At the same time, more and more people are turning to traditional medicine due to the lack of empathy often experienced in public health. These alternative pharmaceutical preparations are generally sold over-the-counter and considered dietary supplements.

Due to its controversial properties, arsenic (As) came early into the sight of humanity. It can occur in many chemical forms, from poisonous to harmless species. However, even small amounts of its toxic forms can have therapeutic and corroborative effects. In soil, the availability of As is mainly determined by the chemistry of iron (Fe). Arsenic phytotoxicity is closely related to the composition and pH of the soil and, last but not least, the tolerance of the plant to As. Biomethylation produces less toxic organic As compounds. The As contamination of many of the aquifers is of geological origin, adversely affecting the everyday life of some 200 million people. In Europe, the south-eastern part of Hungary is mainly affected by naturally occurring As contamination. Recently, significant efforts have been made in Hungary to comply with the 10 µg L⁻¹ health limit for the concentration of As in drinking water set out in European Commission Directive 98/83/EC.¹ However, the choice of technological solution for As removal is primarily determined by the chemical form of As. The pace of development of analytical measurement techniques is extremely fast, and the development of elemental speciation analysis is also supported by the knowledge gained in many other fields of science. In addition to the coupling of separation techniques and atomic spectrometric measurement techniques, speciation procedures include a number of sample preparation procedures that can be a separate source of error. These may occur during sampling in the form of unwanted interconversion of the species, but the interference caused by sample matrix components may
also be significant. In parallel with the need to improve analytical performance, it would be useful to develop cost-effective, speciation methods applicable to different matrices that could be routinely used in analytical laboratories.

The aim of the present review is to give a brief overview on main As speciation methods and to present how to address the challenges represented by As contamination at local and global scales in several environmental (e.g., soil, waters) and biological (e.g., crops, basic and processed food) samples, as well as complementary and alternative medicinal (CAM) products marketed as food supplements with special emphasis on As speciation.

**ARSENIC SPECIES AND THEIR TOXICITY**

Arsenic is a highly toxic metalloid that can occur in soil even at extreme concentration (e.g., 250 g kg\(^{-1}\)) due to both geogenic and anthropogenic activities.\(^2\) Arsenic toxicity depends on its valence and chemical environment. Thus, As(III) species are more toxic than As(V) ones and inorganic As (iAs) compounds are more toxic than organic As ones. The median lethal dose (LD\(_{50}\)) values of the most common As compounds determined by oral exposition to rats have been summarized in Table I.\(^3\)

| As species                              | LD\(_{50}\) (mg kg\(^{-1}\)) |
|-----------------------------------------|-------------------------------|
| AsH\(_3\) (arsine)                      | 3                             |
| As\(_2\)O\(_3\) (arsenic trioxide)     | 20                            |
| Na\(_3\)AsO\(_3\) (sodium arsenite)    | 60                            |
| Na\(_3\)AsO\(_4\) (sodium arsenate)    | 120                           |
| CH\(_3\)AsO(OH)\(_2\) [monomethylarsonic acid, MMA(V)] | 700                            |
| elementary state As (As\(_0\))         | 763                           |
| (CH\(_3\))\(_2\)As (trimethyl arsine, TMA) | 787                           |
| (CH\(_3\))\(_3\)As\(^+\) (tetramethyl arsine, TETRA) | 890                           |
| (CH\(_3\))\(_2\)AsO(OH) [dimethylarsinic acid (cacodylic acid), DMA(V)] | 1600                          |
| CH\(_3\)AsO(ONa)\(_2\) (disodium monomethyl arsonate) | 1800                          |
| (CH\(_3\))\(_2\)AsO(ONa) (sodium cacodylate) | 2600                          |
| (CH\(_3\))\(_3\)As\(^+\)(CH\(_2\))\(_2\)OH (arsenocholine, AsC) | 6500                          |
| (CH\(_3\))\(_3\)As\(^+\)CH\(_2\)COO\(^-\) (arsenobetaine, AsB) | 10000                         |
| (CH\(_3\))\(_3\)AsO (trimethylarsine oxide, TMAO) | 10600                        |

*oral exposition to rats

The high toxicity of arsenite [iAs(III)] – also confirmed by the Pearson acid-base theory – can be explained by its significant affinity for the sulfur (S) atom. Thus, As can bind to the sulfhydryl group of enzymes and inhibits their function, causing cell death. The toxicity of (hydrogen)arsenate ion [iAs(V)] is due to the fact
that its chemical structure is very similar to that of phosphate ion. This latter ion plays a role in the energy balance of living organisms through phosphorylation. Arsenate can be accumulated, mainly in the nails and hair. The iAs(V) taken up by plants, when replaced by phosphate ions, interfere the energy balance.

The iAs species are more toxic compared to organic derivatives such as e.g., arsenobetaine (AsB) and arsenocholine (AsC). However, both dimethylarsinic acid [DMA(V)] and monomethylarsonic acid [MMA(V)] can decrease the levels of hepatic reduced glutathione (GSH) and serum alanine aminotransferase and increase those of cytochrome P450. These events were more intensive in rats at doses of 679 mg kg\(^{-1}\) MMA(V) and 387 mg kg\(^{-1}\) DMA(V) in the liver and lungs. At the same time, organ-specific DNA damage has been demonstrated.\(^4\) Dimethylarsinous acid [DMA(III)] is a toxic intermediate of DMA(V) that can also be detected in urine in the case of As poisoning.\(^5\) Monomethylarsonous acid [MMA(III)] is a carcinogenic and toxic human xenobiotic metabolite formed during the detoxification of GSH and MMA(V) in the liver during acute and minor As poisoning. It is then converted to DMA(V) by oxidative methylation upon reaction with S-adenosyl-L-methionine (SAM)\(^6\) (Figure 1). The major metabolites of the typically non-toxic arsenic sugars, AsB and AsC, are also excreted in the urine (Figure 1). If As enters the human body in large quantities at the same time, it causes vomiting, diarrhea, increased sweating and cramps. The sodium salts of MMA(V) and DMA(V) have also been widely used as insecticides (e.g., currently still permitted in weed control for cotton cultivation).\(^7\) Arsenic has also been used to pickle wood e.g., in the USA.\(^8\) Among the organic As species, roxarsone should be mentioned, used mainly in poultry as a feed additive to increase weight gain and as a coccidiostat. Due to the increased concentration of iAs detected in meat from breeding animals and poultry after metabolization, the use of roxarsone was banned in the US since 2013\(^8\) and also in the EU as early as 1999. However, it is still widely used in China and many developing countries.\(^9\)

Among the human aspects of As toxicity, the harmful effects on health due to the chronic exposure to the consumption of As-containing drinking water in Hungary should also be mentioned. Thus, according to the results of the ASHRAM (Arsenic Health Risk Assessment and Molecular Epidemiology) research project funded by the European Commission and carried out in Hungary between 2002 and 2004, there was a positive correlation between As exposure and skin, lung, bladder and kidney cancer in several settlements located in the Hungarian Great Plain.\(^10\) In the settlements of one the affected regions, for which the As concentration of drinking water exceeded 100 μg L\(^{-1}\), an increase of 1.5 – 2 was observed in the number of spontaneous abortions and stillbirths compared to the control settlements.\(^11\)

![Figure 1. Mechanism of arsenic detoxification in liver for acute inorganic arsenic toxicity. Notations: iAs(III) = arsenite; iAs(V) = arsenate; AsB = arsenobetaine; AsC = arsenocholine; DMA(III) = dimethylarsinous acid acid; DMA(V) = dimethylarsinic acid; MMA(III) = monomethylarsonous acid; MMA(V) = monomethylarsonic acid; SAM = S-adenosyl-L-methionine.](image-url)
ANALYTICAL TECHNIQUES FOR DETERMINATION OF As IN ENVIRONMENTAL AND FOOD SAMPLES AS WELL HYPHENATION POSSIBILITIES FOR As SPECIATION

Several analytical techniques have been proposed for As speciation. Analytical techniques suitable for qualitative and quantitative determination of As discussed in the present review have been compiled in Table II. It is worth mentioning that, in terms of As speciation, hydride generation atomic absorption spectrometry (HG-AAS) and synchrotron radiation X-ray absorption near edge spectroscopy (SR-XANES) allow discrimination between As(III) and As(V). Since the toxicity of As depends significantly on its chemical form, it is advisable to determine not only the total concentration of As in the samples but also that of each species. Thus, several chromatographic methods have been proposed for the separation of As species such as anion/cation exchange, reversed-phase, ion pair, and size exclusion chromatography. Separation of As species has traditionally been performed by high performance liquid chromatography (HPLC) using ion exchange columns. The most common species – iAs(III), iAs(V), MMA(V) and DMA(V) – are separated on an anion exchange column, while AsB and AsC are separated on a cation exchanger. In a recent review by Reid et al., suitability of hydrophilic interaction liquid chromatography, multiple separation mechanisms, and testing of fluorophenyl and graphene oxide stationary phases has also been discussed. Quantification of the separated species is performed using an appropriate atomic spectrometric technique connected to the chromatographic device on-line. Nowadays, inductively coupled plasma mass spectrometry (ICP-MS) is the most commonly used for this purpose.

