Mirza Hasanuzzaman · Kamrun Nahar
Masayuki Fujita *Editors*

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Preface

Arsenic (As) is the most talked about metalloid in the modern world. It is a poisonous metalloid and historically known as "king of poisons," and its toxic potential has been known for millennia. It is the 20th most common element in the earth's crust, and it is present in the terrestrial, marine, and freshwater environments in various chemical forms, usually combined with other metals, sulfur, or oxygen. Arsenic may cause substantial damages to plant and animal kingdom. Therefore, As has become a great concern because of its chronic and epidemic effects on human, plant, and animal health. Naturally occurring As in the water has impacted the lives of millions of people; the situation has been called the "largest mass poisoning of a population in history". For example, in Bangladesh and West Bengal, India, more than 50 million people are drinking water containing As.

Since the last three decades, the toxic effects of As in plants have been investigated widely. Because of its occurrence in all soils and natural waters plants have obviously evolved in the presence of As ions. The complexity of As chemistry and biochemistry confound many efforts to understand the mechanism of toxicity. Different forms of As showed different mechanisms of toxicity. The rate of uptake or accumulation of As also greatly depends on several factors like soil type, plant species, and mechanisms of uptake. Among the cultivated crop plants, rice is the most affected crop from As threats because of the fact that rice is the only major crop grown in waterlogged condition for most of the time, and that rice is particularly efficient at assimilating some forms of As, particularly those generated under anaerobic conditions, and exporting them to grain. In line with the abundance and toxic effects of As in plants the tolerance mechanisms in the plant are being investigated widely. Molecular approaches in revealing the As stress-responsive genes provide effective clues in developing tolerance in plants. Recently, bioremediation technologies using plants and microbes are drawing special attention due to its effective and eco-friendly perspectives. Numerous research works have been carried on different aspects of As chemistry and the mechanisms of toxicity and tolerance in plants. This book presents a collection of 19 chapters written by 57 experts in the field of plant physiology, environmental sciences, and plant biochemistry.

We the editors would like to give special thanks to the authors for their outstanding and timely work in producing comprehensive chapters. We are highly thankful to Dr. Fumiko Yamaguchi, Senior Editor (Editor, Ecology and Animal Science), Springer, Japan, for her prompt responses during the acquisition. We are also thankful to Sivachandran Ravanan, Production Editor of this book and all other editorial staffs for precious help in formatting and incorporating editorial changes in the manuscripts. Special thanks to Dr. Md. Mahabub Alam, Noakhali Science and Technology University, Bangladesh, and Sayed Mohammad Mohsin, Sher-e-Bangla Agricultural University, Bangladesh, for their generous help in formatting the manuscripts. The editors and contributing authors hope that this book will include a practical update on our knowledge of the role of plant nutrients in abiotic stress tolerance.

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Dr. Hasanuzzaman's current work is focused on the physiological and molecular mechanisms of environmental stress tolerance (salinity, drought, flood, and heavy metals/metalloids). Dr. Hasanuzzaman has published over 100 articles in peer-reviewed journals. He has edited six books and written 30 book chapters on important aspects of plant physiology, plant stress tolerance, and crop production. According to Scopus®, Dr. Hasanuzzaman's publications have received over 2700

citations with an h-index of 26. He is editor and reviewer for more than 70 peer-reviewed international journals and was a recipient of the "Publons Peer Review Award 2017 and 2018." He has been honored by different authorities for his outstanding performance in different fields like research and education, and he has received the World Academy of Science Young Scientist Award (2014). He has presented 25 papers, abstracts, and posters at international conferences in several countries (USA, UK, Germany, Australia, Japan, Austria, Sweden, Russia, etc.). Dr. Hasanuzzaman is a member of 40 professional societies and is the acting Publication Secretary of the Bangladesh Society of Agronomy and the Bangladesh JSPS Alumni Association. He is a fellow of The Linnean Society of London.

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is a reviewer of international journals. She is active member of about 20 professional societies. Dr. Nahar attended different international conferences and presented 10 papers and posters in national and international conferences in different countries (USA, Australia, Japan, Austria, Russia, China, etc.).

Dr. Masayuki Fujita is Professor in the Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Kagawa, Japan. He received his B.Sc. in Chemistry from Shizuoka University, Shizuoka, and M.Agr. and Ph.D. in Plant Biochemistry from Nagoya University, Nagoya, Japan. His research interests include physiological, biochemical, and molecular biological responses based on secondary metabolism in plants under various abiotic and biotic stresses; phytoalexin, cytochrome P450, glutathione *S*-transferase, and phytochelatin; and redox reaction and antioxidants. In the last decade, his works were focused on oxidative stress and antioxidant defense in plants under environmental stress. His group investigates the role of different exogenous protectants in enhancing antioxidant defense and methylglyoxal detoxification systems in plants. He has supervised 4 M.S. students and 13 Ph.D. students as main supervisor. He has about 150 publications in journals and books and has edited 8 books.

Chapter 1
Arsenic Uptake and Transportation in Plants

Dariusz Latowski, Anna Kowalczyk, Kamila Nawieśniak, and Stanisław Listwan

Abstract The arsenic uptake and translocation systems in plants are dependent on As species. Uptake of inorganic arsenate [As_{in}(V)] is conducted via specified group of high-affinity phosphate (P_i) transporters belonging to the PHS family, called P_i transporter 1. Recently identified transcription factors involved in the regulation of As_{in}(V) intake in plants are also described in this chapter. The role of other proteins such as mitochondrial proteins localized to the inner mitochondrial membrane and responsible for dicarboxylate exchange between the mitochondrial matrix and the cytosol or P_i transporter traffic facilitator 1 located in the endoplasmic reticulum (ER) of *A. thaliana* is not omitted. Uptake of inorganic arsenite [As_{in}(III)], as well as the organic derivatives of As from environment and distribution in plants, is conducted by channels created by proteins belonging to three of the five plant aquaporin subfamilies called nodulin 26-like intrinsic protein (NIP), membrane (PIP), and tonoplast intrinsic proteins (TIP). The significance of ABC (ATP-binding cassette) transporters which are responsible for transferring of As_{in}(III)-phytochelatin complexes across the tonoplast to the vacuole as well as the role of transporters responsible for inositol uptake in As translocation from the xylem into the phloem is explained. Additionally, the meaning of some elements like S, Si, and Fe in As influx in plants is considered.

Keywords Arsenic species · Ion flux · Metalloids · Phytochelatins · Soil pollution

1.1 Introduction: Uptake and Transport of Arsenic Depend on Soil Properties and As Species

Although no specific As uptake systems have evolved (Stolz et al. 2006), the uptake of this metalloid from As-contaminated soils or water by plants including plant crops such as rice, brussels sprout, or other vegetables is commonly observed

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(Larsen et al. 1992; Cottingham et al. 2013). In the human body, about 40% of As content comes with the food, and plants are the primary route by which As enters in the food chain (BIAM 2002; Colangelo and Guerinot 2006). So, As accumulation in plants and its introduction to the food chain by plants are serious issue. Therefore, identification of As transporters and channels, as well as understanding As transport mechanisms in plants, can be applied in safe cropping and phytoremediation of As-contaminated areas in the future (Zhao et al. 2010a; Zhu et al. 2008). For safe cropping, resistant plants able to prevent accumulation in the harvested plant product are required, whereas for phytoremediation, the resistant plants capable of growing at high As concentrations and accumulate As in harvestable biomass are needed.

Among the three allotropes and nine oxidation states, either organic As (As_{org}) or inorganic As (As_{in}) is available to plants in four main forms of As: inorganic arsenate [As_{in}(V)], arsenite [As_{in}(III)], and their organic derivatives, i.e., monomethylarsonic acid (MMA) and dimethylarsinic acid (DDA) (Fig. 1.1) (Kläning et al. 1989; Norman 1998; Ellis and MacDonald 2004; Janiak et al. 2012). Generally, in the environment, the content of As_{org} is lower than As_{in} (Abedin et al. 2002). Moreover, the concentration of As_{org}(III) is lower than As_{org}(V) because of high volatility of As_{org}(III) (Mestrot et al. 2011).

As availability to plants depends on soil composition, texture, and other physicochemical properties of the soil, whereas As uptake and transportation systems in the plant are strictly connected with As species. In fine-textured soil, low content of As is observed, while coarse-textured soils with little ion exchange capacity and less colloidal material contained more As. Under oxidative conditions, As(V), the oxidized form, dominates As(III), whereas under reducing conditions occurring in such environment as flooded rice paddy fields, more mobile As(III) dominates As(V) (Punshon et al. 2017). Moreover, when anoxic conditions develop, redox potential (E_H), responding to the extent of aeration of the soil drops, electron acceptors are depleted causing reduction and dissolve of iron oxides and oxyhydroxides, thus increasing mobility of As previously strongly bound with these molecules (Fendorf and Kocar 2009; Meharg and Zhao 2012). This reductive dissolution of iron-bearing minerals under anaerobic conditions is the dominant biogeochemical process in the transition from As_{in}(V) to As_{in}(III). Forming of the Fe plaques in the rhizosphere of rice and other plants growing on flooded areas (e.g., water species) is a common mechanism of As uptake limitation (Chen et al. 2005; Seyfferth et al. 2010). Fe plaque consists of ferrihydrite, a widespread on the Earth's surface hydrous ferric

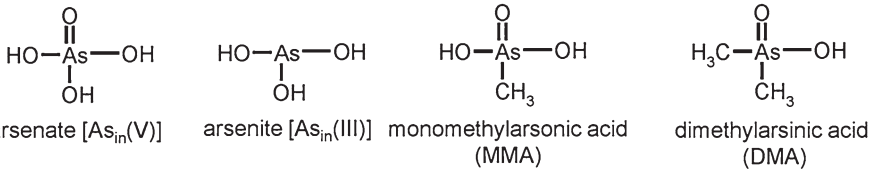

Fig. 1.1 Chemical structures of four main forms of inorganic and organic arsenic species occurring in the environment

oxyhydroxide mineral. It is formed on the root surface as a result of Fe^{2+} to Fe^{3+}oxidation by the oxygen released through aerenchyma of the roots of the plants growing in anaerobic soils. Oxidized iron strongly adsorbs arsenates. Iron plaques on the roots of tested plants contain 70–80% $As_{in}(V)$ and 20–30% $As_{in}(III)$. Additionally, bacteria colonizing the root iron plaque are also able to oxidize As(III) (Hu et al. 2015). It was shown that the concentrations of $As_{in}(V)$ within iron plaques of rice roots were five times higher than in root tissues of rice. Thus, the iron plaque formed on roots surface is natural barrier protecting from the migration of As to the plant. The properties of iron plaque depend on the genotype of plants (Dwivedi et al. 2010) and microbial composition, but also on silica which, as a next factor affecting the formation of iron plaque, controls the As concentration in iron plaque as well as in plants. Results of short-term experiments on As uptake by excised rice roots demonstrated that iron plaques limit $As_{in}(V)$ uptake but increase this process in case of $As_{in}(III)$ (Hansel et al. 2002; Blute et al. 2004; Liu et al. 2004a, b; Chen et al. 2005; Zhao et al. 2009). It was supposed that this relation is associated with $As_{in}(III)$ transporter systems efficiently operating within rice cell membranes. These systems shift the balance of $As_{in}(III)$ binding reaction to the form not associated with iron plaques but quickly absorbed by the roots of rice. $As_{in}(V)$ is stronger bound with soil particles such as aluminosilicates or aluminum hydroxides than $As_{in}(III)$, and thus reduction of $As_{in}(V)$ to $As_{in}(III)$ is one of the most significant factors increasing As bioavailability.

Additionally, silicic acid and silicates as well as phosphate being structural analogues of $As_{in}(III)$ or $As_{in}(V)$, respectively, can facilitate release of As adsorbed on soil particles into the soil solution at their high concentrations (Luxton et al. 2006). Values of pH below 4 and above 9 are another factor releasing As from its strong bonds with soil particles (Meharg and Zhao 2012). At the physiological pH range, predominating forms of $As_{in}(V)$ are deprotonated arsenates ($[H_2AsO_4]^-$), whereas $As_{in}(III)$ up to pH 8.28 exists mainly as protonated arsenous acid (H_3AsO_3). Protonated form of $As_{in}(V)$, i.e., arsenic acid (H_3AsO_4), dominates only below pH 1.31 (Bienert and Bienert 2017). The local alterations in E_H, pH, and the other physicochemical soil properties including the content of organic matter occurring in the rhizosphere and caused by plants and microbes also strongly influence concentrations and bioavailability of As (Acosta et al. 2015; Seyfferth 2015; Andres and Bertin 2016; Xiao et al. 2016). The decrease of E_H and an increase in the level of organic matter foster As methylation in soils (Frohne et al. 2011). Of the four main As forms available for plants, the organic, methylated derivatives are absorbed with the slowest rate and much slower than inorganic forms of As. On the other hand, the mobility of organic As derivatives in plants is greater than inorganic (Carey et al. 2010, 2011; Ye et al. 2010). Besides, DDA is generally better absorbed than MMA. Among of inorganic forms of As, $As_{in}(III)$ is better assimilable by plants than $As_{in}(V)$ (Raab et al. 2007a; Finnegan and Chen 2012).

Methylated As forms, independently on their state of oxidation, as well as $As_{in}(III)$, due to their physicochemical similarities to silicic acid, can be absorbed and translocated in plants via silicon (Si) influx-efflux systems but also by other channels dedicated to transport of small neutral molecules such as glycerol or anti-

monite. Thus the presence of silicic acid, as well as some other small neutral molecules, can competitively inhibit uptake of these As forms from the environment (Bienert and Bienert 2017).

$As_{in}(V)$ as structural analogue of inorganic phosphate (P_i) is absorbed by plants through phosphate transporters (the Phosphate Transporter 1 family of proteins, PHT1). Since the P_i affinity of PHT is higher than for $As_{in}(V)$, it is known that phosphate-supplemented soil usually reduces the uptake of $As_{in}(V)$ by plants. Additionally, increasing or decreasing PHT1 or appropriate Si transporters content in plant plasma membrane, by genetic engineering techniques, can also increase or decrease rate and amount of all main forms of As absorbed by plants (González et al. 2005; Ma et al. 2008; Zhao et al. 2009; Chen et al. 2011; Wu et al. 2011; Cao et al. 2017).

1.2 Arsenite and Arsenic Methylated Derivatives

1.2.1 Uptake and Translocation Systems

Plants use several systems to uptake arsenite [$As_{in}(III)$] and As methylated derivatives independently on their oxidation states from the environment but also to transport them into xylem or phloem and subsequently to particular plant cells and inside them between their subcellular compartments. Moreover, some of these systems can also be used to efflux As. The most researched transport systems of these As species belong to aquaporins (AQPs) (Bienert and Bienert 2017).

AQPs are integral membrane proteins in almost all living organisms excluding only some intracellular bacteria or thermophilic Archaea (Abascal et al. 2014). They exist in the various cellular membranes, including the plasma membrane, the endoplasmic reticulum, the mitochondria, the chloroplast, the vacuole, and even the vesicles involved in the trafficking pathway (Maurel et al. 2015; Bienert and Bienert 2017).

In these membranes, AQPs form pores and thus efficiently facilitate or enable the uptake, translocation, sequestration, or extrusion water and small mainly uncharged solutes. Although AQPs function as homo- or heterotetramers, each monomer can also work as a channel on its own. Additional central pore formed by four monomers closely associates together as tetramer, probably serves as another transport path (Fig. 1.2c) (Yool et al. 1996; Fu et al. 2000). AQP monomers are highly conserved, and two structural segments in each monomer can be distinguished. Each segment consists of three long membrane-spanning α-helices (marked as H1–H3 in the first and H4–H6 in the second segment), one reentrant short α-helix (marked as HB in the first and HE in the second segment), and two interconnecting loops (marked as LA, LB in first and LD, LE in the second segment). The parts of LB and LE, with the conserved and functionally important Asn-Pro-Ala (NPA) motifs, form HB and HE, respectively. Two structural segments of each monomers are connected together by additional, the fourth loop (LC), linking directly helix H3 with H4

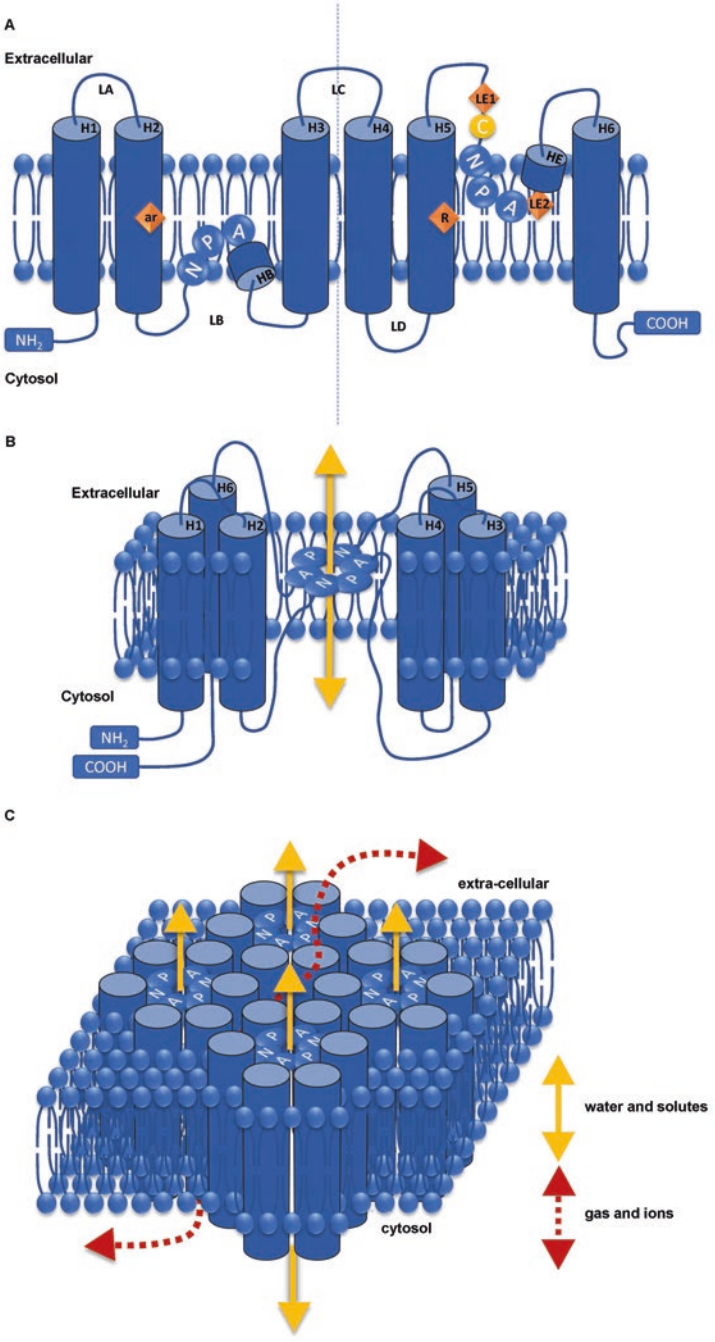

Fig. 1.2 The structures of AQP monomer (**a**, **b**) and homotetramer (**c**). H1–H6, α-helical domains; HB, HL, short α-helices with functionally important NPA (Asn-Pro-Ala) motifs; LA–LE, interconnecting loops; ar, R, LE1, LE2 (in diamonds), four amino acid residues created the functionally important aromatic selectivity filter, termed commonly as ar/R or more precisely as ar/R/LE1–LE2; **c** (in a circle), a cysteine residue (Cys 189) that can bind mercury blocking the AQP function

(Fig. 1.2a, b). α-Helices of AQPs create a solute-conducting channel with length between 20 and 28 Å and diameter from 4 to 6 Å. Although both ends of the channel on the membrane surfaces are widen funnel-shaped, the pore diameter can be constant over its length of the channel or narrow down around the narrowest region of the pore termed as the aromatic selectivity filter (Fu et al. 2000; Sui et al. 2001). The selectivity filter is located about 8–9 Å away from the first NPA motif and together with NPA motifs is the most important and narrowest region of AQPs that underlies their specificity (Törnroth-Horsefield et al. 2006; Frick et al. 2013a, b; Kirscht et al. 2016). The two NPA (Asn-Pro-Ala) motifs form the pore in which Asn residues are responsible for interaction with ligands by hydrogen bonds and probably create effective electrostatic proton barrier in AQPs (Fig. 1.2) (Tajkhorshid et al. 2002; Ilan et al. 2004; Forrest and Bhave 2007). The aromatic selectivity filter, termed commonly as ar/R or more precisely as ar/R/LE1–LE2, is formed with four amino acid residues – two located in second and fifth α-helices at position also termed as H2 and H5 and two others in second NPA box located in loop E at position signed as LE1 and LE2 (Fig. 1.2a) (Fu et al. 2000; Sui et al. 2001; Savage et al. 2003). These four residues include a conserved arginine residue (R) in loop E and less conservative aromatic residues (ar) at H2 α-helice (de Groot and Grubmüller 2001). The selective filter determines the rate of molecules transport by steric blocking too large molecules. Besides, the selective filter and NPA motifs create key interactions, such as van der Waals forces or hydrogen bonds, with transported molecules (Fu et al. 2000; Sui et al. 2001). Moreover, it was concluded that ar/R/LE1–LE2 together with NPA motifs are responsible for disruption of the hydrogen bonds between water molecules in the AQP pore and thus exclusion of the transport of protons via the Grotthuss mechanism (Kosinska-Eriksson et al. 2013; Zeuthen et al. 2013; Kreida and Tornroth-Horsefield 2015). N- and C-termini of each monomer of AQPs are oriented toward cytoplasm, and in some AQPs, these termini are posttranslationally modified and can possess sulfhydryl residues such as Cys, what explains AQP inhibition by mercury and other heavy metals (Fig. 1.2a) (Maurel et al. 2015; Bienert and Bienert 2017). Therefore, also As penetration can be partially inhibited not only by alternative AQP substrates such as mentioned above (glycerol, antimonite, or silicic acid), but also by mercury and other heavy metals which interact with sulfhydryl residues of Cys (Meharg and Jardine 2003).

The selectivity and kinetic parameters of aquaporins are commonly tested in four systems: (i) in isolated organelles, protoplasts, or tissues of living organisms (Ramahaleo et al. 1996; Uehlein et al. 2003; Besserer et al. 2012; Noronha et al. 2014); (ii) in vesicles isolated from cells or their membranes including transgenic cells with expressed aquaporins (Jung et al. 1994; Fang et al. 2002; Niemietz and Tyerman 2002; Schnurbusch et al. 2010); (iii) in liposomes or planar lipid bilayers with purified and reconstituted aquaporin proteins (Ye and Verkman 1989; Zeidel et al. 1992; Weaver et al. 1994; Verdoucq et al. 2008) and; (iv) in heterologous expression systems such as *Xenopus laevis* oocytes and yeast (Preston et al. 1992; Dordas et al. 2000).

Plant aquaporins belong to the ancient superfamily of major intrinsic proteins (MIPs) (Saier et al. 2016). Based on subcellular location and sequence homology, they are separated into five subfamilies:

(i) Plasma membrane intrinsic proteins (PIPs) (Kammerloher et al. 1994)
(ii) Tonoplast intrinsic proteins (TIPs) (Maeshima 2001)
(iii) Nodulin 26-like intrinsic proteins (NIPs) (Wallace et al. 2006)
(iv) Small basic intrinsic proteins (SIPs) (Johanson and Gustavsson 2002)
(v) X intrinsic proteins (XIPs) (Kaldenhoff et al. 2007)

It was documented that As_{in}(III) and/or As methylated derivatives are transported by aquaporins belonging to PIPs, TIPs, and all three functional NIP groups (NIP-I, NIP-II, NIP-III).

Based on permeation function, plant AQPs are divided to three major groups:

(i) Aquaporins that transport water
(ii) Aquaglyceroporins that permeate water and other neutral solutes (Borgnia et al. 1999)
(iii) Aquaporins that conduct ionic species, based on the evidence of human aquaporins (Yool et al. 1996; Fu et al. 2000; Yu et al. 2006)

Arsenous acid [$As(OH)_3$] and methylated As derivatives (Fig. 1.1) like many other molecules including boric acid [$B(OH)_3$] (Takano et al. 2006; Tanaka et al. 2008; Hanaoka et al. 2014), germanic acid (Ma et al. 2006; Hayes et al. 2013), selenous acid (Zhao et al. 2010b, c), and silicic acid [$Ge(OH)_4$ and $Si(OH)_4$] (Ma et al. 2006) are transported by aquaglyceroporins (Bienert et al. 2008a, b; Ma et al. 2008; Kamiya et al. 2009; Xu et al. 2015; Li et al. 2016). Transport of these acids was explained by similarity in the structure and atomic radii of their molecules [$As(OH)_3$: 3.57 Å; $B(OH)_3$: 3.43 Å; $Si(OH)_4$: 4.19 Å; $Ge(OH)_4$: 4.48 Å] as well as several physicochemical and structural characteristics with glycerol which is the canonical NIP substrate (Porquet and Filella 2007).

However, recent studies on NIP2;1 mutants with the changes in specific amino acid residues within the ar/R/LE1–LE2 selectivity filter of rice, *A. thaliana*, and barley suggested that metalloid permeation seemed to be controlled not only by atomic radii of molecules but also by some differences in interactions of metalloids with AQPs preceding interactions with ar/R/LE1–LE2 residues (Ma et al. 2008; Mitani-Ueno et al. 2011; Hayes et al. 2013).

1.2.1.1 NIPs in Arsenic Transport

In 2008, three independent studies for the first time demonstrated that uncharged H_3AsO_3 molecules permeate certain plant NIPs (Bienert et al. 2008a; Isayenkov and Maathuis 2008; Ma et al. 2008). Moreover, applying the heterologous expression of plant NIPs in frog oocytes and yeast cells clearly revealed NIPs as important bidirectional channels both in influx and efflux of As_{in}(III) and organic form of As

(MMA and DDA) (Bienert et al. 2008a, Ma et al. 2008; Li et al. 2009a). Today, six of ten identified *O. sativa* NIPs (OsNIPs:) work as As_{in}(III) channels, similarly to the *A. thaliana* NIPs (AtNIPs) where six of nine identified are permeable to As_{in}(III). Additionally, two NIPs of *Lotus japonica* (LtNIPs) and one of *Hordeum vulgare* (HvNIP) make transport of As_{in}(III) possible.

The major channel for influx and efflux of As_{in}(III), MMA, and DDA in *O. sativa* is OsNIP2;1 which is also the first identified silicon (Si) channel for Si uptake from the environment in higher plants. In spite of OsNIP2;1 as a channel – not a transporter – it is also called the Si transporter (OsLsi1).

The ar/R/LE1–LE2 motif of OsNIP2;1(OsLsi1) comprises Ser at the H5 position, two residues of Gly at positions H2 and LE1, and conserved Arg residue at L2 position. This composition of ar/R/LE1–LE2 is specific for NIP-III subfamily of AQPs. The small-size amino acid residues of ar/R/LE1–LE2 motif form a selective filter with diameter larger than pore diameter of NIP-I and NIP-II (Ma and Takahashi 2002). The differences of the amino acid composition at ar/R/LE1–LE2 are the basis for the division all NIPs into three subgroups: NIP-I which are permeable to glycerol, lactic acid, and water; NIP-II which are less permeable to water than NIP-I, but due to larger pore diameter than pore of NIP-I, they are permeable to larger solutes like boric acid, formamide, and urea; and last, NIP-III subgroup, with the largest pore diameter which apply to the transport of the silicic acid (Abbas et al. 2018).

OsNIPs2;1 (OsLsi1) were indentified in the distal side of epidermal and endodermal membrane cells of rice root. Therefore, they participate in an uptake of uncharged As species (As_{in}(III), MMA, DDA) from environment into the root cells of *O. sativa* as well as cooperate with other channels and transporters facilitating migration of this metalloid species within the plant.

One of these transporters is OsLsi2 – silicic transporter located in the membranes of the same cells as OsNIPs2;1 (OsLsi1) but on the proximal side and is responsible for distribution of As_{in}(III), but not organic As species, from root cells to xylem or stele tissues and as a consequence to accumulation of As_{in}(III) in rice grains. Thus the cooperation of OsLsi2 with OsNIPs2;1 (OsLsi1) and other NIPIII channels is considered to be the main mechanism enhancing accumulation of As_{in}(III) in rice grains (Ma et al. 2006, 2007, 2008). In this cooperation, Si or As penetrate into the cell by NIP-III channels, which are located at the exodermis side of cellular membranes, and leaking from the cells by Lsi2-type transporters located at endodermis side of cellular membranes (Ma and Yamaji 2015). NIP channels and Lsi2-type transporters are located in the membrane of the same cell but with opposite polarity. It is also possible, that these two transporter types are not present in one cell, but in adjacent cell layers (Sakurai et al. 2015).

On the other hand, it is worth remembering that the NIP channels such as OsNIP2;1 (OsLsi1) operate as bidirectional channels. As it was shown in frog oocytes, expression of OsNIP2;1 (OsLsi1) facilitates both the influx and efflux of As_{in}(III) and two tested organic derivatives of As, i.e., MMA and DDA (Ma et al. 2008; Li et al. 2009b). It shows that these As species permeate OsNIP2;1 (OsLsi1) bidirectionally between soil and plant root cells (Khalid et al. 2017). Thus, OsNIP2;1

(OsLsi1) is postulated to be responsible to approximately 20% efflux of As(III) in rice plants (Zhao et al. 2010a).

Lsi2 proteins are not NIP family members, and unlike OsLsi1 (OsNIPs2;1), they do not form the channels but operate as transporters. They are found in many *Liliopsida* and *Magnoliopsida* species including *A. thaliana* (Ma and Yamaji 2015).

The other Si channel, which was reported to strongly cooperate with OsNIPs2;1 (OsLsi1) in Si and probably As distribution in rice, is OsNIP2;2 (OsLsi6). In quantitative trait locus (QTL) analysis OsNIP2;2 (OsLsi6) was identified as contributing to increase of the methylated As level in the grain (Kuramata et al. 2013). The evidence that OsNIP2;2 (OsLsi6) can transport MDA and DDA to grain is still missing; however, it seems very likely that, as on the one side, OsNIP2;2 (OsLsi6) is expressed in the node below the rice panicle after the onset of grain filling (Yamaji and Ma 2009) and, on the other, DDA is translocated into the grain with high mobile in the panicle vascular system (Carey et al. 2010, 2011). Moreover, it was evidenced that OsNIP2;2 (OsLsi6) is polar-localized to the adaxial side of xylem parenchyma cells in the blade and the leaf sheath, and in the shoot, this protein is responsible for the unloading of the silicic acid from the xylem sap into the cytoplasmic leaf space (Yamaji and Ma 2009).

OsNIP3;2, which is expressed mainly in the lateral roots and the stele region of the primary roots, in anthers and suspension cells is another channel which can cooperate with OsNIPs2;1 (OsLsi1) in the distribution of As throughout plant organs (Li et al. 2016). Recently, it was presented that although this protein is involved in As_{in}(III) uptake by lateral roots, its contribution to As accumulation in the shoots is limited (Chen et al. 2017a). The importance of OsNIP2;1 aquaporin both in As_{in}(III) and the organic derivatives of As uptake was presented in *Osnip2;1* knockout rice line. The level of As in shoot of this rice mutant was reduced by 71% and in roots by 53% compared to wild-type plants when these two plant types were exposing an As_{in}(III) (Ma et al. 2008). When plants were treated with MMA and DDA (Fig. 1.1), the level of As in *Osnip2;1* mutant plants was about 50% lower than in wild-type plants (Li et al. 2009a).

The recently identified NIPs member engaged in As_{in}(III) uptake from the environment to rice root cells is OsNIP3;3 (Ali et al. 2012; Katsuhara et al. 2014; Li et al. 2016). Two other channels, i.e., OsNIP1;1 and OsNIP3;1, are also shown to be able to mediate in As_{in}(III) transport in rice, but they probably only support OsNIPs2;1 (OsLsi1) because their expression levels in rice roots are very low (Meharg and Zhao 2012).

In *A. thaliana* as the most important for As_{in}(III) uptake from the environment to the root, AtNIPs were identified: AtNIP1;1, AtNIP3;1, AtNIP5;1, and AtNIP6;1. Additionally, AtNIP3;1, AtNIP5;1, and AtNIP6;1 are involved in transmembrane As_{in}(III) transport and facilitate As_{in}(III) translocation from the root to the stem. Studies with frog oocyte heterologous expression systems demonstrated two additional NIP channels permeable to As_{in}(III), i.e., AtNIP1;2 and AtNIP7;1. In *A. thaliana*, NIP1;2 is strongly expressed in seeds, whereas AtNIP7;1 is selectively expressed in anthers and pollen tissues (Bienert et al. 2008a; Isayenkov and Maathuis 2008;

Kamiya et al. 2009; Xu et al. 2015; Li et al. 2016). Additionally, it was found that regulator of AtNIP1;1 is a calcium-dependent protein kinase (CPK31). The *A. thaliana* mutant of *cpk31* similar to *nip1;1* mutant and the double mutant cpk31 nip1;1 had a higher tolerance to As_{in} (III) than wild-type and cpk31 mutant (Ji et al. 2017).

Applying of yeast heterologous expression systems allowed to identify the other representatives of NIPs subfamily which were shown as permeable to As_{in}(III). There were two proteins of *Lotus japonicus*, i.e., LjNIP5;1 and LjNIP6;1, functioning as bidirectional As(III) channels and three of *Hordeum vulgare*, i.e., HvNIP1;2, HvNIP2;1, and HvNIP2;2 (Katsuhara et al. 2014; Li et al. 2016).

1.2.1.2 PIPs in Arsenic Transport

PIPs are the most abundant, homogenous subfamily of plant AQPs. They form water intrinsic channels in the plasma membrane, and thus they are extremely significant for plants water balance (Maurel et al. 2015; Chaumont and Tyerman 2014). For a few of them, it was evidenced that they are able to transport molecules other than water such as urea, H_2O_2, and CO_2 but also several types of uncharged metalloids including As (Mosa et al. 2012). They are divided into two subgroups, i.e., PIPs1 and PIPs2, with more than 50% sequence identity (Chaumont et al. 2001).

The level of 5 rice PIPs, i.e., OsPIP1;2, OsPIP1;3, OsPIP2;4, OsPIP2;6, and OsPIP2;7, and 13 of *Brassica juncea*, i.e., five PIPs1 and eight PIPs2, was reduced by As_{in}(III) (Mosa et al. 2012; Srivastava et al. 2013). The reduced expression of the mentioned above PIPs genes is in line with a decrease of water content in plants under As_{in}(III) stress, finally resulting in inhibition of seedling growth (Srivastava et al. 2013). On the other hand, at the same time, the increase of the level of reactive oxygen species (ROS) in root plant is observed, and it was shown that ROS also drive to repress of PIP2 genes expression in the root (Wudick et al. 2015). Therefore, it needs to be explained if alterations in level of PIP are the effect of direct As_{in}(III) stress or rather oxidative stress generated by As. Another unresolved question of As_{in}(III) transport by PIPs is molecular mechanism of this transport. It is unclear why orthologous PIP isoforms easily transporting of As_{in}(III) are impermeable to As, in spite of showing 100% similarity in the selective filter and NAP regions and a high degree of overall sequence homology.

1.2.1.3 TIPs in Arsenic Transport

TIPs are subfamily of AQPs commonly located in the plant vacuolar membrane called tonoplast. Among of the other AQPs, they are characterized by highly variable sequences, particularly in selected filter region. The vacuolar subtypes in plants are distinguished on the basis of specific TIP isoforms in the tonoplast. Moreover, the cell differentiation status and the developmental stage of the plant are also related to specific isoforms of TIPs (Jauh et al. 1999).

The high variability in ar/R/ LE1-LE2 sequences results additionally in a broad spectrum of their substrates. It is known that TIPs are permeable to water, and thus they play key role in turgor and widely understood of cellular osmoregulation of plant cells, but besides water, TIPs were as well shown to be able to transport urea (Liu et al. 2003; Soto et al. 2008), NH_3 (Jahn et al. 2004; Loqué et al. 2005), glycerol (Gerbeau et al. 1999; Li et al. 2008), H_2O_2 (Bienert et al. 2007), and various metalloids including As_{in}(III) (Maurel et al. 2015). Up to now, only one TIP was evidenced to be permeable for As_{in}(III). It was identified as TIP4;1 in fern – *Pteris vittata* – the best known As hyperaccumulator. However, although PvTIP4;1 belongs to TIPs subfamily, it is located rather in plasma membranes than in tonoplast. Additionally, it is important to notice that transcription of PvTIP4;1 gene is strongly limited to roots (He et al. 2015). The permeability to As_{in}(III) uptake and translocation were confirmed for PvTIP4;1 in *A. thaliana* where it was constitutively expressed. Additionally, expression of *PvTIP4;1* in yeast cells allowed to show that Arg-Cys substitution in ar/R selectivity filter of PvTIP4;1 made it impermeable to As (He et al. 2015).

