Chapter 8
Arsenic Toxicity and Its Remediation Strategies for Fighting the Environmental Threat

Vishvas Hare, Pankaj Chowdhary, Bhanu Kumar, D. C. Sharma, and Vinay Singh Baghel

Abstract Arsenic (As) is an abundant element found ubiquitously in nature, primarily in the earth's crust and also in the environment. Arsenic is necessary for living beings; however, it is also an emerging issue by virtue of the toxicity it causes in living beings, including humans and animals. Basically, the ground water is badly affected by As contamination, coming from sources including As-affected aquifers, and has severely threatened humanity around the world. Arsenic poisoning is worse in Bangladesh and Uttar Pradesh, where As(III) is found in higher concentrations in ground water, which is used by people. The dissolution process caused by oxidation and reduction reactions leads to the natural occurrence of As in groundwater. There are several review articles on As toxicity and exposure, but with scattered information and no systematic knowledge in a combined way. However, in this chapter we try to compile all the information in systematic manner, which will be helpful for people who are working for As mitigation and removal from environment for sustainable development. This chapter will be helpful in providing detailed knowledge on As occurrence, speciation, factors affecting As toxicity arising because of its biogeochemistry, and various physico-chemical and biological strategies for combating the environmental threats.

Keywords Arsenic toxicity · Accumulation · Arsenic speciation · Health problems · Remediation approaches

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1 Introduction

Arsenic is a harmful metal, found everywhere, i.e. in soil, water, air, and is highly toxic to all living beings. Its distribution among geochemical sources is irregular and is commonly found in the earth's crust. It comes from both anthropogenic and geogenic sources (Smith et al. 1999; Juhasz et al. 2003). However, the worst contamination conditions have been encountered in West Bengal (India) and neighbouring countries such as Bangladesh, and have been created because of natural process (Tripathi et al. 2007). Arsenic is also reported to be invading the food chain, for instance rice, most probably due to contaminated ground water (Meharg et al. 2009; Hare et al. 2017). Arsenic (As) persists in nature for a long time.

Arsenic is found in two forms (organic and inorganic) in nature, but the inorganic As shows higher toxicity than the organic form. Inorganic As is represented by two biological forms {Arsenate As(V) and arsenite As(III)} which can interconvert regulated by the environment, especially redox conditions. Arsenate As(V) interferes with necessary cellular processes such as oxidative phosphorylation and ATP synthesis as it is a phosphate analogue and thus, the main route of arsenate uptake by the roots may be through the phosphate transport mechanism. A little is exported to the shoot through the xylem as the ox anions As(V) and As(III). The toxicity of arsenite is governed by its tendency to bind to sulfhydryl groups, with resultant harmful effects on protein functioning, and As(III) is transported in the neutral $As(OH)_3$ form through aquaglyceroporins (Meharg 2004). Arsenic occurs as oxyanions in soils, predominantly as arsenite As(III) and arsenate As(V). In oxygen-rich environments and well-drained soils, arsenate species dominate ($H_2AsO_4^-$ in acidic soils and $HAsO_4^{2-}$ in alkaline ones), whereas in a reducing environment, for example, regularly flooded soils, As(III) is more stable (Gomez-Caminero et al. 2001). The relative prevalence of the various forms of As in soils depends on the type and amount of adsorbing component of the soil, pH and redox potential (Buschmann et al. 2006). Materials with iron and aluminium oxide surfaces are capable of adsorbing arsenate and arsenite. Humic acids and fulvic acids in soil have been reported to show great affinity for As (Meunier et al. 2011). These may interfere strongly with As adsorption in some situations and As mobility may be increased in soil when they are present (Jackson and Miller 1999). This relationship is postulated to involve bridging metals and deprotonated functional groups within the humic acid (Warwick et al. 2005). Increased As concentration retards usual growth and development of plants if present in irrigation water or in soil with toxicity symptoms such as biomass reduction and yield decreases. Arsenic interferes with plant metabolic system and can inhibit growth, often leading to death when present in higher concentrations (Jiang and Singh 1994). There are several reports on the loss of fresh and dry biomass of roots and shoots, loss of yield and fruit production, morphological changes if the plants were grown in soils treated with As (Mokgalaka-Matlala et al. 2008). Miteva (2002) reported a decrease in growth of the aerial parts and the root system of tomato plants at higher As concentrations. A disease known as "straight head" is a physiological disorder of rice (*Oryzasativa* L.) characterised by the sterility of florets/spikelets leading to a decreased crop yield.

Singh et al. (2010) worked on the effect of As on rice crops in the Indo-Gangetic plains of north-western India and found a positive correlation between the rice growth and As in the irrigation water and soil. There are several reports revealing the positive correlation between rice crops and the fields versus As-contaminated irrigation water (Khan et al. 2009).

Arsenic that has accumulated in plants finally reaches human beings, causing potential health risks such as skin cancer as it become an integral part of the food chain. Arsenic is regarded as a group A human carcinogen by the United States (US) Environmental Protection Agency (EPA). The major health hazards include skin, lung, bladder, and liver cancers, and many other cardiovascular, neurological, haematological, renal, and respiratory diseases (Halim et al. 2009; Johnson et al. 2010; Yadav et al. 2017), mostly ascribed to intake along with contaminated fresh drinking water. Arsenic toxicity in severe stages is marked by symptoms such as dermal lesions (e.g. hyperkeratosis, hyper pigmentation, desquamation and loss of hair (Zaloga et al. 1985), peripheral neuropathy, skin cancer and peripheral vascular disease. The most affected parts are those directly involved in absorption, accumulation and excretion of As, especially the gastrointestinal tract and liver (Yadav et al. 2017). Apart from this, the vascular system and other soft organs such as the heart and kidneys are very sensitive to As. The skin tissues are secondarily affected. The intestinal epithelium is the first barrier against such exogenous inorganic As toxicity.

2 Occurrence/Sources of Arsenic

Arsenic is the 20th most frequently occurring trace element in the soil, 14th in the seawater, and 12th in living systems (humans). Arsenic is abundantly distributed in nature and its derivatives are mobile in the environment. In the environment, the major sources of As include natural/geogenic and anthropogenic sources (Fig. 8.1).

