Review Article

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Research progress on speciation analysis of arsenic in traditional Chinese medicine

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Abstract: Traditional Chinese medicine contains arsenic (As), which in the natural environment accumulates in plants during the growth of Chinese medicinal materials; there are mineral medicines containing As in Chinese patent medicine such as As_4S_4 , As_2S_3 , etc. Due to the toxicity of As-containing compounds and its role in inflammation and treatment of cancers such as leukemia, it is necessary to analyze the chemical form of As. A comprehensive investigation of the compound forms of heavy metals rather than the simple total amount of elements will lay the foundation for the scientific and objective evaluation of the safety of heavy metals. This article summarizes the speciation of As in bulk Chinese medicinal materials and Chinese patent medicines in recent years, and reviews the main research methods of As speciation analysis. The separation and detection combined analysis method focuses on the high-performance liquid chromatography-plasma mass spectrometry and high-performance liquid chromatography-hydride generation-atomic fluorescence spectroscopy, etc. Taking the advanced synchrotron radiation source as the research platform, the use of X-ray near edge absorption fine structure spectrum and micro-area X-ray fluorescence analysis as a microscopic analysis technique supports direct analysis of the As speciation *in situ*. It is the most promising morphological analysis method.

Keywords: traditional Chinese medicine, arsenic, speciation analysis, research progress

1 Introduction

Traditional Chinese medicine embodies profound philosophical wisdom and thousands of years of health preservation concepts and practical experiences in the Chinese nation. It is the treasure of ancient Chinese science and the key to open the treasure house of Chinese civilization. Traditional Chinese medicine is one of China's few industries with international competitive advantages. Since 2005, the domestic growth rate of traditional Chinese medicine industry has basically remained above 20%, exceeding the average growth rate of the pharmaceutical industry. Due to heavy metal residues, etc., China's total export of Chinese medicine currently accounts only for about 1% of the world's sales of botanical drugs [1,2]. According to the ISO international standard of "Traditional Chinese Medicine-Heavy Metal Limits of Chinese Medicinal Materials," the over-limit ratios of heavy metals including lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg) are 3.46, 4.03, 2.91, and 1.41%, respectively [3–7]. Excessive heavy metals have become a key issue that affects the quality and reputation of traditional Chinese medicine and hinders their globalization [8,9].

Through the national "Eleventh Five-Year" science and technology support program project "Comprehensive Control of Soil Pesticide Residues and Heavy Metals in Chinese Herbal Medicines," researcher Guo Lanping from the Chinese Medicine Resource Center of the Chinese Academy of Chinese Medical Sciences sorted out the literature on heavy metals in Chinese medicine from 2000 to 2016. The statistical data of 1,700 samples of 275 kinds of medicinal materials show that among the heavy metals of Pb, As, Hg, and Cd, As has the highest over-limit ratio, reaching 4.03%, of which three batches of seaweed from unknown origin has the highest content, with As content at 81.34–82.55 mg/kg. The figure is followed by 9 batches of honeysuckle (30.80–73.35 mg/kg) from Shandong, Anhui, and Henan, and *Asarum heterotropoides* (33.82 mg/kg) from Fusong, Jilin [10,11]. The 2015 edition of the Chinese Pharmacopoeia stipulates that As in traditional Chinese medicine is ≤ 5.0 mg/kg, and the US Food and Drug

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Administration stipulates that As in traditional Chinese medicine is <2.0 mg/kg [4]. Regarding heavy metal As contamination in Chinese herbal medicines in different medicinal parts, the average As content in algae, fungus, and lichens is 11.77 mg/kg, and the over-limit ratio is 13.64%. The average As content in leaf medicinal materials is 1.10 mg/kg, and the over-limit ratio is 0.71%. The over-limit ratio of As in all other types of Chinese medicinal materials is below 10% [4]. Studies have shown that the heavy metal As has the highest over-limit ratio in the animal category among Chinese medicinal materials, and the heavy metal over-limit ratio is high in flowers, leaves, and whole plants [4]. The quality standards of Chinese medicinal materials have clear stipulations on the total amount of As, but the toxicity of As depends not only on the total amount, but also on its chemical speciation. The paths for heavy metals to enter medicinal plants include: (1) Environmental pollution, environmental factors such as soil, water, and atmosphere constitute important environmental conditions for planting of medicinal materials; (2) The active absorption and enrichment characteristics of medicinal materials during the growth process; (3) Contamination in harvesting, processing, auxiliary materials, packaging, storage, and transportation; and (4) The use of pesticides and fertilizers containing heavy metals in the planting process [12].

As is a common toxic element, which is widespread in nature as one of the main elements of Chinese herbal medicine contamination. The As in the environment exists in inorganic forms, such as As_2O_3 , As_2O_5 , etc., and organic forms including monomethyl arsenic acid (MMA), dimethyl arsenic acid (DMA), and trimethyl arsenic acid (TMA), which is mainly arsenic betaine (AsB) and arsenic choline (AsC) in marine products. The toxicity of As to organisms mainly depends on its speciation. It is generally believed that the toxicity of As is ranked as [13,14]: As hydride and its

derivatives $>$ inorganic arsenite [As(III)] $>$ inorganic arsenate [As(V)] $>$ organic trivalent As compound $>$ organic pentavalent As compound $>$ As element. Methyl As acid has much smaller toxicity than arsenate or As trioxide [15]. The LD_{50} of trimethylarsenic oxide is 8,000 mg/kg, while that of sodium arsenate and As trioxide are 14–18 and 34.5 mg/kg, respectively. The process of inorganic As conversion into methyl As is widespread in microorganisms, plants, animals, and even humans. The difference is that the final product of mammalian methylation of As is dimethylarsinic acid (DMAA) [13,16], while the product of microbial methylation of As is mainly trimethylarsenic.

2 Source of As

With the rapid development of industry and agriculture, including man-made activities such as mining of minerals and burning of fossil fuels, a large amount of As has entered the environment. The As content is 2–82 mg/kg in coal and up to 1,500 mg/kg in lignite. The soil around coal-fired power plants is seriously polluted by As. Over a long time, As compounds have been widely used in agriculture and horticulture as insecticides, disinfectants, fungicides, and herbicides. Some chemical fertilizers also contain a certain amount of As. The domestic amount of As that enters the farmland through the application of organic fertilizers is as high as 1,412 tons. Although many countries have banned the use of these substances in recent years, excessive accumulation of As in the soil until it exceeds the standard has formed a reality in some areas. Since As compounds have excellent wood preservative properties, especially copper chromate arsenic, As is also widely used in forestry and manufacturing, and its global usage is

Figure 1: Biochemical transformation of As in nature. Microbial transformations ($\rightarrow$). MMAA: monomethylarsonic acid; DMAA: dimethylarsinic acid; TMAO: trimethylarsineoxide; MMA: monomethylarsine; DMA: dimethylarsine; TMA: trimethylarsine.

increasing at a rate of 1–2% every year [17]. The biogeochemical cycle of As in the soil and atmosphere is shown in Figure 1 [18].