1.2.1.4 Transport Systems for Arsenite and Methylated Derivatives of As Other than AQPs

AQPs are supported in arsenite and organic derivatives of As transport by other systems including proteins, glutathione, and its oligomers – phytochelatins. Besides the abovementioned silicic transporter OsLsi2 among other proteins, we can indicate the proteins identified in *P. vittata* which are similar to yeast Arsenical Compounds Resistance 3 (ScACR3) permeases active in As_{in}(III) efflux, and therefore called PvACR3 and PvACR3;1 (Indriolo et al. 2010).

ACR3 are included in the family which is one of the bile/arsenite/riboflavin transporter (BART) superfamily (Mansour et al. 2007). Based on operon analyses, it is postulated that these proteins may operate either as primary active transporters, similarly to the ArsB and ArsAB families with ATP hydrolysis, or secondary carriers. Up to now, four of these proteins were functionally characterized, i.e., ACR3 protein of *S. cerevisiae*, also called the ARR3 protein (Wysocki et al. 1997), ArsB protein of *Bacillus subtilis* (Sato and Kobayashi 1998), and PvACR3 and PvACR3;1 (Indriolo et al. 2010). ArsB protein of *B. subtilis* is not related to ArsB of *Escherichia coli* despite the same terminology. ScACR3 and ArsB of *B. subtilis* are plasma membrane carriers which use a proton antiport mechanism to export both arsenite and antimonite however with low affinity (Maciaszczyk-Dziubinska et al. 2011).

PvACR3 and PvACR3;1 are not located in plasma membrane but in tonoplast. Similarly to other known members of ACR3 family, PvACR3 and PvACR3;1 also decrease As_{in}(III) level in the cytosol, but instead of efflux of As(III) from cell to environment, they transfer it into the vacuole. ACR3 seems to be more significant than PvACR3;1. The essential role in As resistance of *P. vittata* was shown by knocking down the expression of ACR3 and ACR3;1 in the gametophyte of this fern species. Only ACR3 mutant results in an arsenite-sensitive phenotype. Moreover,

both in gametophytes and in sporophyte roots, expression of *acr3* was shown to be upregulated by As. Inversely, expression of *acr3;1* is unaffected by As (Indriolo et al. 2010). Recently, PvACR3;1 gene was cloned and expressed in *A. thaliana, Nicotiana tabacum*, and *S. cerevisiae*. In roots of both transgenic plants, increased As retention was observed. The level of As in shoots of transgenic plants was 55–61% lower than in wild-type control under laboratory conditions and in soil experiments with transgenic tobacco of about 22% lower than in control. Additionally, it was shown that PvACR3;1 in transgenic *A. thaliana* is also located in the tonoplast indicating that in plant roots, As_{in}(III) retention is conducted by the same detoxification mechanism as in As hyperaccumulator, i.e., by As_{in}(III) sequestration into vacuoles (Chen et al. 2017b).

It is worth to note that *P. vittata* as As hyperaccumulator contains two copies of ACR3 genes; single copies were identified also in other fern species as well as in moss, lycophytes, and gymnosperms. However, up to now, no ACR3 genes have been detected in angiosperms. Angiosperms are unable to As hyperaccumulation and they usually do not even show the tolerance to As (Indriolo et al. 2010).

Whereas in As hyperaccumulating fern, As_{in}(III) is rapidly transported from roots to fronds where it is stored in vacuoles mainly due to ACR3 proteins, in non-hyperaccumulators most of the arsenite is bound with thiol groups of glutathione or phytochelatins and retained in root cell vacuoles by the action of ABC transporters. Contrary to As nonaccumulator plants, in hyperaccumulators only few thiol complexes with As_{in}(III) are observed (Chakrabarty 2015). ABC transporters are active transporters which hydrolyze ATP to release energy to transport substrates across membranes. They consist of two distinct types of domains. One of them is the nucleotide-binding domain (NBD) also called ATP-binding cassette domain (ABC) from which the name of the whole ABC transporters family comes from. This family belongs to one of the largest and probably one of the oldest superfamily engaged in molecule transport. Besides the NBD (ABC) domain, the transmembrane domain (TMD) is present in ABC transporter structure. Each molecule of ABC transporters consists of at least two TMDs and two NBDs. NBDs (ABC domains) are located in the cytoplasm, they show highly conserved sequence, and they are responsible for ATP binding and hydrolysis. On the contrary, sequences and architecture of TMDs are variable in order to identify and interact with ABC ligands. Besides, TMDs due to energy coming from ATP hydrolysis can undergo conformational changes which make possible transport of ABC ligands across the membrane. In tonoplast of *A. thaliana* and rice cells, two members of ABC transporters family, i.e., ABCC1 and ABCC2, were shown to be involved in the transport of As_{in}(III) complexed with thiol groups of peptides and proteins into vacuoles (Song et al. 2010, 2014).

The rice ABCC1 transporters, localized in tonoplast of phloem and phloem companion cells of nodes, were presented to be responsible for the inhibition of the translocation of As_{in}(III) into grains by transporting thiol-As complexes into vacuoles of phloem cells in node cells. In 2015, it was confirmed that the As_{in}(III) distribution into the grain in rice is limited by nodes which act as As_{in}(III) filter (Chen et al. 2015). It is widely accepted that in nonaccumulator plants, As transport from

the roots to the shoots is highly restricted by As_{in}(III) complexation with thiol groups. In *Brassica juncea* root cells, whole pool of As_{in}(III) was found to be complexed with thiol components, whereas major As transported species within the xylem and phloem was uncomplexed As_{in}(III) (Kopittke et al. 2014). In rice, during 2–4 days of experiment, only 10% of total As_{in}(III) absorbed by plants was detected in shoots and slightly more than 3% in the grains (Zhao et al. 2012). Thus, As_{in}(III) is suggested to be poorly transported by either xylem or phloem, although phloem was considered as the primary route of transport to grains for As_{in}(III). On the other hand, it is postulated that organic As species are transported very efficiently by phloem and xylem (Awasthi et al. 2017).

The most efficient As_{in}(III) loading mechanism into the xylem was detected in As hyperaccumulator, *P. vittata* (Su et al. 2008). Rice was shown to load arsenite into xylem sap more efficiently than other crop plants, e.g., barley or wheat (Su et al. 2010), although As uptake and transfer into rice grains were proven to be strongly dependent on rice cultivar and As bioavailability in soil (Batista et al. 2014).

On the other hand, higher phytochelatin level and reduction of As translocation in the plant are observed in rice exposed to higher As concentrations (Duan et al. 2011).

It is worth noting that ABC proteins can serve as As_{in}(III) transporters but only when the metalloid is complexed with thiol groups. The glutathione and phytochelatin synthesis are induced by cytokinin depletion, and thus cytokinins can influence As transport in plants. Moreover, in 2016 chloroquine-resistance transporter-like transporter (OsCLT1) was identified in plastids of rice as a regulator of glutathione homeostasis and phytochelatin biosynthesis and thus affecting As uptake and distribution in plants. The lack of this transporter in *Osclt1* mutants showed lower level of phytochelatin 2 and As than wild-type plants under exposition both to As_{in}(III) and As_{in}(V) (Yang et al. 2016). Additionally, phytochelatin synthase genes in rice (*Ospcs*1, *Ospcs*3, and *Ospcs*13), as well as ABC transporter genes (Osabcg5, Osabci7_2, and Osabc6), were shown to be upregulated by sulfur. On the other hand, sulfur decreases the expression of other tonoplast transporter gene, i.e., *tip*4;2 especially important in As transport in *P. vittata* (Zhang et al. 2016). Besides, when transport of As_{in}(III) complexes with thiol components in plants is studied, it should also be considered that these complexes are stable within the low pH range 1.5–7.5. At higher pH values, such as pH of phloem sap, they dissociate. Therefore, it is clear that although a high level of thiol components was detected in the phloem sap of *Ricinus communis* or *Brassica napus*, As_{in}(III)-thiol complexes were not identified (Ye et al. 2010).

Another factor affecting As translocation from root to shoot in *P. vittata* is transpiration. It was evidenced that plants with higher transpiration also had a higher level of As in their shoots and, inversely, plants with lower transpiration by 28–67% showed also a lower level of As in shoot by 19–56% (Wan et al. 2015).

Another protein suggested to facilitate As_{in} (III) uptake and its translocation from root to shoot is rice Natural Resistance-Associated Macrophage Protein 1 (NRAMP1). OsNRAMP1 located in the plasma membrane of endodermis and peri-

cycle cells may facilitate As_{in}(III) transfer into xylem and thus xylem movement of As_{in}(III) from root to shoot. OsNRAMP1 gene expression in yeast as well as in *A. thaliana* resulted in enhance of As and cadmium accumulation. In plants, the higher level of As and Cd was detected both in root and shoot (Tiwari et al. 2014). Thus, OsNRAMP1 cooperates with another non-AQP protein, i.e., OsLsi2, and they both help in xylem loading of As_{in}(III) and in root to shoot transportation. As_{in}(III) from xylem sap can also be transported to phloem by inositol transporters (INTs). It was shown that INTs of *A. thaliana* (AtINT2 and AtINT4) which are responsible for inositol uptake from phloem were also involved in the translocation of As_{in}(III) from xylem to phloem and finally into seeds (Duan et al. 2016).

Recently, a putative peptide transporter (PTR7) as a new DMA long-distance transporter from roots to grains was postulated in rice, based on significant expression of *ptr7* in rice roots, leaves and 1st node during ripening of the grain and lack of DMA in grain of rice OsPTR7 mutant, despite grains of wild-type control plant contain 35% As as DMA (Tang et al. 2017).

1.3 Arsenate Uptake and Its Translocation Systems

Arsenate [As_{in}(V)] and phosphate (P_{in}) as structural chemical analogues with similar electrochemical profiles share the same transport pathways in plants. Protein transporters of P_{in} in plants belong to three families. Two of them are members of inorganic phosphate transporter (PiT) family and known as P_{in} transporters 1 (PHT1) and 2 (PHT2). The third group belongs to the ion transporter (IT) superfamily and is termed as phosphate permease family (PHO1). The proteins of PHT1 family are H^+/P_{in} symporters, and they transport P_{in} from the environment into the plant (Bucher 2007; Javot et al. 2007).

The proteins of PHT2 family, in spite of their high similarity to the mammalian phosphate/Na^+ symporter (PNaS) family, in plants, function as H^+/P_{in} symporters and therefore belong to PiT family. Members of PHT2 family occur in plastid membranes of plants (Versaw and Harrison 2002; Bucher 2007).

Proteins belonging to PHO1 family probably transport P_{in} both to the xylem and phloem tissue as well as into cells, such as root epidermal cells, cells of the cortex, or pollen (Wang et al. 2004). Furthermore, on the base of P_{in} uptake kinetics studies, P_{in} transporters are divided in two groups, one with high and another one with low affinity for P_{in} (Dunlop et al. 1997; Misson et al. 2004; Miller et al. 2009). The high-affinity P_{in} transporters, with K_M values in the range of 2.5–12.3 μM, play an important role in the uptake of P_{in}, whereas the low-affinity transporters, with K_M values between 50 and 100 μM (Nussaume et al. 2011), are responsible for translocation of acquired P_{in} (Smith et al. 2001).

Studies with a number of plant species including *P. vittata* (Wang et al. 2002), duckweed (*Lemna gibba*) (Ullrich-Eberius et al. 1989), *A. thaliana* (Clark et al. 2003), velvet grass (*Holcus lanatus*) (Macnair and Cumbes 1987; Meharg and Macnair 1990), and also crop plants such as barley (*Hordeum vulgare*) (Asher and

Keay 1979) or wheat (*Triticum aestivum*) (Zhu et al. 2006) show that $As_{in}(V)$ and P_{in} are absorbed by the roots and transported in plants by the same transporters, which belong to PHT1 family. Furthermore, it was proved that $As_{in}(V)$ competes with P_{in} while ingestion process into cell via PHT1 in many monocots and dicots species, both in As-hyperaccumulators and non-hyperaccumulators plants (Ullrich-Eberius et al. 1989; Meharg and Macnair 1992; Wang et al. 2002; Abedin et al. 2002; Clark et al. 2003; Esteban et al. 2003; Tu and Ma 2003; Bleeker et al., 2003).

PHT1 family was identified in 1996 as a specific family of plant plasma membrane proteins (Muchhal et al. 1996). Up to now, more than 100 PHT1 proteins have been characterized in plants. They are expressed mainly in roots. However, some members of the PHT1 family were also detected in leaves and flowers (Nussaume et al. 2011). Proteins belonging to this family contain conserved amino acid residues sequence, i.e., GGDYPLSATIxSE, although single modifications in amino acid residues in the range of this signature are also observed (Karandashov and Bucher 2005). Proteins of PHT1 family show from 60% to 95% similarity of amino acid sequence between various plant species including *A. thaliana*, rice (*O. sativa*), wheat (*T. aestivum*), potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), tobacco (*Nicotiana tabacum*), *Medicago truncatula*, *Catharanthus roseus*, or *P. vittata* (Ma et al. 2001; Rausch and Bucher 2002; Di Tusa et al. 2016). Moreover, the amino acid sequence of *A. thaliana* PHT1 shares 34% identity and around 50% similarity with yeast PHO84 proteins (Raghothama 1999). One of the two bacterial clusters of phosphate transporters (PiTs) is also close to the PHT1 (Saier et al. 1999).

On the base of hydrophobicity analysis, it was revealed that PHT1 members have 12 hydrophobic membrane-spanning domains (MSDs) each composed of 17–25 amino acid residues. The hydrophobic domains are separated by six extracellular and five intracellular hydrophilic loops. Additionally, as it results from computer analyses, MSDs are divided into two groups of six domains by the longest, hydrophilic loop which is located centrally in the protein molecule (Raghothama 1999). The central loop and the C-terminal and N-terminal of PHT1 members are predicted to be located inside the cell (Fig. 1.3).

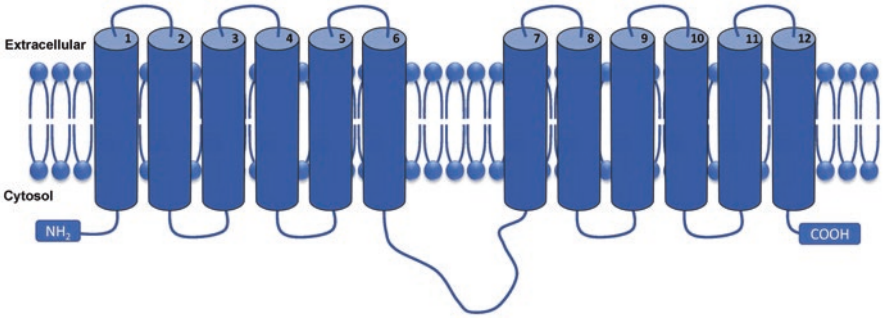

Fig. 1.3 The topology of a plant phosphate transporter with 12 membrane-spanning domains (MSDs), each composed of 17–25 amino acid residues separated by six extracellular and five intracellular hydrophilic loops and centrally located hydrophilic loop

In *A. thaliana* nine PHT1 family members were identified. They contain 520–550 amino acid residues and have a mass of approximately 58 kDa. Six of them besides P_{in} were shown to be involved in the uptake and transport of $As_{in}(V)$ (AtPHT1;1, AtPHT1;4, AtPHT1;5, AtPHT1;7, AtPHT1;8, AtPHT1;9). AtPHT1;1 and AtPHT1;4 are the first identified PHT1 members. They are involved in uptake of P_{in} and $As_{in}(V)$ from environment regardless of the phosphorus level (Shin et al. 2004). AtPHT1;5 supports the distribution of $As_{in}(V)$ and P_{in} from older leaves to young leaves, growing roots, and developing seed organs (Nagarajan et al. 2011; Li et al. 2016). It was also observed that expression of *Atpht1;5*, especially in the flowers, cotyledons, and phloem cells of older leaves, was induced by P_{in} deficiency (Mudge et al. 2002). AtPHT1;7 is another transporter participated in $As_{in}(V)$ distribution in *A. thaliana* plants. *Atpht1;7* is expressed specifically in reproductive tissues.

Two lately identified AtPHT1 members engaged in $As_{in}(V)$ transport are AtPHT1;8 and AtPHT1;9, which dominate in *A. thaliana* roots. Contrary to AtPHT1;1 and AtPHT1;4, they contribute $As_{in}(V)$ uptake from soil under conditions of P_{in} deficiency (Remy et al. 2012).

Recently some WRKY transcription factors of *A. thaliana* such as WRKY6 and WRKY45 were identified, and their influence on $As_{in}(V)$ uptake and distribution by regulation of *AtPht1* gene expression (Castrillo et al. 2013; Wang et al. 2014) was evidenced. These transcription factors were called WRKY because of their almost invariant amino acid sequence, i.e., WRKY, which creates DNA-binding domain. They are transcription factors regulating many processes in plants including responses to biotic and abiotic stress, senescence, seed dormancy, seed germination, and some developmental processes (Rushton et al. 2010).

Additionally, in 2005, it was shown that *A. thaliana* mutant defective in phosphate transporter traffic facilitator 1 (AtPHF1), which is protein conveying the PHT1;1 from the endoplasmic reticulum (ER) to the plasma membrane, is more resistant to arsenate than the wild type. This indicates that AtPHF1 can also affect $As_{in}(V)$ influx to the plant (González et al. 2005).

Although rice (*O. sativa*) is a plant species growing on flooded areas under rather anoxic soil conditions where $As_{in}(III)$ dominates $As_{in}(V)$, 2 of 12 identified P_{in} transporters, i.e., OsPht1;1 and OsPht1;8, were proven to be involved in processes of uptake and translocation of $As_{in}(V)$ in this plant species. Both *OsPht1;1* and *OsPht1;8* are expressed independently on the concentration of P_{in}. As in the case of AtPHT1;1, also OsPHT1;1 transport from ER to the plasma membrane depends on rice PHF1 (OsPHF1). Thus, level of OsPHF1 affects $As_{in}(V)$ uptake and translocation in the plant. Also, transcription factor regulating expression of *OsPht1;8* called P_{in} starvation response 2 (OsPHR2) effected on $As_{in}(V)$ uptake and transport (Jia et al. 2011; Wu et al. 2011; Sun et al. 2012; Kamiya et al. 2013; Li et al. 2016). Recently, three PHT1 transporters of *P. vittata* (PvPHT1) were identified as an effective $As_{in}(V)$ transporters. Studies with yeast heterologous expression system and ^{32}P radiolabeling revealed the significantly higher affinity of PvPHT1;3 to

$As_{in}(V)$ than P_{in}; however, when affinities of PvPHT1;3 and AtPHT1;5 to P_{in} have been compared, they were the same. Furthermore, yeast cells with PvPHT1;3 accumulated more $As_{in}(V)$ than with AtPHT1;5 (Di Tusa et al. 2016).

PHT1 transporters participate not only in uptake P_{in} and $As_{in}(V)$ from environment, but they are also involved in $As_{in}(V)$ translocation to xylem vessels from where it can be distributed between cells or even from one cell compartment to another as well (Catarecha et al. 2007; Zhao et al. 2009; Mendoza-Cózatl et al. 2011; Wu et al. 2011; Finnegan and Chen 2012). For example, $As_{in}(V)$ was found as co-substrate for three mitochondrial protein isoforms localized to the inner mitochondrial membrane and responsible for dicarboxylate exchange with co-substrates such as P_{in}, between the mitochondrial matrix and the cytosol (Palmieri et al. 2008).

1.4 Concluding Remarks

Bioavailability, as well as mobility of As, is strongly dependent on its oxidation states (Rafiq et al. 2017a, b; Shahid et al. 2013). Generally, in the soil and water, the most common form of As is $As_{in}(V)$ although under reducing conditions occurring in such environment as flooded rice paddy fields, the more mobile and toxic As(III) dominates As(V) (Punshon et al. 2017). Rice and other wetland plants developed a protective mechanism against a high level of $As_{in}(III)$ in the environment. Due to their aerenchyma, they can transport oxygen from leaves into roots and then release a part of the oxygen to the rhizosphere. This phenomenon is called radial oxygen loss (ROL) and depends on plants genotype, but also on oxygen availability in soils (Colmer et al. 2006). ROL generates (i) oxidation of Fe^{2+} to Fe^{3+}, which precipitate as iron oxides or iron hydroxides on the root surface as orange iron plaque and (ii) oxidation of $As_{in}(III)$ to $As_{in}(V)$ which is even four times easier sorbed by iron plague than $As_{in}(III)$.

Moreover, iron plaques create scaffoldings for As(III)-oxidizing bacteria that are effective in decreasing As concentration in plant tissues (Hu et al. 2015). It is believed that iron plaques can bind up to 75–89% of inorganic As present in the environment.

Inorganic As(V) enters the root cells, and it is transported in plants sharing the same paths as P_{in}, i.e., unidirectional transporters belonging to PHT1 family. Uptake and transport of inorganic As(III), as well as organic derivatives of this metalloid by plants, are mainly by NIP bidirectional channel. Although $As_{in}(III)$ generally is the most toxic species of As, it is the dominant form of As in plants (Chaumont and Tyerman 2017). However, it is worth to notice that reduction of $As_{in}(V)$ to $As_{in}(III)$ can be considered as detoxification process, because (i) NIPs as bidirectional channel can be engaged in efflux of $As_{in}(III)$ from plant roots to environment and (ii) $As_{in}(III)$ can be bound with thiol groups of glutathione or phytochelatins and be

retained in root cell vacuoles by the action of ABC transporters, what is observed in nonaccumulator or rapidly transported from roots to fronds, where it is also stored in vacuoles mainly due to ACR3 proteins and what is described for hyperaccumulating fern *P. vittata*.

Moreover, it is also important that the rate of uptake for both $As_{in}(V)$ and $As_{in}(III)$ is faster than organic As derivatives such as MMA and DMA, but the mobility of inorganic As species from root to shoot appears to be substantially lower than organic MMA and DMA (Marin et al. 1992; Burló et al. 1999; Abedin et al. 2002; Raab et al. 2007a; Abbas and Meharg 2008; Li et al. 2009c; Carey et al. 2010, 2011). In rice, it was found that DMA(V) was transported to the immature grain approximately 30 times more efficiently than $As_{in}(III)$ (Carey et al. 2010). Moreover, in studies with maize species, the transport of DMA(V) from root to shoot was approximately ten times greater than MMA(V) and three times greater than As(V) (Raab et al. 2007b). The reasons of these differences might be thiol complexation of $As_{in}(III)$ and transport to vacuoles, but also differences between hydrophobicity of inorganic and organic As.

In xylem and phloem sap only pentavalent species of organic As were detected (Li et al. 2009b; Ye et al. 2010). Although organic derivatives species are easier transported within plants than an inorganic form of As, $As_{in}(III)$ dominates both in xylem as phloem sap of plants. In rice it was shown that $As_{in}(III)$ is delivered to the grain mainly through the phloem, whereas DMA(V) is transported to grain with an equal contribution of both phloem and xylem (Awasthi et al. 2017).

Today, we can say that As enters into the root cells by some PTH transporters for $As_{in}(V)$ and NIPs and PIPs and for $As_{in}(III)$ and As organic derivatives or even PTR7, which is postulated as long-distance transporter of DMA. Additionally, *P. vittata* uptake of $As_{in}(III)$ by PvTIP4;1 is observed although normally members of TIP subfamily are present rather in tonoplast than in plasma membranes.

In the root cells, $As_{in}(V)$ is reduced to $As_{in}(III)$ and subsequently, as a complex with thiol molecules, can be located in vacuoles by ABC transporters in nonaccumulator or rapidly transported from roots to fronds, where it is also stored in vacuoles mainly due to ACR3 proteins, e.g., in hyperaccumulating fern *P. vittata*. ABC transporters, are located not only in root cells, but they are also detected e.g. in tonoplast of phloem and phloem companion cells like it was shown for rice ABCC1 transporter.

From root cells, As can be transported as $As_{in}(III)$ or organic derivatives by Lsi2 and NRAMP1 transporters into xylem sap or by Lsi2 or another As effluxer again into the environment. Besides, PHT can also transport $As_{in}(V)$ into the xylem. Into leaf cells, As can be transported by Lsi2 and PHT transporters as $As_{in}(V)$ or NIPs as $As_{in}(III)$ and As organic derivatives or by PTR7 as DMA. In leaf cells, ABC transporters similarly like in root cells can transport $As_{in}(III)$ complex with thiol molecules into vacuoles. To phloem, As is transported by PTR7 as DMA or as $As_{in}(III)$ probably by inositol transporters INT or maybe also as $As_{in}(V)$ by Lsi2. However, still little is known about the phloem and xylem transport of As, as well as a lot has yet to be revealed about the form of As transported and the transporters involved in phloem and xylem loading and unloading.

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Chapter 2
Plant Responses to Arsenic Toxicity: Morphology and Physiology

Vibhuti Chandrakar, Neha Pandey, and Sahu Keshavkant

Abstract Arsenic (As) is a naturally occurring toxic metalloid, ubiquitously present in the environment. It enters the environment from both geogenic and anthropogenic sources. Arsenic accumulates to different edible tissues and thereby enters into food chain. Arsenate and arsenite are two main phyto-available forms of As and are popularly reported to cause toxicity symptoms. Roots are foremost sites of As exposure, which slows down/inhibits extension and proliferation of it. From the roots, As gets translocated to the shoot and inhibits plant growth by slowing/arresting cell division/expansion, biomass accumulation, and plant reproductive capacity. Arsenite is more toxic than that of arsenate, since it has relatively high affinity for sulfhydryl groups of proteins and enzymes thereby alters or inhibits their activities. It interferes with the respiration process by binding to thiol groups of some important respiratory enzymes. Morphological and physiological effects of As include reduced germination and growth, root cell plasmolysis, denodulation and discoloration, leaf wilting, necrosis of leaf tips and margins, reduction in number of leaves and leaf area, distortion of chloroplasts membranes, inhibition in the photosynthetic activity, suppression of starch hydrolyzing enzymes, etc. It is well reported that arsenate replaces phosphate of ATP molecule and hence disrupts cellular energy flow. Arsenic disturbs the uptake of water and nutrients through competition for transporters. Cellular membranes are prime targets of As-induced oxidative stress, as it causes disorganization of membrane structures thereby lipid peroxidation and electrolyte leakage. Membrane damage leads to imbalance in the nutrient uptake and disruption in the stomatal conductance and transpiration process. So, plants have evolved defensive mechanisms in order to protect cells from As-induced oxidative stress through enzymatic and nonenzymatic antioxidants. Binding of As to thiol groups of antioxidant enzymes leads to suppression of defensive system of the plants. Hence, it is necessary to alleviate As from the contaminated areas where

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crops, vegetables, fruits, and pasturages have been cultivated, to protect the health of animals and human beings. Therefore, there is an urgent need to understand the assimilation, metabolism, and toxic effects of As in plants to develop various mitigation strategies against this dreadful contaminant.

Keywords Abiotic stress · Environmental pollution · Metalloid toxicity · Plant growth

2.1 Introduction

Arsenic (As) is one of the nonessential metalloids that poses a serious threat to all living beings including plants and animals (Zhao et al. 2009; Chandrakar et al. 2016a). It is the 20th most abundant ubiquitous element found naturally in the Earth crust (Chandrakar et al. 2016b; Farooq et al. 2016a). The permissible limit of As in groundwater and soil is 10 μg l^{-1} and 20 mg kg^{-1}, respectively (Rosas-Castor et al. 2014; Armendariz et al. 2016). Rosas-Castor et al. (2014) reported the presence of As exceeding 10 μg l^{-1} in groundwater in several countries such as Argentina, Australia, Bangladesh, Chile, China, Hungary, India, Italy, Mexico, Peru, and the United States. In the agricultural soils of Bangladesh and the United States, more than threefold concentration, than the baseline, of As is determined. In large areas of Bangladesh, China, India, and Vietnam, people are dependent on As-contaminated groundwater for irrigation of edible crops (Meharg and Hartley-Whitaker 2002, Panda et al. 2010). Thus, long-term use of As-contaminated water for irrigating the crops might result in the elevated levels of As in agricultural soils (Roychowdhury et al. 2005). Availability of As in the agricultural land is as high as 30,000 mg kg^{-1} soil (Panda et al. 2010). Presence of As in the agricultural area can directly affect the growth and development of plants, reduce the agricultural production, and could add As to the agricultural products; thus, consumption of it via food chain poses an additional risk to human health (Chandrashekhar et al. 2016; Kaim et al. 2016).

The major sources of As are weathering and mineralization of the Earth's crust, mining activities, use of As-based wood preservatives, insecticides, herbicides, and irrigation of crops with As-loaded groundwater (Mirza et al. 2014; Chandrakar et al. 2016b). Human beings are exposed to As directly via drinking of As-contaminated water or indirectly by consumption of crops from the water-soil-plant system (Finnegan and Chen 2012; Rosas-Castor et al. 2014). In Bangladesh, China, and India, half of the total intake of As takes place through consumption of As-contaminated food (Panda et al. 2010). In natural environment, As exist in four oxidation states chiefly, out of which arsenite (AsIII) and arsenate (AsV) are inorganic and more deadly forms, whereas (-III) and (0) are organic as well as less lethal states (Panda et al. 2010; Finnegan and Chen 2012). Among these, AsIII form exhibits more toxicity than the others (Nath et al. 2014; Singh et al. 2017a).

Contamination of As results in deleterious effects in plants that comprise a number of physiological, morphological, and biochemical disorders including reduced

elongation, proliferation and nodulation of roots, stunted growth, wilting, curling, necrosis of leaf blades, reduction in the number of leaves and leaf area thereby inhibiting photosynthesis and accumulation of biomass, stomatal conductance, rate of transpiration, ATP synthesis, losses in the mineral contents, alteration in flow of energy, and poor yield (Chandrakar et al. 2016b; Pandey et al. 2017). Arsenic is absorbed by the crop plants predominantly as AsV (Chandrakar et al. 2016b). Being an analogue of phosphate, As is also transported across the plasma membrane (PM) of root cells via phosphate transporters (Lazzarato et al. 2009). Exposure of crop plants to inorganic As also leads to excessive production of reactive oxygen species (ROS), viz., superoxide radical, hydroxyl radical, and hydrogen peroxide (Rahman et al. 2015). These ROS are also shown to have interrelation with the conversion of AsV into AsIII (Most and Papenbrock 2015). Overproduction of ROS is a probable cause of oxidative injury to important biomolecules like lipids, proteins, nucleic acids, and carbohydrates (Chandrakar et al. 2017a; Yadu et al. 2017a). Maintenance of redox homeostasis and regulated production of ROS are essential for normal metabolism and growth of plants (Bakhat et al. 2017). Plant cells have evolved mechanisms to combat from distressing effects of ROS by using antioxidants enzymes, such as superoxide dismutase (SOD), catalase, guaiacol peroxidase, ascorbate peroxidase, and glutathione reductase, and nonenzymatic antioxidants, such as ascorbate, glutathione, carotenoid, and α-tocopherol (Yadu et al. 2016, 2017b; Xalxo et al. 2017). Apart from the antioxidant machinery, the detoxification of heavy metal/metalloid in crop plants also takes place by chelation of metal ions with ligand or its compartmentalization into cellular organelles as ligand-metal complex (Chen et al. 2017). Both phytochelatins and metallothioneins are groups of thiol-reactive cysteine-rich peptides that bind specifically with toxic metals/metalloids and convert them into nontoxic forms (Gautam et al. 2012).

Therefore, a detailed understanding regarding the mechanisms of physiological and morphological effects of As on crop plants is important for developing appropriate strategies to reduce As uptake, accumulation, and its deleterious effects in plants. Thus, the purpose of this chapter is to collate the so far available information/mechanisms of morphological and physiological responses of plants to As, which will assist the researchers for better understanding and developing important strategies to alleviate the toxic effects of As.

2.2 Uptake and Transport of Arsenic

To understand and manage the devastating effects of As contamination, it is very essential to unravel mechanisms of its uptake, accumulation, and assimilation in plants (Chandrakar et al. 2016b). Roots are the first and foremost organ that comes in contact with As. Therefore, its accumulation is comparatively more in the roots than that of aboveground parts of the plants (Singh et al. 2017b). According to Zhao et al. (2010) and Rai et al. (2015), nodulin-26 like intrinsic aquaporin channels are responsible for uptake of AsIII in *Arabidopsis thaliana* and *Oryza sativa* L. roots

respectively. Also, AsIII is transported by the silicon influx transporter *Lsi1* and the silicon efflux transporter *Lsi2* (Panda et al. 2010). The *Lsi2* plays an important role during transport of AsIII between root and shoot and also for its accumulation in the grains. Under As stress, expression levels of these transporter genes are significantly upregulated which facilitates influx and accumulation of AsIII inside the root cells (Li et al. 2016).

However, phosphate and AsV share the same pathway to cross the PMs of roots (Zhu et al. 2006). Uptake mechanism of AsV also involves co-transportation of phosphate and H^+, with stoichiometry of at least $2H^+$ for each $H_2PO_4^-$ or $H_2AsO_4^-$ (Zhao et al. 2010). About 100 high- and low-affinity phosphate transporters have been identified in the plants. The genes responsible for phosphate absorption are *Pht1* and *Pht2*, which are expressed under phosphate starved condition only (Shin et al. 2004). The *Pht2* gene is involved in the loading of inorganic phosphate into the shoot (Panda et al. 2010). Presence of phosphate in the soil or in growth media decreases the influx of AsV. Similarly, uptake and transportation of AsIII is suppressed by the presence of antimonite and glycerol, but not by phosphate (Chandrakar et al. 2016b).

2.3 Morphological Effects of Arsenic

Presence of As above the permissible limit in soil (20 mg kg^{-1}) and groundwater (10 μg L^{-1}) causes perturbations in the metabolism of plant cells which leads to wilting, curling, necrosis of leaf blades, reduction in the number of leaves and leaf area thereby reduced rate of photosynthesis and accumulation of biomass, losses in the mineral contents, reduced pace of elongation, proliferation and nodulation in roots, stunted growth, and poor yield responses (Talukdar 2013; Chandrakar et al. 2017b) (Fig. 2.1). Binding of AsIII to sulfhydryl groups of both enzymes and proteins leads to distortion in cellular membranes consequently loss of turgor and rigidity which is responsible for wilting of plant parts. Availability of As in the soil limits the uptake of water and important minerals by the root cells thereby creates dehydration condition in the cell which is the major cause for curling of leaves. Deficiency of important nutrients also leads to another symptom of As toxicity, i.e., necrosis, due to which the affected plant parts, especially the green leaves become brown or black in color. Accumulation of As leads to the formation of necrotic patches of circular outline, indicative of cell death occurred in specific regions and seemed to cause the progressive death of the entire leaf. As evidenced by ultrastructure analysis, this necrosis was the resultant of cytoplasm leakage, membrane vesiculation, and cellular disorganization (Farnese et al. 2017). After conducting root bioassay of As stressed *Brassica napus* L. seedlings, Farooq et al. (2017) stated that reduced root length was an outcome of negative effects of As on cell elongation.

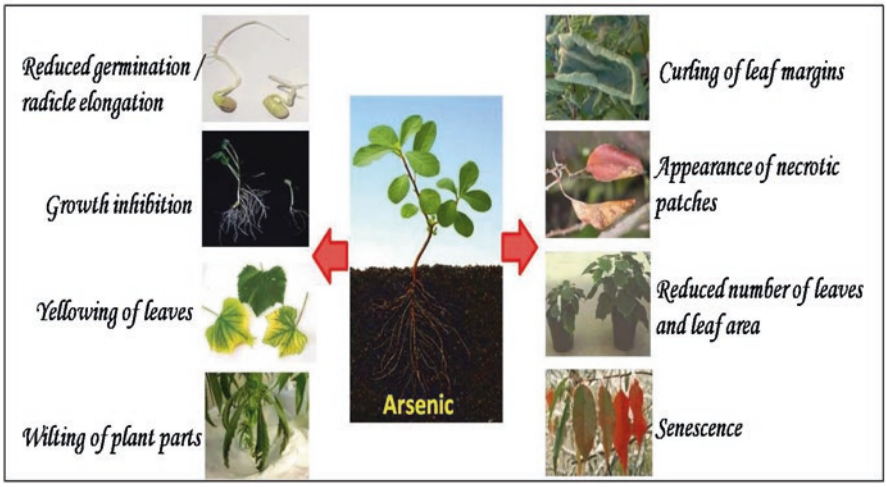

Fig. 2.1 Morphological effects of arsenic in plants

2.4 Physiological Effects of Arsenic

2.4.1 Growth and Productivity

2.4.1.1 Root Plasmolysis

Being a nonessential and toxic metalloid, As affects plants growth and productivity considerably. From the soil, As is taken by the different transporters present in the root cells (Chandrakar et al. 2016b). Roots are usually the first organ of the plants to be exposed to As, where this metalloid causes discoloration and plasmolysis of cells (Shaibur and Kawai 2011). In hyperosmotic solutions such as sucrose, mannitol, or sorbitol, water from the vacuole is extruded out of the tonoplast causing a loss of turgor pressure. If this condition persists for longer time, the protoplast retracts further, which causes the detachment of the PM from the rigid cell wall. Plasmolysis is an active process and well-known characteristic feature of viable cells; therefore, it is used to test the viability of the cells under different environmental stresses (Lang et al. 2014). Exposure of plants roots to As causes increase in root lignification and disintegration of microtubules followed by a steep enhancement in the cell width that results in an increase in root diameter (Lou et al. 2015).