Table II. Analytical techniques discussed in the present review suitable for As determination in environmental and food samples

| Analytical technique | Sample preparation | Sample matrix | Remarks | Speciation | Ref. |
|----------------------|--------------------|---------------|---------|------------|------|
| **Qualitative determination** | | | | |
| ATR-FTIR | – | food waste-derived charcoal added to As-contaminated soil | elucidation of As adsorption mechanism | n.a. | [13] |
| EDXRF | – | Ayurvedic ointment | monitoring of $^{13}$C, $^{121}$Sb and $^{75}$As | – * | [14] |
| LA-DRC-ICP-MS | extraction | rice | off-line with PEI-TLC/OPLC | | [15] |
| SR-XANES | – | xylem sap | LC of standard spectra | discrimination between As(III) and As(V) | [16] |
| **Quantitative determination** | | | | |
| HR-CS-GFAAS | dilution with DW | thermal / well water | off-line with IE-SPE | | [17,18] |
| HG-AAS | dry ashing | solid foods | use of pre-reducing agent (e.g., KI, L-cysteine) | | [19-21] |
| Q-ICP-MS | dilution with DW/MW-assisted acid digestion | aqueous food samples | mathematical correction | [on-line with IE-HPLC & off-line with IE-SPE] | [19] |

Continues on the next page.
### Table II. Analytical techniques discussed in the present review suitable for As determination in environmental and food samples (Continuation)

| Analytical technique | Sample preparation | Sample matrix | Remarks | Speciation | Ref. |
|----------------------|--------------------|---------------|---------|------------|------|
| SF-ICP-MS            | dilution with DW   | water         |         |            | [22] |
|                      | MW-assisted        | aqueous food  | HR when HCl is used | on-line with IE-HPLC & off-line with IE-SPE | [14] |
|                      | acid digestion     | samples; rice; Ayurvedic formulations |         |            |      |
|                      | (+H₂O₂ for organic matter) |               |         |            |      |
|                      | Enzymatic          | rice          | for As speciation |            | [23] |
|                      | extraction with    |               |         |            |      |
|                      | sonotrode          |               |         |            |      |
|                      | –                  | xylem sap     | FIA for total As & R = 4000 |            | [24] |
| TXRF                 | –                  | water         |         | off-line with IE-SPE | [18] |
|                      | MW-assisted        | plant extracts (root, leaves) | for speciation, extraction with NH₄H₂PO₄ (pH=5.6) | off-line with PEI-TLC/OPLC | [25] |
|                      | acid digestion     |               |         |            |      |
| μ-XRF (confocal)     | –                  | rice grains   | individual item analysis |            | [26] |

For acronyms see list of abbreviations; * = phase analysis.

### Speciation of arsenic using solid phase extraction cartridges

One of the pivotal points of elemental speciation is to prevent interconversion of species during sampling and analysis; therefore, it is a great advantage to be able to use an analytical technique that requires minimal and cost-effective sample preparation. Significant efforts in this direction have been seen mainly in the As speciation of water. Cost-efficient separation of iAs species can be successfully performed at the sampling site using solid phase extraction (SPE) cartridges filled with strong anion exchange (SAX) resins. When using SAX resins it is not possible to retain iAs(III) as it is a very weak acid (Figure 2). However, by coupling two miniature columns in series and oxidation by KMnO₄, iAs(III) can also be retained on the microcolumn. Connection in series of SPE cartridges filled with strong anion and cation exchange resins have also been shown to be robust in field studies for speciation of As characterized by samples up to 30 mL of 10 mg L⁻¹ As and complex matrix materials. If necessary, MMA(V) can be determined after selective elution performed with acetic acid, while DMA(V) binds upon passing the fraction not retained on the SAX cartridge through the cation exchanger one (Figure 2).

Alternatively, cartridges filled with Al-containing sorbent may be used. In this case, a breakthrough of the otherwise retained iAs(V) was also observed when ions competing for Al silicate sorption sites were also present in the water. Other SPE cartridges for in situ separation of the methylated forms of iAs have also been prepared. Such sorbents include nanometer-size titanium dioxide particles immobilized on silica gel, octadecyl groups, yeast immobilized on controlled pore size glass, PTFE turnings, cetyltrimethylammonium bromide-modified alkyl silica, 3-(2-aminoethylamino) propyltrimethoxysilane-modified mesoporous silica, macrocyclic materials, hybride resins activated with hydrated Fe oxides and silver chloride, carbon nanofibers, as well as nanotubes and eggshell membrane.
Figure 2. Diagram of a solid phase extraction process involving a cartridge filled with a strong anion exchange for \textit{in situ} separation of arsenic species. (Figure adapted from Ref. 28.) Notations: \textit{iAs(III)} = arsenite; \textit{iAs(V)} = arsenate; DMA(V) = dimethylarsinic acid; MMA (V) = monomethylarsonic acid; $pK_a$ = minus logarithm of base 10 of the corresponding acid dissociation constant.

ENVIRONMENTAL AND FOOD ANALYTICAL APPLICATIONS

\textbf{Arsenic in soil}

\textit{Occurrence of arsenic species in soil}

Arsenic is present in many soil types at an average concentration between 0.1 and 40 mg kg$^{-1}$. As main constituent, it occurs in more than 200 minerals, mostly along with S. Its most important mineral is arsenopyrite (FeAsS), but realgar (\(\alpha\)-As$_2$S$_3$) and auripigment (As$_2$S$_3$) are also noteworthy. In addition, it can occur in many sulfide minerals, mainly as a substitute for S. The concentration of As in igneous rocks is low, averaging 1.5 mg kg$^{-1}$. The concentration range for sedimentary rocks varies from 5 to 10 mg kg$^{-1}$. The concentration of As in the metamorphic rocks reflects the characteristics of the precursor igneous or sedimentary rock. Unconsolidated sediments typically contain 3-10 mg kg$^{-1}$ of As, although this also depends on the texture and constituent minerals.$^{48}$

In soil, the availability of As is mainly determined by the chemistry of Fe. In soils and groundwater, \textit{iAs(V)} dominates. Inorganic \textit{As(V)} are more likely to occur under aerobic, while \textit{iAs(III)} under anaerobic conditions.$^{49}$ Inorganic As species are methylated in soils due to microbial activity (Figure 3).$^{48,50}$ Demethylation converts organic As compounds to \textit{iAs}.$^{48}$ Biomethylated \textit{As(V)} species account for up to 15.5–16.7% of the total As concentration of surface geothermal waters.$^{51}$ Oxyhydroxides of Fe, Al and Mn as well as clays, and mineral oxyanions (e.g., sulfates, phosphates and carbonates) in soil and sediments immobilize As.$^{52}$ Fine particles of clay minerals and (oxy)hydroxides adsorb As due to their high specific surface area.
Figure 3. Simplified mechanism for the illustration of the connection of inorganic arsenic species to iron chemistry as well as bacterial methylation of in soil. Notations: $\text{(CH}_3\text{)}_2\text{As} = \text{dimethylarsine}; \text{MMA}(V) = \text{monomethyl arsonic acid}; \text{DMA}(V) = \text{dimethylarsinic acid}; \text{TMA} = \text{trimethylarsine}.

At regional scale, for example in Hungary, the concentration of As in soils varies between <2.5-230 mg kg$^{-1}$. The limit value of As in Hungarian soils is 15 mg kg$^{-1}$ dry weight (DM). The European Economic Community has classified As a priority risk pollutant, i.e., As is a hazardous substance in all cases. Concentrations of As in floodplain and riverbed sediments in the geochemical regions of Hungary range from 1 to 60 mg kg$^{-1}$.

The origin of As contamination of soils in Hungary has not been well elucidated yet (Figure 4). However, several theories have been suggested. Among the geochemical landscapes of Hungary (Figure 4), the second one is characterized by the accumulation of Ca, Mg and Sr, but As can also be found in the floodplain sediments of the Danube River. Moreover, As occurs in the southern part of the Danube-Tisza interfluve with exceptionally high values, probably due to the fact that As bound to Fe oxyhydroxides during the ice age is mobilized nowadays. In the 3rd geochemical landscape, the dead ground originating from mining non-ferrous and precious metals (Ag, As, Au, Cd, Cu, Pb, Sb, Zn) in Transylvania (Romania) and the highlands of Slovakia, is carried by rivers to the shallow basin of the Great Plain. In this case, As is an accompanying element of non-ferrous metals.

Figure 4. Arsenic containing geochemical landscapes in Hungary. (Figure adapted from Ref. 55.)
Estimation of As concentration in agricultural soils through sequential extraction

First of all, it is important to estimate the total concentration of As in the contaminated soil. Then, it is advisable to perform sequential extraction of As on the soil. In the case of agricultural soils, As is most likely present in the form of iAs(V), so a two-step modified BCR (Bureau Communautaire de Référence) soil extraction procedure is appropriate. Briefly, the water-soluble and carbonate fractions are extracted with 0.11 M acetic acid. This step is followed by extraction of the fraction bound to amorphous Fe(III)/Mn(IV) oxides with 0.5 M hydroxylamine hydrochloride solution at pH = 2. The initial samples as well as the extraction residues can be analyzed by ICP-SF-MS after MW-assisted acid digestion. If appropriate soil CRMs are not available, another atomic spectrometric technique, e.g., total-reflection X-ray fluorescence (TXRF) spectrometric analysis of the same extracts can be used to check the accuracy of the results. To calculate recovery, the concentration of As in the different extracts should be summed and compared to the pseudo-total concentration.