2.1 Natural/Geogenic Sources

Arsenic is ubiquitous in nature, and is distributed throughout the earth's crust, soil, sediments, water, atmosphere and living organisms. Arsenic is reported to be found in soil in higher concentrations than in rocks. Arsenic is naturally found in more than 200 different mineral forms, which contain arsenates (60%), sulphides (20%), sulfosalts and the rest consist of arsenides, arsenites, oxides, silicates and elemental As (20%). On the land, the concentration of As present is around 1.5–3.0 mg kg^{-1}. The concentrations of As in the earth crust of different nations are said to vary within the range from 0.1 to 40 mg kg^{-1} (mean 6 mg kg^{-1}), 1–50 mg kg^{-1} (mean 6 mg kg^{-1}) and mean 5 mg kg^{-1}, but varies considerably among geographic regions (Kabata and Pendias 1984). In natural waters, As is reported to be present at low concentrations. The seawater ordinarily contains 0.001–0.008 mgl^{-1} of As. The

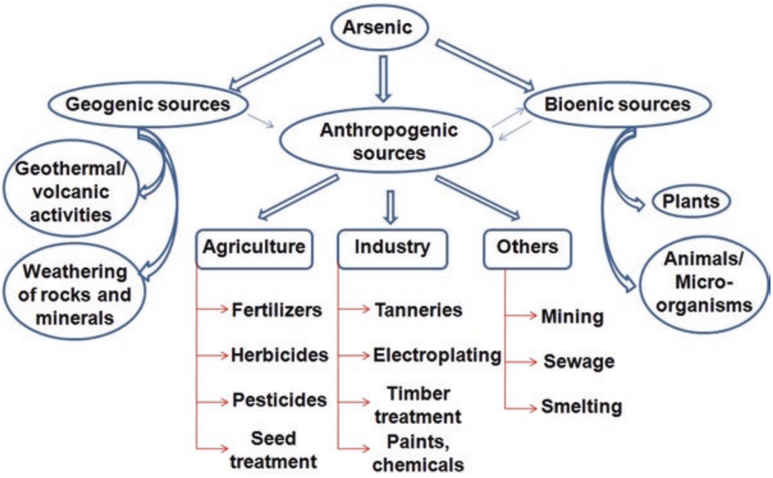

Fig. 8.1 Sources of arsenic contamination in the environment

maximum permissible limits of As in potable water is 50 μgl^{-1} and the recommended value by the EPA and the WHO is 10 μgl^{-1} (WHO Arsenic Compounds 2001; U.S. Environmental Protection Agency 1975). In air, As is present primarily absorbed on particulate matters, is found most of the time as a mixture of As(III) and As(V), with the organic species being of little significance except in areas where the application of As pesticides is frequent. The As content present in air is quite low and ranges from 0.4 to 30 ng m^{-3}; therefore, through air humans generally encounter very low exposure. USEPA estimate that the general public is exposed to a range of approximately 40–90 ng per day by inhalation.

2.2 Anthropogenic Sources

For As, the principal anthropogenic contributions to soils come from the ways of managing municipal solid waste, i.e. primarily combustion, the application of different agricultural support substances, including pesticides such as herbicides, fungicides and insecticides, which contain As. Application of solid waste/sewage sludge into land, river and irrigation waters (Kabata-Pendias and Adriano 1995), the mining and smelting of As-containing ores, combustion of fossil fuels (especially coal), land filling of industrial wastes (pulp and paper, tannery, textile and distillery), the release or disposal of chemical warfare agents (Chowdhary et al. 2017a, b, c; Goh and Lim 2005), manufacturing of metals and alloys, petroleum refining and pharmaceutical manufacturing (Ning 2002; Mishra and Bharagava 2016). Nowadays, for preserving wood chromated copper arsenate (CCA) has become popular, and is another potential source of As in soil (Bharagava and Mishra 2018).

Arsenic is used in various kinds of pesticides, such as lead arsenate, Ca_3AsO_4, copper acetoarsenite, Paris green (copper-acetoarsenite), H_3AsO_4, monosodium methanearsonate (MSMA), disodium methanearsonate (DSMA) and cacodylic acid are used in the production of cotton as pesticides. Arsenic is also used for wood preservatives such as fluor-chrome-arsenic-phenol (FCAP), CCA and ammoniacal copper arsenate (ACA) (U.S. Department of Agriculture 1974). Many As compounds are used for feed additives, such as H_3AsO_4, 3-nitro-4-hydroxy phenylarsonic acid and 4-nitrophenylarsonic acid.

3 Accumulation and Transformation of Arsenic in Edible Crops

Plants vary significantly in their ability to accumulate As and the rice plant is more inclined to As accumulation than other cereals, as it is generally grown under flood conditions where As mobility is elevated. However, wide range of As-resistant microorganisms are present in nature; relatively few of the microorganisms are known to hyper-accumulate As (non-genetically engineered microorganisms) (Xie et al. 2013). Bioaccumulation refers to a process in which As is accumulated in cell membranes and cytoplasm, different from biosorption, in which accumulation takes place at the cell surface (Joshi et al. 2009; Xie et al. 2013). Arsenic transformation in the environment is predominantly biotic (Meng et al. 2011), abiotic transformation of As has been shown to be considerably slower and is believed to be less important than microbially mediated reduction (Jones et al. 2000). The transformation reactions of As include microbial reduction, oxidation, methylation and demethylation, which play a detrimental role in the environmental behaviour of As, because different types of mobility are shown by different chemical forms of As [Methyl As(III)>Methyl As(V)>As(III)>As(V)] (Lafferty and Loeppert 2005), toxicity [Methyl As(III)>As(III)>As(V)>Methyl As(V)] and susceptibility to plant uptake [e.g. uptake by the rice root As(III)>Monomethyl As(V)>Dimethyl As(V)].

3.1 Conversion and Internalisation of Arsenate

In arsenate uptake, the phosphate transporters present in the plant roots play a key role. Confirmation is shown in different physiological and electrophysiological studies exhibiting that arsenate uptake causes an effective inhibition of phosphate, and recent reports show that *A. thaliana* mutants flawed in phosphate transport are more tolerant to arsenate. Reduction of As(V) usually demonstrates an enhancement in the mobility of As in the natural environment, as As(III) has greater mobility than As(V) (Smedley and Kinniburgh 2002). Reduction of As (V) through microbes may occur via respiratory reduction, as microorganisms use arsenate as the terminal electron acceptor in anaerobic respiration (Lloyd and Oremland 2006; Mukhopadhyay et al. 2002; Stolz et al. 2006). Detoxification is the other mechanism of reduction of arsenate to arsenite through microbes (Langner and Inskeep 2000; Stolz et al. 2002). Arsenic detoxification/mitigation has been well documented in *E. coli*, *Staphylococcus aureus* and *Staphylococcus xylosis* and is controlled by "ars" genes that encode for arsenate (Cervantes et al. 1994; Tamaki and Frankenberger 1992). Arsenates detoxifying reducing bacteria were found to play an important role in As mobilisation under oxic conditions. Under flooding conditions, in soil amended with citrate, strong As mobilisation was observed at the commencement of incubation when oxic conditions prevailed (Eh >250 mV) (Corsini et al. 2010).