2.4.1.2 Biomass

In plants, it has been well reported that As causes a significant reduction in the biomass production, which might be due to the fact that roots are the first point of contact with As (Chandrakar et al. 2018). Since As has a high affinity for sulfhydryl

groups of proteins and enzymes present in the radicular membranes, hence causes disruption of root functions and cellular death (Shaibur and Kawai 2011). Reduced germination and growth responses are possibly due to the detrimental effects of As on cellular functioning of the plants where most of the available energy are consumed in the formation of stress linked essentials like phytochelatins, antioxidants, etc. Reduced biomass in the presence of As was possibly an outcome of enhanced permeability of the cell membranes, consequently increased leakage of cellular constituents/ basic nutrients essentially required for energy generation, and optimum growth and development of plants (Farooq et al. 2015). Also, the reduction in biomass accumulation is probably due to the loss of water and decreased water uptake by the root cells under As stress (Gomes et al. 2013). Further, exposure of As leads to degradation of protein because of carbohydrates deficiency, an adaptation of cells which resulted in the reduced protein content and biomass accrual (Agnihotri and Seth 2016). Thus, it is clear that As is well known to affect adversely the plants growth and development upon its accumulation (Chandrakar et al. 2016a). Recently, from the root epidermis and root hairs cells, As-reducing enzymes namely HAC1/ ATQ1 have been identified that provide As stress tolerance to plants by reducing AsV into AsIII (Chao et al. 2014; Sanchez-Bermejo et al. 2014). In Addition, Meadows (2014) also suggested that these enzymes are involved in reducing the As concentration in the different tissues of the plants. Thus, application of genetic engineering manipulates protein expression pattern which may help plants to sustain in As-contaminated area (Farooq et al. 2016a).

2.4.1.3 Cell Division, Elongation, and Expansion

Up on As exposure, reduction/inhibition in the growth may be related with reduced mitotic activity in the meristematic zone of the plant roots that decreased the cell division rate in the apical meristem and reduced the expansion and elongation of the newly formed cells (Mumthas et al. 2010; Farooq et al. 2015). Also, As decreases cellular turgor which leads to an inhibition in cell enlargement in the elongation zone of the roots. Arsenic is also known to suppress the syntheses of proteins involved in cell cycle check points (Reichard and Puga 2010). Exposure of As badly affects the cell division machinery of the plant cells (Sharma 2012). Binding of As to thiol groups leads to an inhibition in activities of enzymes involved in DNA repair system, hence is responsible for the DNA damage (Patra et al. 2004). Genomic analyses of two expansion genes, i.e., *Os01g14660* and *Os04g46650* revealed that their expression was downregulated in the presence of As in *O. sativa* L. (Sharma 2012). Also, As binds to the thiol group of tubulin proteins that leads to disturbance in the spindles thereby affecting cell division. Norton et al. (2008) reported that under As stress, the transcript abundance of two tubulin genes (*Os03g45920* and *Os03g56810*) and two microtubule genes (*Os03g13460* and *Os09g27700*) was strongly repressed in *O. sativa* L. Failure of normal organization and function of spindle apparatus is also due to the induction of lagging in the chromosomes and/or loss of microtubule of spindles, in the presence of As. Fragmented or lagging chromosome also induces the

formation of micronuclei at higher doses of As (Chidambaram et al. 2009). Chromosomal abnormalities and aberrations with lower mitotic index were also observed in response to As in many plants (Patra et al. 2004). One of the adverse effects of As is the chromosome stickiness along with breakage and reunion, which might be due to the formation of bridges. The As-induced chromosomal abnormalities are classified into two groups, viz., clastogenic effects such as fragments, micronuclei, and ring chromosome bridges. Other one is the precocious movement of the chromosome during anaphase which may be attributed to early terminalization and chromosome stickiness (Mumthas et al. 2010).

2.4.2 Photosynthetic System

2.4.2.1 Chlorophyll Synthesis

Plant species when exposed to a phytotoxic amount of As show toxicity symptoms ranging from inhibition of root growth to reduction in photosynthetic rate to cell death (Stoeva et al. 2003). The process of photosynthesis is crucial for the plants to sustain life on this Earth. This important metabolic activity has been carried out by photosynthetic pigments: chlorophyll a is the primary photosynthetic pigment that is responsible for capturing the solar energy, and this process is initiated within the chloroplast, whereas chlorophyll b acts as an accessory pigment during the passage of electron to chlorophyll a. The photosynthetic pigments are very sensitive to As toxicity which may limit the rate of photosynthesis. The decline in the levels of chlorophyll upon As stress might be due to the decrease in ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity and/or its biosynthetic enzymes, δ-aminolevulinic acid dehydratase and protochlorophyllide reductase (Farooq et al. 2016b). Gene expression study of δ-aminolevulinic acid dehydratase revealed that transcript abundance of this gene was low under abiotic stress (Agnihotri and Seth 2016). Limitations in the stomatal conductance and CO_2 fixation and degradation of chlorophyll might be associated with the decreased photosynthetic rate under stress conditions (Stoeva et al. 2003) (Fig. 2.2). Bhattacharya et al. (2012) found that As toxicity impaired the thylakoid electron transport chain which later contributed to distortion in chloroplast and decline in the chlorophyll content. This decrease in chlorophyll synthesis could be due to the generation of ROS that has the potential to damage important components of the cell such as proteins, nucleic acids, and amino acids involved in the biosynthetic pathway of chlorophyll. It can also be suggested that during the course of As exposure, changes in the membrane permeability and root anatomy of plants are possibly responsible for disturbed uptake and transport of water and ions that suppressed the photosynthetic and transpirational rate in *Avena sativa* (Stoeva et al. 2003). Higher dose of As has been shown to activate chlorophyll degrading enzyme, chlorophyllase, which might be attributed to decline in chlorophyll content. Lessening in the photosynthetic efficiency of the plants has been considered as one of the factors for reduced growth and yield under As stress (Mahdieh

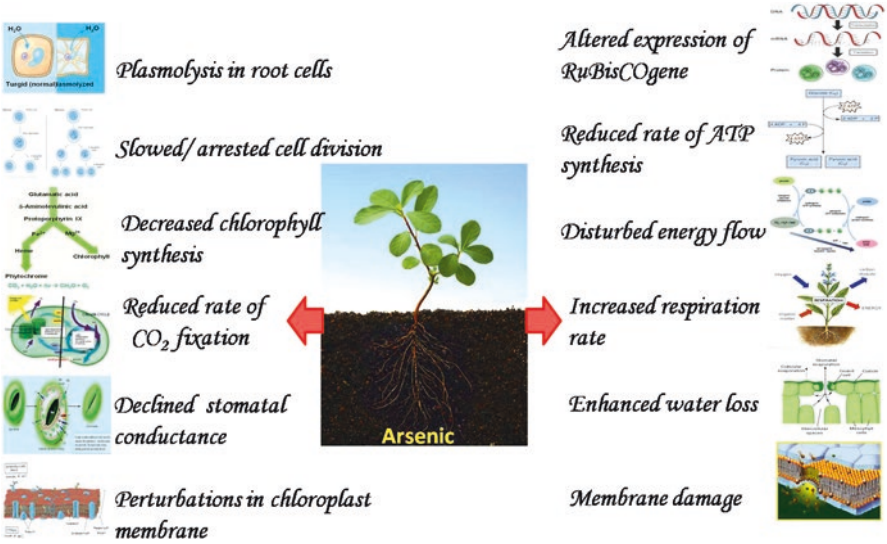

Fig. 2.2 Physiological effects of arsenic in plants

et al. 2013). Also, As-induced acceleration in lipid peroxidation reaction is the causative factor for chloroplast damage and inhibited chlorophyll synthesis (Zavaleta-Mancera et al. 2016). Increased levels of As resulted in the alteration/partial destruction in the chloroplast shape with bending concave membrane along with the variations in the accumulation and flow of assimilates that leads to the deprived chlorophyll contents (Srivastava and Sharma 2013). Thus, in plants exposed to As stress, the major causative agents for declining chlorophyll contents are: (a) breakage and swelling of thylakoid membrane; (b) stimulation of chlorophyllase enzyme; (c) inhibition of chlorophyll biosynthetic enzymes; (d) strong oxidation of photochemical apparatus; (e) reduction in chloroplast density and size; and (f) phosphorus deficiency, or reduction in the transport of magnesium, iron, and manganese.

2.4.2.2 CO_2 Fixation

The net fixation of CO_2 is a crucial step that recycles carbon into the biosphere. It is widely reported that exposure of plants to As reduces the rate of CO_2 fixation thereby photosynthesis (Gusman et al. 2013a). As stated in the previous section, As interferes with the chlorophyll synthesis either through depleting nutrients or by suppressing the biosynthetic enzymes involved in chlorophyll synthesis (Gusman et al. 2013a). Overproduction of ROS in response to As causes distortion in chloroplast membranes that disturbs CO_2 assimilation and photosynthetic process (Stoeva and Bineva 2003). Stoeva et al. (2005) demonstrated that in As-treated *Zea mays* L., the rate of CO_2 fixation was decreased by 2% with a significant reduction in the functional activity of photosystem-II. Exposure of As resulted in considerable reduction

in the chlorophyll fluorescence ratio, rate of CO_2 fixation, the functioning of photosystem-II, and photosynthesis efficiency (Stoeva and Bineva 2003; Chandrakar et al. 2016b). The photochemical efficiency and heat dissipation capacity of a plant are negatively affected by As, thereby leading to the alteration in the rates of gas exchange and fluorescence emission (Gusman et al. 2013a). Also, As contributes in decreasing the amount of large subunit of RuBisCO in *O. sativa* L. leaves which clearly suggests that As interferes not only in CO_2 fixation but also in the expression of chloroplastic DNA (Chandrakar et al. 2016b). Inhibitions/alterations in the photosynthetic activity of the plants are responsible for reduced growth, productivity, and biomass accumulation. The extent of As-induced injuries to photosynthetic carbon assimilation are not yet fully resolute, but it appears that presence of As reduces the availability of carbon to the plant via reduced CO_2 fixation and/or by inhibiting the expression of RuBisCO gene (Chandrakar et al. 2016b). Due to a reduced rate of CO_2 fixation, there is no recycling of $NADP^+$ (final electron acceptor), which could lead to decrease in photosynthetic electron transport. This process culminates into an over-reduction of electron transport rate and excess of excitation energy thereby excessive draining of electrons (Gusman et al. 2013a).

2.4.2.3 Stomatal Conductance

Arsenic is well known to destruct structure of PM. Any damage to the outer membrane leads to an imbalance in the uptake and transport of nutrients, ions, and water in the plant cells that diminishes the stomatal conductance (Paivoke and Simola 2001). Exposure of plants to As results in an alteration in the pigment concentration and stomatal conductance either by inhibiting some key enzymes like δ-aminolevulinic acid dehydratase or by inducing the activity of pigment degrading enzyme, chlorophyllase (Siddiqui et al. 2015a). Exposure of plants to heavy metalloid decreases stomatal opening which is correlated with the deposition of metalloids in the cuticle covering guard cells and its subsidiary cells (Rucinska-Sobkowiak 2016). Moreover, As causes disruption of microtubules that deregulates normal pattern of cell division culminating in the formation of abnormal stomata thereby hampered development of stomata at several stages (Gupta and Bhatnagar 2015). The decline in the rates of photosynthesis, transpiration, and stomatal conductance is probably due to the As-induced deleterious effects on roots, which may affect the uptake of water and ions, resulting in stomatal limitations (Milivojevic et al. 2006). The reduction in the values of photosynthesis and stomatal conductance in As-treated *Lactuca sativa* L. plants suggests a limitation in the stomatal conductance, which results in a decrease in internal CO_2 concentration and as a consequence, reduction in photosynthesis (Gusman et al. 2013a). On the contrary, an increase in internal CO_2 concentration together with a decrease in stomatal conductance is indicative of As promoted direct damages in CO_2 fixation process, probably through a decrease in the number and activity of RuBisCO (Weng et al. 2008). A decrease in RuBisCO activity attributes to a reduction in the phosphorus concentration upon As treatment in plants that results in inhibition of photosynthetic carbon reduction (Miteva and Merakchiyska 2002).

In the photochemical step of photosynthesis, accumulation of As obstructs the electron transporter chain; as a consequence, there are alterations in the formation of NADPH and ATP and rate of energy liberation. Whereas in the biochemical step, As can affect stomatal conductance, confining the CO_2 concentration in the plant or hindering RuBisCO activity (Abedin and Meharg 2002; Rahman and Naidu 2009).

2.4.2.4 Chloroplast Membrane

Chloroplasts are one of the major sites for the production of ROS in cells and are quite sensitive to As imposed damages. Li et al. (2006) and Schneider et al. (2013) observed structural damages in the internal membranes of chloroplasts in the As-exposed leaves of *Pteris vittata* and *Leucaena leucocephala* respectively. At low concentration of As, vesiculation was observed in the outer membrane of chloroplasts, which is an indicator for the beginning of the degradation process of these organelles (Wang and Blumwald 2014). Arsenic triggered degradation of chloroplasts, and alteration in its internal membranes has a strong impact on photosynthesis which is associated with the reduced concentration of photosynthetic pigment and rate of carbon assimilation (Farnese et al. 2017). Exposure of As also leads to perturbations in the structural organization of chloroplast membranes and functioning of integral photosynthetic processes, that culminate into breakage and swelling of thylakoid membranes and decrease in pigment synthesis, due to the lack of proper adaptive adjustments of pigment synthesis system to higher As level (Sharma 2012; Chandrakar et al. 2016b). Arsenic-induced overproduction of ROS and lipid peroxidation reaction might also be responsible for the destruction of chloroplast membranes (Mascher et al. 2002). Under As toxicity, carotenoids serve as antioxidants as it scavenges the free radicals, stabilizes the chloroplast membranes, and repairs the damages of the cell (Farooq et al. 2015). Decline in the contents of carotenoids in response to higher doses of As shows that distortion in the chloroplastic membrane is a common response of As toxicity in plants (Upadhyaya et al. 2014).

2.4.2.5 Expression of RuBisCO Gene

Plants on exposure to an elevated dose of As decreased the pace of photosynthesis due to their direct effect on the photosynthetic apparatus. Fixation of CO_2 is carried out by the catalytic activity of RuBisCO. The catalytic sites are located at the interface of the large subunit of RuBisCO. Arsenic specifically reacts with the larger subunit of RuBisCO and leads to the formation of a ternary stable complex. This complex may further distort the enzyme structure near the catalytic site resulting in total inactivation of the enzyme thereby reducing photosynthesis under As stress (Sudhani et al. 2013). However, an increase in the transcript level of the small subunit of RuBisCO has been observed in As stressed *A. thaliana* (Abercrombie et al. 2008). Downregulation in the expression of RuBisCO gene was responsible for reduced CO_2 fixation and photosynthesis in the leaves of As stressed *O. sativa* L.

(Ahsan et al. 2010). In addition, phytotoxic amount of As can induce alterations in proteins and enzymes of chloroplasts which might affect the photosynthetic efficiency of plants (Farooq et al. 2016b). Moreover, Ahsan et al. (2010) also reported that expression of RuBisCO gene was downregulated in As-treated *O. sativa* L. leaves thereby diminished photosynthetic rate. Thus, higher As stress can induce changes in chloroplastic proteins and enzymes which might affect the photosynthetic efficiency of plants.

2.4.3 ATP Synthesis and Energy Flow

2.4.3.1 Rate of ATP Synthesis

As, AsV and phosphate have parallel chemistry and proportion, some of the phosphate-dependent enzymes of the irreversible metabolic reactions utilize AsV directly as their substrate (Tawfik and Viola 2011). Among these, the most important enzyme is the F_1F_0-type ATP synthase which mediates phosphorylation of ADP to ATP in the inner membrane of mitochondria and the plastid thylakoid membrane (Finnegan and Chen 2012; Chandrakar et al. 2016b). For mitochondrial enzyme, AsV serves as a substrate in a reaction and generates ADP-AsV complex (Chandrakar et al. 2016b). In these reactions, Michaelis constant of the enzymes are similar for both phosphate and AsV, which clearly suggests that the enzymes recognize and react equally well with phosphate and AsV (Moore et al. 1983). Later, Requejo and Tena (2005) reported that there was an upregulation in the expression levels of ATP-synthase and succinyl-CoA synthetase which might be associated with the deleterious effects of As in the rate of ATP synthesis as these enzymes are responsible for catalyzing phosphorylation reactions associated with aerobic catabolism. Furthermore, due to binding of thiols to AsIII, the activity of pyruvate dehydrogenase enzyme also got inhibited. Similarly, suppression in the activity of its homologous enzyme α-ketoglutarate dehydrogenase would lead to a lowering in the formation of its product, succinyl-CoA, which, in turn, is responsible for the upregulation in the expression of succinyl-CoA synthetase gene. Glycolytic enzymes GADPH and aspartate-β-semialdehyde dehydrogenase are other phosphate-dependent enzymes that utilize AsV very efficiently in place of phosphate. In plants, aspartate-β-semialdehyde dehydrogenase plays a crucial role in the biosynthesis of essential amino acids and catalyzes the reversible dephosphorylation of β-aspartyl phosphate to L-aspartate-β-semialdehyde. These enzymes have the same kinetic constants for both Pi and AsV in their respective reactions (Chandrakar et al. 2016b). The replacement of AsV produces highly stable AsV-ester complex that undergoes spontaneous and rapid hydrolysis to form free As and corresponding carbon (Chandrakar et al. 2016b). The rate of hydrolysis of glucose-6-AsV is about 10^5 times greater than the glucose-6-phosphate (Radabaugh et al. 2002).

2.4.3.2 Energy Flow

Being an analogue of phosphate, AsV uncouples the oxidative phosphorylation process by displacing one molecule of phosphate from ATP, thereby forming an unstable volatile ADP-AsV complex (Geng et al. 2006). This causes a disturbance in the cellular energy flow and suppression in the ATP synthesis rate (Meharg and Hartley-Whitaker 2002). This ADP-AsV complex hydrolyzes in the aqueous medium and recycles ADP and As that react at various coupling sites (Bertagnolli and Hanson 1973).

2.4.3.3 Respiration Rate

Application of As promoted increase in the rate of plant respiration. This As-induced increased rate of respiration might be due to the structural similarity between AsV and phosphate; hence, both of these compete for the same active site of ATP synthase, in the mitochondria (Farnese et al. 2017). As a result of this competition, a highly unstable product, AsV-ADP complex, is formed, leading to reduced concentration of ATP. This lower ATP content is a signal triggering enhanced respiratory activity that results in the increased formation of AsV-ADP complex. In this way, input of carbon into cellular metabolism increases due to which rate of net photosynthesis is inhibited in As-contaminated plants. Additionally, As exposure also affected the light-harvesting apparatus which leads to reduction in chlorophyll content and activity of photosystem II. Moreover, As has also been shown to decline photosynthetic electron flow inside the chloroplasts and thereby synthesis of both ATP and NADPH which are crucial for carbon fixation reactions. Also, during stress imposed crises of carbohydrate, chlorophyll can be used as a source of carbon to operate cellular metabolic reactions (Chandrakar et al. 2016b). Hence, it can be concluded that the increased rate of respiration did not contribute to restoring pace of photosynthesis, lower down ROS accumulation, maintain membrane integrity, and/or restore cellular homeostasis. Therefore, increased respiration rate in stressed condition resulted in the occurrence of unusual cycles of AsV-ADP generation that utilize the energy of the cell resulting in the accumulation of more ROS (Finnegan and Chen 2012). Although under As stress the rate of respiration is elevated, it would not hold for longer periods because As itself triggers the distortion in mitochondrial membranes, which would compromise the respiratory process also (Farnese et al. 2017).

2.4.4 Nutrient and Water Uptake

2.4.4.1 Micro- and Macronutrient

The main functions of the plant roots are the absorption of micro- and macronutrients from the soil, supporting the whole plant body, and anchoring it to the ground (Rucinska-Sobkowiak 2016). Also, the roots are the first contact site for As that

accumulates inside the cells and disturbs uptake of the nutrients (Stoeva et al. 2005). Due to adverse effects exerted by As to the root system of the plants, roots are able to change cell membrane selectivity and permeability, resulting in lower nutrients uptake (Gusman et al. 2013b). Sufficient amounts of micro- and macronutrients play a key role in biomass accruals. Accumulation of As may manipulate the uptake of micro- and macronutrients through competition with nutrient ions for binding to transport proteins (Gusman et al. 2013b). Also, As-induced perturbations in the membrane integrity result in altered uptake and transport of ions leading to reduced stomatal conductance. Remarkable alteration in nutrients uptake and their transport were observed in As stressed *Triticum aestivum* L. and *L. sativa* L. which were found to be related closely with a reduced rate of net photosynthesis (Liu et al. 2008; Gusman et al. 2013b). Treatment of As decreases the potassium level which is associated with the agglomeration of plaques inside the root system that reduced the translocation of other important nutrients (Mallick et al. 2011). Upon As exposure, the disturbed status of nutrients might be the reason for the decrease in pigment levels (Siddiqui et al. 2015b). From the soil, plants acquire nitrogen predominantly in the form of nitrate or ammonium. Presence of As disrupts nitrogen assimilation process and downregulates the expression of genes responsible for transport of nitrate and ammonium (Norton et al. 2008). Proteomic studies of RuBisCO revealed that it is degraded by As, as there is a great abundance of nitrogen in the form of amino acids. The drastic change in the amino acid pool upon As exposure was also observed (Dwivedi et al. 2010). Micronutrients such as copper, manganese, and iron are the important constituents of SOD enzyme, which is considered as the first line of defense against oxidative stress. Reduction in the level of micronutrients leads to lessening in the antioxidant potential of the cell (Farnese et al. 2014). Also, As accumulation inside the cells leads to decrease in the uptake of magnesium and sulfur. Since magnesium is the central atom of chlorophyll molecule and a cofactor in enzymes that activate phosphorylation processes, sulfur constitutes a major component of glutathione, phytochelatins, and nonprotein thiols (metabolites and antioxidants) (Kumar et al. 2015). When plants are exposed to high concentrations of As, a decrease in phosphorus uptake was observed, as AsV and phosphorus competes for the same transporter across the membranes of root cells. In plants, phosphorus is a constituent of lipids, nucleic acids, and energy-rich molecules and is also crucial for energy transfer and protein metabolism (Finnegan and Chen 2012; Kumar et al. 2015). Because As can replace phosphorus from ATP, it disturbs the rate of ATP synthesis and energy flow (Garg and Singla 2011). Pathare et al. (2016) studied the expression of proteins that regulate enzymes like H^+ATPases and revealed that its expression is altered under As stress. This enzyme helps in the maintenance of pH and electrical gradient across cell membranes which act as a driving force for the transport of ions and nutrients into and out of the cells (Pathare et al. 2016).

2.4.4.2 Water Loss

Presence of As in the plant cells leads to an alteration in the expression of various transporter genes present in the outer membrane, which disturbs uptake of water from the soil and flow of nutrients and water (Finnegan and Chen 2012). Under abiotic stresses including As, water loss, and decreased water uptake are linked with decreased biomass of the plants (Agnihotri and Seth 2016). Due to the disturbances in the uptake and transport of nutrients and water in the cell, rates of transpiration and stomatal conductance also get disturbed during As exposure (Stoeva and Bineva 2003). Stoeva et al. (2003) have reported that in As-treated plants relative water content has been decreased slightly as compared to their respective controls. Also, As affects the functioning of aquaporins of PM intrinsic protein class, which are major channels for uptake of water from the soil (Siddiqui et al. 2015a). Srivastava et al. (2013a) documented that gene expression of PM intrinsic protein was down-regulated under As stress that lead to a decline in total water content and disturbed water balance, thereby inhibiting growth of seedlings. It is more likely that absorption of water by the plant root cells is indirectly regulated by the alterations in endogenous factors such as root morphology and/or anatomy (Rucinska-Sobkowiak 2016). Farooq et al. (2017) showed numerous alterations due to As accumulation in the ultrastructure cells of the roots. Of note, As-induced structural changes in the root cells lead to inadequate root-soil contact which reduces the capacity of plants to exploit the water from the soil (Rucinska-Sobkowiak 2016). Moreover, metal stress was seen to decrease cross-sectional area available for water transport that reduces xylem conductivity. This decline was caused by partial blockage of xylem elements, progressive decrease in the proportion of xylem tissues available for conduction of water, and a diminution in the size of vessels and tracheids (Rucinska-Sobkowiak 2016) (Fig. 2.3). Abiotic stresses including As toxicity leads to suberization and deposition of callose in the form of "patches" in the cell wall of plant cells (Pirselova et al. 2012; Umar et al. 2013). Callose is mainly localized in

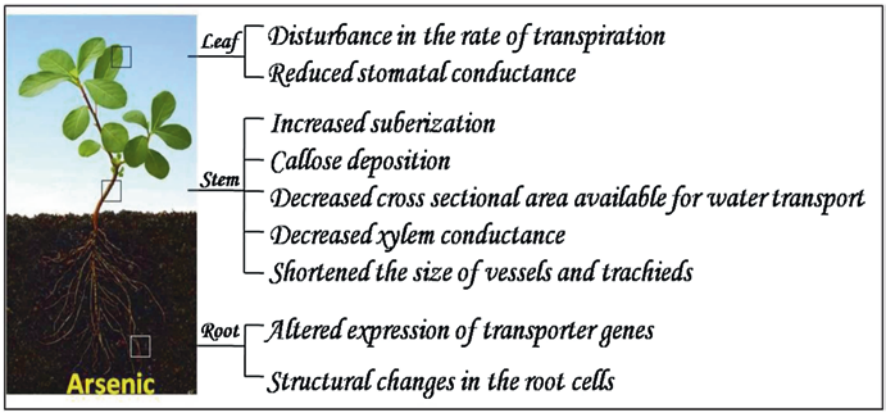

Fig. 2.3 Arsenic-induced changes in transport system which lead to loss of water

cell walls and/or in its surrounding area, which might be responsible for the reduction in the diffusion of water in the apoplast of the cells that surrounds the conducting cells in roots and also limits the intercellular water movement through the plasmodesmata (Rucinska-Sobkowiak 2016). The deposition of heavy metals, blockage of intercellular spaces, and impregnation of middle lamellae might hamper apoplastic water flow. Arsenic-induced decrease in leaf size, the thickness of leaf lamina, intercellular spaces, stomatal density, and the sizes of the stomatal aperture reduce the transpiration rate, thereby affecting plant metabolism. Hence, water balance inside the cells is extremely crucial for plants to survive and sustain growth. Any disturbance in the water homeostasis may affect every aspect of the physiology of plants (Srivastava et al. 2013b) (Fig. 2.3).

2.4.5 Membrane Integrity

2.4.5.1 Cellular Membrane Damage

Apart from physiological changes in mitochondria and chloroplast, the presence of As also disrupts the protoplast of mesophyll cells of the plants. In plants, cellular membranes are very sensitive to damage through As attack (Ismail 2012). Exposure of plants or its parts to As caused increased accumulation of it and consequently loss of membrane integrity (Kaur et al. 2012). The susceptibility of cellular membranes to As toxicity is related to two mechanisms: (1) directly binding of As to the thiol groups of membrane proteins, and (2) As-induced production of ROS that disintegrates membrane stability (Karam et al. 2016). Binding of As to the membrane proteins alters the proper functioning of proteins, thereby membrane damage. Moreover, As imposed ROS regulated membrane lipid peroxidation is held responsible for this loss of membrane integrity (Talukdar 2014). Also, these free radicals are involved in the commotion of the plasma and vacuolar membrane system. Peroxidation of polyunsaturated fatty acids of cellular membranes by ROS attack can lead to chain breakage and, thereby, distorts membrane structure and increases membrane permeability. In the vacuolar membranes, As-induced ROS are involved in the cell signaling cascade and leads to inactivation of the vacuolar enzymes consequently alteration in the structure and collapse of the vacuolar membrane (Farnese et al. 2017). In general, cellular membranes are prime targets of As-induced stress injuries, resulting in denaturation of cytosol, increased permeability, and loss of electrolytes and ions. Depth of injury may closely be linked with measuring membrane stability index. Decline in membrane stability index by As has previously been observed in *Phaseolus aureus* and *Glycine max* L. by Kaur et al. (2012) and Chandrakar et al. (2017a), respectively. Presence of As in the cells modifies lipid-protein interaction in the membranes and also alters the metabolic functions including the activity of crucial enzymes like H^+ATPase, and thereby increased membrane permeability and loss of electrolytes (Pathare et al. 2016).

2.4.5.2 Release of Electrolytes

Arsenic-mediated peroxidation of membrane lipids is also evident from the enhanced release of electrolytes from the cell (Kumar et al. 2014). The abiotic stress-induced injury is commonly recorded in terms of electrolyte leakage (Yuan et al. 2014; Signorelli et al. 2016). Exposure of plants to As resulted in increased electrolyte leakage indicating membrane injury, which is reported to be a direct consequence of As toxicity (Anjum et al. 2016). Elevated leakage of electrolytes has been reported as a useful indicator of As injury in the root tissues of *P. aureus* Roxb., *O. sativa* L., and *G. max* L. (Singh et al. 2007; Begum et al. 2016; Chandrakar et al. 2018). In this context, Talukdar (2013) stated that interaction of As with intracellular components might result in the overproduction of ROS in affected cells. Being an oxidizing agent, ROS initiates lipid peroxidation reaction and gives rise to malondialdehyde (a lipid peroxidized product) that disturbs membrane integrity drastically, consequentially enhancing leakage of cellular constituents (Kaya et al. 2013). Interestingly, As may modify the activities of the enzyme lipoxygenase, which also participate in the alteration of membrane permeability that consequently uplifts the ion leakage (Chandrakar et al. 2017b). Additionally, As-induced inevitable production of ROS and increased activity of lipoxygenase enzyme have direct effects on the disorganization of cell structure through the oxidation of biomolecules (Kazemi et al. 2010). The overall effects of lipid peroxidation reaction increased the permeability of membranes, and thus increased release of those cellular constituents which normally happens through specific channels and membrane proteins, and inactivation of enzymes, receptors, and ion channels (Gill and Tuteja 2010).

2.5 Conclusions and Outlook

Arsenic has gained widespread importance in scientific research because of its deleterious effects on plants and animals. Presence of As adversely affects morphology and physiology of plants. Knowledge of the mechanisms of morphological and physiological responses of plants to As toxicity will facilitate its amelioration through new purposely designed studies. The toxicity symptoms of As include inhibition of growth attributes, distortion in photosynthetic apparatus, inhibition of biomass accumulation, etc. Although considerable attempts have been made to unravel the mechanisms of As-induced injury symptoms in various stages of plant development, our understanding of the subject is still far from the comprehensive. Arsenic imposes oxidative stress via enhanced production of ROS and consequently oxidizes the cellular macromolecules such as lipids, proteins, nucleic acids, and carbohydrates. The resultant products of this oxidation reaction might be interfering with the various metabolic pathways either directly by inhibiting the activities of key enzymes or by creating oxidative stress. Thus, As toxicity has a negative impact on quality and productivity of plants. Plants have developed some strategies to combat the toxic effects of As via several mechanisms, such as sequestration, synthesis of

polyphenols, activation of defensive enzymes, expression of metal binding proteins, accumulation of compatible solutes like sugar, proline, glycinebetaine, mannitol, etc. In recent years, significant progress has been made in understanding the detailed mechanisms of uptake, transportation, effects, speciation, and detoxification of As in plants. However, there are substantial knowledge gaps about the compartmentalization of As in vacuoles as well as its loading in xylem and phloem tissues.

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Chapter 3
Consequences of Paddy Cultivation in Arsenic-Contaminated Paddy Fields of *Lower Indo-Gangetic Plain* on Arsenic Accumulation Pattern and Selected Grain Quality Traits: A Preliminary Assessment

Debojyoti Moulick, Subhas Chandra Santra, and Dibakar Ghosh

Abstract Consequences of paddy cultivation in arsenic (As)-contaminated environment on crop growth, yield, and transmission to food chain through consumption of rice are known to all. Furthermore, the adverse consequences of As toxicity continues upon consumption of As rich rice by human, irrespective of gender and socioeconomic perspective, have been documented in large number of literature. In the current investigation, our prime objectives were to explore (a) As accumulation pattern in different rice varities cultivated in "*As Hot – Spot area*" located in "*lower Indo – Gangetic Plane*" for two successive years, and a least addressed issue (b) how rice grain quality traits get influenced by As contaminated environment. Findings from the current investigation suggest that (i) As content in irrigation water in the study sites were higher than the permissible limit mentioned by FAO; (ii) there is a season-wise, variety-wise, year-wise, as well as location-wise variation in As accumulation pattern noticed; and (iii) variation in grain quality attributes can also be seen. Results also suggest that grain As content were within the range of 0.242 ± 0.007 to 1.222 ± 0.005 mg As per kg. Statistical interpretation indicates that with fluctuation in grain As content significant modulation in grain quality traits like grain weight, head rice recovery % in downward fashion can be observed. Beside these, with variation in grain As content an increase in gel consistency, cooking time, and amylose content were also noted.

Keywords Arsenic uptake · Grain quality · Metalloids · Rice · Yield

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3.1 Introduction

Rice (*Oryza sativa*) is a food crop which belongs to the grass family of Poaceae of the plant kingdom. Domesticated rice has two species, namely, *O. sativa* and *O. glaberrima*. Rice plants are native of a subtropical and tropical part of South Asia and Southeastern Africa (Crawford and Shen 1998). Originally rice was cultivated (most probably) without submersion, but it is conceived that due to mutations, it becomes a semiaquatic plant. Although rice can grow in wide range of environments, it grows vigorously in wet and warm climatic conditions. During cultivation, rice plant develops the main stem (shoot) along with tillers (from 0.6 to 6.0 m in height in floating rice). Tiller which bears a ramified panicle (also known as active tiller) that measures between 20 and 30 cm wide. Each panicle contains 50–300 flowers (floret or spikelet) during the flowering stage, which form the grains. The fruit obtained is a caryopsis (UNCTAD.org). At present rice production represents >30% of the world total cereal production. Over the last three decades, rice production has doubled, due to the introduction of new varieties and development of ancillary cultivation technologies, but its present growth barely follows consumption. According to an estimate in 2025, approximately 4.6 billion people will depend on rice for their daily nourishment. At present, a considerable effort has been directed toward encouraging and developing new technology for the small producers to make the best use of land which is not so favorable for rice cultivation, like brackish/briny soils.

In the fields of *lower Indo-Gangetic plains* as well as in other parts, rice cultivation has been practiced by transplanting nascent seedlings (21–42-day-old) from seedbed to actual field (Ghosh et al. 2016). Bedside these, direct sowing of rice seeds (DSR method) into the actual fields can also be seen, though there are several other methods of paddy cultivation exists such as SRI (systemic rice intensification) and zero tillage etc. were also popular in some parts. During transplanting (in case of transplanted rice), a hill to hill gap of ≈15 cm and a line to line gap of ≈20 cm were maintained along with recommended fertilizer doses and weeding as per norms. In the *lower Indo-Gangetic plains* of West Bengal and adjacent states as well as in Bangladesh (in Ganges-Meghna-Brahmaputra basin), rice cultivation is done in two seasons: (mainly) the *boro* (winter rice) during late December/early January to late April/early May and *kharif* (July to November). Among them *boro* rice is cultivated with intense irrigation, whereas *kharif* rice requires occasional irrigation.

3.1.1 Rice and Economy

Rice has the credit of being consumed for ≈ 5000 years. Rice feeds more than half the entire world population (Carriger and Vallée 2007; Mohanty et al. 2013; Stoop et al. 2009). *O. sativa* accounts for the majority (more than 80%) of world rice cultivation or production. The significant research effort, thus, focused on *O. sativa* itself, except otherwise specified. *O. sativa* has two subspecies, viz., *indica* and

japonica. According to reliable reports of FAOSTAT (Statistical reports of Food and Agriculture Organization) (2008) as well as from IRRI - International Rice Research Institute (IRRI 2013), rice provides 31% of total calorie intake to the Indian population; besides that, rice cultivation also provides employment to ≈1 billion people throughout the world (Dawe 2002). FAOSTAT (2014) suggests that rice has been regarded as the most crucial food crops cultivated and consumed with the credit of having 26% of total cereal production; moreover, in terms, cereal trade rice has the share of 20%. Reports from the Food and Agriculture Organization of the United Nations, i.e., FAO (2011), indicates that in India (alone), rice cultivation covers as much as 44 million hectares, while 157 million hectares of land is used for rice cultivation worldwide. According to findings of Lerche (2011) suggests that agriculture sector employs 57% (with 1/5 of total GDP or gross domestic production) people.