3 Speciation of As in traditional Chinese medicine

3.1 Speciation of As in Chinese medicinal materials

During the growth of Chinese medicinal materials, As in the natural environment accumulates in plants. Liu Xiaojuan of Hebei Agricultural University studied the occurrence of As in Chinese herbal medicines. Using 13 kinds of Chinese herbal medicine in Hebei province as the main objects, including cultivated medicinal materials collected from planting areas, market semi-finished products, and decoction pieces, microwave digestion hydride generation-atomic fluorescence spectrometry (HG-AFS) and high-performance liquid chromatography combined with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) were used to study the content of As in the Chinese herbal medicine samples and its chemical speciations. The results show that in 1% HNO₃ microwave-assisted extraction of As species, the chemical speciations of As in Chinese herbal medicines are mainly inorganic As(III) and As(V), and inorganic As accounts for more than 86% of the total As. DMA was

detected in 8% of the samples, and all of them were decoction pieces; in 20% of the samples of Anemarrhena, 28 µg/kg MMA was detected [19].

Gu Shanyong et al. of Heilongjiang University of Chinese Medicine used HPLC-ICP-MS to detect a total of 103 batches of 17 kinds of commonly used Chinese herbal medicines (16 kinds of botanical drugs and 1 kind of medicinal fungi). Using the high-temperature ultrasonic extraction method, a simultaneous detection method for six As speciation in Chinese herbal medicines was established based on HPLC-ICP-MS, including As(III), As(V), MMA, DMA, AsB, and AsC. The As speciation data of 17 commonly used bulk medicinal materials are shown in Table 1 [20].

Luo Jiaoyang et al. of the Chinese Academy of Medical Sciences and the Institute of Medicinal Plants of Peking Union Medical College established an HPLC-ICP-MS-based analytical method for the analysis of As element form residues in 31 animal medicines. The high-risk varieties of As residues include lumbricus, long-nosed pit viper, zaocys dhumnade, and Aspongopus Chinensis Dallas. The speciation data of As in 31 animal medicines are shown in Table 2 [21]. The detection rates of different As speciations in 31 animal medicine batches are- As(III): 96.77%, As(V): 100%, MMA: 45.16%, DMA: 90.32%, AsB: 93.55%, and AsC: 22.58%.

The quantitation limits of As(III), As(V), MMA, DMA, AsB, and AsC were 0.2, 0.2, 0.2, 0.2, 0.1, and 0.4 µg/L, respectively.

Traditional Chinese medicine is mainly composed of botanical medicine, animal medicine, and mineral medicine.

Table 1: The contents of As speciation in 17 plant Chinese herbal medicines

Types	Origin	Batch no.	As(III)(µg/kg)	As(V)(µg/kg)	MMA(µg/kg)	DMA(µg/kg)	AsB(µg/kg)
American Ginseng	Jilin	8	5.23	4.57	0.89	0.24	0.13
Salvia miltiorrhiza	Sichuan	2	45.66	127.14	1.71	—	—
Acorus tatarinowii	Anhui	1	480.7	1044.30	11	—	1.2
Radix Isatidis	Anhui	2	157.7	—	2.21	—	—
Achyranthes bidentata	Henan	2	5.11	49.15	2.1	—	—
Lily	Gansu	3	51.64	—	1.97	—	—
Yams	Henan	2	87.26	—	1.79	—	—
Poria cocos	Yunnan	3	55.17	25.21	2.05	—	—
Codonopsis pilosula	Sichuan	2	5.86	123.71	1.66	—	—
Notoginseng	Yunnan	3	18.34	175.45	50.07	—	1.14
Honeysuckle	Henan	2	104.13	253.575	2.165	9.765	—
Licorice	Ningxia	3	5.175	126.38	2.03	—	—
safflower	Hunan	1	83.19	250.47	1.75	2.45	1.57
Radix gentianae	Jilin	2	81.71	439.26	2.52	—	4.75
Albizia julibrissin	Hebei	1	90.34	251.81	2.03	—	2.96
Chinese wolfberry	Ningxia	4	5.32	28.98	2.39	—	—
Ginseng	Jilin	4	5.20	12.57	1.97	—	—

—: No As was detected.

Looking at the morphological research data of As in the above traditional Chinese medicine, As(III) and As(V) have a high detection rate, with As(V) as the main detected form. Inorganic As accounts for more than 80%; organic As in plant medicines mainly exists in the form of MMA; organic As in animal medicines mainly exists in the form of AsB, followed by DMA. According to the statistics from the World Health Organization, about 4 billion people worldwide currently use traditional Chinese medicine to treat diseases, accounting for 80% of the world's total population. The safety of traditional Chinese medicine concerns the health and life of the user. Unlike western medicine which has a single and clear material basis, traditional Chinese medicine has extremely complex material basis. Therefore, the morphological structure and valence coordination of As in trace amounts of traditional Chinese medicine determine the patient's dynamic metabolism, dose-effect relationship, toxicity mechanism, etc., after administration.

3.2 Speciation of As in Chinese patent medicines

There are mineral medicines containing As in traditional Chinese medicine preparations, such as realgar (As₂S₂), orpiment (As₂S₃), and As trioxide (As₂O₃). There are 100 Chinese patent medicines containing realgar included in the 2015 Chinese Pharmacopoeia [22]. First published in "The Holy Husbandman's Classic on Roots and Herbs," realgar is often used to treat carbuncle sores, snake and insect bites, and worm accumulation abdominal pain. In recent years, compound preparations containing realgar components such as Liushen pills, Niu Huang Jiedu tablets, Qinghuang powder, compound Qingdai tablets, etc., and single-flavored realgar have been used for clinical treatment of tumors in the blood system and tumors in other parts of the human body [23]. Table 3 shows the speciation data of As in commonly used Chinese patent medicines.