3.2 Conversion and Uptake of Arsenite

Uptake of arsenite by plant roots is mainly in the form of the neutral molecule $As(OH)_3$. Arsenite enters through plant root cells via aquaglyceroporin channels. In higher plants, the nodulin 26-like intrinsic proteins (NIPs) are the structural and functional equivalents of the microbial and mammalian aqua-glyceroporins (Wallace et al. 2006). NIPs are a subfamily of the plant major intrinsic proteins, collectively known as water channels or aquaporins, and arsenite permeability is widespread in different subclasses of NIPs (Zhao et al. 2009; Maurel et al. 2008). Microorganisms tolerate higher As(III) levels by carrying out microbial As(III) oxidation as an effective detoxification process (Paez-Espino et al. 2009; Tamaki and Frankenberger 1992). As(III) oxidation is catalysed by a wide range of microorganisms, e.g. *Alcaligenes faecalis*, *Hydrogenophaga* sp., *A. ferrooxidans*, *T. aquaticus*, *T. thermophilus*. Stolz et al. 2006; Wang and Zhao 2009). The major impact of microbial oxidation of As(III) to As(V) is to reduce As mobility in the environment, as the resemblance of As(V) to mineral solids is usually higher than that of As(III) (Dixit and Hering 2003; Huang et al. 2011; Smedley and Kinniburgh 2002).

3.3 Methylation

Several aerobic and anaerobic microorganisms (Kuehnelt and Goessler 2003) established As methylation. Microbial methylation allows the conversion of aqueous or solid associated inorganic As into gaseous arsines and leaves from the living medium, which is usually regarded as detoxification (Jia et al. 2013). The gaseous arsines endure long distances as they are highly mobile in comparison to aqueous As and may easily travel in the environment (Mukai et al. 1986). The lower adsorption capacity of methylated As than inorganic As has been reported and was considered to be mobilisation owing to the formation of aqueous trivalent and pentavalent methyl As (Huang and Matzner 2006; Lafferty and Loeppert 2005). Low redox potentials (i.e. reducing conditions) encourage the production and mobilisation of methylated As (Frohne et al. 2011). Under reducing conditions, the reduction of As(V) to As(III) may enhance microbial methylation of As followed by enhanced levels of dissolved As in soils and sediments (Bennett et al. 2012; Du Laing et al. 2009).

3.4 Demethylation

Demethylation may occur under oxic and anoxic conditions, but is usually faster under oxic conditions (Huang et al. 2007). Removal of the organic moieties not only induces the general toxicity of As, but also reduces its mobility. Arsenic demethylation usually refers to the degradation of aqueous methylated As. In the atmosphere, the gaseous methylated arsines undergo rapid photo oxidative degradation (Mestrot et al. 2013). Whether the microorganisms found in the environment are able to perform As demethylation is still an open question.

3.5 Nitrate Reduction

Nitrate reduction may not only affect As(V) reduction, but can also influence As cycling under anoxic conditions. For example, nitrate-respiring sediments could reduce As(V) to As(III) once all of the nitrate has been removed (Gibney and Nusslein 2007; Dowdle et al. 1996). In urban lakes, microbial oxidation of Fe(II) and As(III) helped by nitrate may be an important process, leading to the arrangement of particulate ferric-oxide and As(V); an important consequence of enriched nitrate is therefore the presence of As(V) associated with hydrous ferric oxide colloids (Senn and Hemond 2002). Introducing nitrate may support the anoxic oxidation of Fe(II) and arsenite in the subsurface as a means of immobilising As in the form of As(V) adsorbed onto biogenic Fe(III) (hydr)oxides (Sun et al. 2009).

4 Toxicity Profile of Arsenic

West Bengal is most affected by As and it was also first reported here in the 1990s when people started to build up arseniosis, starting with skin rashes and leading to fatal problems, with a toxic effect on organs, for instance, the lungs, kidneys, and bladder (Chowdhury et al. 2000). Related issues were detected in Bangladesh, which is in close proximity to West Bengal and has a similar land pattern based on alluvial and deltaic sediments. Arsenic is not reported to act as nutrient and it is hazardous even at very minimal concentrations to plants and animals (Smedley and Kinniburgh 2002; Buschmann et al. 2008). Toxicity of As could affect different varieties of organisms, including humans (Cervantes et al. 1994). As accumulation in plants affects not only the growth, but also, over time, it becomes a part of the food chain, which eventually causes adverse health problems to human beings such as skin cancer. Arsenic toxicity is largely manifested in the cytoplasm and a common mechanism of detoxifying cytoplasmic metals and metalloids is complexation via sulphur bonds (Tripathi et al. 2007; Rosen 2002).

The chemical forms and oxidation states of As are more significant with regard to toxicity. The mode of toxicity differs between As species. As(V) interferes with phosphate metabolism such as phosphorylation and ATP synthesis, whereas As(III) binds to the vicinal sulfhydryl groups of proteins, affecting their structures or catalytic functions. Because arsenate decreases to arsenite, much of the toxic effect of As(V) may actually be due to its reduction product arsenite. It has been reported that iAs(V) is less toxic than iAs(III) to both animal and humans, but can be toxic to plants. An element is most toxic when it inactivates the enzyme systems, which serves as a biological catalyst. iAs(V) does not react openly with the active sites of enzymes (Johnstone 1963). It first reduces to iAs(III) in vivo before exerting its toxic effect. The enzymes, which generated cellular energy in the citric acid cycle, are harmfully affected. The inhibitory action is based on inactivation of pyruvate dehydrogenase by complexation with iAs(III), whereby the generation of adenosine-5-triphosphate (ATP) is prohibited. Arsenic can inhibit many enzymes, for example the pyruvate oxidase, *S*-aminoacid oxidase, choline oxidase, and transaminase. Although iAs(III) is regarded as the more toxic form of the element, iAs(V) as arsenate can be disruptive as it competes with phosphate. Arsenate is also capable of replacing the phosphorous group in the DNA molecule and this appears to inhibit the DNA repair mechanism. In environments where phosphate content is high, arsenate toxicity to flora and fauna is generally reduced. Arsenic seems to have antagonistic relation to selenium in the body and each counteracts the toxicity of the other. However, As may also interfere with the essential role in metabolism. Arsenic prevents the biological role of selenium, which results in the apparent deficiency of the glutathione peroxidise system (selenium-dependent enzyme).