India's role of being the second largest producer as well as consumer of rice besides the rice-based products (worldwide) is significant in the global economy. Thailand challenged India's position only once in terms of exporting rice, in spite of the fact that India retrieves and maintains its own position from 2011 to 2012 onward. Having the credit of 7% improvement in rice export kissed to 26% (All India Rice Exporter Association or AIREA 2014). Indian rice export, in terms of rice variety(ies), can be grouped into two broad categories, (I) nonaromatic rice and (II) aromatic rice (basmati mainly), and is extended across the entire Middle Eastern countries including Iran, Saudi Arabia, Iraq, etc. Countries like Benin, Senegal, and Bangladesh are the main importers of nonaromatic type of rice. Jha (2014) observed that with the improvement of 7.13 million tons in 2013–2014 from 3.99 million tons in 2011–2012, export of nonaromatic rice varieties has become the most choiceable rice type for importers, whereas aromatic rice have seen a slight increase. Jha (2014) also reported by citing the Codex Standard recommended by the Food and Agriculture Organization (FAO) will going to profit the exporters (Indian) as rice the produced in the USA (other countries) usually contains >0.3 mg $kg^{-1}$of arsenic (As) against Codex Standard for permissible As content of <0.2 mg kg^{-1}. Calpe (2006) coined the global rice market as *thin* market, as there is very precise affection regarding their most sought-after milled rice quality attribute by a wide range of customers often varies from a region to region. On the other hand, "premium quality" a very popular term associated with the grading system of milled rice was found to be influenced by the specific socioeconomic background of consumers (customers), in a noteworthy manner (Deaton and Drèze 2009; Demont et al. 2012).

3.1.2 Rice Grain Quality Attributes from Consumer's Prospective

Rice grain quality is evaluated depending on properties, which can be divided in various ways. Product characteristics could be natural (inherent property), viz., taste, texture, or color, or could be anthropogenic by nature such as method of packaging and trade (brand) name. Besides this classification, variations in search,

cognitive content, and acceptance attributes were also evident. Among the search attributes available for a specific decision ahead to purchase a certain product or selecting specific rice variety(s), such as value, visual appeal (degree of polishing or shine), brand name and packaging are emerge out to influence consumer's choice. Experience constructs can be evaluated (by the consumers) only after the product gets purchased, so even after purchase or after the product is used, certain attributes like taste, texture, quality of cooking, and swelling ability were monitored. Consumers actually rely on other people's or even government opinion or industry assertion while selecting certain rice from local market/sophisticated outlets in urban areas. Findings of Rutsaert et al. (2013) indicate that besides production and post-harvest processing, emphasis on product contents is symbolic such as the mental attitude-type attributes toward the varietal selection. According to the opinion of Minten et al. (2013) that assessment of such properties meticulously is a tough task to execute but rather spoke in favor of relatively high-throughput along with a steady reproducible methodology should have been developed to conduct measurements of a large number of rice quality traits in regular intervals. The visual appearance of milled rice grains (parboiled or non-parboiled) is a crucial search attribute that usually influences consumer's choice and thus is regarded as the first pickup criteria for varietal (crop) improvement programs (Tomlins et al. 2005).

Rice grains consist of mainly amylose and amylopectin starch (starch). From composition point of view, rice is often classified into two well-defined classes: (a) waxy and (b) non-waxy-type varieties. When compared with non-waxy-type rice, waxy rice especially gets sticky upon cooking and used to have negligible amounts of amylose. Authors like Moazzami et al. (2011) found that, when compared with the starch content, rice grains have a much smaller fraction of fat (lipids) than starch, though these fats may make a considerable contribution in processing and nutritional properties. In the early 1960's, a large number of newly released rice varieties were excluded from consumer's preference list. Cereal chemists have developed an appropriate series of cost-effective regular assay methods to evaluate several physical, cooking, and sensory traits of rice, whereas the tests currently employed to evaluate various rice grain quality attributes were designed by Chang and Bardenas (1965), length and width calculation method by Henderson (1957), head rice yield (HRR%) and using I_2 (iodine) binding to measure amylose content by Rao et al. (1952), gelatination temperature or GT by Little et al. (1958) and later by Halick and Kelly (1959), and gel consistency by Juliano et al. (1964). Although there are a huge number of ongoing breeding programs, supported by various governments or private bodies throughout the world, still an even greater emphasis is still required.

3.1.3 Arsenic Contamination in Rice Agroecosystem

As exists geologically in soils as well as in aquifers in a large number of areas throughout the world (Mandal and Suzuki 2002; Nordstrom 2002). In some parts of the world, the As content in soils and in groundwater imposes a serious threat to

human health (Hasanuzzaman et al. 2015; Singh et al. 2015). As has been regarded as a class 1 carcinogen in humans (ASTDR). The As content in groundwater used for irrigation (specially in paddy fields) through shallow wells and for drinking purposes in India and Bangladesh (located in *Ganges-Meghna-Brahmaputra* basin) often surpasses the permissible limits that FAO and WHO (World Health Organization) mentioned (Ahmed et al. 2006; Ahsan et al. 2006; Meharg and Raab 2010). According to an estimate, 50–100 million people (and keeps increasing) are at risk of consuming As-contaminated drinking water residing in the South and Southeast Asia only (Ravenscroft et al. 2009; Fendorf et al. 2010; Flanagan et al. 2012). Findings suggest that ≈ 9.5 million people have been affected by As-induced adverse effects through consuming As-enriched water in nine districts in southern West Bengal, India, itself (Acharyya and Shah 2010). Report published by Chakraborti et al. (2010) indicates that ≈ 36 million (in Bangladesh) and 22 million residents (in India) were found to drink As-contaminated water from tube wells, exceeding permissible limit of As of 10 μg L^{-1} and 50 μg L^{-1}, respectively. So far, As-enriched groundwater has been observed in India (West Bengal), Bangladesh, Burma, Cambodia, and Vietnam (Benner and Fendorf 2010). The actual origin of the As is in the Himalayan mountain range; from here As-rich rocks through weathering and subsequently via erosion, over countless past, released As into the major river systems like the Ganges-Brahmaputra, Irrawaddy, Chao Phraya, Mekong, Red, etc. (Benner and Fendorf 2010). The development of irrigation in the river deltas has caused the mixing of As between shallow and deeper aquifers while also increasing the load of As in agricultural soils (Benner and Fendorf 2010; Erban et al. 2013). Beside these, drinking As-contaminated drinking water and consumption of rice in South and Southeast Asia by the people further amplifies the risk of As-induced toxicities. Among the field crops, rice was found to be an efficient As accumulator (Williams et al. 2007; Meharg and Zhao 2012). Rice plant used to accumulates arsenite (As^{3+}), greater than wheat and barley (as much as two times more) may attributed to its agronomic practice. In most rice-producing agroecosystems, paddy (rice) cultivation takes place in with waterlogged condition with 3–4 cm standing water. Under such anaerobic condition (with low soil pH) soil microflora used to transform As into to more phytoavailable forms to rice plants (Islam et al. 2004; Heikens 2006; Heikens et al. 2007). The uptake of As further mounts up in those areas where As-rich groundwater was used for irrigation (Meharg and Zhao 2012). Arsenic content in paddy field varies widely, depending upon a large number of geogenic as well as anthropogenic factors. Variation of aquifer, previous deposition in soil, soil characteristics, depth and duration of shallow (irrigation practice), slope of the field, etc. were found to significantly influence As load in agriculture (paddy field) soil As pool (Harvey et al. 2006; Brammer 2008; Fendorf et al. 2010; Van Geen et al. 2014). Findings of Stroud et al. (2011) suggest that in the paddy fields of West Bengal (India) and Bangladesh, soil As content differs spatially and substantially. Stroud et al. (2011) further reported that As content in soil and in grain were also varies within rice field itself, might be due to the dynamics of As within soil pore water and surrounding water (stagnant) in the paddy fields.

3.1.3.1 Consequences of Rice Cultivation in As-Contaminated Agroecosystem

Rice cultivation in As-contaminated agroecosystems resulted in inhibition in germination and seedling growth (Moulick et al. 2016a, 2017), reducing growth and yield at harvesting (Moulick et al. 2018a, b; Panaullah et al. 2009; Khan et al. 2010). Beside these, reduction in chlorophyll content in both seedling and flowering stages and enhanced As content in rice grain were also noted (Meharg et al. 2001; Abedin et al. 2002; Williams et al. 2006; Huq 2008, Moulick et al. 2018a, b, c). Furthermore, Lu et al. (2009) and Bhattacharya et al. (2013) observed that enhanced As content in rice grains shares a positive correlation with As content in soils, whereas in Bangladesh authors like Panaullah et al. (2009) and Khan et al. (2010) reported a decreased As load in grain with increasing soil As content. This contradictory relationship between grain and soil As content might be due to variable soil physicochemical and textural properties, As content in groundwater (irrigation), as well as inbuilt As load in soil along with seasonal variability and agronomic practice (BGS/DPHE 2001; Xie and Naidu 2006; Lu et al. 2009; Chowdhury et al. 2017; Khan et al. 2010; Garnier et al. 2015).

Prior to this article, there is hardly any article reported about the impacts of As content in grain (or As stress) and grain quality traits of non-parboiled rice. The current article aims to assess the consequences of rice cultivation in As-contaminated agroecosystem on As accumulation pattern in different plant parts and selected grain quality attributes and further to trace the relationship among the grain As load and quality traits.

3.2 Materials and Methods

3.2.1 Description of Study Sites and Agriculture Practice

We collected mature rice plant from one of the As-contaminated districts of West Bengal. Our study sites located in the *lower Indo-Gangetic plain* came into existence due to the deposition of late Holocene to recent sediments usually carried by the river Ganges (Samal et al. 2011). In our study sites, As content in groundwater usually exceeds >0.01 mg L^{-1} laid by WHO (1992) as a maximum permissible limit. We selected our study sites in such a way (farmers' field) that the actual field site is minimum 0.4 acre or 1.0 bigha of land mass and the land owners provide us information regarding agronomic practice and allow us to take samples. For the years 2013 and 2014, we collected mature rice plant (during harvesting) in both winter (*boro*) and rainfed (*kharif*) seasons from seven sites located in two districts. Landowners or farmers of our study area (all seven study sites) transplanted rice seedlings (21– 42-day-old or 4 – 5 leaf stage) to the actual field from the adjacent seedbed. During the course of transplantation, a 15 × 15 cm and 20 × 20 cm gap between two hill and two lines were maintained, respectively. In the actual field

sites, farmers applied 2000 ± 31 kg FYM (farm yard manure) during the first plow, and ≈1:1:1 urea (N):P_2O_5:K_2O, respectively, per 0.1 ha land were also applied. At maturation, rice plants (with ≈90% turned yellow) are harvested manually and then threshed on wooden board manually (Moulick et al. 2016b).

3.2.2 *Sample Collection, Soil Physicochemical Properties, and As Content Analysis*

During the course of harvest phase of boro and kharif seasons of 2013 and 2014, intact rice plant (with panicle) were uprooted manually, paddy field soil samples were collected (from 10 cm depth by following composite sampling method). During this time irrigation water (86 samples) were also collected from particular owner's field from all the seven study sites. The paddy field soil samples were analyzed for soil physicochemical properties in triplicates. Soil physiochemical properties were determined by adopting the methodologies described by Trivedy and Goel (1986) and Kettler et al. (2001), respectively, whereas, the As content of paddy field soil and irrigation water and in different parts of mature rice plants including in non-parboiled grains (all in triplicate) was determined according to the methodology described by Moulick et al. (2016b).

3.2.3 *Physicochemical Properties and Cooking Characteristics of Milled Rice*

Among the selected physicochemical properties of non-parboiled milled rice considered here, 1000-grain weight (1000 GW) and head rice recovery percentage (HRR%) were computed according to the procedure of Moulick et al. (2016b) and Rao et al. (2013). For determining moisture content (MC), weight of milled grain was noted first ($W_{initial}$) which is then kept in clean premarked glass petri plates (9 cm diameter) and kept inside the hot air oven at 60°C for 48 h. After the designated time, milled rice weight was noted down (W_{final}) and the moisture content of milled rice was calculated by using the formula-

$$MC_{DW} = W_{initial} - W_{final} / W_{final} \times 100 \quad (3.1)$$

Amylose content (AC%) and gel consistency (GC) of milled rice varieties were determined according to the methodology described by Juliano (1971) and Cagampang et al. (1973) respectively. For determining the minimum cooking time (MCT) and water uptake ratio (WUR) first non-parboiled milled rice samples were cooked and then calculated by adopting the methodology of Moulick et al. (2016b) and Yadav et al. (2016).

3.2.4 Data Analysis

All the data except the cooking time were expressed as mean ± SD of three replication formats. Pearson correlation study was carried out using SAS software version 9.3.

3.3 Results and Discussion

3.3.1 Physicochemical Properties of As Content in Paddy Field Soil

Selected physicochemical properties of As-contaminated paddy field soil and irrigation water from three study sites were tabulated in Table 3.1. Findings from the current investigation suggest that during harvesting phase, soil As content in our study sites was in the range of 7.15–11.287 mg kg^{-1} in *boro* season, and for *kharif* season, the range was 5.58–7.842 mg kg^{-1}. The highest soil As content was observed in Bamonbelia during 2014 *boro* and *kharif* seasons. Maximum As content in irrigation water (groundwater) was noted in Chandamari (N) study sites during *boro* 2014, whereas the least As content was recorded from Bamonbelia during *kharif* 2013 season. A considerable variation in paddy field soil physicochemical properties in study sites was observed, with soil acidity (pH of 6.3–7.1) and conductivity (0.41–0.63 μmho/cm). Similar variation was also applicable to available phosphate, nitrate, and organic carbon content (Table 3.1).

3.3.2 As Content in Irrigation Water

As content in irrigation water in our study sites in both the seasons was higher than the permissible limit of 0.01 mg L^{-1} set by FAO (1985) but within the ISI permissible limits of 0.5 mg L^{-1} (39 out of 86 samples). The variation in As content in irrigation water may attribute to rainfall and runoff pattern and depth of water table that have modulated the distribution pattern and mobility of As (Bhattacharya et al. 2002), whereas soil As content lies within the previously established range for As content for nonagricultural soil type and approaching the mean As content of 10.7 mg kg^{-1} (*boro* 2014 in Bamonbelia site) (Kabata-Pendias and Pendias 1992; Roychowdhury et al. (2002). The difference in soil As content with the previous report of Roychowdhury et al. (2002) may be due to the difference in sampling locations, texture, and time of sampling from paddy field (during harvesting phase here). According to a recent report published by Shrivastava et al. (2014) who reported mean soil As content (9.67 mg kg^{-1}) in Chadha block supports the findings from the current investigation carried out Chandamari (N) block that also comes under Chadha block (Table 3.1).

Table 3.1 Selected physicochemical properties of As-contaminated paddy fields (irrigated with As-rich groundwater) of Nadia district in the year 2013 and 2014

Locations	Year	Season (number of samples)	As content		pH	EC (μmho/cm)	Available PO_4^{-3} (mg/100 g)	Available NO_3^{-2} (mg/100 g)	Organic carbon (mg/100 g)
			Soil (mg kg^{-1})	Water (mg L^{-1})					
Chandamari (north)	2014	*Boro* (n = 14)	9.38 ± 0.015	0.76 ± 0.005	6.4 ± 0.3	0.51 ± 0.04	0.582 ± 0.011	1.308 ± 0.019	0.677 ± 0.009
		Kharif (n = 11)	7.34 ± 0.012	0.64 ± 0.002	7.1 ± 0.2	0.41 ± 0.06	0.663 ± 0.007	0.988 ± 0.004	0.584 ± 0.011
	2013	*Boro* (n = 15)	8.18 ± 0.027	0.7 ± 0.006	6.6 ± 0.3	0.57 ± 0.02	0.511 ± 0.008	1.117 ± 0.009	0.520 ± 0.016
		Kharif (n = 13)	6.84 ± 0.033	0.61 ± 0.004	7.0 ± 0.4	0.48 ± 0.05	0.609 ± 0.004	0.874 ± 0.011	0.441 ± 0.010
Kurumbelia	2014	*Boro* (n = 10)	8.751 ± 0.006	0.42 ± 0.004	6.6 ± 0.5	0.63 ± 0.04	0.529 ± 0.008	1.084 ± 0.004	0.589 ± 0.013
		Kharif (n = 09)	6.77 ± 0.032	0.33 ± 0.001	6.8 ± 0.2	0.41 ± 0.05	0.571 ± 0.007	0.877 ± 0.006	0.629 ± 0.006
	2013	*Boro* (n = 10)	7.15 ± 0.008	0.34 ± 0.002	6.5 ± 0.2	0.54 ± 0.03	0.542 ± 0.004	1.073 ± 0.007	0.456 ± 0.014
		Kharif (n = 14)	5.58 ± 0.017	0.27 ± 0.01	6.7 ± 0.1	0.44 ± 0.05	0.613 ± 0.007	0.881 ± 0.009	0.422 ± 0.017
Bamonbelia	2014	*Boro* (n = 09)	11.287 ± 0.16	0.24 ± 0.004	6.5 ± 0.3	0.58 ± 0.03	0.512 ± 0.011	0.986 ± 0.007	0.462 ± 0.014
		Kharif (n = 11)	7.842 ± 0.026	0.18 ± 0.003	7.0 ± 0.3	0.47 ± 0.04	0.546 ± 0.005	0.902 ± 0.007	0.459 ± 0.008
	2013	*Boro* (n = 15)	9.58 ± 0.041	0.17 ± 0.002	6.3 ± 0.1	0.61 ± 0.04	0.476 ± 0.007	0.846 ± 0.012	0.877 ± 0.008
		Kharif (n = 13)	7.14 ± 0.06	0.11 ± 0.03	6.7 ± 0.4	0.56 ± 0.07	0.520 ± 0.016	0.795 ± 0.021	0.645 ± 0.009

All the values represented in mean ± SD format

Selected physicochemical properties (studied here) of paddy field soil form three study sites (in both seasons of the year 2013 and 2014) presented here. The absorption of the two dominant inorganic forms of As, i.e., arsenite/As(III) and arsenate/As(V), by a wide range of metals like iron, alumina, organic carbon, and carbonates is governed by the soil acidity (Ali 2003; Ali et al. 2003). Correlational study carried out among the various physicochemical properties and As content in different plant parts reveals that pH, EC organic carbon significantly influence As absorption by rice root (As content here), supports the view of Bhattacharya et al. (2010). Findings of Shrivastava et al. (2014) regarding the correlation between irrigation water and soil As content support the findings of the current investigation.

3.3.2.1 As Accumulation Pattern in Different Plant Parts of Studied Varieties

Results indicate that As content in mature rice plant follows the order of root ˃ shoot ˃ husk ˃ grain (brown rice here) in all the studied rice varieties irrespective of cropping season and study sites. Among the studied varieties, Swarna variety cultivated in *boro* 2014 season in Chandamari (N) study site of Nadia district accumulated the highest amount of As in its root, whereas the same variety, i.e., Swarna, cultivated in the same season in Kurumbelia accumulated 12.54 ± 0.027 mg kg^{-1} As. Least amount of As accumulation was observed in IR-64 variety (9.673 ± 0.020 mg As kg^{-1}, cultivated in Bamonbelia site among the studied varieties cultivated in *boro* 2014 season. When grain As content were compared among the studied varieties cultivated in *boro* 2014, it suggests that grain (brown rice) As content lies within 0.543 ± 0.012 to 1.222 ± 0.005 mg As kg^{-1}. The same scenario of As content can be seen in *boro* 2013 season among the studied rice varieties. Results suggest that Swarna cultivated in Kurumbelia study site was found to accumulate the highest amount of As in grain, whereas the least As content in grain was noted in M-Shankar variety cultivated in Chandamari (N) variety in the same (0.393 ± 0.012 mg As kg^{-1}) season.

Kharif seasons of both the studied year and in all the study sites present an interesting opportunity to make a comparison among the local/traditional rice varieties and high-yielding rice varieties from grain As accumulation point view. Results (presented in Tables 3.4 and 3.5) indicate that compared to the high-yielding rice varieties, local or traditional rice varieties accumulated relatively lesser As in brown rice fraction, although cultivated in same study site(s). Table 3.4 suggests that in Chandamari (N) site Bdashabhog and Bashkathi accumulated 0.367 and 0.302 mg As kg^{-1}, respectively, whereas Minikit or Satabdi accumulated 0.515 mg As kg^{-1} in grain. The same trend of comparatively lesser As content in grain can be seen among the local varieties than high-yielding varieties studied in other two study sites and cultivated in *kharif* seasons of 2013 and 2014 (Tables 3.4 and 3.5). Among all the rice varieties studied in 2013 and 2014, Khatisha variety cultivated in Chandamri (N) study site had the least grain As content (0.242 mg As kg^{-1}) (Table 3.5). Another very interesting finding emerges out from the current investigation that high-yielding

rice varieties (common) cultivated in both *boro* and *kharif* season had a noteworthy difference in grain As content prospective. Satabdi variety cultivated in Chandamari (N) site had relatively lesser grain As (0.515 mg As kg^{-1}) when cultivated in *kharif* season than in *boro* season (Tables 3.2 and 3.4). The same seasonal difference in grain As content in the common rice varieties can also be seen in Ananda variety (Tables 3.2, 3.4 and 3.5).

People residing in *lower Indo-Gangetic* plain including our current study sites lives / survive on rice and rice-based products. In our study sites, rice is the main crop cultivated at least twice a year basis (in *boro* and *kharif* season). During winter season, i.e., *boro*, rice cultivation demands intense irrigation with As-rich groundwater having As content » FAO and ISI permissible limits (Table 3.1). According to the findings of Williams et al. (2006), drinking As-contaminated water with 0.1 mg L^{-1} is equivalent to consuming rice having 0.08 mg As kg^{-1}. Findings from the current investigation indicate that irrespective of study sites, cropping season and varietal difference, As content soil and irrigation water, As accumulation pattern in mature rice plant lies in the order grain < husk < shoot or straw < root (Table 3.1). These findings are in good agreement with the previous findings of Moulick et al. (2016b) and Shrivastava et al. (2017). As content in root by the studied varieties was in the following range: root, 4.859–12.84; shoot, 2.003–5.63; husk, 0.689–2.598; and grain, 0.242–1.222 mg kg^{-1}, respectively. It is a noteworthy fact that findings from current investigation support the view of Biswas et al. (2014); those varieties cultivated in *kharif* season accumulate comparatively lesser As in grain and other plant parts than that of those cultivated in same sites (same fields actually) in *boro* season. Some varieties like (mostly high yielding) Swarna (MTU-7029), Ratna, Satabdi (IET-4786), IR-64, IET-4094 (Khitis), JS-8, M-Shankar were found to be efficient As accumulators (Tables 3.2 and 3.3). Compared to the high-yielding varieties, local or traditional varieties accumulate less As in grain cultivated alongside in both the studied seasons (Tables 3.4 and 3.5). Furthermore if we consider the

Table 3.2 Bioaccumulation of As in different plant parts of the rice varieties cultivated during 2014 *boro* (winter) season in Nadia District

Study Area	Variety	Root	Shoot	Husk	Grain
Chandamari (N)	Swarna ($n = 3$)	12.84 ± 0.014	5.63 ± 0.036	2.17 ± 0.022	0.783 ± 0.014
	M-shankar ($n = 5$)	11.482 ± 0.011	5.42 ± 0.012	2.38 ± 0.022	0.686 ± 0.019
	Satabdi ($n = 3$)	12.06 ± 0.032	4.46 ± 0.013	2.12 ± 0.015	0.647 ± 0.022
	Ratna ($n = 3$)	11.14 ± 0.024	3.21 ± 0.008	1.886 ± 0.013	0.549 ± 0.003
Kurumbelia	Swarna (L) ($n = 3$)	12.54 ± 0.027	4.67 ± 0.013	2.883 ± 0.026	1.222 ± 0.005
	IR-64 ($n = 4$)	9.673 ± 0.020	3.24 ± 0.011	2.246 ± 0.004	0.677 ± 0.006
Bamonbelia	Satabdi (L) ($n = 2$)	9.852 ± 0.013	3.81 ± 0.0023	1.827 ± 0.023	0.543 ± 0.012
	Khitis ($n = 3$)	10.471 ± 0.026	4.204 ± 0.042	2.534 ± 0.04	0.721 ± 0.011
	Js-8($n = 4$)	10.977 ± 0.03	4.085 ± 0.053	2.336 ± 0.022	0.659 ± 0.004

All values are in mean ± SD format. Arsenic content expressed in mg kg^{-1} DW

Table 3.3 Bioaccumulation of As in different plant parts of the rice varieties cultivated during 2013 *boro* (winter) season in Nadia District

Study Area	Variety	Root	Shoot	Husk	Grain
Chandamari (N)	M-Shankar ($n = 3$)	8.88 ± 0.041	2.844 ± 0.031	0.689 ± 0.004	0.393 ± 0.012
	Satabdi (L) ($n = 3$)	7.68 ± 0.029	2.939 ± 0.014	0.704 ± 0.012	0.637 ± 0.009
	Ananda ($n = 6$)	7.79 ± 0.015	3.744 ± 0.023	0.75 ± 0.0013	0.414 ± 0.016
Kurumbelia	Js-8 ($n = 3$)	8.644 ± 0.024	3.544 ± 0.022	0.786 ± 0.0022	0.477 ± 0.031
	Satabdi ($n = 3$)	6.98 ± 0.0026	3.544 ± 0.012	0.786 ± 0.0015	0.477 ± 0.033
	Swarna (L) ($n = 4$)	11.136 ± 0.015	4.139 ± 0.025	2.598 ± 0.0013	1.009 ± 0.014
Bamonbelia	IET-4094 ($n = 3$)	10.286 ± 0.016	4.772 ± 0.013	2.508 ± 0.016	0.829 ± 0.018
	Swarna (L) ($n = 7$)	9.855 ± 0.0024	3.729 ± 0.028	2.007 ± 0.011	0.907 ± 0.02
	Satabdi ($n = 5$)	10.135 ± 0.011	3.771 ± 0.017	2.115 ± 0.022	0.582 ± 0.009

All values are in mean ± SD format. Arsenic content expressed in mg kg $^{-1}$ DW

Table 3.4 Bioaccumulation of As in different plant parts of the rice varieties cultivated during 2014 *kharif* (Monsson) in Nadia District

Location	Variety	Root	Shoot	Husk	Grain
Chandamari (N)	Badshabhog** ($n = 7$)	5.712 ± 0.003	2.22 ± 0.002	1.134 ± 0.004	0.367 ± 0.001
	Satabdi## ($n = 4$)	6.243 ± 0.002	2.682 ± 0.002	1.826 ± 0.005	0.515 ± 0.003
	Bashkathi**	5.891 ± 0.002	2.183 ± 0.004	0.989 ± 0.001	0.302 ± 0.003
Kurumbelia	Jaya## ($n = 5$)	5.514 ± 0.003	2.645 ± 0.002	1.747 ± 0.004	0.401 ± 0.005
	IR-36## ($n = 4$)	5.442 ± 0.002	2.807 ± 0.004	1.822 ± 0.004	0.498 ± 0.003
Bamonbelia	Satabdi (L) ## ($n = 6$)	5.337 ± 0.004	2.746 ± 0.002	1.766 ± 0.006	0.527 ± 0.002
	Ananda## ($n = 5$)	4.859 ± 0.003	2.477 ± 0.002	1.283 ± 0.004	0.327 ± 0.003

All values are in mean ± SD format. Arsenic content expressed in mg kg $^{-1}$ DW
** traditional variety; ##, high-yielding variety

findings of Halder et al. (2012) and categorize grain morphology with respect to grain As content, the extent of grain As content was found to follow the order short and bold (SB) like Swarna, JS-8, etc. ≫ round bold (RB) Gobindobhog ≫ medium and slender type (MS) ≫ Satabdi, Khitis, IR-64 ≫ extra long and slender (ELS) varieties like Khatisal, Bashkathi, etc. (Tables 3.2, 3.3, 3.4 and 3.5).

3.3.2.2 A Correlative Aspect of Grain as Content and Physicochemical Properties of As-Contaminated Paddy Field Soil

Results of Tables 3.6, 3.7, 3.8, and 3.9 depicted the correlative aspect of grain As content and physicochemical properties of As-contaminated paddy field soil. Results suggest that physicochemical components of As-contaminated paddy field

Table 3.5 Bioaccumulation of As in different plant parts of the rice varieties cultivated during 2013 *kharif* (Monsson) in Nadia District

Location	Variety	Root	Shoot	Husk	Grain
Chandamari (N)	Bashkathi** ($n = 5$)	5.842 ± 0.009	2.462 ± 0.005	0.923 ± 0.004	0.318 ± 0.008
	Badshabhog** ($n = 5$)	7.337 ± 0.007	2.119 ± 0.009	1.147 ± 0.007	0.417 ± 0.005
	Kathishal** ($n = 3$)	6.008 ± 0.010	2.003 ± 0.006	0.908 ± 0.005	0.242 ± 0.007
Kurumbelia	Ratna## ($n = 7$)	7.328 ± 0.006	2.102 ± 0.008	1.088 ± 0.009	0.376 ± 0.009
	Satabdi ##($n = 7$)	5.012 ± 0.004	2.541 ± 0.003	1.259 ± 0.003	0.438 ± 0.005
Bamonbelia	Ananda## ($n = 7$)	6.218 ± 0.003	2.133 ± 0.005	1.106 ± 0.003	0.482 ± 0.008
	Dudh shal** ($n = 6$)	6.557 ± 0.008	2.281 ± 0.006	0.971 ± 0.007	0.279 ± 0.005

All values are in mean ± SD format. Arsenic content expressed in mg kg $^{-1}$ DW
** traditional variety; ##, high-yielding variety; L, lal or dark-brown seed coat color; dry weight

soil significantly influence As buildup in rice plants including in grain. Among the components of physicochemical properties studied, organic carbon content seems to be most influential in As content of brown rice. Organic carbon content shares a significantly positive correlation ($r = 0.752$; 0.895; 0.840) with grain As content among the studied rice varieties cultivated in *boro* 2013 and *kharif* 2013 and 2014, respectively, in Nadia district (Tables 3.7, 3.8 and 3.9). As content of the soil, irrigation water seems to be another important factors that significantly influences As load in different parts (root, shoot, husk) of mature rice plants including grain (Tables 3.6, 3.7, 3.8, and 3.9).

3.3.3 Selected Grain Quality Attributes of Studied Rice Varieties Cultivated in As-Contaminated Paddy Fields

Tables 3.10, 3.11, and 3.13 presented selected grain quality attributes of studied rice varieties cultivated in As-contaminated paddy fields in the study sites in a season-wise and year-wise fashion. Among the studied varieties, a noteworthy variation of grain quality traits can be seen. Considering the example of Swarna and Satabdi varieties cultivated in both *boro* and *kharif* seasons of 2013 and 2014, a noteworthy variation in grain quality traits can be observed (Tables 3.10, 3.11, 3.12, and 3.13). Variation among the quality traits like 1000 GW (grain weight), moisture content, and head rice recovery % of Swarna variety is visible. Swarna variety cultivated in Chandamari (N) and Kurumbelia (in *boro* 2014) had 1000 GW of 15.088 and 15.556 g, respectively; a similar trend is also applicable for the same variety cultivated in *boro* 2013 in Kurumbelia and Bamonbelia, respectively. The variation observed in HRR% among the studied varieties cultivated in *boro* 2013 and 2014 suggests that highest recovery lies in the range of 59.89–76.382% (Tables 3.10 and

Table 3.6 Pearson correlation study among soil physicochemical properties, arsenic content of paddy field soil, irrigation water, and arsenic content in different parts of rice plant studied in *boro* 2014 season in Nadia district

	pH	EC	PO_4^{3-}	NO_3^{2-}	OrC	Soil As	Water As	Root As	Shoot As	Husk As	Grain As
pH	1										
EC	−0.315	1									
PO_4^{3-}	0.307	−0.904**	1								
NO_3^{2-}	−0.087	−0.594	0.557	1							
OrC	−0.489	0.734*	−0.826**	−0.289	1						
Soil As	−0.542	0.502	−0.671*	−0.467	0.600	1					
Water As	0.063	−0.423	0.335	0.575	−0.272	−0.222	1				
Root As	−0.656*	−0.121	0.231	0.523	0.105	−0.164	0.331	1			
Shoot As	−0.412	−0.020	0.131	0.206	0.088	−0.029	0.108	0.729*	1		
Husk As	−0.346	0.700*	−0.572	−0.265	0.789**	0.149	−0.214	0.350	0.283	1	
Grain As	−0.599	0.566	−0.386	−0.210	0.538	0.227	−0.133	0.578	0.385	0.814**	1

EC, conductivity of soil (μmol/cm); PO_4^{3-}, available phosphate (mg/100 g); NO_3^{2-}, available nitrate (mg/100 g); OrC, organic carbon (mg/100 g); arsenic content of soil and plat parts expressed in mg kg^{-1}(dry weight basis); arsenic content of irrigation water, mg L^{-1}

* indicates correlation is significant at the 0.05 level, and ** indicates correlation is significant at the 0.01 level (2-tailed)

Table 3.7 Pearson correlation study among soil physicochemical properties, arsenic content of paddy field soil, irrigation water, and arsenic content in different parts of rice plant studied in *boro* 2013 season in Nadia district

	pH	EC	PO_4^{3-}	NO_3^{2-}	OrC	Soil As	Water As	Root As	Shoot As	Husk As	Grain As
pH	1										
EC	−0.845**	1									
PO_4^{3-}	0.402	−0.036	1								
NO_3^{2-}	0.366	−0.598	−0.250	1							
OrC	−0.797**	0.910**	−0.156	−0.531	1						
Soil As	−0.270	0.515	0.081	−0.350	0.552	1					
Water As	0.009	−0.160	0.184	0.192	−0.314	−0.775**	1				
Root As	−0.235	0.623	0.146	−0.541	0.661*	0.633*	−0.600	1			
Shoot As	−0.480	0.550	−0.078	−0.401	0.352	0.208	−0.170	0.551	1		
Husk As	−0.311	0.652*	0.074	−0.594	0.570	0.383	−0.390	0.910**	0.748*	1	
Grain As	−0.570	0.793**	−0.231	−0.539	0.752*	0.397	−0.327	0.817**	0.680*	0.905**	1

EC, conductivity of soil (μmol/cm); PO_4^{3-}, available phosphate (mg/100 g); NO_3^{2-}, available nitrate (mg/100 g); OrC, organic carbon (mg/100 g); arsenic content of soil and plat parts expressed in mg kg^{-1}(dry weight basis); arsenic content of irrigation water, mg L^{-1}

* indicates correlation is significant at the 0.05 level, and ** indicates correlation is significant at the 0.01 level (2-tailed)

Table 3.8 Pearson correlation study among soil physicochemical properties, arsenic content of paddy field soil, irrigation water, and arsenic content in different parts of rice plant studied in *kharif* 2014 season in Nadia district

	pH	EC	PO_4^{3-}	NO_3^{2-}	OrC	Soil As	Water As	Root As	Shoot As	Husk As	Grain As
pH	1										
EC	0.466	1									
PO_4^{3-}	0.525	0.288	1								
NO_3^{2-}	−0.123	−0.283	−0.338	1							
OrC	−0.488	−0.221	−0.780*	−0.089	1						
Soil As	−0.092	−0.620	−0.518	−0.036	0.365	1					
Water As	−0.003	0.266	0.169	0.308	−0.657	−0.335	1				
Root As	−0.268	−0.728*	0.035	0.049	−0.193	0.603	−0.042	1			
Shoot As	−0.580	−0.091	−0.670	0.024	0.888**	0.108	−0.594	−0.163	1		
Husk As	−0.713*	−0.263	−0.640	−0.055	0.867**	0.208	−0.595	0.043	0.967**	1	
Grain As	−0.600	−0.428	−0.841*	0.0228	0.895**	0.605	−0.570	0.207	0.848*	0.888**	1

EC, conductivity of soil (μmol/cm); PO_4^{3-}, available phosphate (mg/100 g); NO_3^{2-}, available nitrate (mg/100 g); OrC, organic carbon (mg/100 g); arsenic content of soil and plat parts expressed in mg kg^{-1}(dry weight basis); arsenic content of irrigation water, mg L^{-1}

* indicates correlation is significant at the 0.05 level, and ** indicates correlation is significant at the 0.01 level (2-tailed)