Stabilization of arsenic in contaminated agricultural soils

Remediation of soils contaminated with toxic elements (e.g., As) is still a complex task of great importance nowadays, as toxic inorganic constituents cannot be easily removed from contaminated soil. Usually in situ immobilization or stabilization by physical and chemical methods have been proposed for toxic inorganic contaminants. The latter is an attractive alternative as it is a cost-effective and non-destructive process, yet suitable for remediating slightly or moderately contaminated soils. The purpose of immobilization is not only to minimize the mobility of toxic elements, but also to preserve soil structure and fertility. The most commonly used substances for stabilizing heavy metals are various minerals and organic substances [e.g., humic substances, ethylenediaminetetraacetate (EDTA), activated charcoal (AC)].

Despite its many advantages, AC is hardly suitable for soil improvement due to its high production costs involving pyrolysis at 1000 °C for 24 h in an inert atmosphere for charring. Charcoal has been used in agriculture for a long while. However, due to its homonuclear structure, adsorption on charcoal is mainly characterized by van der Waals interaction. The production costs of AC can be significantly reduced by choosing a large amount of available biomass waste as a feedstock. These raw materials do not always prove to be efficient enough, so activation and/or functionalization is required after charring involving mainly sulfuric or nitric acids.

However, As, the most common form of oxyanion, is not easy to stabilize in soil because plant adsorbents have a cation exchange capacity. Depending on pH of the media, iAs(III) and iAs(V) differ not only in valence but also in electrical charge significantly influencing their mobility. Due to the carboxyl and hydroxyl functional groups contained in them, plant-derived ion exchangers are not suitable for binding negatively charged iAs(V) at soil pH. However, biomass wastes (e.g., peanut shells) are available in large quantities. The production of AC from peanut shell was carried out with concentrated sulfuric acid (H₂SO₄) for cost-effectiveness reasons. To increase the mechanical stability of the produced AC particles, composite formation with silicate-containing compounds (e.g., Florisil®) was applied. Composite formation and the binding of As to AC were studied by Fourier transform infrared (FTIR) spectroscopy in attenuated total reflection (ATR) mode. For example, acidic (pH = 5) sandy soil was incubated for four weeks with 15-30 mg kg⁻¹ iAs(V). Freundlich isotherm proved to adequately model adsorption of As. The highest adsorption rate was observed at pH=5 for Florisil®-modified AC. The slope of the line fitted for adsorption was about 30% higher at pH=5 than at pH=8 for Florisil®-AC. However, at pH 8, the rate of As adsorption decreased with Florisil®-modified AC. This suggested that the adsorption of iAs(V) is due to the formation of a covalent bond between the AC carboxyl group and one of the O atoms of iAs(V) while a water molecule is eliminated (Equation 1), as it was confirmed by the ATR-FTIR analysis:

\[ \text{Res} - \text{COOH} + H_2\text{AsO}_4^- \rightarrow \text{Res} - \text{COO} - \text{HAsO}_2^- + H_2O \]  

Equation 1
Formation of this mixed acid anhydride does not occur at alkaline pH, which was also proved by ATR-FTIR analyses.

Depending on the AC modification used, the proportion of immobilized As was approx. 30-40%, of which the soil alone immobilized about 15-20% As. The best results were obtained with Florisil®-modified AC. The amount of immobilizable As increased almost proportionally with increasing AC application dose and doubling the treatment concentration of iAs(V).13

Being electrically neutral over a wide pH range, iAs(III) can be more efficiently stabilized by ACs, e.g., formation of H-bonds. However, oxidation of iAs(III) → iAs(V) must also be taken into account. This cost-effective approach may be successful for acidic agricultural soils slightly contaminated with As.

**Arsenic in water**

**Arsenic in groundwater in aquifers**

The concentration of As in groundwater varies very widely (0.1-5000 µg L⁻¹).48 This is especially problematic if the water body serves as a drinking water base. Several theories have been formulated for the large As contamination in natural water.

1) During the extraction of sulfide ores, As originating from their weathering first binds to Fe(III) (oxy) hydroxides; in parallel with the reduction of Fe(III) to Fe(II), As is released and dissolved in the groundwater in the mining areas.

2) Oxidation of As-rich pyrite or arsenopyrite results in the dissolution of As in groundwater.79 The reductive dissolution of Fe(III) (oxy)hydroxides in groundwater under anaerobic conditions by microorganisms, e.g., may be catalyzed by Acidithiobacillus ferrooxidans and Leptospirillum ferrooxidans.52,80

3) A third theory that is characteristic of the Pannonian Basin in Hungary is that As is brought to the surface by thermal water that erupts from the depths.81

Arsenic can be accumulated in aquifers or highly reducing layers of inland/closed basins (semi-)desert areas.48 Both of these different geological environments contain young sediments in flat, low-lying, and slow groundwater flow areas. Waters with high As concentrations (up to 5000 µg L⁻¹) adversely affect the drinking water supply of some 200 million people worldwide.48 The As contamination of many aquifers is of geological origin. This affects the western part of the North American continent, the vast areas of Chile and Argentina in the southern hemisphere, parts of China, and the Gulf of Bengal and its associated delta region in Asia.48 Studies in the US suggest that changes in As concentration over time cannot be clearly correlated with wet/dry seasons82 or pre- or post-monsoon periods in the Indian subcontinent.83 Arsenic contamination can also be attributed to a lesser extent to anthropogenic activity.84

**Arsenic in surface and drinking water**

In rivers, the As concentration is around 0.1-0.8 µg L⁻¹, which is also influenced by the flow of the rivers, lithology of the river bed and water replenishment of the area. The As concentration in lakes is close to or less than that of rivers.

For the in situ separation of As species from water samples taken from public wells in Hungary, the method described by Le et al.28 was also suitable. For As determination in the samples, ICP-SF-MS was used. High-resolution mode (R = 10000) had to be applied only for As speciation. The sum of the As concentration in the SPE fractions was compared to the values of the total As one obtained for the corresponding original samples. Organic As species [e.g., MMA(V) or DMA(V)] could not be detected in the samples. Even a four-fold enrichment could be applied to the iAs(V)-containing fractions. After regeneration, the SPE microcolumns could be reused at least 15 more times by analyzing repeatedly carbonated mineral water samples with a salt content of 450 mg L⁻¹ between pH 6 and 8 spiked to 150 µg L⁻¹ iAs(V). This approach was applied to determine the ratio of iAs(III) and iAs(V) in water taken from public wells of settlements belonging to different geochemical landscapes of Hungary affected by natural As contamination (Figure 5). The total concentration of As in the samples taken from the public wells of
settlements of the Csongrád plain, located east of the Tisza River and belonging to the 2nd geochemical landscape (Figure 4), reached even 210 μg L\(^{-1}\).

Figure 5. Arsenic distribution between arsenate \([\text{iAs(V)}]\) (blue) and arsenite \([\text{iAs(III)}]\) (red) in water samples taken from public wells in settlements located in the 2nd and 3rd geochemical landscapes affected by natural As contamination in Hungary. Grouping of data according to the relative distance of the settlements from each other. (Figure adapted from Ref. 22.)

The results obtained were in agreement with the geochemical theories on the mobility of As in soil. Thus, mobility of As deposited in flat areas from river sediments situated in the 3rd geochemical landscape, can be attributed to the iAs(V) species. The anaerobic conditions favor the formation of iAs(III) and Fe(II) in the deeper layers. In the southern part of the Danube-Tisza interfluve, located in the 2nd geochemical landscape (Figure 4), mobilization of iAs(V) adsorbed onto Fe(III) (oxo)hydroxides during the ice age could only be achieved if Fe(III) → Fe(II) reduction took place due to anaerobic conditions. The mobility of iAs(III) is due to the increased water solubility of the resulting Fe(II) compounds, and the fact that this species is electrically neutral over a wide pH range. Thus, iAs(III) is believed to be mobilized nowadays.\(^{55,56}\) However, iAs(III) is again converted to iAs(V) species under aerobic conditions.