The Chronic effects of As are well documented. The worst affected organs are those that take part in As absorption, accumulation, and excretion. Chronic As toxicity causes symptoms such as dermal lesions, and loss of hair (Zaloga et al. 1985), peripheral neuropathy, skin cancer and peripheral vascular disease. These symptoms have been identified mostly in populations, used As-contaminated water (Tseng 1977; Zaldivar 1980; Cebrian et al. 1983; Smith et al. 2010). The skin is known to localise and store As because of its high keratin content, which contains several sulfhydryl groups to which As^{3+} may bind (Kitchin 2001). The hazardous health effects of As exposure to different internal and external organs are summarised in Table 8.1.

Table 8.1 Toxicological effects of arsenic in living organisms

Effect	Toxicity	References
Respiratory effects	Tracheae, bronchitis, rhinitis, pharyngitis, shortness of breath, chest sounds, nasal congestion and perforation of the nasal septum	Morton et al. (1994)
Pulmonary effects	1. Non-malignant pulmonary disease	Borgono et al. (1997)
	2. Abnormal skin pigmentation, complained of chronic cough	
	3. Restrictive lung disease	
Cardiovascular effects	1. Cardiovascular abnormalities, Raynaud's disease, myocardial infarction, myocardial depolarisation, cardiac arrhythmias and thickening of blood vessels	WHO Arsenic Compounds (2001)
Gastrointestinal effects	1. Sub-acute arsenic poisoning as dry mouth and throat, heartburn, nausea, abdominal pains and cramps, and moderate diarrhoea	Nagvi et al. (1994)
	2. Chronic low-dose arsenic produces a mild oesophagitis, gastritis, or colitis with respective upper and lower abdominal discomfort	
Haematological effects	1. Anaemia and leukopaenia is reported to result from acute, intermediate and chronic oral exposures	Franzblau and Lilis (1989)
	2. High doses of arsenic are reported to cause bone marrow depression in humans	Environmental Protection Agency (1984)
Hepatic effects	Chronic arsenic induced hepatic changes, including bleeding oesophageal varices, ascites, jaundice, or simply an enlarged tender liver, mitochondrial damage, impaired mitochondrial functions, and porphyrin metabolism, congestion, fatty infiltration, cholangitis, cholecystitis and acute yellow atrophy, and swollen and tender liver	Guha Mazumder (2001) Santra et al. (1999) Chakraborty and Saha (1987)
Renal effects	1. Sites of arsenic damage in the kidney include capillaries, tubules and glomeruli, which lead to haematuria and proteinuria, oliguria, shock and dehydration with a real risk of renal failure, cortical necrosis and cancer	
Dermal effects	1. Chronic exposure to arsenic diffused and spotted melanosis, leukomelanosis, keratosis, hyperkeratosis, dorsum, Bowen's disease and cancer	Southwick et al. (1981)
	2. Chronic doses of 0.003–0.01 mg As kg^{-1} per day	Valentine et al. (1985)
Neurological effects	1. Acute high exposure (1 mg As kg^{-1} per day) often causes encephalopathy with symptoms such as headache, lethargy, mental confusion, hallucination, seizures and coma	Grantham and Jones (1977)
Developmental effects	1. Babies born to women exposed to arsenic dusts during pregnancy have a higher than expected incidence of congenital malformations, below average birth weight	Nordstrom et al. (1979)

(continued)

Table 8.1 (continued)

Effect	Toxicity	References
Reproductive effects	1. Inorganic arsenic readily crosses the placental barrier and affects fetal development	Squibb and Fowler (1983)
	2. Organic arsenicals do not seem to cross the placenta so readily and are stored in the placenta and cause elevations in low birth weights, spontaneous abortions, still-birth, pre-eclampsia and congenital malformations	
Immunological effects	1. Low doses of arsenite (2 × 10–6 M) and arsenate (5 × 10–6 M), phytohemagglutinin (PHA)-induced stimulation of cultured human lymphocytes is increased 49% with arsenite and 19% with arsenate	McCabe et al. (1983)
	2. High doses of arsenite (1.9 × 10–5 M) and arsenate (6 × 10–4 M), PHA-induced stimulation is completely inhibited with an impairment of immune response	
Genotoxic effects	1. Trivalent forms are far more potent and genotoxic than the pentavalent forms	Barrett et al. (1989)
	2. Dimesthylarsinic acid (DMA) is more toxic than monomethylarsonous acid (MMA) in assays using mammalian and human cells and trimethylarsine oxide (TMAO) is more potent at inducing both mitotic arrest and tetraploids	Moore et al. (1997)
Mutagenetic effects	Arsenic promoted genetic damage in large part by inhibiting DNA repair and may cause cancer or problems in the exposed generation	Hoffman (1991)
Carcinogenic effects	1. Patients who received chronic treatment with arsenical medications have greatly increased the incidence of both basal cell and squamous cell carcinomas of the skin	Hutchinson (1887)
Diabetes mellitus	1. Drinking water arsenic exposure	Rahman et al. (1998–1999)
	2. The presence of keratosis as an indicator of arsenic exposure showed elevated risks for diabetes in those exposed to arsenic in their drinking water	

5 Removal Strategies of Arsenic from a Contaminated Environment

Several strategies of As remediation are reported. In-situ treatments are preferred at large sites and are given at the place of contamination itself, whereas ex-situ remediation is done at another location, which involves different treatments of contaminated soil or water (Table 8.2) (Pierzynski et al. 2005).