Table 2: The contents of As speciation in 31 animal medicines

Types	As(III)(μg/kg)	As(V)/(μg/kg)	MMA/(μg/kg)	DMA/(μg/kg)	AsB/(μg/kg)	AsC/(μg/kg)
Bombyx Batryticatus	132.7 ± 100.6	17.28 ± 5.77	ND	<LOQ	3.81 ± 0.58	ND
Cornu Bubali	16.90 ± 4.02	7.16 ± 0.79	9.11 ± 0.73	45.67 ± 5.50	3.76 ± 0.75	ND
Margarita	ND	3.50 ± 0.51	ND	<LOQ	1.80 ± 0.14	ND
Faeces Troglodyterori	49.29 ± 41.29	19.73 ± 6.32	ND	28.34 ± 3.04	16.64 ± 2.40	ND
Scorpio	19.91 ± 4.41	11.67 ± 4.27	ND	2.47 ± 0.48	17.12 ± 7.10	ND
Pheretima	1,883 ± 391.5	431.8 ± 100.1	ND	32.12 ± 2.34	311.6 ± 12.70	ND
Testudinis Carapax et Plastrum	24.16 ± 23.99	3.10 ± 2.07	ND	<LOQ	5.29 ± 0.88	ND
Eupolyphaga Steleophaga	178.2 ± 228.0	30.56 ± 8.23	ND	48.87 ± 5.65	37.96 ± 1.45	ND
Cervi Cornu Pantotrichum	4.39 ± 0.39	3.55 ± 0.98	ND	<LOQ	2.64 ± 0.48	ND
Corii Colla Asini	19.69 ± 0.86	7.46 ± 1.77	ND	<LOQ	1.59 ± 0.36	ND
Cornu Cervi	53.52 ± 45.26	6.00 ± 1.58	ND	<LOQ	2.58 ± 0.35	ND
Ostreae Concha	7.38 ± 5.79	4.42 ± 1.60	ND	ND	1.55 ± 0.77	ND
Cicadae Periostracum	59.45 ± 41.17	21.42 ± 1.46	3.11 ± 1.08	2.79 ± 2.50	11.84 ± 15.22	ND
Margaritifera Concha	18.94 ± 13.16	6.86 ± 5.27	<LOQ	4.24 ± 2.73	10.71 ± 4.19	ND
Trionycis Carapax	27.39 ± 9.39	5.98 ± 0.98	6.91 ± 1.80	4.02 ± 0.90	5.05 ± 1.06	ND
Gecko	7.68 ± 1.94	12.14 ± 4.11	3.62 ± 0.56	10.63 ± 2.97	23.86 ± 19.68	5.10 ± 2.94
Scolopendra	14.29 ± 2.39	9.85 ± 0.81	6.24 ± 1.84	3.78 ± 0.44	99.20 ± 21.94	ND
Hirudo	189.2 ± 73.12	10.29 ± 4.23	7.88 ± 3.68	40.61 ± 15.59	161.1 ± 187.5	19.51 ± 24.22
Zaocys	331.5 ± 202.6	24.95 ± 15.78	6.33 ± 1.79	30.84 ± 18.09	356.0 ± 406.0	120.8 ± 198.7
Hippocampus	12.94 ± 4.69	15.12 ± 3.46	<LOQ	6.80 ± 1.91	61.90 ± 31.94	9.29 ± 1.97
Gekko Japonicus Dumeril et Bibron	97.34 ± 33.27	15.50 ± 4.42	3.51 ± 1.64	27.24 ± 2.29	46.39 ± 4.58	8.45 ± 3.47
Agkistrodon	411.9 ± 85.47	15.45 ± 2.52	1.12 ± 0.64	2.58 ± 1.46	19.50 ± 12.13	ND
Syngnathus	10.94 ± 9.82	7.78 ± 6.01	ND	95.26 ± 32.74	2,016 ± 676.5	36.09 ± 6.60
Nidus Vespa	143.1 ± 173.4	20.63 ± 14.55	5.88 ± 0.62	13.32 ± 1.62	25.23 ± 3.15	ND
Aspongopus	275.5 ± 449.4	27.74 ± 28.38	ND	2.13 ± 0.23	6.28 ± 1.33	ND
Sepiae Endoconcha	36.63 ± 57.81	8.62 ± 8.71	ND	401.5 ± 122.9	1,257 ± 129.5	10.38 ± 0.75
Bufo venenum	10.94 ± 2.13	8.35 ± 4.11	ND	ND	ND	ND
Bungarus Parvus	2.93 ± 1.13	4.90 ± 3.04	ND	62.28 ± 43.54	15.22 ± 17.66	ND
Bovis Calculus	5.79	4.44	ND	ND	ND	ND
Moschus	11.72	19.00	5.16	3.65	9.92	ND
Cordyceps	165.31	219.6	83.12	4.97	11.53	ND

ND: Not detected.

The safety of traditional Chinese medicines containing heavy metal As, such as cinnabar and realgar are often questioned. Table 3 shows that in mineral Chinese patent medicine containing As, As(III) and As(V) have a high detection rate, with As(III) as the main detected form, and the content of organic As is extremely low. As(III) is highly toxic. In fact, after the more toxic inorganic As enters the body, it can be quickly transformed into DMA with very low toxicity and the widest distribution in the blood. This is consistent with the drug distribution required for the treatment of leukemia by Chinese patent medicine with As [26].

4 Analytical method of As speciation

Commonly used methods for the determination of As mainly include atomic absorption spectroscopy (AAS), inductively coupled plasma-optical emission spectroscopy (ICP-OES), inductively coupled plasma-atomic emission spectrometry (ICP-AES), and inductively coupled plasma-mass spectrometry (ICP-MS). Compared with ICP-AES, ICP-MS uses mass spectrometry as the detector, so the detection limit is lower. Due to its ability to measure isotope, it is suitable for simultaneous determination of multiple ultratrace elements [27]. The determination of heavy metals in the Chinese Pharmacopoeia is based on AAS or ICP-MS. In the 2015 edition of the Chinese Pharmacopoeia, the detection of As in realgar and realgar-containing preparations adopts the ancient Chua's method and the silver diethyldithiocarbamate method, and

the As₂S₃ content in realgar is determined by the iodometric method [28].

4.1 Sample pre-processing technology

Element speciation analysis includes three processes of extraction, separation, and detection. In the field of traditional Chinese medicine research, when As is extracted, the common samples mainly include original Chinese medicinal materials, processed Chinese medicinal materials, and traditional Chinese patent medicine. The extraction solvent system includes water, methanol-water, acetic acid-water, hydrochloric acid-water, enzyme, chloroform-methanol-water, artificial gastrointestinal fluid, etc. [29]. According to literature reports, it has been found that when hydrochloric acid-water is used to extract As from animal medicinal materials, there are many types of As; but when the hydrochloric acid solution is greater than 0.5 mol/L, MMA is easily converted to As(III) [30,31]. When methanol-water is used as the extraction solvent, the extraction rate of As(III) is reduced, and the extraction rate of As(V) is increased. Moreover, nitrogen drying or rotary evaporation is required to remove methanol before determination. Otherwise, the total As extraction rate will be significantly high [31]. When water is used as the extraction solvent, the As extraction efficiency is higher than that of other extraction solvents [32]. When acetic acid-water (1:19, volume ratio) is used as the solvent, the standard recovery rate of the five forms of As can reach 83.8–111.7%, and the treatment process is simple. Both organic As and inorganic As can be well

Table 3: The contents of As speciation in Chinese patent medicine

Chinese patent medicine	As(III)/(mg/kg)	As(V)/(mg/kg)	MMA/(mg/kg)	DMA/(mg/kg)	Reference
Niuhuang Qingxin pills	119.3	15.7	ND	ND	[23]
Angong Niuhuang pills	124.1	19.9	ND	ND	[23]
Realgar	5377.8	1046.4	28.1	ND	[23]
Niuhuang Jiedu tablets	1,283	467	BLD	ND	[24]
Bushen tablets	7.5	2.3	0.54	ND	[24]
Twelve Taibao pills	0.40	3.1	BLD	ND	[24]
Bao Ying Dan	0.72	0.38	BLD	ND	[25]
Mingyan pills	0.58	0.62	BLD	ND	[25]
Zhengxin Dan	3.1	9.9	1.4	ND	[25]
Nose Mingqing	0.58	0.90	BLD	ND	[25]
Niuhuang Jiedu tablets	309	286	ND	ND	[25]
Niuhuang Jiedu pills	67.8	33.3	ND	ND	[25]
Children's Zhibao pills	222	20.1	ND	ND	[25]
Children's Qizhen pills	1,908	354	ND	ND	[25]
Angong Niuhuang pills	409	177	ND	ND	[25]
Niuhuang anti-inflammatory tablets	384	98.9	ND	ND	[25]

Nd: not detected; BLD: lower than the detection limit.

extracted [33]. When artificial gastric juice is extracted, the various forms of As are relatively stable without transformation [34].