Table 3.9 Pearson correlation study among soil physicochemical properties, arsenic content of paddy field soil, irrigation water, and arsenic content in different parts of rice plant studied in *kharif* 2013 season in Nadia district

	pH	EC	PO_4^{3-}	NO_3^{2-}	OrC	Soil As	Water As	Root As	Shoot As	Husk As	Grain As
pH	1										
EC	0.242	1									
PO_4^{3-}	0.751	0.281	1								
NO_3^{2-}	0.281	0.710	0.333	1							
OrC	−0.767*	0.167	−0.775*	−0.219	1						
Soil As	−0.328	−0.308	−0.679	−0.401	0.584	1					
Water As	−0.592	−0.408	−0.582	−0.554	0.352	−0.038	1				
Root As	−0.298	−0.132	−0.049	0.196	−0.007	0.0228	−0.187	1			
Shoot As	0.070	0.0313	−0.262	0.213	0.017	0.115	0.126	−0.665	1		
Husk As	−0.743*	0.221	−0.685	0.042	0.721*	0.025	0.678	−0.087	0.272	1	
Grain As	0.870**	−0.200	−0.931	−0.389	0.840**	0.461	0.753*	−0.007	0.158	0.844**	1

EC, conductivity of soil (μmol/cm); PO_4^{3-}, available phosphate (mg/100 g); NO_3^{2-}, available nitrate (mg/100 g); OrC, organic carbon (mg/100 g); arsenic content of soil and plat parts expressed in mg kg^{-1}(dry weight basis); arsenic content of irrigation water, mg L^{-1}

* indicates correlation is significant at the 0.05 level, and ** indicates correlation is significant at the 0.01 level (2-tailed)

Table 3.10 Selected non-parboiled milled rice physicochemical properties and cooking characteristics of rice varieties studied in *boro* season of 2014 from Nadia district

Variety	Study area	1000KW (g)	Moisture content% (MC%)	Head rice recovery% (HRR%)	Amylose content% (AC%)	Gel consistency (mm)	#MCT (min)	Water uptake ratio
Swarna	Chandamari (N)	15.088 ± 0.008	9.055 ± 0.005	64.876 ± 1.118	22.86 ± 0.07	31.025 ± 0.04	23.55	2.62 ± 0.06
M-shankar		16.784 ± 0.011	10.08 ± 0.006	67.843 ± 0.288	26.08 ± 0.04	34.44 ± 0.07	22.35	2.57 ± 0.08
Satabdi		16.408 ± 0.005	10.212 ± 0.006	68.958 ± 1.116	28.64 ± 0.02	35 ± 0.05	22.3	2.75 ± 0.08
Ratna	Kurumbelia	18.422 ± 0.004	13.08 ± 0.05	76.382 ± 0.746	24.58 ± 0.04	36.06 ± 0.03	19.5	2.88 ± 0.07
Swarna		15.556 ± 0.012	8.889 ± 0.02	61.823 ± 1.496	24.27 ± 0.07	41.42 ± 0.03	28.45	1.88 ± 0.06
IR-64		17.289 ± 0.009	12.58 ± 0.004	75.642 ± 0.668	28.87 ± 0.08	44 ± 0.08	21.4	2.46 ± 0.03
Satabdi	Bamonbelia	19.26 ± 0.08	10.79 ± 0.08	73.089 ± 1.202	25.84 ± 0.04	37.02 ± 0.04	19.45	2.54 ± 0.04
Khitis		16.583 ± 0.004	8.996 ± 0.005	66.588 ± 0.744	26.44 ± 0.05	42.05 ± 0.08	20.5	2.29 ± 0.02
Js-8		16.252 ± 0.008	11.395 ± 0.08	70.449 ± 0.825	26.66 ± 0.05	35.04 ± 0.04	20.5	2.37 ± 0.05

Data represented in mean ± SD format
#MCT or cooking time represented in mean value only

Table 3.11 Selected non-parboiled milled rice physicochemical properties and cooking characteristics of rice varieties cultivated in *boro* season of 2013 in Nadia district

Variety	Study area	1000KW (g)	Moisture content% (MC%)	Head rice recovery% (HRR%)	Amylose content% (AC%)	Gel consistency (mm)	#MCT (min)	Water uptake ratio
M-Shankar	Chandamari (N)	17.446 ± 0.012	10.14 ± 0.003	69.41 ± 1.103	22.07 ± 0.03	31.23 ± 0.09	19.25	3.22 ± 0.09
Satabdi		20.02 ± 0.09	11.31 ± 0.08	76.16 ± 1.177	22.89 ± 0.09	32.44 ± 0.07	17.35	2.93 ± 0.05
Ananda		19.88 ± 0.07	10.88 ± 0.06	71.33 ± 0.35	23.77 ± 0.08	31.56 ± 0.18	19.25	2.66 ± 0.06
Js-8	Kurumbelia	16.775 ± 0.011	11.82 ± 0.08	72.369 ± 0.566	23.46 ± 0.09	35.69 ± 0.07	18.55	2.67 ± 0.06
Satabdi		21.26 ± 0.0633	10.08 ± 0.05	71.148 ± 1.022	21.68 ± 0.05	33.26 ± 0.08	19.45	2.35 ± 0.04
Swarna		16.46 ± 0.04	8.021 ± 0.008	59.89 ± 0.08	22.78 ± 0.07	34.88 ± 0.07	27.45	1.79 ± 0.08
Khitis	Bamonbelia	16.203 ± 0.008	8.596 ± 0.005	62.988 ± 0.534	28.25 ± 0.17	43.84 ± 0.11	21.55	2.07 ± 0.04
Swarna(L)		16.138 ± 0.009	9.103 ± 0.005	64.823 ± 1.022	27.05 ± 0.08	32.41 ± 0.06	25.35	2.09 ± 0.05
Satabdi		19.58 ± 0.010	9.33 ± 0.04	68.668 ± 0.896	25.56 ± 0.009	34.81 ± 0.07	21.35	2.26 ± 0.08

Data represented in mean ± SD format
#MCT or cooking time represented in mean value only

Table 3.12 Selected non-parboiled milled rice physicochemical properties and cooking characteristics of rice varieties cultivated in *kharif* season of 2014 in Nadia district

Variety	Study sites	1000KW (g)	Moisture content% (MC%)	Head rice recovery% (HRR%)	Amylose content% (AC%)	Gel consistency (mm)	#MCT (min)	Water uptake ratio
Badshabhog*	Chandamari (N)	19.337 ± 0.008	10.642 ± 0.011	69.884 ± 0.288	20.08 ± 0.09	27.38 ± 0.07	19.35	2.47 ± 0.09
Satabdi#		20.128 ± 0.011	11.037 ± 0.008	64.267 ± 0.866	25.68 ± 0.05	37.43 ± 0.08	22.05	2.72 ± 0.06
Bashkathi*		22.166 ± 0.015	11.32 ± 0.009	65.255 ± 1.1334	21.84 ± 0.06	27.71 ± 0.05	19.45	2.58 ± 0.03
Jaya#	Kurumbelia	20.771 ± 0.006	10.244 ± 0.008	71.322 ± 0.573	25.52 ± 0.08	32.07 ± 0.09	20.15	2.29 ± 0.08
IR-36#		19.204 ± 0.007	10.184 ± 0.006	60.788 ± 0.007	26.63 ± 0.08	31.37 ± 0.05	21.1	2.56 ± 0.05
Satabdi#	Bamonbelia	19.805 ± 0.013	10.891 ± 0.007	63.377 ± 0.521	27.55 ± 0.03	32.78 ± 0.10	23.15	2.06 ± 0.05
Ananda#		21.781 ± 0.008	11.331 ± 0.008	63.022 ± 0.416	20.52 ± 0.07	28.46 ± 0.07	18.05	2.87 ± 0.04

Data represented in mean ± SD format
#MCT or cooking time represented in mean value only
* indicates traditional varieties

Table 3.13 Selected non-parboiled milled rice physicochemical properties and cooking characteristics of rice varieties studied in *kharif* season of 2013 from Nadia district

Variety	Study sites	1000KW (g)	Moisture content% (MC%)	Head rice recovery% (HRR%)	Amylose content% (AC%)	Gel consistency (mm)	#MCT (min)	Water uptake ratio
Bashkathi	Chandamari (N)	19.052 ± 0.029	10.310 ± 0.013	69.532 ± 0.427	23.34 ± 0.06	28.21 ± 0.06	20.45	2.54 ± 0.05
Badshabhog		19.144 ± 0.036	10.217 ± 0.008	67.276 ± 0.311	22.57 ± 0.07	29.08 ± 0.06	22.05	2.19 ± 0.08
Kathishal		20.117 ± 0.026	11.457 ± 0.007	69.101 ± 0.502	19.46 ± 0.08	25.67 ± 0.05	20.45	2.64 ± 0.07
Ratna	Kurumbelia	19.504 ± 0.017	11.785 ± 0.006	72.886 ± 0.647	25.03 ± 0.09	34.19 ± 0.09	20.25	2.34 ± 0.07
Satabdi		19.255 ± 0.019	10.117 ± 0.008	61.522 ± 0.655	24.68 ± 0.07	27.16 ± 011	20.15	3.16 ± 0.06
Ananda	Bamonbelia	20.032 ± 0.010	10.224 ± 0.007	61.411 ± 0.278	26.57 ± 0.09	32.44 ± 0.12	21.25	2.26 ± 0.09
Dudh shal		23.644 ± 0.016	11.641 ± 0.005	65.147 ± 0.339	20.49 ± 0.05	25.93 ± 0.05	17.35	3.012 ± 0.007

Data represented in mean ± SD format
#MCT or cooking time represented in mean value only

3.11), whereas those varieties studied for *kharif* 2013 and 2014 had HRR% that lies in the range of 60.788% (in IR-36) to 72.886% (in Ratna). Similar to physical grain quality traits, variation in chemical grain quality traits was also visible among the studied varieties. Among the studied varieties cultivated in *boro* 2014 and 2013, it is suggested that selected chemical and cooking characteristics were in the range of 21.68–28.87 (AC%), 31.025–44.00 mm (GC), 17.35–28.45 min (MCT), and 1.79–3.22 (WUR) (Tables 3.10 and 3.11). Those varieties cultivated in *kharif season* of 2013 and 2014 had variation in their respective chemical and cooking characteristic traits. Varieties cultivated in *kharif* 2013 and 2014 had 19.46–27.55 (AC%), 25.67–37.43 mm (GC), 17.35–23.15 min (MCT), and 2.06–3.012 (WUR) (Tables 3.12 and 3.13). For the common variety like Ananda cultivated in Chandamari (N) study site in *boro* 2013 and in Bamonbelia study site in *kharif* of 2014 and 2013 seasons, a noteworthy difference in grain quality traits can be observed. In *boro* 2013, Ananda variety cultivated in Chandamari (N) study site had 1000-GW, MC%, and HRR% of 19.88 (g), 10.88%, and 71.33%, respectively, whereas the same variety (Ananda) cultivated in Bamonbelia study site in *kharif* season of the same calendar year had 1000-GW, MC%, and HRR% of 23.644 (g), 11.641%, and 65.147%, respectively. Similar variation in chemical properties and cooking characteristics can also be noticed (Tables 3.11, 3.12 and 3.13).

3.3.4 Statistical Aspect of Grain As Content and Physicochemical Properties and Cooking Characteristics of Non-parboiled Milled Rice

When correlational study carried out among the As the content of non-parboiled grains and selected physicochemical quality traits and cooking characteristics, some interesting facts were emerging out. Our findings suggests that grain As content shares a negative correlation with 1000 GW (r =−0.726), HRR% (r =−0.202), MC (r =−0.536), and water uptake ratio (r =−0.438) respectively, among the studied rice varieties (Fig. 3.1a–c, g). On the other hand, quality traits like AC%, GC, and MCT (r =316, 598, 703) share a positive correlative relationship with grain As content (Tables 3.13, 3.14; Fig 3.1d–f). To our best of knowledge, prior to this article, there is hardly any article available that deals with grain quality aspects of rice cultivated in As-contaminated ecosystem. Moulick et al. (2016b) reported about the impacts of rice cultivation on grain quality traits of parboiled milled rice. Rice grain quality traits are influenced by many QTL's (quantitative trait loci), part of a very complex network. It is assumed that as many as 600 QTLs directly or indirectly modulate rice grain quality traits; details can be found in Gramene Genome Database (http://www.gramene.org/). Furthermore, meta-QTL mapping reveals that quality traits like AC, protein content, organoleptic properties (for aromatic rice varieties), and viscosity were found to be influenced by a large number of QTL located in chromosome 3 and chromosome 6 (Sreenivasulu et al. 2015).

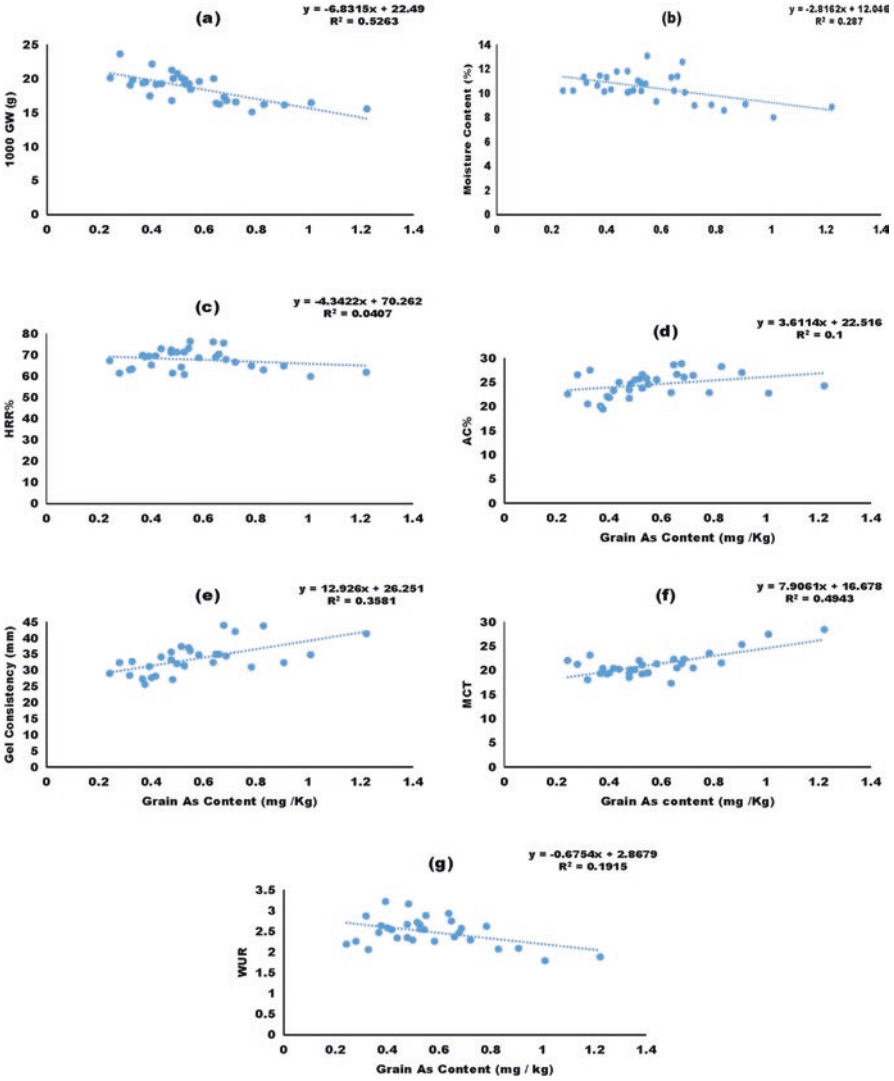

Fig. 3.1 Relationship among grain As content and (**a**) 1000 grain weight or 1000 GW, (**b**) moisture content, (**c**) head rice recovery percentage or HRR%, (**d**) amylose content (AC%), (**e**) gel consistency, (**f**) minimum cooking time (MCT), and (**g**) water uptake ratio (WUR) of the selected milled rice studied

The HRR% is one of the most desirable grain quality traits for milled rice selection (Sakurai et al. 2006; Fofana et al. 2010). The HRR% of milled rice has a great impact on grading rice varieties as well as to its economic value (Futakuchi et al. 2013). An HRR% of $\leq 50\%$ is often considered as an undesirable trait as it implies that $\leq 50\%$ of the milled rice is either thrown away as husk/bran or be considered as brewer's rice (use in a brewery) after the milling process. Results from current

Table 3.14 Pearson correlation study carried out among grain As content of non-parboiled milled rice and physicochemical properties and cooking characteristics of rice varieties cultivated in Nadia district

	As	GW	MC%	HRR%	AC%	GC	MCT	WUR
As	1	−0.726*	−0.536	−0.202	0.316	0.598*	0.703*	−0.438
GW		1	0.325	0.041	−0.249	−0.478	−0.472	0.156
MC%			1	0.646*	−0.094	−0.131	−0.616*	0.500
HRR%				1	−0.058	0.105	−0.561	0.334
AC%					1	0.654	0.277	−0.276
GC						1	0.305	−0.377
MCT							1	−0.675*
WUR								1

Grain As, total arsenic content in milled rice; 1000 K.W, weight of 1000 intact kernel; MC, moisture content; HRR%, head rice recovery percentage; AC, amylose content; GC, gel consistency; MCT, mean cooking time; WUR, water uptake ratio

* indicates correlation is significant at the 0.05 level, and ** indicates correlation is significant at the 0.01 level (2-tailed)

investigation show the HRR% of each of the studied varieties. HRR% of common studied varieties indicates a declining trend in HRR% with an increase in grain As content. Statistical interpretation with grain As content and HRR% shares an inverse relationship ($r = -0.202$; $R^2 = -0.0407$). The downward trend in the HRR% observed might be due to the enhancement of grain chalkiness which occurs due to irregular grain filling process (Fofana et al. 2011) or due to As stress. The effects of adverse environmental condition on rice yield and quality have been reported by various authors, such as reduction of yield by 6%, head HRR% by 9–14% for enhancement of 1 °C temperature, delay in transplanting (Peng et al. 2004; Welch et al. 2010; Lyman et al. 2013; Ghosh et al. 2004). We can assume that As stress cause variation physical traits by modulating starch modulating enzymes, specially wide range of variation in 1000 GW, and traits like HRR% supports the findings of Counce et al. (2005) partially who reported that high temperature (heat stress) can significantly modulate enzymes responsible for starch metabolism can influence grain morphology along with milling traits. Results from the current investigation support the above view (Tables 3.10, 3.11, 3.12 and 3.13). From economical or consumer's point of view, rice varieties with greater HRR% often fetch a higher price in the market (Sakurai et al. 2006) (Fig. 3.1a–c, g).

Among the chemical attributes of selected grain quality traits studied here, AC% and GC are the two most important characteristics that modulate cooking as well as eating characteristics. According to the opinions of Juliano et al. (1965) and Cagampang et al. (1973), AC% and GC have been treated as fundamental indicants directly related to rice eating quality characteristics. Buyers often pick the rice varieties having an intermediate AC% along with GC value (Huang et al. 1998). Findings from the current study show that As stress (latter accumulated in grain) positively stimulates AC% ($r = 0.316$; $R^2 = 0.1$) and GC ($r = 0.598$; $R^2 = 0.358$) among the studied varieties. Earlier investigations suggest that fluctuation in AC%

often imposes modulation in rice grain quality attributes (Krishnasamy and Seshu 1989). Findings of the current study reveal that AC% has a negative correlation with 1000 GW, HRR%, MCT, and WUR milling traits of grain before and after cooking, which supports the view of Lyon et al. (2000) and Moulick et al. (2016b). To explain the above trend of fluctuating AC% and other traits including cooking characteristics, findings of Dhaliwal et al. (1986) can be considered, who suggested that physicochemical properties (HRR%, yield, AC) and cooking characteristics (CT or MCT) including starch composition (due to reduction in starch transport) were negatively influenced shifting in climatic condition and delayed transplantation. It can be assumed that paddy cultivation in As-rich soil might influence physicochemical traits by altering starch transport which resulted in alter HRR%, AC%, and MCT in a variety, location-specific manner. Our findings support the view of Moulick et al. (2016b) regarding variation in MCT, who reported that grain As content significantly enhances cooking time ($r = 0.703$). Similar to correlational study carried out among the grain As content and grain quality attributes of all the studied varieties, regression was also carried out. Regressional interpretation carried out among grain As content and respective grain quality attributes reveals some interesting facts. Regressional study indicates that with a fluctuation of a unit of As content in grain decreases 6.831 (g) grain weight 4.34% (HRR%), 0.67% WUR, beside these an increase in gel consistency, MCT, AC by 12.93 mm 7.91 (min) ($\approx$ 8 min), 3.6% can be assumed. These huge variation in grain As content and their respective quality traits may arise to due variation in cropping season, varietal difference, and climatic condition (Table 3.14; Fig. 3.1a–g). The present study describes preliminary observations on grain quality traits (along with statistical interpretation) of a large number of varieties, cultivated in three locations for two successive years. The locations have a wide variation in soil physicochemical properties, As content in soil, irrigation water, etc. Our intention was to assess the grain quality attributes of rice varieties cultivated in As-contaminated agroecosystem to present a scenario that will provoke the plant breeders to pay special attention to quality attributes, besides less As accumulation capability. A series of comprehensive investigations on molecular justification among As stress and rice grain quality attributes will not only ensure food security for the nation but also enhance financial aspect related to rice.

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Chapter 4
Arsenic-Induced Oxidative Stress in Plants

Anna Kostecka-Gugała and Dariusz Latowski

Abstract Oxidative stress is a common phenomenon in organisms that are exposed to arsenic (As), as well as many other abiotic or biotic stresses. This chapter describes the influence of As on the production of individual reactive oxygen species (ROS) in various pathways of a plant cell. Inorganic As(V) disrupts the phosphorylation metabolism, interfering with, inter alia, the flow of cellular energy. During As(V) to As(III) reduction, the electron leakage leads to ROS formation, and the accompanying redox-driven methylation contributes further to more ROS generation. Inorganic As(III) reacts with sulfhydryl groups of proteins, glutathione (GSH), and phytochelatins, affecting several important cellular functions including those related to the oxidative stress. The description of As toxicity includes the As-induced ROS reactions with macromolecules: lipid peroxidation and protein and nucleic acid damage. Some cellular processes are affected by As, e.g., As-induced ROS are involved in the activation of MAPK signaling cascades resulting in targeting transcription factors and the gene expression. Redox imbalances influence the enzymatic antioxidant system and mobilize the cell to synthesize low-molecular-weight antioxidants which are important in the prevention of ROS-induced damage. Other metabolic consequences of As-induced oxygen stress in the plant cell are also described in the chapter.

Keywords Antioxidant defense · Oxidative stress · Metalloids · Reactive oxygen species · Soil pollution

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4.1 Introduction

The oxidative stress is defined as a dynamic state within the cell when natural forces are mobilized to overcome the effects of increasing level of reactive oxidants. The main reactive oxidants naturally produced in aerobic metabolism are reactive oxygen species (ROS). They are by-products of the respiratory and photosynthetic electron transport chains and more generally of many reactions occurring in cellular compartments with strong electron flow such as mitochondria or chloroplasts but also in peroxisomes, endoplasmic reticulum, plasma membrane, and apoplast (Apel and Hirt 2004; Li et al. 2009; Sharma et al. 2012; Choudhury et al. 2013). Most of the stress factors such as temperature, light, nutrient availability, and various kinds of contaminations, including arsenic (As), can also generate the oxidative stress and ROS production in the cell. To the present, the As-induced ROS production is best documented in mammalian cells (Huang et al. 2004). So far very few studies have been carried out on the source and especially on mechanisms of ROS generation in plants exposed to As (Abercrombie et al. 2008; Sharma 2012).

Most of ROS are free radicals. They are inherently unstable because of the presence of one or more unpaired electrons. As a result, they are highly reactive, although this varies from radical to radical. Their interactions with non-radical molecules result in a free-radical chain reaction with the generation of new free radicals, such as lipid peroxides, proteins, and nucleic acids (Halliwell 1994; Betteridge 2000). In general, the imbalance between ROS production and antioxidant system response is the direct cause of oxidative stress (Sahay and Gupta 2017).

The role of ROS is considered to be so essential that almost every disorder in cellular homeostasis leads to a change in their steady-state level (Mittler et al. 2011). In addition, the cell homeostasis is closely related to the presence of many other reactive species, such as reactive nitrogen species (RNS), reactive carbonyl species (RCS), reactive sulfur species (RSS), and halogens.

On the other hand, ROS, despite their destructive activity, act as second messengers, and their signals interfere with normal physiological responses (Schieber and Chandel 2014). Arsenic toxicity is caused partly by ROS-dependent regulation of cell signal transduction pathways. Our knowledge of these processes, regulated by As, remains incomplete.

4.2 Sites and Pathways of ROS Generation in Plants

In plant cells, the main organelle of ROS formation is chloroplasts, where ROS are generated in several sites of electron transport chains of both photosystems (PS). Under normal conditions, the electron flows from the excited photosystem centers to $NADP^+$ reducing it to NADPH. NADPH finally reduces CO_2 in the Calvin cycle. Under various stress conditions when CO_2 fixation is limited, overloading of electron transport chain results in leakage of electrons from ferredoxin to O_2 reducing it to

superoxide anion radical ($O_2^{\bullet-}$) in the process called Mehler reaction (Elstner 1991; Sharma et al. 2012). This radical is considered as the initial species in many reactions in which various ROS are produced. The electron leakage to O_2 may also occur between Q_A and Q_B plastochinones in electron transport chain of PSII and iron–sulfur (Fe–S) protein clusters of PSI (Cleland and Grace 1999; Sharma et al. 2012).

It is well documented that light-harvesting antennae produce singlet oxygen ($^1O_2$) when light-excited chlorophylls adopt the triplet state (Chl^3) and then reduce triplet oxygen (Krieger-Liszkay et al. 2008). The generation of $^1O_2$ is enhanced when the downstream components of the electron transfer chain are over-reduced, and the electrons cannot leave chlorophylls. $^1O_2$ readily reacts with lipids, proteins, and photosynthetic pigments. It is also rapidly quenched by water, so it operates locally; however, it is also involved in some signaling reactions (Shapiguzov et al. 2012).

The respiratory electron transport chain is the source of ROS in mitochondrial membranes. In flavoprotein region of NADH dehydrogenase segment (complex I), O_2 is directly reduced to $O_2^{\bullet-}$. When substrates for reaction conducted by complex I are limited, the transport of electrons occurs from the complex II to complex I, and this reversed electron flow is known to increase ROS formation in complex I (Turrens 2003). The ubiquinone–cytochrome c_1 region (complex III) is also a site where oxygen can be reduced to $O_2^{\bullet-}$. Highly reducing ubisemiquinone radical, the formation of which is the result of the electron transfer from fully reduced ubiquinone to cytochrome c_1, is considered to contribute to $O_2^{\bullet-}$ generation (Murphy 2009; Sharma et al. 2012). Additionally, several enzymes of mitochondrial matrix, e.g., aconitase, can produce ROS directly, while some others as 1-galactono-γ-lactone dehydrogenase (GAL) can supply electron transport chain with electrons (Andreyev et al. 2005; Rasmusson et al. 2008).

Peroxisomes, endoplasmic reticulum, plasma membranes, and cell wall are other organelles also engaged in ROS production and conversion in plant cells (Table 4.1).

Both $O_2^{\bullet-}$ and H_2O_2 are only moderately reactive to organic molecules. Furthermore, they are intensively eliminated by the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidases as well as nonenzymatic antioxidants, including GSH, ascorbic acid (AsA), tocopherols, flavonoids, carotenoids, and many others (Valko et al. 2007; Fridovich 1995). Due to its charge, $O_2^{\bullet-}$ is not thought to cross membranes passively, so most of it is consumed in the organelle where it is formed. It is assumed that the most $O_2^{\bullet-}$ is converted to H_2O_2. There are also some enzymes in the cell that can produce H_2O_2, e.g., D-amino acid oxidase and acyl-CoA oxidase. Although H_2O_2 is a non-radical species and it is relatively stable compared to other ROS, it can cause oxidative damage at a relatively low concentration (Sharma et al. 2012). It is well dissolved in water and can easily penetrate the membranes, so it can cause oxidative damage far from the site of formation.

One of the most harmful reactions initiated by ROS in the cell is chain peroxidation of polyunsaturated lipid fatty acids which causes uncontrolled electrolyte leakage through the bilayer. In the presence of trace amounts of transient metals, e.g., iron, due to Fenton reaction, H_2O_2 is converted into hydroxyl radical (OH•), an

Table 4.1 Types of reactive oxygen species (ROS) with sites of their origin on the subcellular and biochemical level other than chloroplasts and mitochondria

Organelle	Type of ROS	Process of ROS production	Source
Peroxisomes	$O_2^{\bullet-}$ H_2O_2	Fatty acid chain β-oxidation	López-Huertas et al. (1999), Baker and Graham (2002), and Sharma et al. (2012)
		Glycolate oxidase reaction during photorespiration	
		Enzymatic reaction of flavin oxidases	
		Dismutation of $O_2^{\bullet-}$ by catalase	
		Xanthine and hypoxanthine oxidation to uric acid in peroxisome matrix	
		Reactions in peroxisomal membranes	
Endoplasmic reticulum	$O_2^{\bullet-}$	NAD(P)H-dependent electron transport involving cyt P_{450}; an organic substrate (RH) reacts with cyt P_{450} and then is reduced by a flavoprotein to the radical intermediate (cyt P_{450} R^-). In the reaction with oxygen, it forms cyt P_{450} ROO^- which can be reduced by cyt *b* or occasionally release $O_2^{\bullet-}$ during decomposition	Mittler (2002) and Sharma et al. (2012)
Plasma membranes	$O_2^{\bullet-}$	Electron transfer from NAD(P)H to oxygen, forming $O_2^{\bullet-}$ by NAD(P)H oxidase (NOX). This enzyme is proposed to play a major role in the production of ROS in plants under stress conditions. NOX is also considered a key factor in the immune system of plants	Lynn et al. (2000), Torres et al. (2002), Kwak et al. (2003), Apel and Hirt (2004), and Sharma et al. (2012)
Cell wall	H_2O_2	NADH oxidation catalyzed by a cell wall peroxidase (NADH–POD)	Mäder et al. (1980), Elstner (1991), Apel and Hirt (2004), Šimonovičová et al. (2004), Stoeva et al. (2005a), Heyno et al. (2011), and Sharma et al. (2012)
		Reduction of diamines or polyamines catalyzed by diamine oxidases	
		Oxidation of oxalates by oxalate oxidase	

extremely reactive species which generates alkoxyl (RO•) and peroxyl (ROO•) radicals, as well as organic hydroperoxides (ROOH), responsible for reactions of the membrane destruction (Halliwell and Gutteridge 1986). In consequence, this process profoundly affects the membrane enzymatic and transport activities. In mitochondrial membranes $O_2^{\bullet-}$ has been found to initiate morphologic changes, reducing their integrity and potential (Valko et al. 2005; Jomova et al. 2011). ROS-induced changes have also been observed in membranes of the chloroplast, which subsequently cause swelling and breakage of thylakoids. These processes have been well described for a moss *Sphagnum nemoreum* and fern *Pteris vittata* (Simola 1997; Li et al. 2006).

4.3 ROS Generation Under Plant Exposure to As

Formation of ROS is a common effect of As appearance in the cells (Sharma 2012; Finnegan and Chen 2012; Islam et al. 2015). Arsenic toxicity and its biochemical consequences have been mostly evaluated in ferns and mosses as well as in several macrophytes and higher plants. Many studies have allowed concluding that observed modulations, mainly in the antioxidant system, are not specific to As stress because such changes have also been reported for heavy metals (Kumar et al. 2015).

After entering the cells, As being a redox-active metalloid readily generates ROS, such as $O_2^{\bullet-}$ (Lynn et al. 2000), H_2O_2 (Wang et al. 1996; Barchowsky et al. 1996; Chen et al. 1998), OH•, nitric oxide (NO) (Gurr et al. 1998), as well as dimethylarsinic peroxyl radical [$(CH_3)_2AsOO\bullet$] and dimethyl As radical [$(CH_3)_2As\bullet$] (Yamanaka et al. 1997, 2001). Elevated production of ROS has been found in many plant species under As-induced oxidative stress. For example, H_2O_2 has been extensively generated in grass *Holcus lanatus* (Hartley-Whitaker et al. 2001a), red clover (*Trifolium pratense*) (Mascher et al. 2002), mung bean (*Vigna radiata*) (Singh et al. 2007), and rice (*Oryza sativa*) (Shri et al. 2009). It has been reported that in the presence of As, elevated production of $O_2^{\bullet-}$ is generated by NAD(P)H oxidase (NOX) due to As-dependent upregulation of p22phax subunit responsible for the electron transfer (Lynn et al. 2000; Hunt et al. 2014; Chou et al. 2004) (Table 4.1). However, there are suggestions that As can cause oxidative stress by inducing glycolate oxidase, whereas NOX are not the main source of ROS although NOX may be critical in regulating the antioxidant defenses as well as the transport and translocation of As, P, K, S, Ca, Cu, Zn, and Fe (Gupta et al. 2013).

Recent studies have reported that ROS induction mechanisms differ among the As species. Plants face As predominantly in two anionic forms of arsenate [As(V)] or arsenite [As(III)] (Tangahu et al. 2011). Both of them reveal cytotoxic effects; however, the mechanism of their action within the cell is different. It has been observed that As(V) activates the antioxidant system in cells of macrophyte *Hydrilla verticillata*, while As(III) increases the production of chelators, such as phytochelatins (PCs) (Srivastava et al. 2007). The significant differences in antioxidant and amino acid alterations, especially in the induction of PCs, have been observed in As-accumulating rice plants of two contrasting genotypes (Dave et al. 2013). As(V) toxicity seems to be a result of the ability to substitute phosphate groups in phosphorylation reactions leading to the formation of As(V) adducts that are often unstable (Finnegan and Chen 2012). Replacing phosphate in many reactions, it can disrupt several phosphate-dependent aspects of metabolism (Cozzolino et al. 2010). It can form Glc-6-arsenate; however, it has been demonstrated only in vitro. As(V) inhibits the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) which catalyzes the only glucose oxidation reaction of glycolysis and finally leads to an impairment of cellular respiration and switching off ATP formation. GAPDH is also known for its multiple functions, for example, it is involved in the vesicle transport from the endoplasmic reticulum to the Golgi apparatus and in maintaining cellular iron homeostasis (Boradia et al. 2014).

A lot of evidence reports that iAs(V) in the cytoplasm may hinder oxidative phosphorylation replacing phosphate in ATP (adenosine triphosphate) and form ADP–As (adenosine diphosphate–arsenate) and thus disturbing the cellular energy flow. The bond formed by As in ADP–As is less stable in comparison with the corresponding bond in ATP. Its rapid hydrolysis results in premature uncoupling oxidative phosphorylation (Gresser 1981; Chakrabarty 2015). Although phosphate and arsenate are structurally similar, recent studies on bacteria show that As does not replace phosphorus in DNA or RNA (Erb et al. 2012; Reaves et al. 2012). Elevated As(V) levels are also the cause of activation of antioxidant enzymes, such as SOD, ascorbate peroxidase (APX), and guaiacol peroxidase (GPX). This stimulation is especially marked in high As-accumulating plants (Dave et al. 2013).

The second way of As(V) transformation in the cells is its reduction. The conversion of As(V) to As(III) in plant cells leads to the ROS generation (Mascher et al. 2002). Following uptake, As(V) is reduced rapidly, and most plants have a high facility for As(V) reduction. This reaction occurs both enzymatically and nonenzymatically (Finnegan and Chen 2012); in the nonenzymatic pathway, GSH (Delnomdedieu et al. 1994) and GSH reductase (Foyer and Noctor 2011) can be engaged. It has also been found that at least 90% of the As reduction capacity in the root protein extracts of *Holcus lanatus* is enzymatic (Bleeker et al. 2006). Several studies have confirmed that the main enzyme involved in As(V) reduction in plants is arsenate reductase (ACR), using GSH as an electron donor (Ellis et al. 2006; Zhao et al. 2009; Sánchez-Bermejo et al. 2014; Meadows 2014). The reaction is coupled with NAD(P)H oxidation via GSSH reduction catalyzed by glutathione reductase (GR) (Ellis et al. 2006; Sharma 2012). The data show that other enzymes, e.g., cytosolic triosephosphate isomerase (cTPI) and mitochondrial glutaredoxin (GLR5), can also be directly or indirectly involved in this process (Rathinasabapathi et al. 2006; Sundaram et al. 2008; Zhao et al. 2009). The As reduction may be followed by methylation regarded as a detoxification process because the pentavalent methylated As metabolites like monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)) are generally known to be less toxic and excreted more readily than iAs(III) (Flora et al. 2007). In algae cells, arsenite methyltransferase is involved in the reactions of both MMA(V) and DMA(V) formation (Tang et al. 2016). To date, the origin, pathways, and enzymology of As methylation in plants have not been sufficiently explained (Zhao et al. 2009).