Table 8.2 Arsenic bioremediation proposed in the literature to date

Microbial process	Comments	References
Biosorption	Fe(III) treated *Baccilus subtulis* has 11 times higher As(V) sorption capacity than that of the native bacteria	Yang et al. (2012)
	The maximum biosorption capacity of living cells of *Bacillus cereus* for As(III) was found to be 32.42 mg g^{-1} at pH 7.5, at optimal conditions of contact time of 30 min, biomass dosage of 6 g L^{-1}, and temperature of 30 °C	Giri et al. (2013)
	Bacillus cereus strain W2 retained As(III) and As(V) up to 1.87 mg As g^{-1} of dry cell weight and dry cell removal capacity up to 0.18 mg As g^{-1}	Miyatake and Hayashi (2011) and Prasad et al. (2011)
	The biosorption capacity of the *Rhodococcus* sp. WB-12 for As(III) was 77.3 mg g^{-1} at pH 7.0 using 1 g L^{-1} biomass with the contact time of 30 min at 30 °C	
Bioaccumulation	Engineering of phytochelatin producing, As transporter GlpF co-expressing and an As efflux deletion *Escherichia coli* showed a 80-fold more As accumulation than a control strain, achieving an accumulation level of 16.8 μmol g^{-1} (dry cell weight)	Singh et al. (2010)
	Saccharomyces cerevisiae was engineered for 3- to 4-fold greater As(III) uptake and accumulation by overexpression of transporters genes FPS1 and HXT7 responsible for the influx of the contaminant coupled with and without high-level production of cytosolic As sequestors (phytochelatins or bacterial ArsRp)	Shah et al. (2010)
	Engineered *Escherichia coli* expressing ArsR accumulated 50–60 times higher As(III) and As(V) than control	Kostal et al. (2004)
Bioreduction	The co-presence of anthraquinone-2,6-disulfonate with As(V) respiratory-reducing bacteria (*Bacillus selenatarsenatis* SF-1) improved the removal efficiency and can be an effective strategy for remediation of As-contaminated soils	Yamamura et al. (2008)
Biomethylation	Engineering the soil bacterium *Pseudomonas* putida expressing the As(III) S-adenosylmethionine methyltransferase gene has the potential for bioremediation of environmental As	Chen et al. (2013)
	Soil microorganism, e.g. *Trichoderma* sp., sterile mycelial strain, *Neocosmospora* sp. and *Rhizopus* sp. Fungal strains could be used for soil As bioremediation via biovolatilisation	Srivastava et al. (2011)

(continued)

Table 8.2 (continued)

Microbial process	**Comments**	**References**
Biomineralisation	The nitrate- and sulphate-plus-lactate-amended microcosms with sediment from an aquifer with naturally elevated As levels decreased effective soluble As levels from 3.9 to 0.01 and 0.41 μM via sorption onto freshly formed hydrous ferric oxide and iron sulphide	Omoregie et al. (2013)
	The biogenic Mn oxides generated by *Marinobacter* sp. MnI7-9 oxidised the highly toxic As(III) to As(V) and decreased the concentration of As(III) from 55.02 to 5.55 μM	Liao et al. (2013)
	Arsenic immobilisation by biogenic Fe mineral formed by *Acidovorax* sp. BoFeN1, an anaerobic nitrate-reducing Fe(II)-oxidising ß-proteobacteria	Hitchcock et al. (2012)
	Microbial calcite precipitated by an As(III) tolerant bacterium *Sporosarcina ginsengisoli* CR5 to retain As	Achal et al. (2012)
	Bioremediation strategy based on injecting nitrate to support the anoxic oxidation of Fe(II) and As(III) in the subsurface as a means of immobilising As in the form of As(V) adsorbed onto biogenic Fe(III) (hydr)oxides	Sun et al. (2009)
Other process	**Comments**	
Phytoextraction	This uses pollutant-accumulating plants to extract and translocate pollutants to the harvestable parts. It can be sub-divided into phytoextraction using hyperaccumulator plants and chemically induced phytoextraction for the accumulation of metals to plants. Induced phytoextraction, however, has not yet been applied to As	Fitz and Wenzel (2002) and Salt et al. (1998)
Phytostabilisation	Uses pollutant-tolerant plants to mechanically stabilise polluted land to prevent bulk erosion, reduce air-borne transport and leaching of pollutants. In contrast to phytoextraction, plants are required to take up only small amounts of As and other metals to prevent transfer into the wild-life food chain	Fitz and Wenzel (2002)
Phyto-immobilisation	The use of plants to decrease the mobility and bioavailability of pollutants by altering soil factors that lower pollutant mobility by formation of precipitates and insoluble compounds and by sorption on roots	Fitz and Wenzel (2002)
Volatilisation	The use of plants to volatilise pollutants. Volatilisation of As is known to occur in the natural environments	Frankenberger Jr and Arshad (2002)
Rhizofiltration	The use of plants with extensive root systems and high accumulation capacity for contaminants, to absorb and adsorb pollutants, mainly metals, from water and streams	Salt et al. (1998)
Phytofiltration	The use of plant uptake contaminants into the biomass, thus removing the pollutant	Raskin and Kumar (1994)
Rhizodegradation	Transformation of the contaminant in the rhizosphere can occur in soil organisms such as fungi or bacteria or via enzymes exuded from microorganisms or plants	Schultz et al. (2001)

5.1 Physico-Chemical Strategies for Arsenic Remediation

5.1.1 pH

Arsenic (V) adsorption reduces with increasing pH, and highest absorption occurs at above pH 8.5, adsorption of As(III) increases with increasing pH. The degree of influence of pH on As adsorption varies from soil to soil. The highest adsorption of As(V) is at pH 4, whereas for As(III) the adsorption maxima are found at approximately pH 7–8.5 (Fitz and Wenzel 2002; Mahimairaja et al. 2005).

5.1.2 Phosphate

Phosphate (PO_4^{-3}), an analogue of As(V), acts as an essential factor for the nature of As in oxygen-rich soils (Mahimairaja et al. 2005; Williams et al. 2006). The effect of PO_4^{-3} additions to aerobic soils on the uptake of As therefore dependS on the balance between competition for sorption sites and competition for uptake. As(III) is not an analogue of PO_4^{-3}; hence, making the presence of PO_4^{-3} most likely less relevant to the behaviour of As under flooded conditions (Takahashi et al. 2004). The role of PO_4^{-3} in the rhizosphere is not known, where aerobic conditions may prevail under flooded situations. There are many factors that affect the biogeochemistry of As (Table 8.3).