At present, the commonly used As speciation extraction methods include water bath extraction, ultrasonic-assisted extraction, and microwave-assisted extraction. Various pre-processing techniques for As speciation analysis are shown in Table 4.

Generally speaking, ultrasonic-assisted extraction and microwave-assisted extraction are used in many applications. The ultrasonic process can break up the solid particles, increase the contact area between the solvent and the particles, disperse the insoluble substances into the extractant, and also dissolve the substances wrapped in the particles. In microwave extraction, the extractant and target components can be rapidly “heated” under the action of microwave, and the use of low-power microwave extraction can avoid long-term high-temperature decomposition of the sample [42].

4.2 Detection method

4.2.1 AAS

AAS has the advantages of low detection limit (flame method can reach $\mu\text{g}/\text{cm}^3$ level), high accuracy (relative error is less than 1% compared to flame method), good selectivity (that is less interference), fast analysis speed, and wide application range (the flame method can analyze more than 30/70 elements, the graphite furnace method can analyze more than 70 elements, and the hydride generation method can analyze 11 elements). However, for the detection of As, AAS has low sensitivity

and big interference. Zheng Zhiyuan *et al.* detected As in 4 Tibetan medicines (25 flavor Coral pill, 25 flavor Tophus pill, Renqing Changjue, and Renqing Mangjue) by AAS. Accurately absorbed 50 μL of As standard solution into a 10 mL volumetric flask, diluted to the mark with HCL at 1% mass concentration, and thus prepared 5 $\mu\text{g}/\text{mL}$ As solution as a reference substance. The detection limit was 0.012 $\mu\text{g}/\text{mL}$, and the standard recovery rate was 96.67–99.87% [43].

4.2.2 ICP-AES

The ICP-AES method has high sensitivity and shorter duration. It can measure trace elements and micro elements at the same time, with a wide linear range and obvious comprehensive advantages. Xiao Xinyue *et al.* detected As in deerhorn gum by ICP-AES, extracted it by microwave digestion, and prepared standard As solution at a mass concentration of 0.3 mg/L as the reference using 2% nitric acid. The measurement conditions of the ICP-AES instrument include the incident power of 1.4 kW, the cooling gas as argon, the cooling gas flow rate of 12 L/min, the carrier gas flow rate of 1 L/min, and the auxiliary gas flow rate of 0.8 L/min, and the sample lift volume of 1 mL/min. Finally, the detection limit of As was 0.27 mg/kg, and the adding sample recovery rate was 90–112% [44].

4.2.3 HPLC-ICP-MS

The combination of HPLC-ICP-MS has the advantages of ultra-high sensitivity and simultaneous measurement of multiple elements, which is one of the most effective tools for the laboratory to study As speciation. Table 5 shows

Table 4: Extraction method of As speciation

Sample	Extraction method	Solvent	Extraction efficiency (%)
Soil [35]	Heated at 100°C for 3 h	1 mol/L phosphoric acid and 0.12 mol/L ascorbic acid	74.6–90.4
Seaweed [36]	Heated at 100°C for 2.5 h	0.3 mol/L HNO_3	66–86
Atmospheric particles [37]	Ultrasound extraction, 40 min	1 mol/L phosphoric acid	90.0
Plants [38]	Ultrasound extraction, 30 min, twice	0.15 mol/L HNO_3	84.2–104
Mussels [39]	Ultrasound extraction, 30 min, twice	50% Methanol-1% HNO_3	96–102
Seaweed [40]	Microwave-assisted extraction, 80°C, 1.5 h	2% HNO_3	83
Sediment and sewage sludge [41]	Microwave-assisted extraction, 40 W, 20 min	0.3 mol/L phosphoric acid	37–97

Table 5: Application of HPLC-ICP-MS in speciation analysis of As in traditional Chinese medicine

Tested drugs	Ar species	Operating parameters of HPLC	Operating parameters of ICP-MS	Detection limit	Recovery (%)	Proportion of different species
American Ginseng, Salvia miltiorrhiza, Acorus tatarinowii, Radix Isatidis, Achyranthes bidentata, Lily, Yams, Poria cocos, Codonopsis pilosula, Notoginseng, Honeysuckle, Licorice, safflower, Radix gentianae, Albizia julibrissin, etc. [20]	As(III)	Dionex™ IonPac™ AS7 (250 mm × 4 mm i.d.) anion exchange column; protective column; thermo scientific™ Dionex™	RF power 1,550 W; auxiliary gas flow: 0.80 L/min; carrier gas flow: 1.10 L/min; cooling gas flow: 13.98 L/min; sampling depth: 5.0 mm; atomization chamber temperature: 2°C; peristaltic pump speed: 40 rpm; auxiliary gas flow: 0.80 L/min, He gas flow: 5 mL/min; sampling cone aperture: 1.0 mm; interception cone aperture: 0.4 mm	As(III) 0.10 µg/L	84.2–121.5	The detection rates of As(III) and As(V) were high, some DMA, MMA, and AsB were detected, AsC was not detected
	As(V)			As(V) 0.10 µg/L		
	MMA	column; thermo scientific™ Dionex™		MMA 0.25 µg/L		
	DMA	IonPac™ AS7 (50 mm × 4 mm i.d.);		DMA 0.15 µg/L		
	AsC	mobile phase A: 2 mmol/L ammonium carbonate solution, mobile phase B: 80 mmol/L ammonium carbonate solution; flow rate: 1.0 mL min ⁻¹ ; injection volume: 20 µL, gradient elution		AsC 0.10 µg/L		
	AsB			AsB 0.20 µg/L		
Cordyceps sinensis [45]	As(III)	Dionex™ IonPac™ AS7 (250 mm × 4 mm, 5 µm); column temperature: 25°C; balance time: 30 min; mobile phase: 5 mmol/L ammonium carbonate solution in phase A and 100 mmol/L ammonium carbonate solution in phase B, gradient elution	RF power is 1,550 W; peristaltic pump speed: 40 rpm; auxiliary air flow: 0.78 L/min; carrier gas flow: 0.82 L/min; collision gas flow of Q cell He: 4.95 mL/min; vacuum of analysis chamber <5 × 10 ⁻⁷ mbar; measure mode: Kinetic Energy Discrimination (KED)	As(III) 0.453 µg/kg	83.3–115.9	It mainly exists in the form of As(III) and As(V). Inorganic arsenicAs = 1 mg/kg AsB <0.02 mg/kg DMA <0.008 mg/kg AsC and MMA: not detected
	As(V)			As(V) 4.122 µg/kg		
	MMA			MMA 0.365 µg/kg		
	DMA			DMA 0.564 µg/kg		
	AsC			AsC 0.041 µg/kg		
	AsB			AsB 0.561 µg/kg		
Honeysuckle, Alisma orientalis, Caulis Spatholobi [46]	As(III)	Hamilton PRP-X100 (250 mm × 4.1 mm, 10 µm) anion exchange chromatographic column; mobile phase: 0.05 mol/L NH ₄ H ₂ PO ₄ (ammonia adjusted the pH value to 5.8); injection volume: 10 µL	RF power: 1,500 W; sampling mode: time resolved analysis, peak mode: TRA; sampling element: 75 As, peristaltic pump flow rate: 0.3 r/s	As(III) 0.01 mg/kg	105.1–108.4	Alisma orientalis: small amount of As(III) and As(V) Honeysuckle: small amount of As(III), DMA, and As(V); Caulis Spatholobi: no As(III), DMA, MMA, and As(V)
	As(V)			As(V) 0.01 mg/kg		
	MMA			MMA 0.01 mg/kg		
Bombyx Batryticatus, Bubali Cornu, Margarita, Faeces Trogloterori, Scorpio, Pheretima, Testudinis Carapax et Plastrum, Eupolyphaga Steleophaga, Cervi Cornu Pantotrichum, etc. [21]	DMA			DMA 0.01 mg/kg	86.9–116.6	As(III) 96.77% As(V) 100% MMA 45.16% DMA 90.32% AsB 93.55% AsC 22.58%
	As(III)	Dionex™ IonPac™ Analytical AS7 (250 mm × 4 mm i.d.); protective column:	RF power: 1,550 W; Ar flow rate of cooling gas: 14 L/min, Ar flow rate of atomizing gas: 1.09 L/min, Ar flow rate of auxiliary gas: 0.8 L/min, KED mode; flow rate of collision gas He: 5.075 mL/min; peristaltic pump speed: 40 rpm.	As(III) 0.2 µg/L		
	As(V)	Dionex™ IonPac™ guard AS7 (50 mm × 4 mm); mobile phase A: 2 mmol/L ammonium carbonate solution, mobile phase B: 100 mmol/L ammonium carbonate solution; flow rate: 1.0 mL/min, gradient elution		As(V) 0.2 µg/L		
	MMA			MMA 0.2 µg/L		
	DMA			DMA 0.2 µg/L		
	AsC			AsC 0.4 µg/L		