The studies show that dimethylarsine (DMAH, $(CH_3)_2AsH$) can react with oxygen to form DMA radicals and superoxide anions and in this way contribute to increasing ROS concentration in plants (Sharma 2012; Chakrabarty 2015). Exposure to monomethylarsonous acid (MMA(III)) results in the formation of ROS mainly in mitochondria, whereas dimethylarsenous acid (DMA(III)) affects ROS generation in other organelles as well as arising of DNA damage symptoms. This suggests the different mechanisms of ROS induction among the three arsenicals, i.e., iAs(III), MMA(III), and DMA(III) (Naranmandura et al. 2011). Moreover, it has been reported that DMA is involved in the Fe-dependent oxidative stress induction by iron ion release from a ferritin molecule (Shi et al. 2004). Recent theoretical studies show that the order of increasing favorability for arsenical activation by ROS is

$O_2 < O_2^{\bullet-} <$ HO•, and the oxidation of DMA(III) to DMA(V) is highly exoergic in many reactions with concomitant formation of free radicals. This is followed by the least favorable MMA(III) and iAs(III) (Zamora et al. 2014).

Although As(III) may be a product of As(V) reduction within the cell, it can directly enter plant cells (Islam et al. 2015). In a result of the oxidative stress induced by As(III), many proteins are damaged, especially those rich in cysteine, because As(III) binds to their sulfhydryl (SH–) groups. Oxidation of cysteine leads to protein conformational changes and may modify active centers of enzymes resulting in the loss of their function. For example, binding of As(III) to thioredoxin reductase disrupts the redox balance (Wang et al. 2007) and to NO synthase decreases bioactive NO content (Sumi et al. 2005). The activity of several enzymes can also decrease due to inactivation of their dithiol cofactors. By binding to sulfhydryl groups, As(III) inhibits interaction of lipoic acid, a cofactor for pyruvate dehydrogenase (PDH), with its apoenzyme. PDH catalyzes the oxidation of pyruvate to acetyl-CoA with NAD^+ (Hunt et al. 2014). Acetyl-CoA participates in several cycles and pathways, e.g., the citric acid cycle and the glyoxylate cycle. Inhibition of its synthesis decreases ATP production and affects energy metabolism critically (Bergquist et al. 2009). Inhibition of glucose synthesis from fatty acids *via* acetate is harmful especially in a germination stage of oil plants. Moreover, synthesis of isoprenoids, including steroids, carotenoids, and abscisic acid, also requires acetyl-CoA as a precursor. Carotenoids in plants protect light-harvesting complexes against light overexcitation and photooxidative damage. It is well documented that they dissipate the excess of absorbed energy, scavenging Chl^3, thereby preventing the formation of ROS such as $^1O_2$. They can also quench ROS directly once formed during photooxidative stress (Young and Britton 1990; Strzałka et al. 2003). Acetyl-CoA deficiency can also decrease the synthesis of zeaxanthin by that diminishing photoprotective significance of the xanthophyll cycle. More generally, an insufficient amount of carotenoids deepens the symptoms of oxidative stress, eventually leading to bleaching of plant tissues (Young and Britton 1990).

The endoplasmic reticulum as a site for the synthesis and packing of proteins is strongly coupled to disulfide bond formation. A large number of redox carriers participate in the delivery of redox equivalents to achieve a correct formation of protein disulfide bonds (Espinosa-Diez et al. 2015). The proteins with a zinc finger motifs in their structure, e.g., numerous enzymes involved in transcription and DNA repairing, are particularly sensitive to redox potential changes. The studies have shown that As(III) binds to the proteins which have three or four cysteine residues in the zinc-binding site. When oxidizing the SH– groups responsible for zinc binding, the Zn molecule is substituted by As(III) resulting in the enzyme inactivation (Kröncke and Klotz 2009). Arsenic(III)-substituted molecules are especially sensitive to oxidation by ROS, and during this reaction As(III) is released and can again enter the cycle of protein damage (Zhao et al. 2012; Zhou et al. 2015).

As(III) binds to a large number of free GSH molecules which are then secreted into the vacuole or from the cell by ATP-binding cassette (ABC) transporters. Although this process leads to a decrease in As(III) concentration in the cell and

hence to cellular detoxification, this results in a depletion in GSH pool, thereby reducing the defense against oxidative stress (Flora et al. 2007). It is obvious that GSH insufficiency decreases the activity of many enzymes using it as a substrate, e.g., of glutaredoxins, which are electron carriers in GSH-dependent syntheses, involved in signaling of salicylic acid, as well as of adenylyl-sulfate reductase, an enzyme of the sulfur assimilation pathway. GSH is also a main precursor of phytochelatins (PC), small peptides of general structure $(\gamma\text{-Glu–Cys})_n$–Gly ($n$ = 2–11), although $(\gamma\text{-Glu–Cys})_2$–Gly ($PC_2$) and $(\gamma\text{-Glu–Cys})_3$–Gly ($PC_3$) are the most common. As metallothioneins III, they chelate silver, As, cadmium, copper, and mercury ions (Raab et al. 2005) and help to secrete them into the vacuole, thus functioning in the first step of their detoxification (Schmöger et al. 2000; Song et al. 2010; Wojas et al. 2010). Since GSH plays such an essential role in the maintaining of the physiological balance between pro- and antioxidants, it is not surprising that increased GSH oxidation introduces disturbances in the cellular redox regulatory system.

Metal-induced lipid peroxidation was described in plants a long time ago. For estimation, the scale of this process the content of one of its main products, malondialdehyde (MDA), is the most frequently analyzed. In the study of Gupta et al. (2013), MDA content has increased in the 1- and 5-day As-exposed *Arabidopsis* leaves, although statistically significant differences have only been observed after longer treatment. Both malondialdehyde (MDA) and electrolyte leakage have significantly increased in leaves and roots of legume fenugreek (*Trigonella foenumgraecum*) when plants have been exposed to As in the highest concentration used (30 mg/kg soil) (Talukdar 2013). Recent studies of Singh et al. (2017) reveal the increase of by-products of lipid peroxidation, thiobarbituric acid reactive substances (TBARS), including MDA as one of the main products, with increase in As concentration up to 200 μM in roots and shoots of the new potential hyperaccumulator, perennial grass vetiver (*Vetiveria zizanoides*). It is worth noting that after 14 days of the experiment, the increase in TBARS concentration has been significantly larger.

As(III)-induced ROS and NO accumulation within the cell causes much damages to nuclear acids (Lynn et al. 2000). The modification of nitrogenous bases (e.g., 8-hydroxy-2'deoxyguanosine (8-OHdG) forming) can result in substitution in apurinic and apyrimidinic sites, nucleotide deletion, *dissociation* of proteins covalently bound to DNA, and single-stranded DNA cracks (Lynn et al. 2000; Jena 2012; Mucha et al. 2017). Studies on yeast genome have shown that As can induce double-stranded DNA breaks in all phases of the cell cycle and this process may be carried out both directly and through the ROS generation (Litwin et al. 2013). In As-stressed pea (*Pisum sativum*) plant, growth impairment due to a chromosome or mitotic microtubule damages has been observed (Dho et al. 2010).

As(III) induces morphological changes in the mitochondrial outer membrane, causing it to break and thus ROS escape (Jomova et al. 2011; Hosseini et al. 2013). Damaged mitochondria are removed by autophagocytosis, and normally this process prevents the spread of ROS. However, data show that As(III) is able to stop autophagocytosis, probably by inhibition of autophagosome and lysosome fusion. In a consequence, negative effects of ROS acting accumulate (Lau et al. 2013; Qi et al. 2014; Mucha et al. 2017).

In addition to ROS, reactive nitrogen species (RNS), including NO, NO_2, N_2O_3, N_2O_4, $ONOO^-$, S-nitrosoglutathione, and S-nitrosothiols (Sahay and Gupta 2017), can also be produced extensively in multiple cell sites during exposure to As (Ding et al. 2005; Sharma 2012) and therefore cause the nitrosative stress (Jomova et al. 2011) manifested in reactions such as lipid nitration, S-nitrosylation, metal nitrosylation, tyrosine nitration, etc. (Sahay and Gupta 2017).

One of the most ubiquitous RNS, a lipophilic free radical with relatively long half-life NO, is known for both the beneficial and damaging functions in the plant cell. NO is a key signaling molecule which plays a broad physiological and pathophysiological roles (Islam et al. 2015). It reacts rapidly with ROS, thiols, and proteins inducing signals that directly and indirectly regulate enzymatic activities (Siddiqui et al. 2011; Sandalio et al. 2012), hormonal responses, ROS production and degradation, immune defense mechanism to pathogens, cellular transport, and cell death (Wink et al. 2001; Palmieri et al. 2008; Domingos et al. 2015) and influence germination, flowering, ripening of fruits, maturation, and senescence of organs (Arasimowicz and Floryszak-Wieczorek 2007). NO prevents oxidation damage induced by heavy metals and metalloids by promoting the conversion of $O_2^{\bullet-}$ into H_2O_2 and O_2 as well as by enhancing activity of H_2O_2-scavenging enzymes protecting cells against H_2O_2-mediated cell death, alkyl hydroperoxides, and xanthine oxidase (Wink et al. 2001; Lamattina et al. 2003; Shi et al. 2005; Zheng et al. 2009). On the other hand, depending on its concentration and location in plant cells or tissues, NO acts as an antioxidant and scavenger of several other reactive intermediates (Oz et al. 2015), including $O_2^{\bullet-}$ (Gross et al. 2013).

Recent studies have confirmed the importance of NO in decreasing As toxicity by regulating antioxidant defense systems mentioned above (Singh et al. 2009, 2016). In plants exposed to As, NO has been found to decrease the oxidative damage as an activator of antioxidant enzymes (SOD, APX, and CAT) and a stimulator of synthesis of other antioxidants (Huang et al. 2002; Song et al. 2006; Jin et al. 2010). Moreover, it is believed that NO can delay or prevent programmed cell death, probably due to its influence on ROS concentration (Beligni et al. 2002); however, recent studies have not confirmed As participation in programmed cell death initiated by NO (Farnese et al. 2017). The addition of exogenous NO to As-treated leaves of *tall fescue* (*Festuca arundinacea*) significantly reduces ROS levels, increases antioxidant enzyme activities, and prevents lipid peroxidation (Jin et al. 2010). NO supplementation to As(V)-exposed plants of rice helps maintain a high GSH/GSSG ratio (Singh et al. 2016; Duan et al. 2011).

Under As-induced oxidative stress, NO depletion has been observed (Kumagai and Sumi 2007). This effect is probably caused by interaction of NO and ROS generated by As (Kumagai 2009; Hunt et al. 2014). Moreover, one of the methylated As compound, MMA(III), readily binds to thiol groups of NO synthase (NOS), the enzyme responsible for NO formation in animals and probably also in plants (Domingos et al. 2015). Animal NOS can also be indirectly inhibited by As during the reduction of tetrahydrobiopterin/sapropterin (BH4), a cofactor of NOS enzyme complex (Kumagai and Sumi 2007; Kumagai 2009; Hunt et al. 2014). No data in this field in plants have been presented so far.

4.4 As-Induced Oxidative Stress in Selected Cellular Processes

Plant cells have evolved various signaling mechanisms that activate their defense systems, thus facilitating survival under As-induced oxidative stress. Signaling molecules involved in the reduction of As toxicity are proteins (numerous kinases, G-proteins), hormones, transcription factors, ROS, NO, and Ca^{2+} (Chan et al. 2016). The pathways by which the stress signals in plants are perceived are still poorly researched (Islam et al. 2015).

Many pieces of evidence point to ROS as central sensors for activation of signaling cascades resulting in gene expression and targeting the transcription factors (Mittler et al. 2011). Ultimately, they affect plant acquisition of tolerance to biotic and abiotic stress, including As stress (Torres et al. 2002; Miller et al. 2008), but also such basic processes like gravitropism (Joo et al. 2001), cell stomatal closure (Neill et al. 2002; Kwak et al. 2003; Yan et al. 2007), cell division and differentiation (Mittler et al. 2011), and programmed cell death (Mittler 2002; Bethke and Jones 2001). Overproduction of ROS, as a consequence of the abiotic stress in plants, is regarded to be one of the determinants of autophagy (Wang et al. 2017); however, there is no research into the process suggesting it could be directly induced by As. In leaves of tobacco (*Nicotiana tabacum*) exposed to high concentration of H_2O_2, upregulation of 175 genes out of which many are known to be involved in signaling activities has been observed (Vranová et al. 2002). The studies of Desikan et al. (2000, 2001) on *Arabidopsis* plants have confirmed the contribution of H_2O_2 in cellular signaling, indicating that the oxidative stress leads to change in expression of genes involved in signal transduction. Alternatively to the signaling function, ROS can also directly oxidize the components of signaling pathways. This complex phenomenon proves a huge specificity of ROS interaction with certain small peptides, hormones, and lipids (Mittler et al. 2011).

Being potentially harmful to the cell, ROS in low or moderate concentrations are important initiators or transducers of molecular signals. It is well documented that in chloroplasts ROS have been involved in retrograde signaling, i.e., chloroplasts emit signals that regulate the expression of nuclear genes, e.g., of programmed cell death or adjustment of photosynthesis to altered conditions (Nott et al. 2006; Galvez-Valdivieso and Mullineaux 2010). Now we also know that the photosynthetic functions during plant acclimatization can also be regulated by cell wall signals (Padmanabhan and Dinesh-Kumar 2010). Rapid accumulation of ROS in the wall in response to abiotic and biotic stimuli is called apoplastic oxidative burst, and it depends on several enzymes such as cell wall peroxidases (Wojtaszek 1997; Bindschedler et al. 2006) and NOX (Wojtaszek 1997; Torres et al. 2002; Suzuki et al. 2011). The latter ones are commonly known as respiratory burst oxidase homologs (Rboh) (Torres and Dangl 2005), and they are transmembrane flavoproteins that oxidize cytoplasmic NADPH, translocate electrons across the plasma membrane, and reduce extracellular oxygen to $O_2^{\bullet -}$ in the wall. Due to its charge, $O_2^{\bullet -}$ cannot penetrate the lipid bilayer passively, so most of it remains in the apoplast,

where it is rapidly converted into H_2O_2, either spontaneously or in a reaction catalyzed by SOD, a first defender among antioxidant system (AOS) enzymes. The stability and ability to long-distance diffusion make H_2O_2 a good candidate for the signaling molecule involved in the precise regulation of specific cellular processes (Muller-Delp et al. 2012). H_2O_2 can be imported into the cytoplasm via channel proteins, aquaporins (Soto et al. 2012; Shapiguzov et al. 2012), or react with extracellular apoplastic protein (AP) or transmembrane proteins (including receptor-like kinases, RLKs) ultimately resulting in changes in gene expression through intracellular signaling pathways, involving MAPKs (mitogen-activated protein kinases) which are signaling transducers which amplify signals through reversible phosphorylation at Tyr and Thr residues and impact many processes, including cell proliferation (Shapiguzov et al. 2012).

There are many pieces of evidence that signaling cascade which involves MAPKs is activated by ROS and NO when the plants grow under As stress (Pfannschmidt et al. 2003; Foyer and Noctor 2005; Kiffin et al. 2006; Jin et al. 2010). Analysis of *Arabidopsis* transcriptome has shown that under As(V)-induced stress, genes involved in secondary cell wall biogenesis, cell cycle, and oligopeptide transport have been mainly downregulated. Several genes of proteins which participate in signaling, such as MAPK, MAPK kinase, and Ca^{2+}-dependent protein kinase (CDPK), have been upregulated (Huang et al. 2012). It has been proved that As(III)-induced H_2O_2 is involved in activation of several MAPKs such as MPK3 and MPK6 (Jonak et al. 2002; Gupta et al. 2009). In As-stressed *Arabidopsis*, H_2O_2 has induced the increase in nucleotide diphosphate (NDP) expression which activates many of MAPKs (Hancock et al. 2001; Moon et al. 2003; Zhang et al. 2006). When As is applied to rice plants, different components of this signaling cascade (e.g., OsMPK3, OsMPK4, and OsMKK4) have been upregulated (Rao et al. 2011; Huang et al. 2012). As MAPKs are a part of plants' innate immunity, it is probable that As-induced oxidative stress impacts this poorly known aspect of plant functioning (Asai et al. 2002). Until now, a complete analysis of MAPK gene family involved in As-induced signal transduction in plants has not been performed (Rao et al. 2011).

In plant cells, ubiquitous intracellular second messengers are calcium ions. They are involved in regulation of ROS production in the apoplast and chloroplasts. As stress has been found to be responsible for the increase of Ca^{2+} concentration in Tex-Mex tobacco (*Nicotiana plumbaginifolia*) (Price et al. 1994) and *P. vittata* (Li et al. 2006). Under abiotic stress, Ca^{2+} is involved in regulation of multifunctional intermediate Ca^{2+}-binding protein, calmodulin (Yang and Poovaiah 2003; Maksymiec 2007; Islam et al. 2015). Rice shoots exposed to As(V) showed the upregulation of 15 calmodulin as well as calmodulin-like genes responsible for metabolism, transport, and plant tolerance to heavy metals (Huang et al. 2012).

There are not many studies on hormones of plants growing under oxidative stress caused by As; however, links between plants' exposure to heavy metals and modulations in phytohormone concentrations are being considered (Islam et al. 2015). It is known that ROS in low concentrations are the second messengers in plant hormone (auxins, gibberellic, abscisic, jasmonic, and salicylic acids) response, influencing root gravitropism, seed germination, programmed cell death, stomata

closure, lignin biosynthesis, hypersensitive responses, and osmotic stress (Sharma et al. 2012). The research of Farooq et al. (2016) has revealed that the application of methyl jasmonate (MJ) into rape (*Brassica napus*) leaves minimizes the oxidative stress through the decrease of ROS synthesis (H_2O_2 and OH) and by the maintenance of GSH and AsA in their reduced forms. Enhanced enzymatic activities and gene expression of antioxidants (SOD, APX, CAT, and glutathione peroxidase), as well as secondary metabolites, suggest that MJ plays an effective role in the regulation of multiple transcriptional pathways involved in oxidative stress responses. Genes for jasmonic acid (JA) synthesis have been found to be upregulated both in roots and shoots upon As exposure in rice (Yu et al. 2012; Islam et al. 2015). Increased JA synthesis in As-treated plants such as *Arabidopsis* promotes the expression of certain genes necessary for signaling and response to stress and those important in GSH metabolism (Xiang and Oliver 1998; Agrawal et al. 2003; Islam et al. 2015). Transcriptomic analysis of As-treated plants has confirmed upregulation of JA, abscisic acid, and ethylene signaling genes (Islam et al. 2015). The responses mentioned above suggest that hormonal signaling can significantly influence physiological processes in plants when they are exposed to this metalloid (Huang et al. 2012).

4.5 Effect of As on the Enzymatic Antioxidant System

It is reasonable that the imbalance of redox state in any of cellular compartments results in alterations in the activity of antioxidant enzymes as well as other enzymes engaged in the redox balance and these processes lead to oxidative stress induction. The opinion on the importance of individual enzymes in alleviating oxidative stress is divided as studies on the activity of several enzymes have yielded partially divergent results. One of the probable reasons could be too narrow ranges of As concentrations used in some experiments, i.e., the concentration-dependent effects observed for the low range may be only a fragment of the overall picture of As influence on the antioxidant system.

According to You and Chan (2015), the main ROS-scavenging enzymes involving in abiotic stress in plants include SOD, APX, CAT, glutathione peroxidase (GPX), MDHAR, DHAR, glutathione reductase (GR), glutathione *S*-transferase (GST), and peroxiredoxin. Many investigations have shown that SOD, CAT, GST, glutaredoxin, and/or peroxidase activities often increase in response to As exposure (Mylona et al. 1998; Srivastava et al. 2005; Geng et al. 2006; Abercrombie et al. 2008; Norton et al. 2008; Chakrabarty et al. 2009; Finnegan and Chen 2012; Pandey et al. 2015). In 2004 Cao et al. have demonstrated that the activities of SOD, CAT, APX, and GPX in the As hyperaccumulator, Chinese brake fern (*P. vittata*), have increased in plants growing in soil with lower As concentration (up to 20 mg kg^{-1}) but decreased in those growing in the higher concentrations. According to Foyer and Noctor (2005, 2011) as well as Noctor et al. (2012), APX, DHAR, and GR operating within ascorbate (AsA)–glutathione (GSH) cycle as well CAT and GST outside this

cycle are the predominant enzymatic antioxidant defense components against ROS-induced oxidative imbalance in the cell. On the other hand, several reports deny such a significant role of CAT in ROS detoxification in As-treated mung bean, moss *Taxithelium nepalense*, *Arabidopsis*, and lentil (*Lens culinaris*) (Singh et al. 2007; Gupta et al. 2013; Talukdar and Talukdar 2014; Talukdar 2016). According to proteomic analysis, several enzymes have been identified as important for cellular redox homeostasis (e.g., three SODs, two GPX, one peroxiredoxin, and one *p*-benzoquinone reductase) besides four additional, functionally heterogeneous, proteins have been identified as highly responsive to As in maize roots (Requejo and Tena 2005). Transcriptomic analysis of ROS-related genes reveals the regulation of alternative oxidase (AOX), GR, GST, and class III peroxidase (PRX) and the upregulation of glutaredoxin and thioredoxin in rice roots with As(V) treatment (Huang et al. 2012). Also in rice, at least ten GST genes are upregulated in response to As(V) exposure, while no more than two are downregulated (Norton et al. 2008; Chakrabarty et al. 2009). On contrary, changes in GST gene expression do not appear to have such a significant role in the plant response to As(III) (Chakrabarty et al. 2009). Since $O_2^{\bullet-}$ is the initial product of aerobic metabolism, the enzyme responsible for its reduction plays a key antioxidant role in the cell. It is superoxide dismutase (SOD) (EC 1.15.1.1) which catalyzes the reaction of dismutation (disproportionation) of $O_2^{\bullet-}$ to longer-lasting H_2O_2 and O_2. Removal of $O_2^{\bullet-}$ is essential to avoid inhibition of several enzymes including those of Calvin cycle (Tanaka et al. 1982; Sharma 2012). Studies performed on As-stressed plants have shown a divergent SOD response to the excessed amount of this metalloid. In maize, As-sensitive clones of grass *Holcus lanatus*, and As hyperaccumulator Chinese brake fern (*P. vittata*), the enzyme is induced by low concentration of As and either stays at the same level or decreases in activity at higher amounts (Mylona et al. 1998; Hartley-Whitaker et al. 2001a; Cao et al. 2004; Finnegan and Chen 2012). On the other hand, Talukdar (2013) has observed a concentration-dependent strong induction of SOD in fenugreek (*Trigonella foenum-graecum)* leaves and shoots treated with As in three concentrations (10, 20, and 30 mg As/kg soil). According to Meharg and Hartley-Whitaker (2002), the variation in SOD activity may be a consequence of its molecular structure; SOD isoforms contain metal cofactors: Cu, Zn, Mn, and Fe (Cu/ZnSOD, MnSOD, and FeSOD, respectively). However, the explanation may also be at the level of gene expression. In *Arabidopsis*, transcripts for genes encoding a cytosolic and chloroplastic Cu/ZnSOD isoform have been induced more than twice by As(V) treatment, while transcripts for FeSOD have been suppressed for about fivefold (Abercrombie et al. 2008). The upregulation of Cu/ZnSOD has been reported in rice seedlings (Shri et al. 2009) as well as one MnSOD and two major Cu/ZnSOD isoenzymes in red clover, both exposed to an elevated concentration of As (Mascher et al. 2002; Sharma 2012). Recent studies of Singh et al. (2017) revealed induction of SOD in vetiver grass (*V. zizanoides*); in its roots, SOD activity has significantly increased when exposed to As for 14 days, whereas in the shoots, higher SOD activity has been observed both after 7 and 14 days of the experiment.

A product of $O_2^{\bullet-}$ decomposition, H_2O_2, can then be converted into O_2 and H_2O without consuming reducing equivalents by catalase (EC 1.11.1.6), a tetrameric

evolutionarily conservative enzyme found in peroxisomes, glyoxysomes, cytosol, mitochondria, and root nodules (Sharma 2012). Increased activity of CAT is reported as a common result of As exposure to plants (Mylona et al. 1998; Cao et al. 2004; Srivastava et al. 2005; Geng et al. 2006; Duman et al. 2010; Nath et al. 2014). Higher activity of CAT has been reported in As-tolerant Chinese brake fern (*P. vittata*) than in As-sensitive slender brake fern (*Pteris ensiformis*) and Boston fern (*Nephrolepis exaltata*) (Srivastava et al. 2005; Sharma 2012). Conversely, an As-induced decrease in CAT activity has been discovered in mung bean, *Taxithelium nepalense*, *Arabidopsis*, and lentil (Singh et al. 2007; Gupta et al. 2013; Talukdar and Talukdar 2014; Talukdar 2016). The CAT induction in fenugreek (*T. foenum-graecum*) leaves and shoots treated with As in three concentrations (10, 20, and 30 mg As/kg soil) as well as with accompanying phosphorus (200 mg P/kg soil + 30 mg As/kg soil) has been observed only in leaves and shoots treated with As in the moderate concentration. In the highest concentration of As, the enzyme decrease has been evident, however not in the plants additionally treated with P (Talukdar 2013). In vetiver grass (*V. zizanoides*), CAT upregulation has been observed, especially after longer (14 day) As exposure at the highest (200 μM) concentration of the metalloid in the roots and shoots (Singh et al. 2017).

Ascorbate peroxidase (APX, E.C.1.11.1.11) is another H_2O_2 decomposing enzyme containing heme and located in plastid membranes and stroma. Its function is to catalyze H_2O_2 reduction to H_2O and MDHA. This reaction requires AsA and GSH regeneration system, i.e., AsA–GSH cycle. APX activity seems to be crucial for plants since chloroplasts do not contain CAT (Sharma 2012). During exposure to elevated amounts of As, increased activity of this enzyme has been detected in mung bean (Singh et al. 2007) and common bean (*Phaseolus vulgaris*) (Stoeva et al. 2005b). Ghosh et al. (2016) have shown the increase in APH activity in maize; however, it is probably inadequate to scavenge all H_2O_2 produced under As(V) stress. Talukdar (2013) has observed alterations in APX content in leaves of fenugreek (*T. foenum-graecum)* treated with As in three concentrations (10, 20, and 30 mg As/kg soil). In the roots of this plant, the concentration-dependent decrease in the enzyme content has been noticed. Singh et al. in the recent work (2017) have demonstrated a concentration- and time-dependent induction of APX in As-treated roots and shoots of vetiver grass (*V. zizanoides*).

Guaiacol peroxidases (EC 1.11.1.7), the group of enzymes located in the cytosol or being bound to the cell wall, are also responsible for H_2O_2 reduction using GSH as the electron donor. Guaiacol peroxidase upregulation has been observed in As-exposed mung bean (Singh et al. 2007) as well as in vetiver grass (*V. zizanoides*) after 7 and 14 days of As exposure on the shoots and 14-day treatment of the roots (Singh et al. 2017).

The cell redox state is regulated by different antioxidant enzymes widespread in the cytoplasm, mitochondria, and plastids like GR (EC.1.6.4.2). Its main role is a reduction of glutathione disulfide (GSSG) and consequently the regeneration of the GSH pool (Sharma 2012). During As-induced oxidative stress in rice seedlings, the increased requirement of the antioxidant, GSH, is achieved by GR stimulation (Shri et al. 2009). Srivastava et al. (2005) have observed enhanced GR activity in roots of

ferns: *P. vittata*, *P. ensiformis*, and *Nephrolepis exaltata*; however, the activity of this enzyme in fronds and rhizome of the latter two species has turned out to be higher than in roots of the As hyperaccumulator *P. vittata*. In the study of Gupta et al. (2013), time- and concentration-dependent increase of GR activity has been noticed in As-treated *Arabidopsis* leaves.

Another family of ubiquitous enzymes in plants, glutathione *S*-transferases (GST, EC 2.5.1.18), using GSH as a cosubstrate or coenzyme and catalyzing the conjugation of GSH with different substrates to detoxify them, is induced by toxic metals, As, and other oxidative stress factors (Sharma 2012). GST stimulation in response to As stress has been confirmed in maize and sugarcane (*Saccharum officinarum*) (Mylona et al. 1998; Ghelfi et al. 2011). In studies of Mokgalaka-Matlala et al. (2009), As(III)-treated roots and leaves of mesquite (*Prosopis* sp.) have revealed significantly higher GST activity than the roots and leaves treated with As(V). The roots of both As(III)- and As(V)-treated plants showed an initial increase in GST at low As concentration (5 mg/L), followed by significant inhibition up to 50 mg/L. The differences in GST activities prove that the particular organs and tissues differ in their response to As and thus in detoxification ability. In their recent work, Singh et al. (2017) report that GST activity increases with the As concentration both in roots and shoots of vetiver grass (*V. zizanoides*), and this effect is dependent on the time of As exposure.

The functions of GPX (EC 1.11.1.9), which play the essential role in H_2O_2 neutralization in animals, are still unclear in plants (Bela et al. 2015). These multiple isoenzymes considered as part of plant antioxidant system (Sahay and Gupta 2017) are distinguished by distinct subcellular locations and differential responses to the environmental stress (Bela et al. 2015). According to Ozyigit et al. (2016), GPX and APX probably work together in various pathways such as antioxidant and secondary metabolite metabolism, redox homeostasis, and stress adaptation in plants.

4.6 Effect of As on Nonenzymatic Antioxidants

In response to abiotic stress, plants can synthesize nonenzymatic antioxidants such as AsA, GSH, phytochelatins (PCs), carotenoids, phenolics including anthocyanins, and other low-molecular compounds which can reduce the level of H_2O_2 and other ROS. It has been reported that low-molecular-weight antioxidants generally accumulate during As treatment (Schmöger et al. 2000; Hartley-Whitaker et al. 2001b; Bleeker et al. 2003, 2006; Khan et al. 2009; Song et al. 2010; Nath et al. 2014). Furthermore, the contents of nonenzymatic antioxidants follow similar trends such as As concentrations in the plant, increasing with soil As concentration. The larger nonenzymatic antioxidant contents have been observed in the fronds than in the roots, especially when exposed to higher As concentrations (Cao et al. 2004).

Two low-molecular compounds, i.e., AsA and GSH, are coupled in AsA–GSH cycle that operates in the cytoplasm, plastids, mitochondria, and peroxisomes. Among the enzymatic antioxidant systems, this cycle occupies a vital place such as

AsA, GSH, and NADPH which are present in high concentrations in plant cells. It is assumed that it plays an essential role for H_2O_2 detoxification (Noctor and Foyer 1998; Nath et al. 2014). In AsA–GSH cycle, H_2O_2 is reduced to H_2O by APX using AsA as the electron donor. The oxidized AsA (MDHA) is regenerated by MDHAR and DHA by DHAR with GSH. Finally, GSSG is regenerated by GR using NADPH as an electron donor. The studies show that the enzymes involved in the recycling of GSSG and oxidized AsA are often induced in plants under exposure to As (Ahsan et al. 2008; Khan et al. 2009).

Moreover, AsA being a substrate for APX and GSH for GPX are independently involved in H_2O_2 decomposition. The reduction of As(V) to As(III) is also a GSH-dependent process. Namely, it is coupled with NAD(P)H oxidation via GSSH reduction catalyzed by GR. The product of this reaction, GSH, is then an electron donor for arsenite reductase (Ellis et al. 2006; Sharma 2012). The studies show that rapid As(V) influx results in GSH depletion and PC production in the cell of *H. lanatus* (Hartley-Whitaker et al. 2001b), probably due to GSH consumption during As(V) reduction. Similarly, a decrease in GSH content has been observed in red clover exposed to As (Mascher et al. 2002). On the other hand, a significant increase in GSH and PCs upon exposure to this metalloid has been noticed in As-tolerant macrophyte *H. verticillata* (Srivastava et al. 2007). According to Singh et al. (2006), protection from the oxidative damage by a higher level of AsA–GSH pool is related to As(V) tolerance in As-hyperaccumulator *P. vittata*. ROS produced during As treatment typically induce an increase in the oxidation state of GSH and AsA pools in favor of GSSG and DHA over GSH and DHA (Singh et al. 2006; Abercrombie et al. 2008). Such a shifting in the redox state toward oxidative imbalance may be a result of several reactions, i.e., both GSH and AsA can be directly oxidized by $O_2^{\bullet-}$ and •OH or, alternatively, H_2O_2 can oxidize GSH and AsA by specific peroxidases or, in the case of GSH, also GST and glutaredoxin, which catalyze disulfide reduction in the presence of NAD(P)H and GR (Abercrombie et al. 2008).

Many studies have shown that the oligomerization of GSH to produce PCs is induced during plant exposure to As (Schmöger et al. 2000; Geng et al. 2006; Singh et al. 2006; Khan et al. 2009). As forms various complexes of PC–As(III), especially binding to PC_2 and PC_3 (Raab et al. 2004). One of the methylated As(III) compound, namely, monomethylarsonic acid (MMA(III)), has been found to be chelated by phytochelatin 2 (PC_2) in plants of sunflower (*Helianthus annuus*) (Raab et al. 2005). Since the increased synthesis of PCs can result in more effective detoxification, the depleting cellular GSH pool decreases the antioxidant capacity of the cell (Hartley-Whitaker et al. 2001b; Finnegan and Chen 2012). It has been reported that increased PC synthesis in *Arabidopsis* and tobacco and high levels of PC in vacuoles have resulted in increased tolerance to As (Li et al. 2004; Wojas et al. 2010; Zanella et al. 2016). On the other hand, Sung et al. (2009) show that while GSH levels in *Arabidopsis* increase like in the As-tolerant variety of *B. juncea*, the PC levels decline. According to Zhao et al. (2003), As-hyperaccumulator *P. vittata* has a rather limited capacity to accumulate PCs in response to As exposure. Ghosh et al. (2016) have found the increase in total PC level in maize root as an early response to As stress.

AsA is not only a substrate for APX but also the most abundant low-molecular-weight antioxidant in plants, present in the stroma of chloroplast, apoplast, cytosol, and vacuole, which plays the important role in growth and development (Singh et al. 2006). So far, a small number of reports on the reaction of AsA during As-induced oxidative stress are available (Sharma 2012). Singh et al. (2006) have demonstrated the significant increase in AsA concentration and the ratio of its reduced/oxidized form in fronds of As hyperaccumulator, *P. vittata*, after exposing to different concentrations of As for 1, 5, and 10 days and lower increase in sensitive *P. ensiformis* exposed to As in medial concentration after 5 and 10 days of experiment. According to Czech et al. (2008), AsA concentration increases in hypocotyls whereas decreases in roots of cucumber (*Cucumis sativus*) plants exposed to this metalloid.

Carotenoids as isoprenoids are hydrophobic, so they are located mainly in thylakoid membranes, where, as described previously, they are involved in ROS quenching during photosynthesis. The decrease in carotenoid content has been noticed in many plants, e.g., in oat, bean, and red clover growing in As-contaminated soil (Mascher et al. 2002; Stoeva and Bineva 2003; Stoeva et al. 2005a). This effect is probably one of the reasons for the decrease in the efficiency of PSII, because As does not damage PSII proteins (Farnese et al. 2017). Swelling and breakage of thylakoids, as well as starch accumulation in chloroplasts, have also been observed as common effects of As treating (Simola 1997; Sharma 2012). Interestingly, Singh et al. (2006) have observed an increased carotenoid concentration induced by As in hyperaccumulator, *P. vittata*, while the reverse effect in As-sensitive *P. ensiformis*, both growing under the same conditions. Talukdar (2013) has reported that in fenugreek (*Trigonella foenum-graecum*) leaves treated with As in three concentrations as well as with accompanying phosphorus, only slight fluctuations in the total carotenoid content are present. Recent studies show a small increase in carotenoid content after 7 days of As treatment of vetiver grass (*V. zizanoides*) and a significant increase after 14 days of the experiment (Singh et al. 2017).

Other hydrophobic antioxidants involved in the antioxidant response to metal stress are tocopherols and tocotrienols. Both the compounds are known to be effective quench $^1O_2$ and peroxyl radicals, protecting the photosynthetic apparatus from oxygen toxicity and lipid peroxidation (Lushchak and Semchuk 2012).

As-treated plants also show dramatic changes in amino acid pools (Dwivedi et al. 2010; Pavlík et al. 2010; Finnegan and Chen 2012), including proline, the effective $^1O_2$ and •OH scavenger, and cysteine, an antioxidant and the precursor of GSH and PCs (Yadav 2010).