For in situ separation of As species, the SPE method described by Le et al.\(^{28}\) was also applicable to samples taken from a geothermal well.\(^{17}\) The As content of the samples was determined by high resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS-GFAAS) using an Xe arc lamp as a continuous-spectrum primary radiation source equipped with a transversely heated graphite tube atomizer (THGA).\(^{17}\) A linear response to As in the concentration range of 5 – 200 μg L\(^{-1}\) was obtained by external calibration. In addition, the absorbance values up to an As concentration of 500 μg L\(^{-1}\) could be properly fitted with a quadratic polynomial equation. The LOD value of the method was 0.6 μg L\(^{-1}\). This method was characterized by adequate RSD and recovery. When the 150 μg L\(^{-1}\) of an iAs(III) fraction acidified with HNO\(_3\) and stored for up to 30 days was analyzed by HR-CS-GFAAS, the oxidation rate of As(III) → As(V) was less than 10%. The iAs speciation could be performed with sufficient accuracy (± 5%)
up to pH 8. A mass balance could be set up for the As species determined by SPE-HR-CS-GFAAS. The total As concentration was about 400 µg L⁻¹. Chloride and sulphate ions in natural waters above 100 and 50 mg L⁻¹, respectively, caused interference in the determination of As by the continuum source GFAAS method. The effect of high concentration of sulphate and chloride ions generated by magmatic gases in volcanic water on the As measurements was minimized by two-fold dilution of the samples before HR-CS-GFAAS analysis. The proportion of iAs(III) in the geothermal water was about 55%. However, it was reported that the proportion of iAs(III) can be more than 70% in geothermal waters. Slow cooling of the water below 66 °C did not change the proportion of As species in the samples. The SAX-SPE has been shown to be effective for As speciation in water with high salinity at about 85 °C. Due to the total concentration of As (i.e., 400 µg L⁻¹) in the water sample, almost half of which was iAs(III), the analyzed water cannot be used for recreational and/or aquaculture purposes.

For the separation and enrichment of iAs species in tap, lake and well waters, the same Dowex 1-X8 SAX-type SPE charge using electrothermal AAS was suitable for the determination of As in the fractions eluted from the cartridges. Arsenic speciation of surface and groundwater was performed using silica gel-based SAX-SPE- HG-AAS hyphenated technique.

**Arsenic removal technologies from drinking water**

Arsenic has a complex water chemistry and exists in a variety of inorganic and organic forms in waters, depending on pH, salinity, acid dissociation constants (Kₐ) of As oxyacids, as well as redox potential of the iAs(V)/iAs(III). Chemical oxidation, co-precipitation, adsorption, ion exchange, reverse osmosis, and membrane filtration are used to remove As from (waste)water.

From a technological point of view, As removal processes can be divided into three major groups: 1) conventional technologies (coagulation, Fe-Mn removal, lime softening); 2) sorption processes (ion exchange, activated Al); 3) membrane technologies (reverse osmosis, nanofiltration, micro- or ultrafiltration). The removal efficiencies and main characteristics of the six most commonly used As removal technologies for the two iAs species present in water are summarized in Table III.

### Table III. Main features of some arsenic removal technologies

| Technological solution | Removal efficiency (%) | Notes |
|------------------------|------------------------|-------|
|                        | iAs(III) | iAs(V) |                               |
| Coagulation with Fe, sedimentation/filtration | 60 | 95 | 1. filtration through 0.1 or 1.0 µm pore size  
2. pH dependent |
| Coagulation with Al, sedimentation/filtration | 15 | 80 | also suitable for removal of fluoride |
| Lime softening | 70 | 99 | 1. for hard water  
2. soft water must be acidified |
| Ion exchange | - | >95 | 1. independent of pH;  
2. SO₄²⁻ interference |
| Activated Al oxide | - | 98 | 1. iAs(V) exchanges OH⁻ on the surface of Al₂O₃  
2. pH dependent |
| Reverse osmosis | 60 | >95 | treated water not suitable for human consumption |

iAs(III) = arsenite; iAs(V) = arsenate.

As shown in Table III, each of the technologies is more efficient for the iAs(V) species, so oxidation of iAs(III) is often applied first. Oxidation by simple direct aeration is slow, but there are several chemical agents that can speed up the process such as chlorine gas, hypochlorite, ozone, KMnO₄, hydrogen peroxide, Mn oxides, and UV radiation.
The main water treatment steps applied in the waterworks operating settlements located in the southern regions affected by natural As contamination in Hungary are summarized in Table IV.

Table IV. Steps of drinking water quality improvement technologies applied in the waterworks of the monitored settlements

| Step | Water treatment step | Target compound | Notes |
|------|----------------------|-----------------|-------|
| 1    | Gas removal by purging with air | CH₄ |       |
| 2    | Flocculation and oxidation | iAs(III), Fe(II), Mn(II) | 2-4 w/w% KMnO₄ + 40 w/w% Fe₂(SO₄)₃ |
| 3    | Quartz sand filtration | products of oxidation cf. step 2 |       |
| 4    | Pre-chlorination | NH₄⁺ | 5 mg L⁻¹ Cl₂ |
| 5    | Granular activated carbon filtration | by-products of step 4 |       |
| 6    | Post-chlorination (Cl₂, ClO₂, NaOCl) | — | 0.3-0.5 mg L⁻¹ free Cl₂ concentration after chlorination |

Water samples were also analyzed by HR-CS-GFAAS and TXRF after in situ separation of the As species by SPE. The total concentration of As in the raw water samples taken from the wells of the waterworks varied between 42.9 and 118 μg L⁻¹. In well waters, iAs(III) (> 80% of total As) was the predominant species in each settlement. After the addition of oxidizing and flocculant and filtration on quartz sand, the total concentration of As decreased below 10 μg L⁻¹ during the applied water treatment technology. This value was typically about 5 μg L⁻¹. Regarding the proportion of species, iAs(V) was the predominant species. Arsenite was not detected in the samples taken after water treatment. Application of As speciation allowed fine tuning of the reagent use (e.g., KMnO₄) for the oxidation step.

In addition, application of TXRF has the advantage to provide multi-element information. Thus, changes in the concentration of other elements of interest in Directive 98/83/EC in water samples could be monitored after the addition of the oxidizing (KMnO₄) and flocculant [Fe(III) sulfate]. Thus, it was demonstrated that the concentrations of Mn and Fe in drinking water fell far short of the values laid down in Directive 98/83/EC. After the addition of the oxidizing agent and the flocculant, the Fe, Mn and S constituents were retained by the SPE microcolumn, but the cartridges could be regenerated with HNO₃ solution.

Moreover, dilution of contaminated water with that of adequate quality to reduce As contamination of drinking water is an accepted practice, as well. For example, the proportion of iAs(V) in the drinking water of some settlements established on the floodplain soil of the Ráckeve branch of the Danube belonging to the 2nd geochemical landscape with a total As concentration larger than 10 mg L⁻¹ was found to be decreased after repeating sampling and analysis after 5 years. However, the proportion of As species was almost the same as in the samples originally taken. This result means that the reduction of the As concentration was achieved by dilution with water of adequate water quality.

**Arsenic in plants and the importance of xylem sap analysis**

The first studies on the accumulation of As in plants were published first in the 1970s. It was found that, the As content in plants grown on As-contaminated soils was slightly higher than in lightly contaminated soils (0.01-5 mg kg⁻¹ As), but As accumulation was not observed. The fern native to China (*Pteris Vittata* L.) was the first plant to be found to be capable of hyperaccumulation of As (> 20 g kg⁻¹ DM). Arsenic can also be taken up in larger amounts by rice (*Oryza sativa* L.). However, the As content of carrots (*Daucus carota* L.) can also reach up to 1 mg kg⁻¹. Arsenic is found primarily in the roots of plants, and
its concentration can be up to 75-fold higher than that determined in the shoot.\textsuperscript{98,99} The vast majority of As is localized in the apoplastic space of the root.\textsuperscript{100} In the rice plant grown under reducing conditions, 1% of the total As concentration (2.3 mg kg\textsuperscript{-1}) was transported to the shoot. In the root, 60% and 39% of the apoplastic and non-apoplastic fractions contained As, respectively.\textsuperscript{100} In addition, the hyperaccumulative fern contained about 1/6 of the total As in the apoplast.\textsuperscript{101}

Arsenic species found in terrestrial and aquatic plants, lichens, and fungi are divided primarily into hydrophilic and lipophilic compounds.\textsuperscript{102} In terrestrial plants, mainly inorganic species of As, namely, iAs(V) and iAs(III), are detected, although methylated derivatives of iAs(V), DMA(V) and MMA(V), may be present in small amounts.\textsuperscript{103}

Since iAs(V) is the most stable species in the soil under aerobic conditions, it is expected that plants will absorb it mainly in this form. Iron deposition on the root surface can significantly bind iAs(V) species.\textsuperscript{100} In the reducing environment of root cells, iAs(V) is readily reduced to iAs(III). This reduction is thought to be an essential process in the detoxification of As, as the resulting more toxic iAs(III), being electrically neutral over a wide pH range, is transported to senescent leaves.\textsuperscript{104} It has not been clearly demonstrated whether organic As compounds detected in plant tissues can be taken up directly from the soil. There is also no clear evidence that iAs species are methylated by microorganisms living in root symbiosis or by the plants themselves.\textsuperscript{103}

The MMA(V) species is converted to DMA(V) in the root or xylem sap of the As hyperaccumulative fern (\textit{Pteris Vittata} L.).\textsuperscript{105,106} However, microorganisms may also be involved in methylation. Study of As in terrestrial plants provides a two-fold information since: (i) it may indicate the bioaccessibility of As, and (ii) As taken up by plants may enter the food chain. The phytotoxicity of As also depends on the pH, phosphate, composition (e.g., Fe, Al and organic matter content) of the soil and the tolerance of plants for As.\textsuperscript{107} Since various As species have different toxicities, the information obtained from their identification and distribution within the plant may provide an answer to the plant metabolism of As and an assessment of the health risks posed by the introduction of As into the food chain.