(Continued)

Table 5: Continued

Tested drugs	Ar species	Operating parameters of HPLC	Operating parameters of ICP-MS	Detection limit	Recovery (%)	Proportion of different species
Cordyceps sinensis and its products (capsules and tablets) [47]	As ⁽ⁱⁱⁱ⁾	Dionex TM IonPac TM As19 columns (250 mm × 4 mm, 4 µm); mobile phase: buffer solution containing 10 mmol/L sodium acetate, 3 mmol/L potassium nitrate, 10 mmol/L potassium dihydrogen phosphate, and 0.2 mmol/L disodiummethylenediamine tetraacetate (pH = 10 adjusted by ammonia); absolute ethanol (99:1); flow rate: 1.0 mL/min; injection volume: 25 µL; column temperature: 30°C	Measure mode: KED pattern; number of test quality <i>m/z</i> : 75(As); atomization chamber temperature: 2°C; carrier gas: high purity argon; collision gas: high purity He	The detection limit of AsB, MMA, DMA, and AsO ₃ ³⁻ is about 0.25 µg/L, the detection limit of AsO ₃ ³⁻ is about 0.15 µg/L	89.8–109.6	It mainly exists in the form of As ⁽ⁱⁱⁱ⁾ and As ^(v) . Organic arsenicAs AsB is detected, but MMA and DMA are not detected
	As ^(v)					
	MMA					
	DMA					
	AsB					
Chenxiang Huaqi pill [48]	As ⁽ⁱⁱⁱ⁾	ICS5000 ion chromatograph (Thermo Fisher Scientific Co., Ltd) AS7 anion chromatographic column (250 mm × 4 mm i.d.); mobile phase: 5 mmol/L ammonium carbonate (A), 100 mmol/L ammonium carbonate (B), gradient elution, 0–2.0 min, 100% A; 2.0–5.5 min, 100% B; >5.5–10 min, 100% A; flow rate: 1.0 mL/min; injection volume: 25 µL		As ⁽ⁱⁱⁱ⁾ 0.108 µg/L As ^(v) 0.055 µg/L MMA 0.011 µg/L DMA 0.108 µg/L AsC 0.027 µg/L AsB 0.053 µg/L	92.4–105.8	AsB, DMA, MMA, As ⁽ⁱⁱⁱ⁾ and As ^(v) were detected, AsC was not detected. Among the five detected forms, As ⁽ⁱⁱⁱ⁾ and As ^(v) were the main ones
	As ^(v)					
	MMA					
	DMA					
	AsC					
	AsB					

the application of HPLC-ICP-MS in As speciation analysis of Chinese herbal medicines.

4.2.4 Capillary electrophoresis combined with ICP-MS (CE-ICP-MS)

Compared with other chromatographic separation technologies, CE-ICP-MS has the advantages of high separation efficiency, low sample consumption, and fast analysis speed, while ICP-MS has the advantages of element-specific detection and extremely low detection limits. Zhao Yunqiang et al. used capillary electrophoresis and ICP-MS to determine the speciation of As in algae, using microwave-assisted extraction method, with radio frequency power (RF power) at 1,300 W and outer plasma gas flow rate at 15 L/min. CE conditions: The capillary column was an uncoated fused silica capillary with an inner diameter of 75 μm and a length of 60 cm (Hebei Yongnian Optical Fiber Factory); separation voltage of 18 kV; electrokinetic injection with sampling time of 10 s; running buffer of 50 mmol/L H_3BO_3 -12.5 mmol/L $\text{Na}_2\text{B}_4\text{O}_7$ (pH 9.0); the flow rate of pump 1 being 12 $\mu\text{L}/\text{min}$, and the flow rate of pump 2 being 120 $\mu\text{L}/\text{min}$. ICP-MS parameters: plasma auxiliary gas flow rate of 0.90 L/min; carrier gas flow rate of 0.75 L/min; and atomization chamber supplementary gas flow rate of 0.30 L/min. The detection limits of As(III), As(V), DMA, MMA, AsB, and AsC are 0.08, 0.12, 0.12, 0.09, 0.09, and 0.12 $\mu\text{g}/\text{L}$, respectively. The average standard recovery rate was 90–103% [49]. Chen Farong et al. used CE and ICP-MS to determine the speciation of As in the blue-spotted mackerel, digested it with a microwave digester, and then directly used ICP-MS to determine the total As amount in the sample. CE conditions: separation voltage: 18 kV; capillary column: uncoated fused silica capillary tube, 75 μm i.d. $\times$ 60 cm; buffer: 40 mmol/L H_3BO_3 -10 mmol/L $\text{Na}_2\text{B}_4\text{O}_7$ (pH 9.10); electrokinetic injection: sampling time of 15 s; pump speed: 36 $\mu\text{L}/\text{min}$; separation time: 30 min. ICP-MS conditions: RF power: 1,350 W; sampling cone: platinum cone; sampling depth: 7.6 mm; plasma gas flow rate: 15.0 L/min; carrier gas flow rate: 1.2 $\mu\text{g}/\text{L}$; auxiliary gas flow rate: 1.0 L/min; atomization chamber temperature: 2.0°C; mass number: 75As; integration time: 1.0 s. Detection limit: 0.1, 0.3, 0.7, 0.2, 0.8, and 1.1 $\mu\text{g}/\text{L}$, respectively, for AsC, AsB, As(III), DMA, MMA, and As(V), and the standard recovery rate was 93–98% [50].