5.1.3 The Effect of Iron Hydroxides

As(V) and As(III) adsorb primarily to iron-hydroxides (FeOOH) of the soil and As(V) has stronger bonds compared with As(III). The behaviour of FeOOH primarily depends on its redox conditions; thus, iron redox chemistry play as an important parameter in regulating As behaviour (Fitz and Wenzel 2002; Takahashi et al. 2004). Under anaerobic conditions, the dissolution of FeOOH readily occurs, which results in the release of As in the soil. Such As occurs chiefly as As(III) (Takahashi et al. 2004). FeOOH serves as a sink for As and is quietly insoluble under aerobic conditions. Fe and As behaviour is closely related and very dynamic in lowland paddy fields. In comparison with sandy soils, FeOOH more frequently occurs in clayey soil (Fitz and Wenzel 2002; Mahimairaja et al. 2005). At the same concentration, the clayey soils are found to be less toxic in comparison with sandy soils owing to the strong bond of As in clayey soils.

5.2 Microbial-Based Arsenic Remediation

Arsenic is very toxic to the biological system, although microorganisms have evolved several mechanisms to deal with this problem. The key modes of microbial remediation of As-affected soils are: oxidation and reduction, biosorption,

Table 8.3 Factors affecting the biogeochemistry of arsenic

	Factor	Effects
1.	pH	Arsenic mobility increases at a very low pH (pH <5). As(III) solubility increases as the pH decreases within the range (pH 3–9). In contrast, the pattern is reversed in the case of As(V)
2.	Speciation	As speciation in soil is essential to assess the As toxicity in plants. Among all species toxicity order of As: As(III) > As(V) > MMA > DMA
3.	Redox condition	Increased solubility of As in reducing environment
4.	Fe-plaque	Fe-plaque decrease As uptake by plants
5.	Organic matter	High amount of OM can reduce As solubility and lead to less As availability for plants
6.	Soil texture	Arsenic is five times more in sandy and loamy soils than in clay soils
7.	Fe-Mn oxides	Under aerobic conditions, the Fe-Mn phases decrease As mobility. However, under flooding conditions, these phases can release As mobility, leading to more available As for plant uptake
8.	Heavy metals	Heavy metals can form ternary complexes with arsenate on Fe and Al oxide surfaces, thus lowering the bioavailability of arsenic
9.	Phosphate	Phosphate PO_4 is a chemical analogue of arsenate and its increasing concentration decreases As content in Fe-plaques
10.	Silica	High silica availability in soil reduces the arsenite uptake
11.	Sulphate	The application of sulphur significantly reduces As accumulation in rice
12.	Nitrate	Nitrate (NO_3) is a strong oxidiser and its reduces As uptake
13.	Irrigation practice	Under aerobic water management practices, rice takes up less As (0.23–0.26 ppm) than under anaerobic practices (0.60–0.67 ppm)
14.	Seasonal variation	Winter season crops have a high accumulation of As in comparison with monsoon season crops
15.	Genotype	The genotype variation in As uptake may be due to differences in root anatomy, which controls root aeration and Fe-plaque formation on the root surfaces or differences in the arsenic tolerance gene

complexation, biomethylation, sequestration, solubilisation, and microbe-mediated phytoremediation. Microbial oxidation and reduction involve various metal ions that drastically affect As mobility. Microbes evolved their biochemical pathways to utilise As oxyanions, either as an electron acceptor [e.g. As(V)] for anaerobic respiration, or as an electron donor [e.g. As(III)] to support chemoautotrophic fixation of CO_2 into cell carbon (Santini et al. 2000; Rhine et al. 2006).

5.2.1 Arsenic Remediation by Bacteria

Bacterially mediated remediation involved the reduction of As oxyanions. Stolz and Oremland (1999) decode the stepwise reduction of As oxyanions that selected bacteria perform. Up to 80% As removal was reported by researchers. Many Gram-negative and Gram-positive bacteria employ common ars operon (typically ars RDABC)-based mechanisms for resistance against As. The operon may be encoded either on chromosomes or on plasmids (Xu et al. 1998).

5.2.1.1 Oxidation/Reduction

The energy for microbial growth is derived from the oxidation process. Arsenite oxidase is the enzyme that plays a pivotal role in arsenite oxidation. The enzyme was identified and sequenced and found to belong to the DMSO reductase family. Like many arsenite oxidases, the one (AoxAB) isolated from *Hydrogenophaga* sp. strain NT-14 is made up of a heterodimer (from the gene aoxAB) containing iron and molybdenum as a part of their catalytic unit. The bio-oxidation contributes to As non-availability by immobilising it, alleviating its toxicity in flowing acidic water and neutralising it by environmental alkalinity.

The reduction or conversion of As(V) to As(III) in an anaerobic environment is mediated by mixed populations of anaerobes such as methanogens, fermentative, sulphate- and iron-reducing bacteria. Microbial reduction of As(V) could even occur when As is found bounded with iron hydroxides (Langner and Inskeep 2000). Zobrist et al. (2000) reported that the reduction of As(V) may lead to its mobilisation, without dissolving the sorbent phase. The anaerobically incubating As(V) is co-precipitated with Al hydroxide in the presence of *Sulfurospirillum barnesii*. Moreover, the microbial reduction of As(V) to As(III) under aerobic conditions in As-contaminated soils may occur relatively quickly, resulting in excessive As mobilisation and transport from contaminated soils to groundwater (Macur et al. 2001). Arsenate reduction is a part of anaerobic arsenate respiration in some bacteria (e.g. *Shewanella* sp. strain ANA-3) (Krafft and Macy 1998), where arsenates serve as a terminal electron acceptor.

5.2.1.2 Methylation/Demethylation

The methylation of As occurs via the reduction of pentavalent As to trivalent As and the addition of a methyl group. The conversion of As (V) to small amounts of volatile methylarsines was first described in *Methanobacterium bryantii* (McBride and Wolfe 1971). Methylation is considered to be detoxification. However, not all methylated As products are less toxic (Bentley and Chasteen 2002); As(V) can be rehabilitated to mono- and di-methylarsine by *Achromobacter* sp. and *Enterobacter* sp., and to mono-, di-, and trimethylarsine by *Aeromonas* sp. and *Nocardia* sp. (Cullen 1989). Methylation and demethylation also play critical roles in the toxicity and availability of As in soils and groundwater (Wang and Mulligan 2006). Methylation of As(III) and As(V) sometimes forms volatile species, which lead to the escape of As from water and soil surfaces. The oxidation of methylated forms of As may convert them back to the oxidised As(V); however, demethylation of mono- and microbes could use dimethyl As compounds and methylated arsenicals as possible carbon sources (Maki et al. 2004).