The study of xylem sap playing a central role in the transport of toxic and essential elements and nutrients to plant shoots, is particularly advantageous for elemental speciation, as it requires minimal sample preparation. Xylem sap from cucumber plants (\textit{Cucumis sativus} L.) is easily collected and a single sample preparation step consisting of filtration through 0.22 μm membrane filters should be used for chromatographic studies.\textsuperscript{108-118} The roots of most plants should be placed in a pressurized chamber filled with an inert gas (e.g., nitrogen gas) to enhance exudation. This, together with the fact that interconversion of As species can occur relatively easily, makes it difficult to perform As speciation in xylem sap. Pickering \textit{et al.}\textsuperscript{114} confirmed that only a small amount of As was transported to the shoot of Indian mustard (\textit{Brassica juncea} L. var. Czern) applying a treatment of 250 μmol L\textsuperscript{-1} iAs(III) or iAs(V) by XANES measurements. The iAs(V) taken up was stored by the plant both in the root and shoot mainly in the form of As(III) tris(thiolate) complexes. Kertulis \textit{et al.}\textsuperscript{106} have been detected mainly iAs(V) in the xylem sap of \textit{P. vittata} L. treated with either iAs(III) or iAs(V) at concentrations up to 50 mg L\textsuperscript{-1}, regardless of the type of treatments used. In the case of treatments with MMA(V) or DMA(V), As was transported to the shoot in these methylated forms. However, iAs(III) has always been detected at the apex of the fern leaf, suggesting that reduction of iAs(V) occurs in the plant shoot.

Plant growth experiments conducted in nutrient solution have shown that the concentration of As(V) (2–10 μmol L\textsuperscript{-1}) and the iAs(V):phosphate ion concentration ratio of the hydroponics (1:100, 1:1, and 100:1) play an important role in the development cucumber plants. This phenomenon is due to the similar structure of hydrogen arsenate and hydrogen phosphate ion, taken up and transported within the plant by the same mechanism. To eliminate competition between iAs(V) and phosphate, it is advisable to set their concentration ratio in the medium to 1:1. However, the seedlings showed a severe phosphate deficiency at a phosphate ion concentration of 2 μmol L\textsuperscript{-1}, which could only be eliminated at a concentration of 250 μmol L\textsuperscript{-1}. Therefore, in plant growth experiments, it is advisable to keep the phosphate concentration at this value and to reduce it to the As concentration only at the start of As treatment.\textsuperscript{24}
Speciation analysis of the nutrient solutions also confirms the different uptake mechanisms of the two species – electrically neutral iAs(III) and ionic iAs(V) at physiological pH. As the pKₐ values of H₃AsO₄ and H₃AsO₃ differ significantly, i.e., 9.3 and 2.3, respectively, and taking into account the pH of the hydroponics (pH 4.7–5.9), the electrical charge of these two species is significantly different. The iAs(V) uptake is similar to that of phosphate ions, with electrically neutral iAs(III) being taken up through aquaporin.

When sampling xylem sap from cucumber plants treated with As, the rate of exudation with respect to sampling time and fresh root weight (FRW) was very similar for both As treatments: 12.4 and 11.2 µL h⁻¹ FRW⁻¹ for iAs(V) and iAs(III) treatments, respectively. Three As species – iAs(III), DMA(V) and iAs(V) – could be identified in the xylem sap. Regardless of the As treatment, the following concentration order was determined: iAs(III) > iAs(V) > DMA(V). The proportion of DMA(V) in the xylem sap did not reach 5%. The mass of the As species in the xylem sap collected for 1 h taking into account the exudation rate, was very similar: 17.65 and 16.55 ng h⁻¹ for the iAs(V) and iAs(III) treatments, respectively. Arsenobetaine and AsC were not detectable in the samples by cation exchange HPLC-ICPSF-MS. The predominance of iAs(III) in xylem sap of cucumber plants grown in As containing hydroponics was consistent with literature data. Zabludowska et al. found that As present as iAs(V) in aerobic soils was taken up by the phosphate transport system of ferns. Subsequently, a portion of the iAs(V) is reduced to the iAs(III) specific in the root by the arsenate reductase and further translocated to the shoot via the xylem. Reduction is an important process in As tolerance, as iAs(III) can react with highly reactive compounds containing SH groups, such as thiol compounds, e.g. phytochelatins or GSH – and is thus immobilized by complex formation. The iAs(III) ratio was also greater than 60% in xylem sap of rice, tomatoes, wheat, cabbage, and barley. However, methylated As species are rarely detected. The presence of iAs(V) in the xylem sap of plants treated with the iAs(III) species can be attributed to the partial oxidation of iAs (III) to iAs (V) in the hydroponics.

Cucumber plants also take up DMA(V) species in an unaltered way. Zhao et al. found that not only in cucumber plants, but also in xylem sap or different tissues of common velvet grass (Holcus lanatus) and sunflower (Helianthus annuus) grown in iAs-containing hydroponics, methylated As species could be determined only in traces. This provided indirect evidence that the biomethylation capacity of the plants was low.

Arsenic containing xylem saps can also be studied by XANES for As speciation purposes. The normalized profile of the absorption edge of the As K shell for a xylem sap sample taken from cucumber plants (Cucumis sativus L.) treated with iAs(V) is shown in Figure 6. These studies also confirmed that iAs(III) is the predominant species in xylem sap regardless of the chemical form of As used for plant growth.

**Figure 6.** Spectra of a xylem sap taken from cucumber plants (Cucumis sativus L.) treated with iAs(V) as well as those of arsenite (red line) and arsenate (blue line) standards by near near-edge X-ray absorption spectroscopy. (unpublished figure)
The XANES spectra of nutrient solutions and xylem saps can be simulated by linear combinations (LC) of XANES spectra of iAs(III) and iAs(V) standards. Thus, LC calculations confirmed that >80% of As detected in xylem sap corresponds to the iAs(III) species regardless of the type of As treatment. This is consistent with the results obtained by HPLC-ICP-MS, as the proportion of iAs(III) in those similar samples was 86%.

As speciation studies on cucumber plants can be performed by applying off-line polyethylene imide thin layer chromatography – total-reflection X-ray fluorescence (PEI-TLC-TXRF) spectrometric method using acetone: acetic acid:water. The elution of the different As species was as follows: iAs(V) (Rf = 0.06) < iAs(III) (Rf = 0.38) < MMA(V) (Rf = 0.59) < DMA(V) (Rf = 0.91). Due to the reproducible operating conditions ensured by overpressured layer chromatography (OPLC) such as controlled flow rate, external application of pressure and accurate flushing volume, as well as doubling of the running distance, the separation was faster and resolution was better for each 25 ng of iAs(III), iAs(V), MMA(V), and DMA(V) expressed in elemental As in a multicomponent standard solution (Figure 7).

![Figure 7. Off-line PEI-OPLC-TXRF chromatogram of multicomponent standard solution of arsenite [iAs(III)], arsenate [iAs(V)], monomethylarsonic acid [MMA(V)] and dimethylarsinic acid [DMA(V)] containing 25 ng of As each.](https://doi.org/10.1016/j.sab.2006.08.011 Copyright © (2022), Elsevier.)

The efficiency of As extraction from the roots of cucumber plants treated with iAs(III) species was 92%. To quantify As by the developed off-line PEI-OPLC-TXRF method, the extract should have been applied in triplicate onto the plate. However, due to the high concentration of nutrient cations in the root samples and the electrostatic repulsion between positively charged PEI cellulose, this was not feasible. Only iAs(V) and iAs(III) species could be detected by applying 4 μL of the root extract. The presence of iAs(V) in the samples is due to the oxidation of iAs(III) to iAs(V) in the nutrient solution during treatment. In addition, the time-consuming extraction process could not be performed in an inert gas atmosphere.

A faster As speciation method can be achieved by introducing the different As species separated on the PEI-TLC layer into the ICP-MS by laser ablation (LA). Even much smaller amounts of As (different As species containing 3 ng As each) can be detected with this hyphenated technique than with the PEI-TLC/OPLC-TXRF method. The Rf values calculated for the separated species also showed a good agreement with the data obtained by the PEI-TLC-TXRF method. Arsenobetaine and AsC were detected together at the sampling point. The resolution calculated for each adjacent peak ranged from 1.5 to 2.5, and no OPLC separation was required. The method has significant potential for rapid identification of As-contaminated samples, which was verified on enzymatically extracted NIST SRM 1568a rice flour. Thus, up to 10 pg...
MMA(V) could be distinguished from the background value. This proposed TLC-LA-ICP-MS method was later modified for silica gel-based high-efficiency TLC plates for Cr speciation. It has recently been further optimized using an iAs(V)-imprinted polymer to separate iAs(III) species. The developed µ-TLC-LA-ICP-MS method has been successfully applied to mine water spiked with iAs(III) and iAs(V) species. Using the optimal conditions, the detection limit of As was 0.3 μg L⁻¹.