4.2.5 LC-AFS

LC-AFS has the characteristics of short analysis time, low reagent consumption, high precision, and high recovery

rate. The application of LC-AFS in As speciation analysis of Chinese herbal medicines is shown in Table 6.

4.2.6 High-performance LC combined with HG-AFS (HPLC-HG-AFS)

HG-AFS has the advantages of high sensitivity, small interference, and simple operation. Cao Xiaogang et al. used HPLC-HG-AFS method to detect four kinds of As compounds in *Cordyceps sinensis*. AFS conditions: negative high voltage of 285 V; lamp current of 100 mA; auxiliary cathode lamp current of 45 mA; carrier gas (Ar) volume flow rate of 400 mL/min; auxiliary gas (Ar) volume flow rate of 600 mL/min. HPLC column, guard column PRP-X100 (25 mm $\times$ 2.3 mm, 12–20 μm); anion column PRP-X100 (250 mm $\times$ 4.1 mm, 10 μm); mobile phase $(\text{NH}_4)_2\text{HPO}_4$ solution (20 mmol/L, adjusted to pH 6.0 with 10% formic acid); volume flow rate of 1.0 mL/min; injection volume of 100 μL ; and column temperature of 30°C. The detection limits of As(III), DMA, MMA, and As(V) were determined to be 0.01, 0.005, 0.005, and 0.01 mg/kg, respectively. The sample recovery rate was between 87.0–94.0% [32]. Wang Sufen et al. used HPLC-HG-AFS combined technology to analyze different speciations of As in bee pollen. Instrument conditions: negative high voltage of 285 V; total current of 100 mA; auxiliary current of 45 mA; carrier gas flow rate of 400 mL/min; shielding gas flow rate of 600 mL/min; and peristaltic pump speed of 80 rpm. Chromatographic conditions: Hamilton PRP-X100 chromatographic column (250 mm $\times$ 4.1 mm, 10 μm); mobile phase of 15 mmol/L $(\text{NH}_4)_2\text{HPO}_4$ solution; injection volume of 100 μL ; flow rate of 1.0 mL/min. The detection limits of As(III), DMA, MMA, and As(V) were 1.0, 2.1, 1.2, and 3.1 $\mu\text{g}/\text{kg}$, respectively, and the standard recovery rates were between 79 and 100% [54].

Through comparison of several methods, it is found that the commonly used As speciation detection methods are ICP-MS and HG-AFS. In particular, ICP-MS has the advantages of low detection limit, wide linear range, and ability to measure a variety of element forms, while HG-AFS cannot measure certain forms of elements. At present, in the analysis of As speciation in the field of traditional Chinese medicine, separation technology is often combined with detection technology. More commonly used methods are HPLC-ICP-MS, HPLC-HG-AFS, etc. The mobile phases of liquid chromatography usually include KH_2PO_4 , K_2HPO_4 , $(\text{NH}_4)_2\text{CO}_3$, $(\text{NH}_4)_2\text{HPO}_4$, etc. The concentration and pH value of the mobile phase, and ionic strength have a significant effect on the separation effect and retention time.

Table 6: Application of LC-AFS in speciation analysis of As in traditional Chinese medicine

Tested drugs	As species	Operating parameters of LC	Operating parameters of AFS	Detection limit	Recovery (%)	Proportion of different species
Earthworm, oyster, clamshell, chicken's gizzard-membrane, leech [51]	As ⁽ⁱⁱⁱ⁾	Mobile phase: mixed solution of 5 mmol/L Na ₂ HPO ₄ and 45 mmol/L KH ₂ PO ₄ (pH 5.9); reductant: 2% KBH ₄ + 0.5% KOH solution; current carrying 5% HCl	Atomizer height: 8 mm, carrier gas flow rate: 400 mL/min, shielding gas flow rate: 900 mL/min, total As lamp current: 60 mA, auxiliary lamp current: 30 mA, As form lamp current: 70 mA, auxiliary lamp current: 50 mA, negative high voltage: 320 V	As ⁽ⁱⁱⁱ⁾ 2.68 µg/kg As ^(v) 7.66 µg/kg MMA 3.58 µg/kg DMA 5.38 µg/kg	93.2–104.0	The detection rate of As ⁽ⁱⁱⁱ⁾ and As ^(v) is high. For MMA and DMA, the detection rate is low
	As ^(v)					
	MMA					
	DMA					
Ganoderma lucidum spore powder [52]	As ⁽ⁱⁱⁱ⁾	Separation column Hamilton PRPX-100 anion exchange column (250 mm × 4.1 mm, 10 µm); protective column Hamilton PRPX-100 anion exchange column (10 mm × 4.1 mm, 10 µm); mobile phase: 15 mmol/L ammonium dihydrogen phosphate (pH 6.0); volume flow: 1.2 mL/min; injection volume: 100 µL	Negative high voltage of 270 V; As lamp current of 60 mA; carrier gas: high purity argon; current carrying 5% HCl; reducing agent: 0.5% potassium hydroxide .5% potassium borohydride solution	As ⁽ⁱⁱⁱ⁾ , As ^(v) , MMA, and DMA <0.2 µg/L	96.8–100.3	The detection rate of As ⁽ⁱⁱⁱ⁾ and As ^(v) is high. For MMA and DMA the detection rate is low
	As ^(v)					
	MMA					
	DMA					
Centipede grass [53]	As ⁽ⁱⁱⁱ⁾	Injection mode: manual injection, injection volume: 100 µL. Leaching method: isocratic leaching, mobile phase pump speed: 1 mL/min,	Shielding gas/carrier gas Ar 900 mL/L/ Ar 300 mL/L, negative high voltage: 280 V, main lamp current: 60 mA (total), 80 mA (form), auxiliary lamp current: 30 mA (total), 40 mA (form), atomization height: 8 mm, carrier HCl (5.6%), reductant: KBH ₄ (2%), KOH (0.5%)	As ⁽ⁱⁱⁱ⁾ 0.5129 µg/L	92.7–108.4	As mainly exists in the inorganic form of As ⁽ⁱⁱⁱ⁾ , and As ^(v) , MMA and DMA are less
	As ^(v)					
	MMA					
	DMA					

4.3 Synchrotron radiation to study the chemical speciation of As

Synchrotron radiation (SR) technology is a series of collectively referred technologies based on synchrotron radiation devices. Synchrotron radiation is the electromagnetic radiation emitted by charged particles at a speed close to the light speed along the tangent direction of the track when they are moving in a curve. It acquired its name after first use on the synchrotron in 1947. So far, the SR device has experienced three generations of development, and the research on the fourth generation of light sources is currently being actively carried out internationally. The third-generation SR source is characterized by a large number of inserts, and its brightness is at least 100 times higher than that of the brightest second-generation light source, thus enabling SR applications to achieve spatial and temporal resolution. There are currently three SR devices in China: Beijing Synchrotron Radiation Facility, National Synchrotron Radiation Laboratory, and Shanghai Synchrotron Radiation Facility, with energies of 2.2, 0.8, and 3.5 GeV, respectively. China is actively promoting the construction of high-energy SR devices with energy of 5.0 GeV to make up for the deficiency of domestic high-energy light sources.