4.7 Concluding Remarks

The specificity of As involvement in the oxidative stress induction is related to the substitution of phosphate by arsenate (As(V)) and to the high susceptibility of arsenite (As(III)) to binding to SH– groups. Reactions mentioned above, as well as

methylation accompanying the reduction of As(V) to As(III), contribute to the overproduction of ROS responsible for the generation of the oxidative stress. The response of plant cells to the oxidative stress has several common features, regardless of its initial factor. Certain differences may be related to the presence of specific ROS such as DMA radical, different ratio between individual species, and simultaneous occurrence of non-radical reactions associated with the increased As content.

In general, the As-induced ROS formation stimulates the synthesis of antioxidant metabolites and activates enzymes involved in the antioxidant defense. Modification of several metabolic pathways such as GSH synthesis, which occurs in response to the oxidative stress, increases plant tolerance to As. Large species-dependent variations in plant sensitivity to As allow for expecting that additional metabolic pathways related to As tolerance will be discovered in the near future.

Over the last decades, many reasons have arisen for which ROS are currently considered to be signaling molecules in many processes, e.g., in the response of organisms to various types of stress. The new concept has been based on two recently discovered features of ROS interactions with proteins: their specificity and reversibility. Since the mechanism of As-dependent signaling in plants is still poorly investigated, the identification of genes involved in the oxidative stress response will be essential for the development of technologies enabling to safe cropping as well as allowing for effective phytoremediation of this harmful metalloid.

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Chapter 5
Plants Response and Tolerance to Arsenic-Induced Oxidative Stress

Anindita Mitra, Soumya Chatterjee, and Dharmendra K. Gupta

Abstract Arsenic (As) is a toxic metalloid of global concern derived from natural, geothermal, and anthropogenic sources. Arsenic has deleterious effects in all forms of life including plants. Between the two inorganic forms, the highly oxidized pentavalent arsenate (As^{V}) is prevalent in the aerobic environment, while the highly reduced trivalent arsenite (As^{III}) is the predominant form in an anaerobic environment. The main route of As^{V} uptake in plants is through the phosphate transporters, while As^{III} and methylated As species enter through nodulin 26–like intrinsic protein (NIP) or aquaglyceroporins. After entering into the plant cell As can severely impede plant metabolism which leads to various physiological disorder. Subsequently, growth of the plants is subdued, and it results in delaying or restraining accrual of biomass and induces loss of fertility, yield, and fruit production. Exposure to inorganic As in plants promotes oxidative stress by generating reactive oxygen species (ROS) during their conversion from As^{V} to As^{III}. Plants have a well-organized antioxidant defense system to combat As stress. In plants, As intoxication triggers the activation of enzymatic antioxidants like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), glutathione *S*-transferase (GST), and glutathione peroxidase (GPX); synthesis of nonenzymatic antioxidants, such as ascorbate and γ-Glu-Cys-Gly-tripeptide glutathione (GSH); and accumulation of anthocyanin in the leaves. As tolerance in plants is achieved by the production of phytochelatin following As exposure which is derived from GSH. This chapter aims to provide current updates about the molecular mechanism involved in uptake of the inorganic and organic species of As, their translocation, and the As-induced stress in plants with a special emphasis on oxidative stress.

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Keywords Uptake · Translocation · Oxidative stress · Antioxidative enzymes · Phytochelatin · Glutathione

5.1 Introduction

Arsenic (As) is a toxic metalloid. Naturally, it exists in four oxidation states (-III), (0), (+III), and (+V) (Rathinasabapathi et al. 2006). Mostly available chemical forms of As having diverse physicochemical properties are: arsenite (As^{III}), arsenate (As^{V}), trimethylarsine (TMA), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), arsenosugars, arsenocholine (AsC), arsenobetaine (AsB) (Panda et al. 2010). Due to various factors including dumping of industrial wastes and dust from smelters, As contamination in soils is indiscriminate in many parts of the world (Chatterjee et al. 2017a). Depending on the redox status, inorganic arsenite or arsenate is primarily present in soil solution, which is the most phytoavailable form (Meharg and Hartley-Whitaker 2002). Among the inorganic As species, trivalent state of As is most toxic in comparison to the pentavalent state (Gupta and Chatterjee 2017), whereas the organic As has less toxicity than inorganic species (Chung et al. 2014). As^{V} is present in aqueous solution in the form of H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, and AsO_4^3, whereas As^{III} exists in reducing form, for example, H_3AsO_3 in anaerobic groundwater (Panda et al. 2010). Arsenic may also associate in nature with several other metals like copper, cobalt, nickel, silver, and lead (Gupta et al. 2017).

Arsenic concentration usually varies from noncontaminated soil to contaminated soils from 10 mg kg^{-1} to 30,000 mg kg^{-1}, respectively (Adriano 1986; Vaughan 1993). Terrestrial plants grown in noncontaminated soil show less than 10 mg As kg^{-1} in tissues, but a typical threshold of 40 mg kg^{-1} of As has been reported from different tissues of crop plants (Matschullat 2000). High-affinity phosphate transporters help plants to readily take up arsenate (being an analogue of phosphate) from the soil (Meharg and Macnair 1992). Incorporation of As to the food chain via the groundwater-soil-plant system due to the use of high As contaminated groundwater in agriculture and bioaccumulation of As in crop plants are potentially hazardous to public health (Rahman et al. 2008; Patra et al. 2004).

Arsenic has no known biological function in plants. The exposure of plants to a higher level of As^{III} and As^{V} induces the production of reactive oxygen species (ROS) (Gupta et al. 2013a). Transformation of arsenate to arsenite within plant cell produces ROS directly through Haber–Weiss reactions (Mascher et al. 2002, Mithöfer et al. 2004). Heavy metal interaction with the antioxidant system generates oxidative stress in plants (Srivastava et al. 2004), either indirectly through disruption of electron transport chain (Qadir et al. 2004), creating disorders in the essential elemental metabolisms, or directly through ROS-mediated cellular damages, enhanced lipid peroxidation, and membrane leakage (Dong et al. 2006). Arsenic-induced negative effect in plant development is a well-known fact (Islam et al. 2015), where significant interspecific variation and also among cultivars within the same species (like, *Oryza sativa*) are reported (Lei et al. 2013; Lemos Batista

et al. 2014; Begum et al. 2016). Shorter length and lower biomass, mainly in roots, accompanied by oxidative stress of a plant, signify arsenic triggered stress symptoms (Abercrombie et al. 2008; Shri et al. 2009; Talukdar 2011; Upadhyay 2014).

Plants have evolved several mechanisms to combat As-induced stress such as suppression of high-affinity phosphate/arsenate transporter and to bind the metal to extracellular exudates and cell wall constituents thereby reducing uptake, sequestration of metals in the vacuole, complexation of metalloids by different substances, activation or modification of plant metabolism, and synthesis of antioxidant enzymes (Duquesnoy et al. 2010). Antioxidative defense is achieved either by nonenzymatic antioxidants with low molecular mass (like GSH, glutathione, and ascorbate (AsA)) and enzymatic antioxidants like ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione reductase (GR), and catalase (CAT) (Finnegan and Chen 2012; Sharma 2012; Talukdar 2013a).

Although a number of reports are available on the morphological and physiological mechanism of As uptake and accumulation in plants, however, oxidative information on stress induced by As and related defense mechanisms are still poorly recognized. The present chapter is an attempt to focus on oxidative stress in plants induced by As and antioxidant defense mechanisms relating to As uptake, translocation, and phytochelatin (PC)-mediated As detoxification mechanism.

5.2 Uptake of Different Arsenic Species by Plant

5.2.1 Arsenate Uptake

The pathways of As uptake in plants have been extensively investigated by several authors (Tripathi et al. 2007; Zhao et al. 2009, 2010; Mitra et al. 2017a). Physiological and electrophysiological studies revealed that as the oxyanion structure of arsenate (As^V) is analogous to inorganic phosphate (Pi), both are transported through shared transporter in higher plants (Meharg et al. 1994; Gupta et al. 2011). During uptake of each phosphate ($H_2PO_4^-$)/arsenate ($H_2AsO_4^-$) molecule, two protons ($2H^+$) are co-transported across the membrane (Ullrich-Eberius et al. 1989). Although hundreds of phosphate transporters are recognized in higher plants, the PHT1 family of Pi transporter present in the roots is likely to be involved in As^V transport (Ullrich-Eberius et al. 1989; Wu et al. 2011). Studies reported that Pht protein transports As^V in As hyperaccumulators (Wang et al. 2002; Tu and Ma 2003; Cesaro et al. 2015), As-tolerant non-hyperaccumulators (Meharg and Macnair 1992; Bleeker et al. 2003), and also in As-sensitive non-accumulators (Esteban et al. 2003). However, different phosphate transporters present in hyperaccumulator plants show greater affinity for As^V than non-accumulator species of plants (Wang et al. 2002; Poynton et al. 2004). Double mutant *Arabidopsis thaliana*, for two high-affinity Pht1 isoform Pht 1;1 and Pht 1;4, was found to be resistant for arsenate than wild-type plants, which strongly supports the role of Pht 1;1 and Pht 1;4 in arsenate transport (Shin et al. 2004). Magnitude of phytotoxicity was greater following increasing

uptake in soil with low levels of Pi as PHT transporters have higher affinity for phosphate than arsenate; therefore, As^{V} may outcompete Pi for entry through the root (Meharg et al. 1994). This can be overcome by applying larger amounts of phosphates that compete with arsenate at root surfaces to decrease uptake and phytotoxicity (Tu and Ma 2003; Titah et al. 2013). Some of the As-tolerant plants species such as *Holcus lanatus* and *Cytisus striatus* can grow in soil with higher As concentration without exhibiting any toxicity, which can be achieved by restricting the inflow of As by constitutive suppression of high-affinity phosphate/As^{V} transporter (Meharg and Macnair 1992; Bleeker et al. 2003).

5.2.2 Arsenite Uptake

In reducing environment, like swampy areas, arsenite (As^{III}) is the predominant As species (Marin et al. 1993; Chatterjee et al. 2017b). In plants, members of nodulin 26–like intrinsic proteins (NIPs) commonly known as aquaporins are known to involve in As^{III} transport through the root cells (Isayenkov and Maathuis 2008; Ma et al. 2008; Mitra et al. 2014). Additionally, NIPs also facilitate the transport of multiple uncharged solutes including glycerol, urea, ammonia, boric acid, and silicic acid, hence called aquaglyceroporins (Wallace et al. 2006) but impermeable to water (Bienert et al. 2008). The other three plant aquaporins comprise tonoplast intrinsic protein (TIPs), plasma membrane intrinsic protein PIP), and small basic intrinsic protein (SIPs) (Chaumont et al. 2005; Maurel et al. 2008). In contrast to arsenate, arsenite uptake is repressed by glycerol and antimonite instead of phosphate (Zhao et al. 2009). Aquatic macrophytes take up As either through phosphate transporter by active transport or passively through aquaglyceroporins and/or physicochemically adsorb in the root (Rahman and Hasegawa 2011; Mitra and Chatterjee 2016).

Ma et al. (2008) have isolated an arsenite transporter OsNIP 2;1, also known as *Lsi1* in the rice root, which primarily transports silicon. Efflux of arsenite directed from the root toward xylem is mediated by another arsenite transporter *Lsi2* also described by Ma et al. (2008). Role of *Lsi2* gene was confirmed from the observation of *Lsi2* mutant rice species in which As^{III} accumulation was found much lower in the shoots or xylem sap in comparison to those xylem sap of wild species (Ma et al. 2008). Recently, two transporters OSNIP 3;3 and HvNIP1;2 have been reported to involve in As^{III} transport in the yeast cell (Katsuhara et al. 2014).

5.2.3 Uptake of Organic Species of Arsenic

Organic forms of As such as MMA^{V} and DMA^{V} are in very small proportion in soil and may derive from the previous application of arsenical pesticides and herbicides or may be synthesized by the microorganism. The organic As compounds are less efficiently taken up by plants than that of inorganic As species (Carbonell-Barrachina

et al. 1998; Raab et al. 2007). Very little information is available about the mechanism involved in the uptake and transport of methylated As species by plants. In aquatic plants, As^{III} is transported passively through aquaglyceroporin channel in the form of dimethylarsinic acid (DMAA) and monomethyl arsinic acid (MMAA) (Rahman and Hasegawa 2011). The aquaporin *OsLsi1* is involved in the uptake of MMA^V, and the loss of function in rice *OsLsi1*led to an 80% reduction in MMA^V uptake and 50% for DMA^V compared to wild species (Li et al. 2009). Although rate of uptake of MMA^V and DMA^V by plant roots occurs very slowly than that of arsenate or arsenite (Abbas and Meharg 2008; Li et al. 2016), greater mobility of MMA^V and DMA^V was found within the plant tissue than that of inorganic As species (Li et al. 2009; Carey et al. 2010,2011). Involvement of *OsLsi1* was confirmed in the uptake of organic As species, but no role is played by *OsLsi2* in plants in the efflux of the MMA^V and DMA^V (Li et al. 2009).

5.3 Translocation of Arsenic

Arsenic hyperaccumulators have greater mobility of As relating to translocation from roots toward shoots in comparison to non-hyperaccumulator. The less efficient translocation of As directing toward shoot from root tissue in non-hyperaccumulators is indicative of the low ratios of shoot As to root As concentrations (Burlo et al. 1999) and thereby justifying the phenomenon that the reduction of arsenate to arsenite occurs rapidly in roots, following complexation with thiols and insulation within the root vacuoles. In *A. thaliana* knocked out *AtACR2* gene (arsenate reductase) using RNAi leads to increased accumulation of As in the shoots (Dhankher et al. 2006). Blocking *AtACR2* leads to more arsenate available for xylem transport to the shoots from root via the phosphate transport pathway. Among all As species, DMA is translocated more proficiently from roots to shoots, although root uptake is less efficient compared to other As species (Raab et al. 2007). The inorganic form in which As is transported from root to shoot is questionable. Some authors reported that arsenite prevalently exists in the xylem sap, accounting for 60–100% of the total As (Zhao et al. 2009). *A. thaliana* mutant for phosphate transporter, defective in xylem loading of phosphate but showed no effect on As distribution to the shoots (Quaghebeur and Rengel 2004), suggests that As is not loaded into the xylem as phosphate analogue arsenate. Duan et al. (2005) also support that majority of the transported As is in arsenite form as AR activity was solely confined within the roots. In contrary, a number of reports showed that arsenate is present in the xylem as it is being loaded by PHT protein, into the xylem vessels (Catarecha et al. 2007; Zhao et al. 2010; Mendoza-Cózat et al. 2011; Wu et al. 2011). However, methylated As is detected very meager amount in xylem sap as DMA was found in xylem sap of cucumber (*Cucumis sativus*) and tomato plants only at <4% of the total As (Mihucz et al. 2005; Xu et al. 2007).

5.4 Arsenic-Induced Oxidative Stress in Plants

Arsenic exposure leads to abiotic stress which emanates to oxidative stress at cellular level by producing reactive oxygen species (ROS) (i.e., singlet oxygen, $^1O_2$; superoxide, $O_2^{\bullet -}$; hydrogen peroxide, H_2O_2; hydroxyl radical, $OH^{\bullet}$) that surpass the pace of their metabolism (Gill and Tuteja 2010; Mallick et al. 2011). Arsenic induces ROS production by blocking the activity of key enzyme system along with electron drainage during As^{V} to As^{III} conversion (Sharma 2012). The reduction of As^{V} is succeeded by methylation of inorganic As, a redox-directed process that may also give rise to ROS (Zaman and Pardini 1996). Methylated As species such as dimethylarsinic acid (DMA) causes iron-dependent oxidative stress which is based on iron released from ferritin. DNA damage takes place by reactive oxygen species which are generated directly from DMA^{3+} (Shi et al. 2004). ROS induces chain like peroxidation of polyunsaturated fatty acid in membrane lipids, damaging the proteins, amino acids, nucleotides, and nucleic acids (Noctor et al. 2016; Moller et al. 2007). Malondialdehyde (MDA), a product of lipid peroxidation resulting from membrane damage, is considered as an indicator of oxidative stress (Shri et al. 2009). Lipid peroxidation also increases thiobarbituric acid-reacting substances (TBARS) and H_2O_2 content in *H. lanatus* (Hartley-Whitaker et al. 2001), *Trifolium pratense* (red clover) (Mascher et al. 2002), *Vigna radiata* (mung bean) (Singh et al. 2007), and *Oryza sativa* (rice) (Shri et al. 2009). In *Pteris vittata* and *Sphagnum nemoreum*, As exposure leads to alteration of chloroplast membrane structure and subsequent rupture and enlargement of thylakoid membranes (Simola 1997; Li et al. 2006). Elevated and nonmetabolized cellular H_2O_2 is responsible for severe damages to biomolecules such as cellular lipids and proteins and consequent interruption of key cellular functions (Gill and Tuteja 2010; del Río 2015).

Differential modulation in the antioxidant system occurs in the plant under As stress as reported from several studies (Dwivedi et al. 2010; Tripathi et al. 2012). Activated antioxidant system and increased levels of PC production in different plants like *Hydrilla verticillata* and *C. demersum* suggest that specific proteins are responsive to As stress (Srivastava et al. 2007; Mishra et al. 2008; Dave et al. 2013a). Similarly, enhanced activities of antioxidative enzymes such as superoxide dismutase, APX, peroxidase (POD), and GR indicate As exposure generates oxidative stress (Shri et al. 2009; Dave et al. 2013b). The first line of defense in higher plants includes activation of CAT, SOD isozymes, and the AsA-GSH cycle in response to As stress. To mitigate the negative effects of excess ROS, the plant defense system functions in a coordinated manner under adverse environmental circumstances in the different cell compartments and organs (Airaki et al. 2015). However, following exposure to higher As level, ROS production reaches too high that the antioxidant defense mechanisms may be devastated, leading to cellular damage which ultimately leads to cell death (Van Breusegem and Dat 2006).

5.5 Arsenic-Induced Metabolic Alterations in Plants

The potential of As^{V} to substitute for Pi and the aptness to bind and alter the activities of fundamental enzymes and hazardous effects of ROS have a direct and significant effect on plant metabolism. Arsenic vulnerability leads to changes in the levels of various compounds like starch and sugars and modulates the activities of the key enzymes of interrelated metabolic pathways like RNase, protease, and leucine aminopeptidase in plants (Mishra and Dubey 2006; Choudhury et al. 2010). Productivity was severely hindered due to significant disruption of carbohydrate metabolism in plants growing in As-contaminated soil and may be due to the rise in the level of soluble sugars in the tissues, especially sucrose and hexoses, the end products of the photosynthesis (Mishra and Dubey 2013). A comparative transcriptomic analysis revealed variation in the lipid metabolism and phytohormone signaling in plants under $As^{(III)}$ stress (Yu et al. 2012).

To encounter the ROS generated by the As exposure, plants need to produce sufficient metabolites, and such response predominantly impacts on carbon, nitrogen, and sulfur metabolism of plants (Finnegan and Chen 2012). Promoting accumulation of AsA is the main effect of As^{V} on plant carbon metabolism to reinforce protection against ROS (Srivastava et al. 2005; Singh et al. 2006; Khan et al. 2009). However, genomic analysis on carbon metabolism proved no changes in transcriptional profiles as observed both in *Arabidopsis* and *Oryza sativa* (Abercrombie et al. 2008; Norton et al. 2008; Chakrabarty et al. 2009). Exposure to As^{V}, As^{III}, and MMA^{III} are able to interfere the photosynthetic process in different ways like decrease in chlorophyll content (Duman et al. 2010; Gupta et al. 2013b) or Photosystem II activity (Stoeva and Bineva 2003) which may perturb photosynthetic electron flow across the membrane of thylakoid sinking the efficiency to produce ATP and NADPH, both of which are essential to fuel the carbon fixation reactions.

Arsenic exposure has the potential to strongly reduce the nitrogen fixation in alfalfa roots as observed when alfalfa growing in As-contaminated soil had less than half of the total number of root nodules formed in the absence of As (Carrasco et al. 2005; Pajuelo et al. 2008). Transcriptomic analysis by Lafuente et al. (2010) reported that As^{III} exposure prevents the gene expression required for early nodule development. As a result, soil contaminated with As shows lower potential for N_2 fixation in ecosystem involving legume-rhizobium symbiosis as evidenced from alfalfa. Considerable changes in the amino acid pool have been reported to occur after As exposure (Dwivedi et al. 2010; Pavlík et al. 2010). A number of the study reported that the RuBisCo, an abundant protein having the capacity to store nitrogen, can be a target for disruption in As^{V} treated plants (Duquesnoy et al. 2009; Ahsan et al. 2010; Bona et al. 2010). Thus, As exposure that accompanies lower protein content in plants may be due to As-induced diminution in carbohydrate metabolism that would deter the biosynthesis of amino acids (Finnegan and Chen 2012).

The major As detoxification pathway, that is, binding of As with the thiol group of GSH and PC, indicates the crucial importance of sulfur metabolism regulating plant survival in As-contaminated soils. Adequate supplies of the GSH building blocks Glu, Cys, and Gly are required immediately after As exposure. According to Munoz-Bertomeu et al. (2009), cysteine is the limiting substrate for GSH biosynthesis in Arabidopsis. Decreasing cysteine pool following As exposure (Sung et al. 2009) signifies that higher Cys biosynthesis is needed to support GSH and PC generation that is also steered from sulfur metabolism (Finnegan and Chen 2012). Plants that overproduce the enzymes mediating GSH and PC biosynthesis were found to maintain higher levels of nonprotein thiols than wild species (Guo et al. 2008). Sulfur is acquired from the soil in the form of sulfate to sustain biosynthesis of GSH and PC at high rate. Both species of As induces the expression of sulfur transporter genes. Norton et al. (2008) observed that in rice subsequent to As^{V} treatment upregulation of five sulfate transporter genes, but Sung et al. (2009) reported that in *Arabidopsis* at least one gene is upregulated. Similarly, As^{III} treatment in *B. juncea* and rice seedlings at least one sulfate transporter gene was found to be upregulated (Chakrabarty et al. 2009; Srivastava et al. 2009). However, Takahashi et al. (2011) suggested that small number of transporters may be adequate to direct the mobility of sulfate from the soil toward the plants root.

5.6 Enzymatic Antioxidative System

5.6.1 Superoxide Dismutase

Superoxide dismutases or SODs are metalloenzymes that play key roles in protecting cells from oxidative stress by catalyzing the dismutation of $O_2\bullet-$ to H_2O_2 (Li et al. 2017). Superoxide dismutase enzyme requires metals as cofactors. SOD associated with Cu/Zn is found in the cytosol, plastid, peroxisomes, and root nodules. Mn-SOD is confined in the mitochondria, and Fe-SOD is localized in the plastids. In maize root, the proteomic analysis reveals Cu/Zn SOD as one of the highly responsive enzymes to As which is involved in cellular homeostasis during redox disturbance (Requejo and Tena 2005). SOD activity was found to significantly increase in response to As toxicity as evidenced from As hyperaccumulator and sensitive fern species (Srivastava et al. 2005), in maize (Mylona et al. 1998) and in the grass *H. lanatus* (Hartley-Whitaker et al. 2001); in contrast, high concentration of As inhibits the accumulation of SOD mRNA, thus reducing its activity (Gong et al. 2005; Gunes et al. 2009). The inhibition of SOD activity in response to high As exposure could be attributed to inactivation of the enzyme by H_2O_2 produced in different cellular compartments where SOD neutralizes $O_2^{\bullet-}$ (Khan et al. 2009). ROS- detoxifying enzymes are induced during abiotic stress but are also susceptible to oxidative damage (Dietz et al. 1999). Hydrogen peroxide itself is a highly reactive oxidizing agent that undergoes detoxification by CAT and the AsA–GSH cycle, both regulates H_2O_2 level (Shigeoka et al. 2002; Fig. 5.1). The equilibrium between

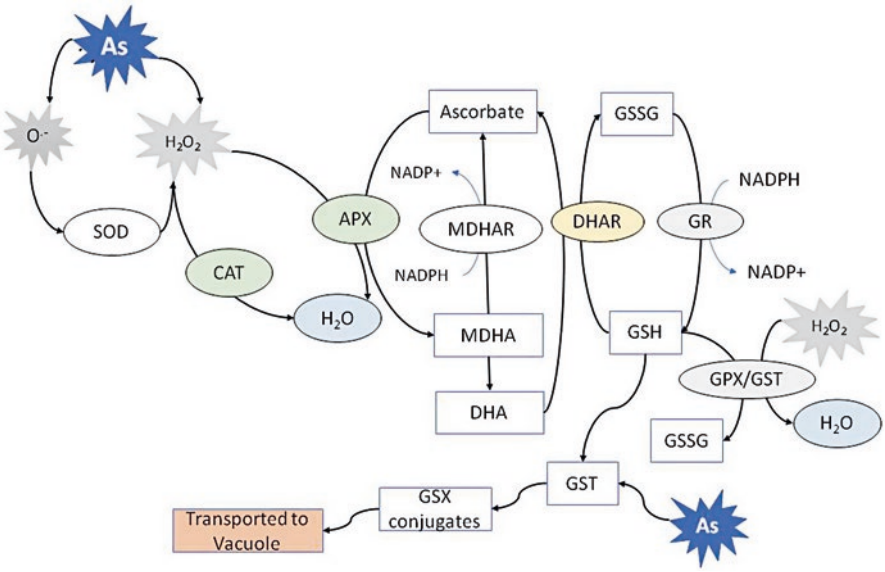

Fig. 5.1 Antioxidant defense system in plants after As exposure including enzymatic and nonenzymatic antioxidants (modified from Hasanuzzaman et al. 2012). As, arsenic; SOD, superoxide dismutase (in peroxisomes and plastids); *CAT* catalase (in mitochondria, peroxisomes, cytosol), *APX* peroxidase (in mitochondria, peroxisomes, cytosol, chloroplast), *MDHA* monodehydroascorbate, *DHA* dehydroascorbate, *DHAR* DHA reductase, *GSH* glutathione, *GSSG* glutathione disulfide, *GR* glutathione reductase (in chloroplast, mitochondria, cytosol), *GPX* glutathione peroxidase (in cytosol, chloroplasts, mitochondria, peroxisome, apoplast), *GST* glutathione sulfo-transferases (in cytosol)

the activity level of SOD and enzymes involved in AsA–GSH cycle and sequestration of metal ions promotes to maintain the steady-state level of O_2 and H_2O_2 and play crucial role by inhibiting formation of the ROS via the metal-dependent Haber–Weiss or Fenton reactions (Mittler 2002).

5.6.2 Catalase

Catalase is another H_2O_2 scavenger, located mainly in peroxisomes, glyoxisome, cytosol, mitochondria, and root nodules (Shugaev et al. 2011; Sharma et al. 2012; Su et al. 2014). This tetrameric, heme-containing enzyme degrades hydrogen peroxide promptly into water and molecular oxygen without utilizing cellular reducing supplements, thereby, protecting the cell by removing hydrogen peroxide by saving energy (Sharma 2012). Following As exposure, an upsurge of CAT activities was found in *Zea mays* (Mylona et al. 1998). As-tolerant Chinese brake fern (*P. vittata*) displays higher degree of CAT activity than As-susceptible slender brake fern (*P. ensiformis*) and Boston fern (*Nephrolepis exaltata*) (Srivastava et al. 2005). In contrast, As-induced

deterioration of CAT has also been reported by Singh et al. (2007) in *Vigna radiata* (mung bean) and in moss, *Taxithelium nepalense*. The association of a heme prosthetic group with CAT has been established by the irreversible inhibition of CAT by cyanide, azide, and hydroxylamine, all of which are hemeprotein inhibitors (Anjum et al. 2016). In addition, existence of a thiol group close to the active center of CAT contributing in the CAT-mediated reactions has been proven from the inhibition of CAT by thiol inhibitors like aminotriazole and mercaptoethanol.

5.6.3 Ascorbate Peroxidase

An alternative mechanism to detoxify H_2O_2 by peroxidase through AsA-GSH pathway is found in higher plants that require AsA as a reductant to reduce hydrogen peroxide into water (Fig. 5.1; Mehlhorn 1990). APX are class I heme-peroxidases, which function as active scavengers of H_2O_2 in higher plants and prevail as cAPX or cytosolic isoforms, mit APX or mitochondrial isoforms, and also in microbodies as mAPX, including peroxisomal and glyoxysomal isoforms, and ch APX or chloroplastic isoforms (Miyake and Asada 1996; Yadav et al. 2014; Anjum et al. 2016). Isoforms are unlike in their molecular weight, stability, and substrate specificity optimal pH and have been refined and characterized from several plant species including *Pisum sativum* (Caverzan et al. 2012), *Camellia sinensis* (Chen and Asada 1989), *Gossypium hirsutum* (Bunkelmann and Trelease 1996), *Cucumis sativus* (Battistuzzi et al. 2001), *Nicotiana tabacum* (Madhusudhan et al. 2003), *Oryza sativa* (Sharma and Dubey 2004), *Olea europaea* (Lopez-Huertas and del Rio 2014), and *Ziziphus mauritiana* (Yadav et al. 2014). APX catalyzes the reduction of hydrogen peroxide into water and two molecules of monodehydroascorbate (MDHA; Noctor and Foyer 1998). APX activity has been upregulated after As exposure as observed in maize (Miteva and Peycheva 1999), beans (Stoeva et al. 2005), mung bean (Singh et al. 2007), and rice seedling (Shri et al. 2009).

5.6.4 Glutathione Reductase

Glutathione reductase (GR, NADPH: oxidized glutathione oxidoreductase) is another key component of ROS scavenging system, located predominantly in chloroplast but also in mitochondria and cytosol in a small amount (Gill and Tuteja 2010). Glutathione reductase reduces glutathione disulfide (GSSG) to GSH using NADPH as reducing equivalent (Fig. 5.1), and thus conserves the cellular redox levels by retaining a high ratio of intracellular GSH/GSSG and AsA/dehydroascorbate (AsA/DHA) during oxidative stress (Anjum et al. 2012). Two genes, namely GR1 and GR2, have been distinguished to encode GR in plants; both are expressed in plastids and mitochondria (Jozefczak et al. 2012). A range of biotic and abiotic stress factors such as heavy metals and metalloids affect the activity of GR in plants (Anjum et al. 2010, 2011a, b; Gill and Tuteja 2010). Unfortunately, there is paucity of reports

about the active role of GR in higher plants during oxidative stress induced by As. It is found in rice seedlings that higher level of GSH required during As-induced oxidative stress is achieved by the activation of GR (Shri et al. 2009). Similar reports, that is, elevated level of GR activity has been observed in roots of *P. vittata*, *P. ensiformis*, and *Nephrolepis exaltata* after As exposure (Srivastava et al. 2005).

5.6.5 Glutathione Peroxidases

Glutathione peroxidase (GPX) belongs to large peroxidase family with broad substrate specificity, localized in cytosol, chloroplasts, mitochondria, peroxisome, and apoplast of plant cell, and catalyzes the reduction of H_2O_2, organic and lipid hydroperoxides consuming GSH pool as a reducing substrate, thereby protecting the cells from ROS (Anjum et al. 2010, 2011b). Some authors opined that, plant GPXs are actually peroxiredoxins (Prx) as they can use both GSH and thioredoxin (Trx) as electron donor, but Trxs act as more efficient reductants (Herbette et al. 2002; Iqbal et al. 2006; Navrot et al. 2006; Noctor et al. 2011). Millar et al. (2003) identified a family of protein isoforms called AtGPX1–AtGPX7 in *Arabidopsis* among which AtGPX1and AtGPX7 are present in chloroplast providing antioxidant protection and synchronizes salicylate, and ROS triggered plant immune responses (Chang et al. 2009). The other GPXs isoforms are found in the cytosol, mitochondria, and the endoplasmatic reticulum (Milla et al. 2003). Arsenate stress induced to increase the GPX activity in dose-dependent manner as observed in mung bean and in rice (Singh et al. 2007; Singh et al. 2015). A study carried out in *P. vittata* reported that a rise in GPX activity has occurred up to 20 mg kg^{-1} As and then declined with the increasing As concentration (Cao et al. 2004).

5.6.6 Glutathione S*-transferase*

Glutathione *S*-transferases (GSTs) found in plant cytosol are major phase II, ROS-detoxifying enzymes (Sheehan et al. 2001) and dependent on GSH for catalyzing the conjugation of GSH via the sulfhydryl group to diversified electrophilic centers of hydrophobic compounds (Marrs 1996; Fig. 5.1). This reaction renders the compound more polar and facilitates its transport to vacuole or apoplast (Mylona et al. 1998). GSTs perform versatile roles where GSH serves as a co-substrate or coenzyme (Ghelfi et al. 2011). Like other antioxidant enzyme GST activity increases in plants after As exposure (Mylona et al. 1998; Srivastava et al. 2005; Norton et al. 2008; Mokgalaka-Matlala et al. 2009; Chakrabarty et al. 2009). As for example, upregulation of at least 10 GST genes has been observed in rice in response to As^{V} exposure, while not more than two GST genes are downregulated (Norton et al. 2008; Chakrabarty et al. 2009). However, no noticeable changes in GST transcript were found in response to As^{III} (Chakrabarty et al. 2009), focusing that two inorganic As forms have differential effects on cellular metabolism.

5.7 Nonenzymatic Antioxidants

5.7.1 Ascorbate

Ascorbate (AsA) is the most abundant antioxidant in plants, present in cytosol, apoplast, and in the stroma of chloroplast. Synthesis of AsA occurs in the cytosolic region chiefly from the transformation of d-glucose (Hasanuzzaman et al. 2012). AsA reacts with a range of ROS such as H_2O_2, $O_2^{\bullet -}$, and $^1O_2$ and is the most important reducing substrate for the removal of H_2O_2 (Singh et al. 2006) and restore membrane-bound carotenoids and α-tocopherol via the AsA-GSH cycle in plant cells (Sharma 2012). In the AsA-GSH cycle, two molecules of AsA (reduced) are utilized by APX to reduce H_2O_2 to water with the concomitant generation of oxidized form MDHA that immediately disproportionates into DHA and AsA (Gapper and Dolan 2006) by MDHAR or ferredoxin with the electron donor NADPH in the chloroplasts (Gapper and Dolan 2006). Recycling of AsA (reduced) from dehydroascorbate (DHA) is a GSH-dependent pathway catalyzed by dehydroascorbate reductase (DHAR) that consumes NADPH as a reducing agent. A report from the study by Singh et al. (2006) showed that following As exposure an upsurge of AsA (reduced) concentration and the ratio of AsA/DHA occurs in the fronds of As-hyperaccumulator *P. vittata* and As-sensitive *P. ensiformis*.

5.7.2 Glutathione

The potential detoxification mechanism found in plants for combating heavy metal induced phytotoxicity is by synthesizing low molecular weight thiols having high affinity for the toxic metals (Bricker et al. 2001). GSH is one of the vital low molecular weight tripeptide thiol associated with sulfur and found as reduced (GSH) and oxidized (GSSG) forms. GSH takes part in a slew of cellular processes including defense against ROS, sequestration and complexation of heavy metals, control of cell division, in budding, and in transport and storage of reduced sulfur (Vernoux et al. 2000; Cobbett and Goldsbrough 2002; Freeman et al. 2004; Ogawa et al. 2004; Foyer and Noctor 2005; Mullineaux and Rausch 2005). Formation of GSH involves two ATP-dependent enzymes namely γ-glutamylcysteine synthetase (GSH1) and GSH synthetase (GSH2). In the first reaction, synthesis of γ-glutamylcysteine (γ-EC) occurs through a peptide bonding between the carboxyl group of glutamate and the amino group of cysteine by the catalytic action of GSH1. In the second reaction, ligation between glycine residue and γ-EC is catalyzed by GSH2 to form GSH. GSH1 plays major role in the regulation of GSH biosynthesis (Yadav 2010).

During As detoxification, coupling of the reduction of arsenate to arsenite and NADPH oxidation occurs where GSH (reduced) is serving as the electron donor for arsenate reductase (Ellis et al. 2006). In plants, As is transported as oxyanion arsenate which is reduced to arsenite and sequestered as thiol–peptide complexes in

vacuoles. Transgenic *A. thaliana* overexpressing both arsenate reductase (arsC) and GSH1 together showed substantially greater As tolerance than GSH1 transgenic and wild-type plants (Dhankher et al. 2002). One protective role of GSH in plants to As exposure is relieving from ROS. Supplementation of exogenous GSH and cysteine to plants under As stress reduced oxidative stress was observed, and the growth of rice seedlings was restored (Shri et al. 2009). Another important role of GSH is to serve as a precursor for the synthesis of phytochelatins a set of novel heavy metal-binding peptides.