**Dietary As intake and As speciation in rice grains using simple kitchen technology operations**

For the population not exposed to As-contaminated drinking water, nutrition is the most significant source of iAs intake. A close to linear relationship was observed between the As concentration of foods containing more than 40% water (e.g., soups, soft drinks, soda water, beer) and that of the water used for their production. Moreover, the As concentration of the samples with high water content was positively correlated with that of the water used (i.e., Pearson’s correlation coefficient was 0.830-0.923). However, in Bangladesh, it has been shown that a partial reduction of As contamination in drinking water was not sufficient to significantly reduce the amount of As ingested. In Europe, the population consumes an average of 153 g of vegetables per day, compared to around 250 g in Asia. In Chile, the total As and iAs content of cooked vegetables from an As-contaminated area was studied. During the study it was found that the concentration of As in the drinking water used for cooking varied between 41 and 572 μg L⁻¹. The results also showed that foods made a significant contribution to iAs intake, which also posed a higher health risk for those 15 years of age and younger. Exposure of school children (n=55) to As-contaminated well water was assessed in several areas of Mexico. The considerable As concentration in the water showed a clear correlation with that of determined in the hair samples of children. According to the authors, As-contaminated water used for cooking may have been the main source of As exposure. The use of poor-quality glass and unglazed earthenware can also cause As poisoning. The concentration of As dissolved by soaking in unglazed earthenware varied from 30.9 to 800 μg L⁻¹, while the As concentration obtained in the same experiments with glazed earthenware often fell below the detection limit (<0.5 μg L⁻¹), but maximally reached only 30.6 μg L⁻¹. Valentine et al. compared the dietary habits of the population in two As-contaminated areas (Mexico and California) where there are geographical differences in the prevalence of skin diseases. Although the population exposure to As was similar to that of drinking water, there was a difference in the diet (vitamin A consumption) for the groups studied. The issue of dietary intake has been studied in detail in Latin America. The dietary exposure of the population to As has been extensively studied in different regions of France and Catalonia as well as in Hong Kong. The main route of bioavailability of As is the food chain (diet and drinking water consumption). According to Yost et al., 21-40% of As ingested by terrestrial foods in North America is of inorganic origin. The average concentration of As in vegetables is around 20 μg kg⁻¹. One of the staple foods for human consumption, rice (Oryza sativa L.) is also often contaminated with As, at concentrations of up to 280 μg kg⁻¹. Also in the United States, rice consumption is a significant source of children exposure to As. According to the Food and Agriculture Organization (FAO), the People’s Republic of China is the largest rice producer in the world. China is followed by India, Indonesia, Bangladesh and Vietnam. In many areas of these countries, groundwater is highly contaminated with As. The relatively high As content of rice suggests considerable uptake and translocation of this element. According to Liang et al., the rice grains are first husked by grinding (and friction), i.e. the husks (bran layers) covering the grains are removed. The rice grains, stripped of their husks, are polished with a (conical) grinder and bleached with glucose or talcum. The 8-10% of the initial weight of brown rice is removed, mainly in the form of bran. As with most cereals, the distribution of the amounts of elements within the rice grain is heterogeneous. Consequently, it is worth examining the effect of milling rice grains on element content. Of the trace elements accumulating in rice, special attention is paid to the highly toxic Cd. However, several other toxic elements have already been determined in various milled rice cultivars. Rice plants are basically classified into two classes. We distinguish between short and long grain rice. The two most common subspecies are indica and japonica. Short-grained and cooked rice is
a subspecies of *japonica*, while long-grained rice is of Indian origin. *Indica* subspecies are usually grown on water-flooded arable land, while *japonica* species can be grown on dry soil. In general, rice crops and grains grown by the flood method are studied. However, Rascio *et al.* studied Vialone Nano rice grown in the province of Verona (Italy) used to make risottos.

The rice grains must be subjected to solvent or enzymatic extraction before chromatographic separation. Methanol is often used for solvent extraction. Thus, e.g., 76% of the concentration of As in the NIST 1568a in the rice sample could be recovered by extraction with methanol-water (1:1 v/v) using ultrasonication. In the case of extraction with trifluoroacetic acid (TFA) solution at 100 °C for 6 h, the recovery in As-spiked rice samples was quantitative as opposed to methanol extraction. In the latter case, the recovery varied between 31 and 72%. However, a reduction in iAs (V) was observed using TFA. According to Huang *et al.*, quantitative extraction could be achieved by using dilute HNO₃ solution for As speciation in rice grains. By extracting As species from freeze-dried rice produced at the IRMM with water or methanol:water mixtures of different compositions (1:1, 9:1 and 1:1 then 9:1 v/v), Pizarro *et al.* found that about 80% of the total concentration of As can be extracted with a 1:1 v/v mixture of methanol:water using a single extraction step. Accelerated solvent extraction (ASE) is similar to conventional solid-liquid extraction, but it is performed at higher temperatures and pressures, reducing the time required for extraction. However, depending on the type of solvent, the temperature and the pressure used, ASE causes the rice grains to swell. Nevertheless, ASE has been successfully used to extract As species of marine fish and seaweed.

The biggest disadvantage of enzymatic extraction methods is their high time demand, which can result in the interconversion of As species. To break down starch, Heitkemper *et al.* used α-amylase for As determination in rice prior to extraction with methanol:water (1:1 v/v). Under these conditions, an extraction efficiency of 59% was achieved for white rice grains. However, for the NIST 1568a rice sample, this value was found to be 97%. Although the best results were obtained using TFA, it was found that iAs(V) was partially reduced to iAs(III) during extraction. In addition, Pizarro *et al.* rejected the use of TFA for As-speciation from rice by HPLC-ICP-MS because in this case poor chromatographic resolution of the extracted As species was observed. Focused ultrasonic treatment coupled with enzymatic hydrolysis has been successfully applied to the extraction of As from rice grains for speciation purposes as well as to other samples of biological origin.

Rice is dominated by iAs(III), iAs(V), DMA(V), and MMA(V). Arsenic speciation can be performed in rice seedlings as well as in rice grains. In raw rice grains, iAs(III) is the predominant species, accounting for about 50% of the samples. The other major As species present in the samples is iAs(V), the percent of which varied between 25 and 33%. At the same time, the percent of DMA(V) ranged from 8 to 10%, while that of AsC from 6 to 10%. In 2015, the European Commission revised Directive 2006/1881/EC on maximum levels for iAs in food. Thus, the sum of the concentrations of iAs(III) and iAs(V) in rice used for food processing in the case of infants and young children under Regulation (EC) No 2015/1006 may not exceed 0.1 mg kg⁻¹. Study of 24 high-As rice samples grown in the United States revealed that they contained mainly iAs(III) and DMA(V) species. The DMA(V) content increased proportionally with the total As content, while the concentration of iAs(III) (approximately 0.1 mg kg⁻¹) did not change significantly. From this, the authors concluded that rice can be divided into two groups according to its As content: DMA(V) and iAs rice. According to this theory, varieties grown in the United States belong to the first category, while those growing in Europe and Asia to the second one. It was suggested that this difference was due to plant genetic reasons. Although this theory cannot be completely ruled out, the type of soil composition and irrigation is more likely to be responsible for the high DMA(V) content. Furthermore, the recent use of pesticides containing DMA(V) and MMA(V) in the central and southern parts of the United States may play a role.

It is common practice to perform As speciation of rice in ground form. This is understandable since rice flour is used for cooking in Asia and also from an analytical chemistry point of view, as the homogeneity of the sample is a key issue to obtain reliable results. In China, rice is usually cooked with small aliquots
of water. In this way, rice absorbs the available water. However, this approach does not provide real information for estimating the As exposure from rice consumption for example, in the so-called Western societies, where rice is usually consumed as a side dish, so it is cooked in plenty of water and the remaining water is poured off. Thus, there is a need to perform As speciation also according to this latter kitchen technology methods. On the other hand, milk rice, risotto, etc. requires a different approach. The As speciation performed on rice cooked in As-containing water diluted with varying degrees of deionized water (DW) (1:1 to 4:1 v/v) demonstrated the ability of rice grains to absorb As during cookin.167 Bae et al.168 reached to a similar conclusion by determining the total As concentration of rice cooked with local water in Bangladeshi households.

Extraction of rice grains with room temperature and boiling DW provides valuable information on the removal of toxic elements in them. By simulating two simple kitchen technologies (washing and cooking) corresponding to one of the possible ways of preparing rice, it is possible to determine the extent to which the As content of the rice grains can be removed.26 The minimum cooking time was chosen so that the rice grains would soften in line with the recommendations for cooking instant and long grain rice. The total As concentrations of the investigated rice samples did not exceed 171.3 ± 7.1 µg kg⁻¹, which was also in agreement with previous literature data.96 When determining the As content of polished rice samples taken from different geographical areas of ten countries, seven-fold differences were found in the median value. Samples from Egypt (0.04 mg kg⁻¹) and India (0.07 mg kg⁻¹) had the lowest As concentration, while the United States (0.25 mg kg⁻¹) and France (0.28 mg kg⁻¹) had the highest one.169 Since 2014, the health limit value for iAs set by WHO/FAO is 0.2 mg kg⁻¹ in polished rice.170 According to the water extraction results, 8–17% and 29–42% of As could be removed from the studied rice grains by washing room with temperature DW and cooking, respectively.23 These results are also consistent with results reported by Bae et al.,168 according to which 57–81% of As remained in varieties of rice cooked in Bangladeshi households.