The interaction between SR light and matter can be divided into three categories: (1) Absorption, the corresponding SR technologies include X-ray absorption spectroscopy (XAS), infrared (IR) spectroscopy, ultraviolet visible (UV-Vis) Spectroscopy, soft/hard X-ray imaging technology scanning transmission X-ray microscopy (STXM), etc.; (2) Scattering, the corresponding SR technologies include X-ray diffraction (XRD), protein X-ray crystal diffraction (PX), small angle X-ray scattering (SAXS), etc.; and (3) Secondary particle excitation, corresponding SR technologies include X-ray photoelectron spectroscopy (XPS), X-ray fluorescence spectrometry (XRF), etc. The following will focus on the application of XAS and XRF technologies in the study of As chemical speciation.

After years of development, SR technology has gradually become an ideal tool for studying element species and its composition characteristics [55]. According to the different energy positions of different types of atomic absorption edges, X-ray absorption fine structure (XAFS) generated by SR, including X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS), supports convenient identification of the neighboring atoms around the element of interest in the sample, and provides information on the structure of atomic clusters in a small range. The XAFS method only needs a small amount of samples in measurement, without

requirement for separation and purification, so even non-destructive analysis is possible. XAFS has been widely used to study metal elements and their species in low-concentration samples, such as the species and distribution of Cd in waste incineration fly ash [56], the species and distribution of Fe in atmospheric particulates [57], valence state, and distribution of elements such as Ni and S in oil residue fly ash [58]. XANES contains the electronic structure information of the substance. By comparison with the standard substance, the valence state of the absorbing atom, its coordination atom, and other “fingerprint information” can be accessed. However, EXAFS can acquire structural information such as bond length and coordination number without acquisition of crystal samples, so it is very suitable for the speciation analysis of As in environmental samples [59]. For As, the excited K-layer electron energy of 11.867 keV is often used for sample analysis.

The basic principle of XAFS is to measure the absorption coefficient of the sample under different characteristic wavelength X-ray energies and obtain the absorption spectrum. Spectrum shape is related to the chemical speciation of the element. As(III), As(V), MMA, DMA, TMA, AsB and AsC have significantly different characteristics in XANES spectrum. The absorption edges (maximum absorption) of As(III)-GSH, As(III), and As(V) are 11869.8, 11871.4, and 11874.8 eV, respectively. Using the difference in the absorption spectrum, *in situ* analysis of As speciation in complex environmental media can be made to quantitatively provide the proportion of each form of compound in the sample, as shown in Figure 2 [60]. Through the analysis of four kinds of birds in the mining area, Wei Chaoyang project found that the main speciation of As were DMA, As(III) and As(V), and As(III)-GSH.

Fluorescence analysis is to excite the molecules or atoms of a substance after irradiation by an external light source. In retreat, they emit fluorescence. The wavelength of fluorescence is related to the energy level structure of the molecules or atoms. Therefore, by measuring the wavelength and intensity of fluorescence, it is possible to determine the type and content of the element that emits the fluorescence, thereby determining the composition of the sample. The X-ray fluorescence analysis method based on SR has high sensitivity and low detection limit. It can detect multiple elements at the same time (theoretically, all elements between Na and U can be detected) [61], and can perform element distribution analysis and micro-area *in situ* determination of substances. It is a highly sensitive, fast, and non-destructive multi-element simultaneous analysis method. By scanning the sample, it is possible to access the spatial

Figure 2: XANES raw data (---) and fitting (—) for model As species and samples.

distribution information of the elements in the tissue or organ slices at the micrometer scale [62]. As shown in Figure 3, Fu and Luo scanned the distribution of elements in human hair samples collected from mining areas by μ -XRF technology and found that Pb and As were mainly distributed along the central axis of the hair, which gradually increased from hair root to tip [63]. Andreas et al. used Micro-XRF and EXAFS spectroscopy to study the distribution and morphology of As in polluted riverbank soil and plant roots, and at the same time, visually revealed the distribution relationship of multiple elements in the soil [64].

4.3.1 Research on the speciation of As in environmental samples by SR technology

Soil is both a sink and a source of As. The SRXAS method does not require pre-treatment of the sample, which avoids the transformation of As speciation during the pre-treatment process. Mandaliev et al. used XAS to exploit river soil rich in arsenopyrite ore and found that the main speciation of As was As(v). Although As(III) was not detected, there was a large amount of As(I), which was determined to originate from arsenopyrite in the

Figure 3: Micro-XRF distribution map of Pb and As in a single hair.

mining ruins [65]. Ono FB et al. used μ -XANES to analyze the soil of a gold mining area in Brazil, and found that the As speciation mainly exists in As(v). Further analysis by μ -XANES and μ -SXRF showed that As mainly exists in the form of arsenopyrite (FeAsS) and weathering products [66]. Acosta et al. analyzed the soil near the gold mine by XANES technology and found that As mainly existed as As(v) [67].

The speciation of As in As-contaminated soil is different from that in mining areas. Liu Yongxuan used EXAFS to determine the surface soil in the mining area of the Diaojang River Basin in Guangxi. The results showed that the upper reaches were mainly composed of arsenopyrite, As(v), and As(III), with percentages of 15, 82, and 3%, respectively; the middle reaches were mainly composed of Arsenopyrite and As, with percentages of 8 and 92%, respectively, and the lower reaches were mainly composed of As(v) [68]. Gautier Landrot et al. used micro-area X-ray fluorescence (μ -XRF) spectroscopy, micro-focused X-ray absorption spectroscopy (μ -XAS), and EXAFS to study the soil in a park in Washington, which has always been an important leather production

center on the Atlantic coast of the United States. The analysis found that the soil As speciation is mainly As(v) with a small amount of As(III). Using μ -XRF, it is found that As mainly coexists with Al, and a small amount of As coexists with Fe. Through characteristic toxicity experiments, it is inferred that arsenate is adsorbed on aluminum oxide and will be converted into arsenoside structure compound after a long time [69].