5.2.1.3 Biofilm and Biosorption

There are several microorganisms that can form biofilms and in a biofilm, microniches could coexist with variable physiological needs, enabling the coexistence of spatially separated conflicting redox processes simultaneously (Labrenz et al. 2000; Van Hullebusch et al. 2003). This is evident by the enrichment of As in biofilms. The As concentrations in rock biofilm may reach up to 60 mg kg^{-1} of dry weight (Drewniak et al. 2008).

5.2.1.4 Biostimulation and Bioaugmentation

Bioaugmentation is the process that exploits metal-immobilising microbial population(s) to convert highly toxic metal into a less toxic state at the site itself. In biostimulation, a stimulus is provided to microorganisms pre-existing at the site. The stimulus may be a nutrient, growth substrates with or without electron acceptors or donors. This could be achieved by delivering nutrients (biostimulation) or microbial populations (bioaugmentation) into the soil using biosurfactant foam technology (Hug et al. 2011). The biosurfactants enhance the availability of metal ion [Fe(III), As etc.] to the microorganisms by reducing the interfacial tension and formation of micelles.

5.2.1.5 Biomineralisation

In biomineralisation, the As is immobilised by its precipitation, e.g. scorodite and As sulphide. More than 300 compounds of As are known to occur in various environments (Drahota and Filippi 2009). In As-contaminated suspended aquifer, the availability of free Ca^{2+} control the As mobility by digenetic precipitation of calcium arsenates (Martinez-Villegas et al. 2013).

5.2.2 Arsenic Remediation by Algae

Certain species of algae take up copper, cadmium, chromium, lead, and nickel (Qiming et al. 1999) from aqueous solutions by releasing a protein called metallothioneins. Metallothioneins bind the metal as a defence mechanism to remove/mitigate the toxic effect of metal by its regular cellular activity. The biosorption by algae is a viable mechanism for the treatment of metal waste and could work efficiently for multi-component metal systems. Algae respond to metal ions by synthesising low molecular weight compounds such as glutathione and carotenoids, and initiate the synthesis of several antioxidants and enzymes (superoxide dismutase, catalase, glutathione peroxidise and ascorbate peroxidise). *Chlorella* and *Scenedesmus* are the two most frequently exploited species used for metal uptake. *Scenedesmus* sp. has much greater metal binding potentials than *Chlorella* sp.

Suhendrayatna et al. (1999) exposed *Chlorella* sp. to various concentrations of arsenite ranging from 0 to 100 µg As cm^{-3}. They found that the cell growth of *Chlorella* sp. was affected at concentrations higher than 50 µg As cm^{-3} At concentrations greater than 50 µg As cm^{-3} The cell growth of *Chlorella* was suppressed. There is a lack of studies on wastewater treatment plants where mixed bacteria culture or the alga *Scenedesmus abundans* was used for the removal of As. Available literature indicates that arsenite As(III) is a more toxic form and thus more difficult to remove using conventional treatment methods (Zouboulis and Katsoyiannis 2005).

5.2.3 Arsenic Remediation by Yeast

In yeast, As(V) enters in the cells through high-affinity phosphate transporters such as Pho84, whereas As(III) influx occurs through the aquaglyceroporins Fps1 (Wysocki et al. 2001). In addition, glucose permeases are also involved in As uptake by yeasts (Liu et al. 2004). Expression of yeast hexose carriers in mutant *D*fps1 restored As sensitivity, which was As(III)-tolerant earlier (Liu et al. 2006). Arsenic tolerance in yeast is encoded by the gene cluster *ACR1*, *ACR2* and *ACR3*. *ACR1* encodes a putative transcription factor that regulates the transcription of *ACR2* and *ACR3*, possibly by directly sensing cellular As levels. *ACR2* encodes an arsenate reductase and *ACR3* encodes a plasma membrane-expressed As(III) efflux transporter. This gene cluster provides a process for the sensing, reduction and efflux of As. In addition, a second pathway is also present in yeasts, in the form of vacuoles. Cytosolic As(III) complex with glutathione can be sequestered through an ABC-type transporter, *YCF1*, that also transports conjugates of other toxic compounds (Ghosh et al. 1999).

5.2.4 Arsenic Remediation by Fungi

Fungi present in polluted soils play an important role in the maintenance of indigenous diversity and protect them against the uptake of toxic heavy metals (HMs) by plants. Sharples et al. (2000) showed that the ericoid mycorrhizal fungus *Hymenoscyphus ericae* significantly reduces the uptake of As by *Calluna vulgaris* when grown in As-affected soil. Various mycorrhiza-based phosphate transporters have been reported to increase expression in arbuscular mycorrhizae (AM). They are StPT3 in *Solanum tuberosum* LePT3 and LePT4 in *Lycopersicon esculentum* (Nagy et al. 2005; Xu et al. 2007), and MtPT4 in *Medicago truncatula* (Javot et al. 2007). Contradictorily, mycorrhizal association increases As accumulation in the fern *P. vittata* (Liu et al. 2005). The study by Uppanan (2000) exposed the *Alcaligenes* spp. from soil that could oxidise arsenite to arsenate and taken up by the plant via phosphate transporter (Meharg and Hartley-Whitaker 2002). In AM-mediated phytoremediation of As, contradictory results have been reported on As uptake by plants, e.g. the addition of rhizo-fungi in As-contaminated soil, and the uptake and the accumulation of As were induced in *P. vittata* (Leung et al. 2006).

5.2.5 Arsenic Remediation by Genetic Engineered Microbes

The genetically engineered microbes may serve as selective biosorbents and lay the foundation of green technology for the eco-friendly, low-cost, efficient removal of HMs such as (Singh et al. 2008a). For the development of As-metabolising/accumulating microbe, the microbe should comprise the ability to modify its naturally occurring defence mechanisms and be able to develop a novel pathway. The bacterial enzymes arsenate reductase (ArsC) and GSH synthase (g-ECS) were successfully expressed in the plant *Arabidopsis thaliana*. These enzyme systems confer the ability to accumulate arsenate as GSH–As complexes. In the same way, YCF1 protein of yeast expressed in *A. thaliana* enhanced As storage in the vacuole (Song et al. 2003). These reports open up the path of genetically engineered microbes and their metabolic pathways for successful As sequestration. The phytochelatin (PC) synthase of *A. thaliana* was expressed in *E. coli* (Sauge-Merle et al. 2003) and produced PC, leading to moderate levels of As accumulation. However, the level of GSH, a key precursor of PC, acts as a limiting factor for high PC production and As accumulation. The PC synthase of *S. pombe* (SpPCS) when expressed in *E. coli* resulted in higher As accumulation than the wild type (Singh et al. 2008b). Significant levels of PC were obtained by co-expressing As transporter GlpF; this led to a 1.5-fold higher accumulation of As. On deletion of As efflux in the *E. coli* strain, the highest As accumulation of 16.8 mmol g^{-1} cells of *E. coli* was recorded.