For removal of elements by washing and cooking, it also plays a role if metal contamination affects the surface of the rice grains or its deeper layers.171 Cooking may increase the diffusion rate of these ions.171 Confocal µ-XRF analysis provides an opportunity to track the extraction of the elements and interpret the results obtained by measurements in bulk phase (e.g., ICP-MS). By confocal µ-XRF analysis, normalization of the intensity confirmed that the intensity of the X-ray fluorescent signal of most elements was higher in the near-surface part of the studied rice grains.26 Thus, elements (e.g., As) were enriched from the surface of the rice grain to a depth of about 80 µm.26

In terms of As speciation in washed and cooked rice, the main extractable chemical form of As was also iAs(III), with proportions ranging from about 40 to 70% in white rice grains at room temperature and between 60 and 75% in boiling DW extracts.23 Arsenocholine, DMA(V) and iAs(V) could also be determined in these samples. In contrast, cooked samples were generally dominated by the presence of iAs(V), suggesting that iAs(III) was partially oxidized during cooking.23 Pal et al. reached to the same conclusion by performing As speciation following cooking of rice grown in As-contaminated areas of West Bengal in distilled water.172

**Arsenic intake by breastfeeding**

Exclusive breastfeeding is recommended for the first six months after birth for infants, because of providing optimal conditions for the immunological and psychological development of the newborn. However, potential inorganic contaminants in breast milk (e.g., As) are transmitted to the neonate through breastfeeding.173 Toxic elements are absorbed to a greater extent in neonates than in adults, and bile excretion is low in the first weeks after delivery.174 Arsenic occurrence in breast milk is mainly due to a diet rich in marine fish and seafood.175 The As concentration of breast milk in endemic As-contaminated areas (e.g., aquifers) was found to be lower than expected.176 According to the Joint FAO/WHO Expert Committee on Food Additives, the lower limit of the benchmark dose (BDML₀.₅) with iAs-induced lung cancer as the end point of toxicity effect is 3 µg kg⁻¹ BW.177
Twenty-four-hour pooled samples taken from the same mother\textsuperscript{178} would be ideal for elemental analysis in breast milk. Since this is not possible, usually, samples are collected from only one mammal gland that has not been involved in breastfeeding for 2-3 hours prior to sampling.\textsuperscript{178} The most common sample preparation procedure is MW-assisted acid digestion using a mixture of concentrated HNO\textsubscript{3} and hydrogen peroxide solutions. If freeze-drying is applied before analysis,\textsuperscript{178-180} the sample demand is smaller.\textsuperscript{180} The digested samples are nowadays analyzed by ICP-MS.\textsuperscript{181-183}

A recent study conducted in Hungary on the determination of As in breast milk of non-smoking, exclusively breastfeeding volunteers with an average body mass index and with no occupational exposure to As in the early stages of breastfeeding, the mean concentration of As in the samples was 0.41 ± 0.20 µg L\textsuperscript{-1}. However, the median values of the As concentrations determined for all samples were 0.35 µg L\textsuperscript{-1}, respectively, indicating an almost symmetric distribution of the concentration dataset.\textsuperscript{184} The median As concentration in the abovementioned study was approximately 45% larger than in the Hungarian mature milk samples published in 1991 and analyzed by neutron activation analysis.\textsuperscript{185} Unlike in 1991, the results of the recent study showed that As was detectable in all samples. This is due not only to the advances in instrumental analysis, but also to the changes in the dietary habits of the last 30 years. Today, more poultry meat is consumed, because it is considered healthier, and a larger selection of seafood and marine fish is available in Hungary. Lovreglio \textit{et al.}\textsuperscript{186} found that less toxic AsB and AsC occur even in poultry meat. Concentration values of As in samples taken in Taiwan and Sweden were also similar to those obtained in the aforementioned recent study.\textsuperscript{187} Higher As concentrations have been reported from Greece.\textsuperscript{175} Outside Europe, higher values have been reported from Pakistan (0.5 µg L\textsuperscript{-1}),\textsuperscript{188} Iran (0.85 µg L\textsuperscript{-1}),\textsuperscript{189} Bangladesh (1 µg L\textsuperscript{-1}),\textsuperscript{190} Japan (1.4 µg L\textsuperscript{-1})\textsuperscript{191} and Lebanon (2.3 µg L\textsuperscript{-1}).\textsuperscript{192} In mature breast milk samples analyzed in Portugal, As concentration was about 5.8 µg L\textsuperscript{-1}, presumably due to regular consumption of fish and seafood, while the Pb concentration was lower.\textsuperscript{193}

Estimation of the intake of As in breast-fed infants can be performed by the method applied by Rebelo and Caldas\textsuperscript{194} consisting of expressing the ratio between the determined concentration and the benchmark dose limit (BMDL) value. The value thus obtained is called margin of exposure (MOE). Its estimated value is inversely proportional to the risk to public health posed by a certain toxic element.\textsuperscript{195} In the US, this value is typically above 50 for As.\textsuperscript{196} According to literature data, the MOE values for As in Europe are about 50 or higher.\textsuperscript{194} No significant relationship could be found between the age of the volunteers and the As concentrations in breastmilk samples.\textsuperscript{184} In return, the MOE values calculated for Pb proved that this element continues to pose a potential risk to infants.\textsuperscript{184} Moreover, the Pb concentration in breast milk was significantly correlated with the age of the mothers.\textsuperscript{184,197}

Organs playing an important role in reproduction and offspring care are protected against As, As-speciation is difficult to achieve.

\textit{Potential threat represented by As in complementary and alternative medicinal products}

\textit{General considerations}

According to the WHO, traditional medicine is defined as CAM. This category includes traditional Chinese medicine, the Ayurvedic or Indian medicine system, the Tamil Siddha, the Persian - Arabic Unani, and homeopathy. Of these, Ayurveda is only surpassed in popularity by traditional Chinese medicine. The main difference between these two systems is that Ayurvedic preparations can not only contain herbs but also minerals and elementary state elements (e.g., Hg, S). From an analytical chemistry point of view, Ayurvedic preparations can be classified as herbal, herbomineral and animal ones. Of the metals used to make Ayurvedic preparations, Hg is of primary importance. Among the minerals, those containing As such as realgar (\(\alpha\)-As\textsubscript{4}S\textsubscript{4}) and auripigment (As\textsubscript{2}S\textsubscript{3}) are common constituents. Through the current numerous infocommunication channels, these products have become available worldwide. Online sales of Ayurvedic products have recently skyrocketed.\textsuperscript{198} By performing elemental analysis of 193 Ayurvedic preparations purchased at random from the Internet, the authors of the study found that Pb, Hg or As were detectable in 21% of the products. The order of detection frequency of these toxic elements in the investigated...
products was: Pb > Hg > As. Heavy metal and As contamination was twice as common in herbomineral products as in other types of products. In the Netherlands, about 20% of the labels of 292 traditional herbal preparations used in Ayurveda, traditional Chinese and Tibetan medicine, indicated the presence of Hg, As or Pb. These elements could be determined by ICP-MS in 64% of the samples. In about 20% of the investigated samples (n = 59), the amount of these elements exceeded the maximum allowable intake for humans with a single recommended dose. For 26 products, the estimated weekly As intake was 0.53 and 427 mg.\(^{199}\)

The European Commission classifies Ayurvedic preparations only as food supplements under Directive 2002/46/EC. In the United States, where Ayurvedic preparations are subject to the requirements of the Food Supplements Act 1994 (DSHEA - Dietary Supplement Health and Education Act), there is no need to demonstrate the safe use or efficacy of these products.\(^{198}\)

The large number of scientific communications on Ayurvedic research published in peer reviewed journals contain relatively less information from analytical chemistry point of view. Elemental content of Ayurvedic products has been studied by neutron activation analysis,\(^{200}\) voltammetry,\(^{201}\) XRF spectroscopy\(^{199,202}\) or ICP-MS.\(^{199}\) The advantage of XRF is that it is a non-destructive analytical technique, although not suitable for the determination of trace elements, while ICP-MS has extremely low detection limits, its acquisition and maintenance costs are much higher, and sample preparation is a much more time-consuming process. Literature data are difficult to be compared due to the wide range of Ayurvedic products. An attempt is presented hereby. Khandpur \textit{et al.}\(^ {203}\) investigated eight different Ayurvedic formulations, the As concentrations in these products were ranging from 5 to 248 mg kg\(^{-1}\). The concentration of As in a blood sample from a patient undergoing Ayurvedic treatment was 0.202 mg L\(^{-1}\), although the normal value is generally <0.06 μg L\(^{-1}\). Saper \textit{et al.} studied 70 Ayurvedic formulations purchased in stores and online\(^ {204}\) in the United Stated by X-ray fluorescence spectrometry. Of the 70 samples investigated, Pb, Hg, and As were quantifiable in 14. The concentration of As ranged from 0.037 to 8.13 g kg\(^{-1}\).\(^ {204}\)

In a recent study, analysis by ICP-SF-MS after MW-assisted acid digestion of six different Ayurvedic products purchased online from India and one purchased in Hungary showed that the As concentration was relatively low, i.e., 0.41 ± 0, 05 mg kg\(^{-1}\) in the plant-derived sample (containing \textit{Centella Asiatica}).\(^ {14}\) The concentrations of As in the rest of the investigated samples ranged from 0.04 to 771 mg kg\(^{-1}\). The concentration of As (106 ± 13 g kg\(^{-1}\)) was the highest in an As\(_2\)O\(_3\)-containing preparation.\(^ {14}\) Due to its significant As content, an Ayurvedic ointment was only worth analyzing by energy dispersive X-ray fluorescence (EDXRF). Thus, the spectra of the ointment recorded by EDXRF confirmed that As was the major component of the sample.