4.3.2 Research on the chemical speciation of As in biological samples by SR technology

As naturally exists in marine ecosystems, and mining activities may cause pollution to these ecosystems. Whaley-Martin et al. mastered the distribution of As in blue mussels by HPLC-ICP-MS and XAS analysis techniques. The XAS spectrum shows that As(III) compounds are mainly concentrated in the digestive glands. However, AsB found in the digestive glands and surrounding tissues was in very small concentration and high similarity, which indicates that AsB can be used for all physiological purposes of mussel cells, such as intracellular penetrants [70]. Hong et al. investigated the pollution of As in water and sediments in a highly industrialized area in Pohang City, South Korea. Through HPLC-ICP/MS and μ -XANES analysis, it was found that AsB was mainly present in fish, bivalves, crabs, and shrimps, while As(III) was mainly present in freshwater snails, and As compounds were mainly distributed in the intestines of mullets and clams [71].

Mining and smelting activities are the main sources of increased soil As content, and soil As pollution also causes a certain degree of harm to the ecosystem structure. Enzo Lombi et al. used XANES technology to study the morphology of As in *Pteris vittata*, a fern, and found that As in plant leaves mainly exists in the form of As(III) [72]. Webb et al. combined the study of EXAFS and found that in fresh leaves, trivalent As mainly exists in the form of hydrated arsenite ions, that is, non-coordinating arsenite. However, with the aging and drying of fresh leaves, the speciation of As in the leaves gradually changed from As(III) to As(v) [73]. Through multimedia sampling in Shimen realgar mining area, Yang et al. found that plants absorb As(v) from the soil and reduce it to As(III) in the body; while the As in earthworms contains only a small amount of AsB in addition to inorganic As. The soil, plants, and litter basically do not contain AsB. It is thus inferred that AsB in earthworms is mainly derived from the transformation of As by the earthworms [74]. In addition, analysis of high-trophic biological birds in the mining area, including sparrows, *radix pulsatillae*, thrushes, and magpies, found

that the As content in the bird muscles ranges from 1.13–4.95 mg/kg, and the main speciations of As are DMA, As(III), and As(v). In addition, there are a small amount of AsC and AsB, as well as As(III)-GSH, an unextractable and unidentified speciation of As in the HPLC-ICP-MS data [60]. Bird feathers can be used as a marker to reflect environmental pollution, and its As content can reach up to 51.65 mg/kg, and its main As speciation is inorganic As. Compared with other similar studies, the As content is significantly higher in the muscles and feathers of birds in this study, indicating that long-term As pollution in the mining area will lead to excessive As enrichment in birds.

4.3.3 Research on the distribution and transformation of As by SR technology

Different chemical speciations of As are distributed differently in animals and plants. For plants, it is necessary to separate different tissues and determine the As content; for animals, it is necessary to determine the As content in different organs after the experimental animals are sacrificed, so that the distribution of As in the body can be determined.

Schaller et al. studied the adaptation and survival mechanism of *Gammarus pulex* in a high As environment. Based on the process of feeding, digesting, and storing As from the leaves, it was found that As is mainly distributed along its intestinal system [75]. Pearce et al. used μ -XRF and μ -XANES to analyze the micro-area distribution and morphology of As in the toenail slices of children in the mining area, and found that the main speciation of As in toenails is As(III), which is coordinated with sulfur and methyl. In the toenail samples processed by mine waste, the upper edge mainly exists as As(v) and is coordinated with O; the lower edge mainly exists as As(III) and is coordinated with sulfur and methyl [76].

Tong-Liang Wu et al. found through μ -XRF and XANES that most of As is distributed and concentrated in rice husk, bran, and embryo. The main As species in rice is DMA (average >60%). The high correlation between As and Fe in soil indicates that iron-related materials may be a potential way to effectively control As pollution [77]. Chen Tongbin analyzed the super-enriched plant ciliate desert-grass by SRXRF and found that As in the leatherleaf midrib of ciliate desert-grass presents a strong tendency to transport like the mesophyll on both sides, which has a strong wood unloading ability. Through analysis of other elements, it is found that the most mobile element K in the plant has most similar distribution as As, while the

distribution of Fe and Ca with weaker mobility is opposite to that of As [78].

4.3.4 Application of SR technology in As removal

Microorganisms play an important role in the biogeochemical cycle of As. Zeng *et al.* used HPLC-HG-AFS and XANES to study the form transformation of As and the inflow and outflow of arsenite on fungal cell membranes. As(III) can be transported into the cell and adsorbed outside the cell by the cell walls of *Trichoderma asperellum* (SM-12F1), *Penicillium janthinellum* (SM-12F4), and *Fusarium oxysporum* (CZ-8F1). As(III) in some cells can be oxidized and methylated to produce As(V), MMA, and DMA. Some intracellular As(III) can exude from fungal cells. Fungal strains show different responses to different As species. It is feasible to use these fungal strains to repair the environment contaminated by As(III) in the future [79].

Plant stabilization is a cost-effective long-term bioremediation technology for fixing metal tailings. However, the biogeochemical problems that affect the stability and mobility of metal molecules in the root zone are still difficult to solve. Mei L used EXAFS to study the effects of regulation of sulfur metabolism in the body on As and sulfur speciations and the accumulation of As by ciliate desert-grass. Studies have found that in ciliate desert-grass, sulfhydryl groups can act both as a reducing agent and a chelating agent for As, and it is speculated that under these conditions, the ratio of sulfhydryl groups to As may be a factor that determines the specific role of sulfhydryl groups in ciliate desert-grass [80].

μ -SXRF and XAFS have been widely and thoroughly applied in element distribution analysis and morphological analysis, bringing new discoveries to the understanding of migration characteristics and transformation laws of elements in the environment and organisms, revealing important clues regarding effectiveness and toxicity of elements. With the continuous development of SR technology, it has become possible to use *in situ* X-ray absorption to study the morphological structure, valence state coordination, dynamic metabolism, and dose-effect relationship of the heavy metal As in Chinese medicinal materials.

5 Conclusion and prospect

Heavy metal pollution in traditional Chinese medicine is an important factor affecting the quality of traditional

Chinese medicine and its internationalization. The binding mechanism of heavy metal pollutants and soil aggregates, the path, and mechanism of their entry into traditional Chinese medicinal plants are research hotspots in many disciplines such as environmental science and traditional Chinese pharmacology. As is one typical heavy metal pollutant in soil and Chinese medicinal materials. The speciation analysis of As may allow us to clarify the way for As to enter the environment and the nature of migration and transformation process. At present, the mainstream analysis method in the laboratory is separation and detection combined analysis method, of which the most widely used are HPLC-ICP-MS, LC-AFS, and HPLC-HG-AFS. With high sensitivity, they belong to apparent morphology research method, which can enable indirect analysis of As speciation. How to maintain the stability of As speciation in the pre-treatment process has always been a difficult point in analysis. The pre-treatment method with high extraction rate that does not change the speciation of As demands further study. Taking the advanced SR source as the research platform, the use of X-ray near edge absorption fine structure spectrum and micro-area X-ray fluorescence analysis as a microscopic analysis technique supports direct analysis of the As speciation *in situ*. It is the most promising morphological analysis method to explore the path of As pollutants into medicinal plants, the mechanism of element form transformation, and influencing factors from the molecular level.

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