Sulphur-reducing bacteria naturally precipitate As(V) by the formation of an insoluble sulphide complex with H_2S (Rittle et al. 1995). Recently, a yeast strain was engineered that was coexpressing AtPCS and cysteine desulfhydrase, to elevate the accumulation of As by the formation of PC metal–sulphide complexes (Tsai et al. 2009). The approach to exploiting the resting cells of high-affinity biosorbent property for As had also been exploited. To achieve this, AtPCS were expressed in *S. cerevisiae,* which naturally has a higher level of GSH; the engineered yeast strain accumulated high levels of As in resting cell cultures (Singh et al. 2008b). Arsenic accumulation in *E. coli* was achieved by over-expressing the As-specific regulatory protein ArsR. These engineered resting cells were able to remove 50 ppb of As(III) per hour (Kostal et al. 2004). The concept has been extended to using a naturally occurring As-binding MT (Singh et al. 2004). These resting cells were able to remove 35 ppb of As(III) in 20 min. New irrational approaches such as directed evolution, genome shuffling and metagenomic studies could also be used to develop new As-removing pathways suitable for As remediation. It was demonstrated by the modification of As resistance operon by DNA shuffling (Crameri et al. 1997). The cells with optimised operon can grow in 0.5 M arsenate, showing 40-fold enhancement in As resistance. Meanwhile, Chauhan and coworkers constructed a metagenomic library of sludge obtained from industrial effluent treatment plants, and identified a novel As(V) resistance gene (*arsN*) encoding a protein similar in action to acetyltransferases. Its over-expression resulted in higher As resistance in *E. coli* (Chauhan et al. 2009).

5.3 Plant-Mediated Arsenic Remediation

Phytoremediation, the plant-mediated bioremediation of polluted soil, water and air, is an emerging cost-effective, non-invasive and widely accepted method for removing/mitigating environmental pollutants (Boyajian and Carreira 1997; Singh et al. 2003; Bharagava et al. 2017). Plants have a natural ability to accumulate inorganic and organic contaminants. The organic pollutants are metabolised and microbial degradation of organic pollutants is promoted in the rhizosphere. Phytoremediation involved the cultivation of metal-tolerant plants able to concentrate the metal in their tissues. The plant biomass thus produced is harvested and dried and is deposited in a landfill or added to smelter feed (Kramer 2005). The exceptional ability of *Pteris vittata* to accumulate As could be explored to design an efficient phytoremediation strategy, although greenhouse studies demonstrate the promising potential of As extraction by *P. vittata*. However, the results of two small-scale field trials are less promising owing to low biomass production (<1 tonne dry biomass ha^{-1}) (Salido et al. 2003). In 2 years of growth, the total As removal by *P. vittata* was about 1% of As in top soil (30 cm in depth) (Kertulis-Tartar et al. 2006).

6 Challenges

- Chronic As poisoning is a major threat to large sections of the global population and food consumption is one of the biggest contributors to human As exposure.
- Contamination of As in paddy soils is a widespread problem because of the irrigation of As-laden groundwater in the south southeast, including India.
- Anaerobic conditions in flooded paddy soils are conducive to the mobilisation of As, leading to much enhanced As bioavailability to rice plants (Lee et al. 2008; Xu et al. 1998). Rice is a major source of inorganic As for populations based on a rice diet and not exposed to high As in drinking water (Kile et al. 2007; Meharg et al. 2009).
- Accumulation of As in paddy soil can cause phyto-toxicity to rice plants and a significant reduction in grain yield, thus threatening the long-term sustainability of the rice cropping system in the affected areas (Khan et al. 2009).
- Research concerning microbial As is still in its infancy; therefore, a thorough understanding of the true As behaviour in the surface and subsurface environments under the influence of microbial activities is still very challenging.

7 Conclusion

The following points have been concluded in this study:

- This chapter has attempted to summarise As content in edible crops, its relationships with soil and irrigated groundwater and the factors controlling As mobilisation and uptake in edible crops.
- Arsenic accumulation in plants is largely influenced by a variety of factors, including soil physicochemical parameters; other elements such as iron, phosphorus, sulphur and silicon concentrations; and environmental conditions that control As availability and uptake in the soil–rhizosphere–plant system.
- Environmental conditions can be managed by changing irrigation practices. For example, the flooding of the paddy soil mobilises As in the soil solution and can increase As accumulation in rice.
- Therefore, changing agricultural practices to aerobic rice cultivation throughout the entire season may be a viable strategy for mitigating this problem. However, there are arguments in certain cases because of flood conditions.
- Arsenic is present as arsenite, which cannot compete with phosphate; furthermore, phosphate increases As mobility because it competes with arsenate for the adsorption site of Fe oxides/hydroxides.
- The use of vegetation directly or indirectly to remove contaminants from water or soil is an important innovative remediation technology potentially applicable to a variety of contaminated sites.
- Selection of the appropriate plant species is a critical process for the success of this technology.
- Approaches to reducing As uptake in crops, especially in the edible parts, would provide a viable alternative.
- Many natural substances are expected to exhibit substantial effects on the microbial processes and subsequently change the environmental behaviour of As, either directly or indirectly.
- Researching microorganism–As interactions also provides the opportunity to study As remediation taking advantage of microbial activities.
- Arsenic concentration in the agricultural field soil was below 20.0 mg kg^{-1} (the maximum acceptable limit for agricultural soil, recommended by the European Community (EC)) and 1.0 mg kg^{-1} dry weight of As (the permissible limit of As in rice according to WHO recommendations).
- The total amount of As in raw rice is not taken into the human body because of its distribution in the following order: root>straw>husk>grain. An appreciably high efficiency in the translocation of As from shoot to grain was observed compared with the translocation of As from root to shoot.

Thus, this chapter covers all As-oriented problems and their fate in the environment and treatment technology for the sustainable development of the environment and environmental safety.

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