**Arsenic bioaccessibility in Ayurvedic complementary and alternative medicines**

Knowledge of the total concentration of a toxic element (e.g., As) is necessary but not sufficient to estimate adverse health risks, so it is important to quantify the amounts of element(s) that could be assimilated after ingestion of a certain dose. However, application of \textit{in vivo} experiments raises ethical issues. Thus, several \textit{in vitro} procedures have been developed to simulate the effects of the human digestive process.\(^ {205}\)

Data from only a few complex analytical studies on bioaccessible concentrations of toxic elements in Ayurvedic formulations have been reported.\(^ {206-208}\) Giacomino \textit{et al.}\(^ {209}\) used a mixture of hydrochloric acid, sodium chloride and pepsin to simulate gastric juice. Pancreatin, potassium dihydrogen phosphate and sodium hydroxide solution were used to simulate duodenal fluid. However, As\(_2\)O\(_3\) and As sulfides can hardly be dissolved in HCl due to their (thio)acid anhydride properties, so their dissolution is expected only in alkaline media. However, due to their high initial concentration, As uptake by the human body may still be significant. For samples containing As\(_2\)O\(_3\)/cinnabar red and α-As\(_2\)S\(_4\)/HgS, the estimated daily intake exceeded the WHO recommended daily dose of 3.0 μg iAs BW kg\(^{-1}\) by a factor of 11.1 to 21.4.\(^ {14}\)

After incubation of 17 products purchased from pharmacies and local markets in Italy and India, respectively, in suspensions of gastric and duodenal fluids, a remarkable dissolution was observed for As.\(^ {209,210}\)

Koch \textit{et al.}\(^ {206}\) characterized the elemental content of CAMs by ICP-MS and XANES, focusing on the bioaccessibility of As and Pb, respectively, by incubation in a model suspension containing pancreatic
and bile extract, respectively. Interestingly, bhasma formulations characterized by a strong covalent bond between As(III) and S atoms increased the bioaccessibility of As. The authors explained this phenomenon by the oxidation of As(III), which was confirmed by XANES measurements. In addition, the estimated dietary intake using the bioaccessible As concentration values in several cases was greater than the daily tolerable limit set by California Proposition 65. Bolan et al. investigated bioaccessibility of As-containing rice and some Ayurvedic products of plant origin by using an exhaustive sequential extraction method proposed by Tessier et al. as well as in simulated gastric and duodenal fluids. The concentration of bioaccessible As was highly dependent on the procedure used. However, pancreatin is one of the most preferred compounds for simulating duodenal fluid. Extraction efficiencies determined by modeling bioaccessibility resulted in large differences. Therefore, it would be important to develop and adopt an appropriate extraction method.

Arsenic speciation in phosphate-rich bioaccessible fractions of Ayurvedic preparations

Arsenic speciation in bioaccessible fractions is not a routine procedure. Until now, elemental speciation has been performed directly on Ayurvedic samples in solid state of matter using X-ray-based analytical techniques (e.g., XANES, extended X-ray absorption fine structure, EXAFS) due to the high toxic element concentration of the formulations. In herbal preparations, the predominant As species were organic, while in mineral-containing Ayurvedic products, As occurs in inorganic form.

Two Ayurvedic formulations containing As in moderate concentration were studied after spiking to 150 µg kg⁻¹ iAs(V) standard solution, incubation in a pH = 6.8 suspension simulating duodenal fluid and SAX-SPE separation. The highest recovery of about 80% for the iAs(V) species was obtained when the model solution did not contain phosphate ions. The presence of sodium hydroxide did not affect the recovery of iAs(V). Oxidation of iAs(III) was observed using optimal SPE conditions for two Ayurvedic (herbo)mineral preparations with high As content. Thus, 6.3% of the iAs(III) in the bioaccessible fractions were oxidized in the sample containing As₂O₃ and cinnabar red, compared to 33% in the formulation containing realgar and HgS. Spiking a simple homeopathic sample matrix containing theoretically As₂O₃ with a 1 µg L⁻¹ iAs(V) standard solution, recovery of total As and iAs(V) of samples subjected to SAX-SPE were about 97% and 95%, respectively. The recovery of total As and iAs(V) was approximately 90% and 80%, respectively, when the homeopathic product was spiked to 10 µg L⁻¹ iAs(V) and then incubated with the duodenal modeling suspension without the addition of phosphate ions. Study of the homeopathic remedy has also shown that it was mainly phosphate ions that prevent the separation of As species with SPE and not the matrix constituents. Breakthrough results in this area with efficient As-speciation methods have yet to be published.

CONCLUSIONS

In recent decades, several As speciation methods have been developed, many of which are cost-effective as alternatives to the original on-line hyphenated techniques consisting of an HPLC separation followed by atomic spectroscopic detection. In the case of TLC, the OPLC-TXRF method provides reproducible operating conditions, resulting in faster separation and better resolution. Laser ablation for PEI-TLC plates followed by ICP-MS measurements proved to be suitable for the screening of biological samples. In situ separation of As species in water with SAX-SPE can be easily accomplished from matrices up to pH<9 with higher enrichment and/or dilution, followed by laboratory analysis of acid-preserved samples. The cost-efficient TXRF spectrometry allows the simultaneous determination of several other trace elements in drinking water. At the regional level (e.g., in Hungary), the exposure of the population to As has been significantly reduced since 2017 by drilling new wells and establishing new waterworks in settlements of poor water quality. To the best of my knowledge, however, As speciation has not been widely used in the development of As removal technologies. To date, there is no real breakthrough in the use of As speciation methods. Arsenic contamination is challenging since inorganic As species cannot easily be decomposed compared to purely organic contaminants. Thus, the removed As is concentrated in hazardous waste disposal, which is also costly. Another possibility is to immobilize As e.g., in the soil. In this respect, bio-
waste is a promising raw material after conversion to AC with concentrated H\textsubscript{2}SO\textsubscript{4}. In order to increase mechanical stability and adsorption, it is advisable to use a silicate-containing material after charring to stabilize As (e.g., Florisil\textsuperscript{®}). Although Florisil\textsuperscript{®} is a widespread chromatographic adsorbent, it is expensive, but simple charring processes can keep the cost of producing ACs low (e.g., no application of energy-demanding pyrolysis).

In the lack of a definitive solution for the removal of As from drinking water, it is important to estimate the exposure of the population in areas with extensive natural As pollution. Dietary intake is the major source for nonoccupational exposure to As. Washing and cooking rice in plenty of water is advisable in regions affected by As-contaminated water. In this way, large amounts of As can be removed. According to a recent study, significant efforts are still being made in several parts of the world (e.g., West Bengal) to develop intervention procedures to reduce As pollution of waters.\textsuperscript{218}

The estimated intake of As from the bioaccessible fractions of Ayurvedic formulations poses a health risk. If public health employees were more empathetic to their patients, or if a significant portion of the contemporary society did not reject the results of scientific research, there would be no demand for such dubious quality drugs. A recent review paper\textsuperscript{219} highlighted the need to (i) elucidate the mechanisms of incorporation of toxic elements into CAM formulations, (ii) to understand the biogeochemical cycles of these elements and in particular their metabolites; (iii) to develop appropriate speciation methods to study the \textit{in vitro} bioaccessibility of toxic elements; and (iv) conducting clinical trials and developing stricter legislation. In accordance with the current practice, elemental speciation in Ayurvedic preparations containing high concentrations of toxic elements is performed directly in the solid state by X-ray-based analytical techniques (XANES, EXAFS). Although the success of X-ray measurement techniques is undeniable, more accurate information could be obtained using appropriate \textit{in vitro} methods to study bioaccessibility, but extreme pH and complex sample/extraction matrix represent real obstacles.

Even today, several reviews deal with the estimation of health risks caused by dietary As intake\textsuperscript{220,221} and the development of As speciation methods.\textsuperscript{222} Regarding the reduction of As contamination in environmental compartments and food items, many questions still should be addressed in the future.

**Conflicts of interest**

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