5.8 Role of Phytochelatin in Detoxification and Arsenic Tolerance

The most common method of detoxification of heavy metal/metalloid in plants is by synthesis of PC. PC is synthesized by the catalytic action of PC synthase (PCS) from GSH by transpeptidation of (γ-glutamyl-cysteinyl) n-glycine (Gasic and Korban 2007). PC has the capability of binding via sulfhydryl and carboxyl residues to a range of metals like Zn, Cu, Cd, as well as As (Gupta et al. 2013c). Studies support the occurrence of PCs throughout the plant kingdom, in gymnosperms, angiosperms, and bryophytes (Clemens 2006). As tolerance in As-non-hyperaccumulating plants is achieved through considerable increase in the production and procurement of GSH and phytochelatins (PC) following exposure (Schat et al. 2002; Grill et al. 2006). The presence of heavy metal ions and metalloid such as Pb, Cd, Hg, Ag, Cu, Zn, As, etc. is required for the constitutively expression of PCS gene (Vatamaniuk et al. 2004). The presence of As^{III}-GSH or As^{III}-PC complexes has been recognized in various plants such as Indian snakeroot (*Rauvolfia serpentina*), in perennial grass commonly known as Yorkshire fog (*H. lanatus*), *sunflower* (*Helianthus annuus*), *Indian mustard* (*B. juncea*), and in Cretan brake fern (*Pteris cretica*) (Pickering et al. 2000; Schmoger et al. 2000; Montes-Bayon et al. 2004; Raab et al. 2004). In sunflower plants (*H. annuus*), following As exposure, synthesis of 14 different As complexes have been reported including GS-As^{III}-PC_2, As^{III}-PC_3, As^{III}-$(PC_2)_2$, As^{III}-GS_3, and MMA-PC_2 (Raab et al. 2005). Schulz et al. (2008) reported that short chains of PCs instead of long chain dominate in As-tolerant plants. Study of cad1-3 mutant *A. thaliana*, lacking the functional enzyme for PC synthesis, ascertained the predictable role of PCs in As detoxification; the mutant was unable to produce functional PCs and was found to be more sensitive (10–20 fold) to arsenate than the wild-type plants (Ha et al. 1999). Finally, As is detoxified within root and shoot tissue vacuoles by sequestrating As^{III}-PC complexes (Tripathi et al. 2007) thus unable to interfere with the cellular metabolism (Mitra et al. 2017a). In rice leaves, PC-arsenite complexation restricts the mobility of As from leaves to grains (Mitra et al. 2017b). In *Arabidopsis*, ABC transporter MRP1/ABCC1 and MRP2/ABCC2 are involved in the transport of As^{III}-PC conjugates (Song et al. 2010). In rice, transcription-level upregulation of homologous ABCC2 transporter gene was found after As exposure

(Chakrabarty et al. 2009). A report of Mendoza-Cózat et al. (2011) has proven the presence of ABCC transporter in different plant species sharing homology with *Arabidopsis* ABCC1 and ABCC2 transporter. In non-hyperaccumulator plants, phytotoxicity is reduced by rapid formation of As-PC complexes and sequestration within vacuoles of root cells where acidic pH (5.5) is favorable to stabilize the complex following As uptake, thereby restricting the As transport from the root to shoot (Liu et al. 2010; Mendoza-Cózat et al. 2011). The predominating form in which As is transported from root to shoot is controversial. In sporophytes of *P. vittata*, As is directed to the shoot mainly as As^{V} form and accumulated in the fronds as As^{III} as reported by Zhao et al. (2003). In contrast, Duan et al. (2005) suggested that As is translocated mostly in reduced form (As^{III}) and thus supporting the restriction of AR activity within the roots. Dissimilar with non-hyperaccumulators, where most of As is detoxified by the formation of As-PC complexes, hyperaccumulators like *P. vittata* and *P. cretica* were found to store 60–90% of arsenic as arsenite (As^{III}) form in the vacuole of fronds (Pickering et al. 2006; Su et al. 2008) with little complexation with PC in the roots and fronds (Zhao et al. 2009).

5.9 Conclusion

In recent years, researchers are trying to decipher the As uptake and transport in plants through studying molecular and physiological mechanisms. In plant tissue, oxidative stress produced due to ROS production and disorders of antioxidant defenses have been considered a significant matter in As toxicity. In this chapter, an attempt has been made to compile the updated information about As toxicity specifically on oxidative stress and the antioxidant defense system in plants. Although As is a non-redox active metalloid, excessive ROS is produced during valency conversion and methylation in plant. Common manifestations of As-induced phytotoxicity are growth inhibition, shortening of roots (than shoots), and severe effects on anatomical structures, photosynthetic apparatus, and antioxidant defense activities are found. As a result, agricultural productivity worldwide is hugely affected by As. Therefore, an urgent need is to find As-tolerant plant variety to increase agriculture productivity in affected areas. ROS scavenging are vital for plant defenses, and overexpression of gene coding for ROS-detoxifying enzymes helps to increase tolerance against environmental abiotic stresses. Transgenic plants that overexpress gene coding for ROS-detoxifying enzymes may be a prospective item to grow plants with improved tolerance against As. Another way is to apply exogenous chemical protectants like glycinebetaine, proline, Se, and signaling molecules like NO to alleviate oxidative stress (Hasanuzzaman et al. 2011a, b; Hasanuzzaman and Fujita 2011). Meharg and Meharg (2015) reported that adequate silicon fertilization greatly boosts rice yield by alleviating biotic and abiotic stresses and improving grain quality through lowering the content of inorganic As. Nitric oxide (NO), the gaseous free radical, is a widespread intracellular messenger and has regulatory roles in plant physiological processes (Neill et al. 2002). Though the NO-mediated amelioration against As-induced oxidative stress appeared to be synchronized by modulating antioxidant enzyme

activities, NO itself has the capacity to detoxify ROS directly (Talukdar 2013b). Therefore, an integrated approach by producing transgenic plants overexpressing genes related with antioxidant along with exogenous protectants may be implemented in order to achieve greater tolerance to As stress.

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Chapter 6
Arsenic Toxicity in Crop Plants: Responses and Remediation Strategies

Lakita Kashyap and Neera Garg

Abstract Arsenic (As), a naturally occurring nonessential metalloid, has a potential to affect plant and human health negatively. It enters the environment by mineralization of rocks and by activities of microorganisms that enhance its mobilization. Human interventions have accelerated As concentration in the soil to the levels exceeding the hazardous threshold. As occurs in both organic and inorganic forms, with inorganic form more toxic. Inorganic species comprise of arsenate (As V) and arsenite (As III), where As V is prevalent in aerated soils, while As III occur in anaerobic soils, with As III more toxic and mobile than As V. Once inside the plants, As V is converted into As III with the help of arsenate reductase. Plants exposed to As stress exhibit severe toxic effects on root growth which further decrease nutrient acquisition and disturb metabolic processes. As V is taken by plants' roots through phosphate transporter (PHT1) and interferes with oxidative phosphorylation. On the other hand, plants uptake As III through aquaporins and hamper enzyme activities by reacting with thiol groups. Various tools have been used by scientists in the last decade for the alleviation of metal stress in plants, among which, use of biological materials such as arbuscular mycorrhizal (AM) fungi and silicon amendment has gained importance due to their ability to restrict metalloid uptake. This chapter highlights recent advances concerning (1) As speciation in the environment and their uptake mechanisms, (2) impact of As species on plant growth and metabolism, and (3) use of AM and Si in mitigating As stress.

Keywords Arsenic toxicity · Silicon · Arbuscular mycorrhiza · Remediation

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6.1 Introduction

Arsenic (As) is a potent toxicant that has influenced plants as well as human health dramatically since ages. The word As is derived from Persian word *zarnikh*, which means yellow orpiment, and it was adopted by Greeks as *arsenikon* meaning masculine or potent (Henke 2009). It was discovered by Albert Magnus, a German scientist, by heating soap with orpiment (As trisulfide, As_2S_3) in 1250 (Mandal et al. 2011). It is a metalloid with characteristics of both metals and nonmetals which belongs to group Va of the periodic table with atomic number 33, atomic weight 74.91, and electronic configuration [Ar]3d $^{10}4s^2 4p^3$. It principally occurs as covalent complexes and in the anionic pieces of salt. Arsenic has more than 33 isotopes with an atomic mass ranging from 60 to 92. Arsenic shares characteristics with metal like bismuth and nonmetals like nitrogen and phosphorus (Flora 2015; Smith 2016). It is present naturally in the environment, yet human interference has amplified its proportion throughout the globe. It is, therefore, necessary to comprehend As sources, geochemistry, and different mechanisms related to As discharge into the earth (Flora 2015).

As concentration in the soil has become a matter of great concern due to its prolonged and widespread impacts on plant and animal health (Hughes et al. 2011). Its concentration in groundwater and soils has been identified as a serious threat to plants' and animals' health in countries like Bangladesh, Thailand, Taiwan, Ghana, Chile, Zimbabwe, Argentina, South Africa, China, Mongolia, Mexico, Canada, the United States, and India, where Bangladesh leads the list among the most contaminated countries (Bissen and Frimmel 2003). As is a nonessential and toxic element which shares similar transport pathways as that of some of the essential elements (such as As V for phosphate and As III for aquaporins; Li et al. 2016). Plants exposed to As display visible symptoms of toxicity such as chlorosis, necrosis, wilting, stunted plant growth, and reduced productivity (Campos et al. 2014). Roots are the first organs exposed to As stress exhibited inhibition of root extension and proliferation due to plasmolysis of roots (Carbonell-Barrachina et al. 1998). In addition, once inside the plants, As interfere with major metabolic functions (photosynthesis, respiration, and carbon metabolism) and influence the nutrient acquisition by competitive inhibition (Srivastava et al. 2009). It has also been reported to react actively with thiol (SH-) group of the enzymes and disturb the various biochemical processes, thereby ensuing reduced crop productivity (Sharma 2013). As have been reported to stimulate the generation of ROS either by conversion of one form of As to the other or by malfunctioning of cellular processes, thus resulting into peroxidation of lipids which disturb membrane stability and hence inhibit nutrient transport pathways (Gomes et al. 2012; Liu et al. 2012). To combat the As-induced toxic effect stress, plants are inherited with various detoxification mechanisms to keep the metalloid content to minimum level. Damaging effects of As caused by ROS are alleviated through activation of antioxidative defense system [enzymatic superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APOX); nonenzymatic ascorbate, proline, carotenoids, anthocyanins, flavonoids, glutathione (GSH), phytochelatins (PCs)] (Srivastava et al. 2009).

6.1.1 Arsenic in Environment

As is a naturally occurring ubiquitous element that ranks 20th in abundance on the earth's crust, 14th in the seawater, and 12th in the human body (Mandal and Suzuki 2002), with its average concentration ranging from 1.5 to 5 mg kg^{-1} (as reviewed by Sharma et al. 2014). It enters naturally into the soil through processes such as weathering and disintegration of As-bearing rocks as well as via volcanic eruption (Punshon et al. 2017). In non-contaminated soils, As concentration may range from 0.1 to 55 mg kg^{-1} with the total amount of As in oceans approximately 3.7×10^6 kt (kiloton), 9.7×10^5 kt in earth's crust, 25×10^9 kt in sediments, and 8.12 kt in the atmosphere (Bissen and Frimmel 2003) on a global basis. As is naturally present in groundwater of countries like India, Chile, Hungary, Vietnam, Mexico, Taiwan, Romania, Argentina, and many parts of the United States (Bissen and Frimmel 2003). However, As concentration in different terrestrial lands may vary with 50–3200 μg L^{-1} in India, 0.01–626 mg kg^{-1} in China, 3–51 mg kg^{-1} in Bangladesh, 2.5–15 mg kg^{-1} As content in Germany, 1.0–20 mg kg^{-1} in the United States, and 1.8–60 mg kg^{-1} in Italy (Shrivastava et al. 2015). Atmosphere serves as a repository for As which stores approximately 1.2×10^7 kg As $year^{-1}$, with 8.9×10^6 kg As $year^{-1}$ emitted by volcanic eruptions, 2.1×10^7 kg As $year^{-1}$ contributed by microbial volatilization (Jang et al. 2016), and 3345 t by natural forest fires (*Jeke* 1994*;* Bissen and Frimmel 2003).

With the globalization and industrialization, human interference is increasing with each advancing day, thus disturbing the natural environment. Elevated As concentration in soil and groundwater could be attributed to anthropogenic activities like mining, refining of ores, coal burning, influx of As-laden fluid and wastes from modern plants, water system with As-polluted water, and utilization of As-based pesticides, herbicides, and fertilizers, with concentrations as high as 100–2500 mg kg^{-1} (Sharma et al. 2014). With increasing population, usage of chemical fertilizers and pesticides has increased exponentially to improve crop production. These fertilizers contain As as one of the adulterants along with the other toxic heavy metals, which tend to accumulate in the soil and change soil properties (Savci 2012). Agriculturists use inorganic As salts like calcium arsenate, lead arsenate, sodium arsenate, etc. as bug sprays/pesticides for debarking trees, in steers and sheep to control ticks, bugs, and lice and furthermore in sea-growing weed control, resulting into widespread As contamination in the environment (Hartley et al. 2003). As per data available, approximately 1000 metric tons $year^{-1}$ of As is distributed via irrigation through tube wells during the dry season (Saha 2006). One of the main reasons for As contamination is digging of tube wells deep inside the crust which leads to weathering of rocks and therefore release of As into the groundwater. This As-contaminated water is then cycled for drinking as well as irrigating the fields (Huq et al. 2006). Another source of As contamination in the environment is through combustion of coal and fossil fuel which causes long-term and persistent deposition of As in the local and encompassing territories (Matschullat 2000). In addition to this, global release of As has been reported from the smelting of ores, sludge, and

flue dust which was estimated to be 53.5 kt in 2008 (USDI 2009). As accumulation close to a lead smelter was estimated to be 2 (g kg^{-1}), close to a copper smelter 0.55 (g kg^{-1}), and near gold smelter 0.5–9.3 (g kg^{-1}), where 80% of As is emanated by Cu refinery and coal combustion (as reviewed by Bissen and Frimmel 2003). Furthermore, domestic waste and sewage from the treatment plants further contribute to As addition to the terrestrial environment (Beesley and Dickinson 2010) as well as to the groundwater. All these factors lead to elevated As accumulation in the environment and thereby exceed the permissible limit of As in drinking water (10 μg L^{-1}) and agricultural soils (20 mg kg^{-1}) (Atker and Naidu 2006).

6.1.2 Geochemical Processes and Physicochemical Behavior of Arsenic in Soil

As is an important component of more than 300 minerals (Hudson-Edwards and Santini 2013) and is associated with sulfides, arsenides, oxides, and hydroxides of aluminum (Al), iron (Fe), manganese (Mn), copper (Cu), and cobalt (Co) (Jang et al. 2016). The amount of As in soil and water greatly depends upon soil physical and chemical properties that influence adsorption-desorption processes. As-bearing sulfides are considered as primary minerals generated by mesothermic, hydrothermic, or diagenetic processes (Hudson-Edwards and Santini 2013). Primary and most abundant As-containing minerals present in the environment are arsenopyrite ($FeAs_5$), realgar (As_4S_4), orpiment (As_2S_3), and enargite (Cu_3AsS_4) (Ravenscroft et al. 2009). Other than this, secondary As-bearing minerals include scorodite and kankite (Fe arsenates), tooeleite (Fe sulfoarsenates), yukonite (Ca-Fe arsenates), pharmacolites (Ca-Mg arsenates), nickeline (NiAs), loellingite ($FeAs_2$), tennantite ($(Cu, Fe)_{12}As_4S_{13}$), and arsenolite (As_2O_3) (Mandal and Suzuki 2002; Ravenscroft et al. 2009). Temperature is an important factor in controlling the As mineralogy (Smedley and Kinniburgh, 2002; Henke and Hutchison 2009). At 400–450°C, As may precipitate as arsenopyrite (Luengo et al. 2007), and at a temperature between 150 and 250, As is precipitated as orpiment, realgar, or the elemental form As(0).

6.1.3 Different Arsenic Species Present in the Environment

As exist in the environment in five oxidation states, which are denoted as +1, 3, 0, +3, and +5 (Sharma et al. 2014). On the basis of oxidation states, As can be classified into organic and inorganic form, where trivalent oxidation states of As (organic as well as inorganic As species) are more toxic than those in the pentavalent oxidation state (Patel et al. 2005). The major chemical forms of As present in the environment are arsenate (As V), arsenite (As III), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenocholine, and arsenobetaine (Tangahu et al. 2011). Inorganic species of As, i.e., As V and As III, are more prevalent and toxic

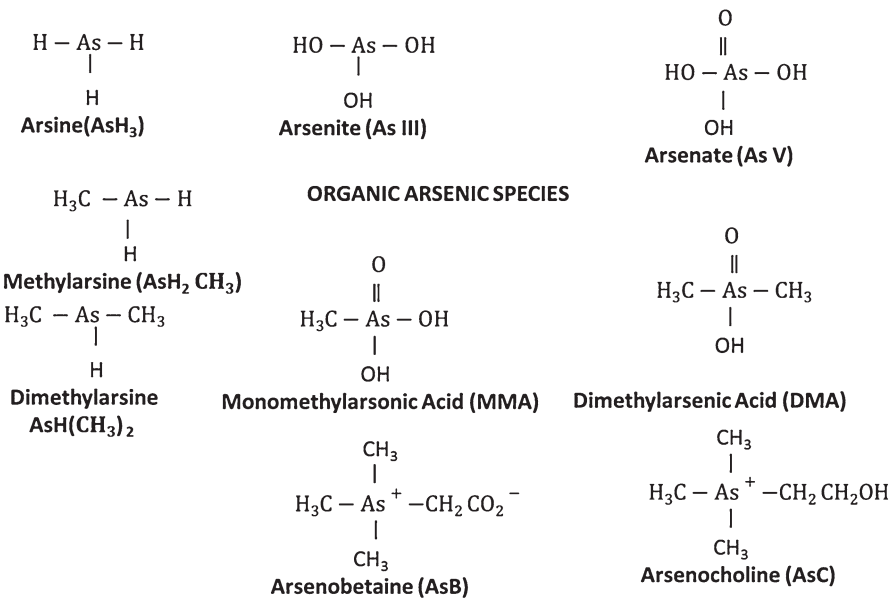

Fig. 6.1 Inorganic arsenic species

than organic species (Fig. 6.1; Sharma 2013). Since As is redox-sensitive metalloid, with the fluctuation of redox or pH in the soil ecosystem, interconversion between different As species takes place (Georgiadis et al. 2006). As V is prevalent in aerated soils, while under reducing conditions, As III is predominant (Mitra et al. 2017). However, under anaerobic environmental conditions, highly toxic inorganic As species could be reduced to less toxic, volatile, and easily oxidizable methylarsines. In oxic conditions with soil pH ranging 4–8, As V and As III are the most abundant and thermodynamically stable species present in the terrestrial environment (Stazi et al. 2015). As toxicity depends upon its oxidation state which decreases in the order of (Hindmarsh et al. 1986) arsine >arsenite >arsenoxides >arsenate >arsonium compounds >arsenic. Contrary to this, Vega et al. (2001) reported As toxicity as arsenite (As III) >monomethylarsine oxide (MMAOIII) > dimethylarsinous glutathione (DMAIIIGS) > dimethylarsinic acid (DMAV) > monomethylarsonic acid (MMAV) > arsenate (As V).

6.1.4 Factors Affecting Arsenic Absorption and Availability in Soil

As sorption in the soil particles is the essential process that immobilizes this metalloid in the soil. The affinity of As for oxide surfaces is well known and can be affected by several biogeochemical factors such as soil pH, redox minerals, organic content, soil texture, and ionic strength (Adriano 2001).

6.1.4.1 pH

Bioavailability and solubility of As is generally dependent on soil pH which determines As speciation and leachability (Adriano 2001; Quazi et al. 2011; Sahoo and Kim 2013). As in the form of As V gets strongly adsorbed to the oxide minerals under aerobic and neutral to acidic conditions. This adsorption of As to the mineral surface prevents As leachability and solubility and thus protects the environment from As-induced harmful effects (Smedley and Kinniburgh 2002). It has been reported that maximum adsorption of As V takes place at pH 5.0 and As III between 3 and 9 pH (Aide et al. 2016). Under oxidizing environment (aerobic condition), As dominates the soil in the form of As V (H_3AsO_4) below pH 2; however, as the pH increases from pH 2 to 11, H_3AsO_4 gets dissociated to $H_2AsO_4^-$ and further to $HAsO_4^{2-}$ due to ionization of one or more protons (Flora 2015). As V is documented to adsorb strongly to the hydroxides, oxides, and sulfides in the soil under acidic and neutral conditions, while desorption takes place when the environment is alkaline. This dependence of pH could be validated by the fact that increased soil pH generally increases the negative surface charge of hydroxyl particles which encourage the desorption of As from Fe oxides (Marin et al. 1993; Streat et al. 2008) and promote mobility and exchangeable capacity, thus leading to higher availability of As to plants (Fitz and Wenzel 2002). Despite the fact that majority of the studies documented that high pH leads to the release of As to the environment, García et al. (2009) observed coprecipitation of As either with sulfates and carbonates to produce oxyhydroxides and sulfates or can precipitate to form calcium arsenate. Due to these reasons, it could be demonstrated that soils with pH close to 10.5 have maximum As V retention (Goldberg and Glaubig 1988). However when pH dips below 2.5, As V turns out to be totally protonated (Zhang and Selim 2008), rendering it with less tendency to be held by soil particles. Low pH prompts to increase As retention due to the shift in mineral surface charges from negative to positive which subsequently makes As less mobile. However, at pH less than 1, all As species exist in a neutral form where As is not sorbed and becomes mobile again (Flora 2015). As III predominates the soil below pH level 6 and has a neutral character in the soil which reduces its adsorption capacity when compared to As V (Moreno-Jiménez et al. 2012).

6.1.4.2 Redox Potential

The redox potential of As II, I/V of soil, is an imperative parameter that influences As speciation and adsorption. The redox states of soils generally range from around +500 mV (surface soils) to around −300 mV (extremely reducing conditions). The redox potential in soils depends upon partial pressure of oxygen and other redox-sensitive components like soils rich in sulfides, oxides, and microbial populations (Grafe and Sparks 2006). Under reducing conditions with redox potential ranging from 0 to 200, As is present in the +III oxidation state (As III). At this redox range, As III and sulfides are most stable, and therefore, the mobility of As is reduced due

to its precipitation with sulfides such as iron sulfide to form arsenopyrite (FeAsS). It is reported that about 65–98 % As is found as As V in acidic and neutral conditions with redox potential ranging from +500 to +200 mV (Masscheleyn et al. 1991). Low redox is reported to account for mobilization of As in the soil substrate, while under high redox potential, As forms complexation with oxides and sulfides present and gets retained in the substrate medium.

6.1.4.3 Organic Matter and Soil Composition

Organic matter is a heterogeneous compound that constitutes a series of various organic mixes of variable weights and differentially polymerized. Soils are generally constituted with elements like carbon, nitrogen, hydrogen, phosphorus, nitrogen, oxygen, and sulfur and are accompanied by function groups such as carbonyls, alcohols, hydroxides, amines, etc. The level of polymerization and molecular weight of the organic mixes generally influence the solubility and accessibility of As to plants. Reports on the effect of organic matter on metalloid availability are inconsistent. It is observed that organic compounds with larger molecular weights had more functional groups and could retain more trace elements (Moreno-Jiménez et al. 2012). Walker et al. (2004) found soils amended with organic matter declined heavy metal availability to the plants by transforming bioavailable forms to complexed forms associated with carbonates and oxides. In another study, Das et al. (2008) observed that organic mixes decreased the As accessibility through the formation of stable and insoluble arseno-organic complexes by their adsorption on to the organic colloids of the soils. Furthermore, Gadepalle et al. (2007) observed in As mobility decreases due to the formation of stable complexes when supplemented with organic matter. Contrary to this, Weng et al. (2009) observed reduced adsorption capacity of As V to goethite in the presence of fumic acid and humic acid in which could be due to retention capacity electrostatic competition between As V and organic matter. Studies conducted by Redman et al. (2002) and Buschmann et al. (2006) also demonstrated that higher organic matter enhanced the desorption of As from the soil particles due to increase in microbial activity as well as a decrease in soil redox potential through the release of As from Fe-oxyhydroxides (Harvey et al. 2002).

As behavior in the soil is greatly determined by the nature and composition of the soil. Clay soils have higher retention capacity due to the presence of oxides which have a finer texture and large surface area than the sandy soil (Fitz and Wenzel 2002; Sahoo and Kim 2013). It is observed that inorganic As species has a high affinity for hydrous oxides of Fe, Mn, and Al in corrosive soils. If iron and aluminum hydrous oxide concentrations in the soil are low, As has a tendency to be more mobile. Reduction in As availability in soil is primarily believed to be due to less-dissolvable mineral and ionic structures that are firmly adsorbed to soil particles or coprecipitated with other different minerals (Turpeinen et al. 2001; Turpeinen et al. 2002; Shrivastava et al. 2015).

6.1.4.4 Soil Microorganisms

Bioavailability, speciation, and solubility of As are largely dependent on microorganisms present in the soil. Numerous authors have accounted for microbial methylation of As by bacterial species (Bentley and Chasteen 2002; Shrivastava et al. 2015). Prokaryotes have developed metabolic strategies to tolerate As stress either by excluding As or binding As within the cell (Aide et al. 2016). Blue-green algae, the primary producers were observed to reduce As V to As III before methylation to MMA and DMA (Fitz and Wenzel 2002; Heikens et al. 2007). Masscheleyn et al. (1991) proposed that microbial mineralization of organic matter by utilizing As V as an electron acceptor reduces As V to As III, which is then effluxed out of the cells (Yin et al. 2011; Zheng et al. 2013). Srivastava et al. (2011) observed that fungi such as *Rhizopus* sp., *Trichoderma* sp., and *Neocosmospora* induce volatilization of As, which is then released into the atmosphere in the less toxic form. Another detoxification mechanism adopted by microorganisms is microbial oxidation of As III to less toxic As V with the help of arsenate oxidase (Páez-Espino et al. 2009; Zheng et al. 2013).

6.1.5 Arsenic Uptake from Soil to Plant Roots

As is fundamentally taken up by terrestrial plants through roots, while some aquatic plants can take up As from water using their leaves (Wolterbeek and Meer 2002). As V and As III are the two principal accessible forms present in the soil. An ability of plants to take up As is highly dependent upon As species as well as plant species (Fig. 6.1).

6.1.5.1 Arsenate Uptake

Numerous electrophysiological and physiological studies have demonstrated that As V, an analog of phosphorus (P), shares similar chemical and physical properties with P and, thus, competes for the same uptake transporters (Zhao et al. 2009; Li et al. 2016). Due to the high resemblance between the two anions ($H_2AsO_4^-$ and $H_2PO_4^-$), plants become vulnerable to As V as it gets effortlessly fused into cells through the high-affinity Pi transport system (Catarecha et al. 2007). Asher and Reay (1979) were the first to document that phosphate was able to strongly inhibit As V uptake. Meharg and Macnair (1990) studied the uptake mechanism in *Holcus lanatus* genotypes and observed that both As V and phosphate were taken up by similar transporters, although transporters had a greater affinity for phosphate. More than 100 members of phosphate transporters (Pht1) have been characterized in plant roots which are responsible for phosphate uptake from the soil. Genes belonging to PHT1 family (Pht1 genes) have been identified in *Arabidopsis thaliana* (9 genes, Pht1;1 and Pht1;4), *Oryza sativa* (13 genes), *Zea mays* (6), *Hordeum vulgare* (8),

and *Triticum aestivum* (2 genes) (Rausch and Bucher 2002'; Gonzalez et al. 2005; Hasan et al. 2016). Furthermore, Gonzalez et al. (2005) detected phosphate transporter traffic facilitator 1 (PHF1) in the endoplasmic reticulum of roots, senescing leaves and flowers of the plants grown in P-deficit soils. They further suggested that mutation of PHF1 in *A. thaliana* hampered the Pht1;1 trafficking from the endoplasmic reticulum to the plasma membrane and increased the As V resistance in mutant type when compared to wild type. Overexpression of AtPht1;1 (expressed in vegetative tissues) or AtPht1;7 (expressed in reproductive tissues) increased As V uptake and accumulation, hence aggravated As V sensitivity to the plant (LeBlanc et al. 2013). The tremendous increase in As V uptake and translocation was reported when OsPht1;8 was overexpressed in rice plants (Jia et al. 2011; Wu et al. 2011). Wu et al. (2011) further documented that mutation in rice OsPHF1 gene had less capacity for uptake as well as transport of P and As V from roots to shoots in rice plants. In *Saccharomyces cerevisiae*, five phosphate transporters were identified, two of which were high affinity, i.e., Pho84 and Pho89, and three were low-affinity phosphate transporters, i.e., Pho87, Pho90, and Pho91 (Persson et al. 2003). Knocking out PHO84 and PHO87 genes in yeast resulted in reduced As V uptake and improved As V resistance, indicating the role of phosphate transporters in As V uptake (Bun-Ya et al. 1992; Maciaszczyk-Dziubinska et al. 2012). However, no such responses were observed in the case of As III, indicating a different pathway for its uptake (LeBlanc et al. 2013).

6.1.5.2 Arsenite Uptake

As III is a neutral inorganic As species predominant in the anaerobic soil. Microbes (*E. coli* and yeast) and mammals were observed to transport neutral molecule such as glycerol through aquaporin channels (Bhattacharjee and Rosen 2007, Sharma et al. 2014) which could also transport As III. Ma et al. (2008) tested the members of NIP family for As III transport such as NIP1 (OsNIP1;1), NIP II (OsNIP3;1), and NIP III (OsNIP2;2-Lsi6 and OsNIP2;1-Lsi1) and observed NIP I and II could not transport silicic acid, while As III was transported through all NIPs (Mitani et al. 2008). Bienert et al. (2008) cloned isoforms of NIPs from *O. sativa (*OsNIP2;1 and OsNIP3;2), *Lotus japonicus (*LjNIP5;1 and LjNIP6;1), and *A. thaliana* (AtNIP5;1 and AtNIP6;1) which were expressed in yeast for metalloid transport were reported to have bidirectional As III transport across the plasma membrane, indicating the involvement of these proteins in toxicity via influx and detoxification via efflux of the toxic compounds. Xu et al. (2015) knocked out the gene responsible for NIP3;1 expression and observed less accumulation of As III in shoots of mutated *A. thaliana* when compared to wild type. However, the double mutant (loss of function) NIP3;1 and NIP1;1 in *A. thaliana* exhibited much higher As III tolerance and improved root and shoot growth under As III stress than their wild types. These findings suggested that NIP3;1 and NIP1;1, members of aquaporin protein family in *Arabidopsis*, play a vital role in As III uptake as well as As III translocation to aboveground parts of the plants. Various NIPs such as NIP5;1, NIP6;1, and NIP7;1

have been characterized to efflux As III out of cells (Bienert et al. 2008; Isayenkov and Maathuis 2008). Similarly, OsNIP2;1 (Lsi1) found that rice plant had an ability to extrude As III out of root cells to an external medium (Zhao et al. 2010), thus indicating bidirectional movement of As III (Xu et al. 2015). As III is the most predominant form of Ma et al. (2008) mutated OsNIP2;1-Lsi1 and Lsi2 decreased As III uptake in rice plants which greatly reduced As III accumulation in shoots and grains of rice. Besides NIP, plasma membrane intrinsic protein (PIP) such as OsPIP2;4, OsPIP2;6, and OsPIP2;7 was also found to be permeable to As III (Mosa et al. 2012). Downregulation of these PIP genes in roots and shoots of rice plants strongly reduced the As III uptake (reviewed by Li et al. 2016). Mathews et al. (2011) studied the impact of glycerol and antimonite (Sb III)—the As III analogs—and silver nitrate, an aquaporin inhibitor, on As take-up in *Pteris vittata* plants. They found no impact on As accumulation in the presence of glycerol and Sb III, yet decline was reported in the presence of silver nitrate in As III-treated plants.

6.2 Phytotoxic Effects of Arsenic on Physiological and Biochemical Attributes in Plants

Phytotoxic symptoms of As species depend upon soil texture, soil pH, organic matter, As species, and plant species (Sharma et al. 2014). Plants exposed to As inhibit seed germination (Li et al. 2007), plant biomass (Garg and Singla 2012), necrosis, chlorosis, nutrient deficiency, plant reproductive capacity, crop yield (Garg and Kashyap 2017), and ultimately plant death (Sharma et al. 2014). However, differential responses were observed among the various plant species in response to As V and As III stress (Abedin and Meharg 2002) which are explained as under.

6.2.1 Effect of Arsenic on Seed Germination, Growth, and Productivity

Plant growth is a component of complex interaction among sources and sinks, the root, and the shoot system, thus building harmony between them (Anjum et al. 2011). The streamlining of growth performance and harvest maintenance under ecological stress conditions depends upon the vegetative and reproductive development of the plants (Murtaza et al. 2016). Metal toxicity to plants not just depends on the concentration and the type of metal but also on life stage, i.e., germination, the emergence of seedlings, or vegetative developmental stage. It has been reported that both As V and As III have a negative correlation with germination as well as the seedling establishment in various crop plants. Li et al. (2007) observed that As under low concentration (0–1 mg kg^{-1}) induced seed germination and plant growth in wheat seedling, while under increasing As concentration (5–20 mg kg^{-1}), all the

factors declined gradually, indicating its toxicity. A significant decline in germination rate, seedling shoot length, seedling root length, primary leaves area, and biomass has been observed in mung bean seedlings when subject to 5μM, 10μM, and 20μM As V in petri dish experiments (Swarnakar 2016). Similarly, Akhtar and Shoaib (2014) also witnessed metalloid sensitivity to *T. aestivum* seedlings due to the accumulation of As in plant tissues. Furthermore, Singh et al. (2007) observed a significant decline in the root (up to 82%) and shoot length of *Vigna mungo* seedling exposed to different concentrations of As. As-induced inhibition in seed germination and seedling growth has also been reported in wheat (Zhang et al. 2002), rice (Rahman et al. 2007), and *Helianthus annuus* L. (Imran et al. 2013). Abedin and Meharg (2002) observed differential responses of As V and As III in eight rice varieties in terms of seed germination and early seedling growth in rice varieties. At organ level, As V had higher detrimental effects on roots, while As III affected the seed germination more. In another study, a significant decline in root length, shoot length, germination percentage, and amylase activity was observed under As V and As III in a concentration-dependent manner where As III declined all the endpoints more markedly than As V (Liu et al. 2005a, b). Furthermore, Bhattacharya et al. (2012) observed a significant reduction in seed germination in *Cicer arietinum* when exposed to varying concentrations of As V and As III concentrations (200 ppb, 400 ppb, 600 ppb, and 800 ppb), with As trioxide having more negative effects. Similarly, Sanal et al. (2014) also reported a significant reduction in α-amylase activity, relative shoot, and root length as well as seed germination in barley seedlings exposed to As V and As III stress, with higher detrimental effects observed under As III stress. This As-induced reduction in seedling growth could be due to dehydration of seedlings which comes as an instant effect of abiotic stresses and resultant inhibition of cell division. In addition, inhibition of seed germination due to As stress could also be attributed to a reduction in α-amylase activity which is the key enzyme for starch degradation (Liu et al. 2005a, b).

As stress is one of the significant deterrents for increasing crop production in the cultivated area. One of the underlying impacts of As stress on plant growth includes alteration of root architecture, inhibition of nutrient and water acquisition, degradation of photosynthetic enzymes, and ultimately reduction of crop yield (Garg and Singla 2012; Garg and Kashyap 2017). High concentration of As in the soil inhibits plant growth due to interference with various metabolic processes which eventually lead to reduced crop productivity (Mokgalaka-Matlala et al. 2008; Srivastava et al. 2009). Roots are the first site that comes in contact with this nonessential metalloid and are reported to have morphological alterations (inhibition of lateral root growth, reduced stelar system), consequently reduced roots and shoots growth (Hasanuzzaman et al. 2015). Besides the negative impacts, one of the interesting facts about As toxicity is that it has been reported to induce plant growth at a low concentration while lethal at higher concentrations (Garg and Singla 2011; Finnegan and Chen 2012). Mishra et al. (2008) observed that As accumulation and toxic effects in *Ceratophyllum demersum* escalated linearly in a concentration (As V, 0, 10, 50, 250 μM) and time-dependent manner (1, 2, 4, and 7 days), with no visible symptoms of toxicity at 4 days while a significant reduction in plant biomass with

an increase in As concentration. In another study, Sultana et al. (2012) reported that *V. mungo*, when irrigated with As-contaminated water (1, 2, 5, and 10 ppm), displayed better growth and productivity (pod and seed number) at 2 ppm, while with increase in As concentration up to 10 ppm, these parameters affected drastically, signifying induction of As toxicity increases in a concentration-dependent manner. As have also been noticed to induce morphological anomalies such as the disintegration of vascular structures in the roots as indicated by loosening of pith, cortex, and vascular bundles and lack of root hairs (Talukdar 2013). Furthermore, Srivastava and Sharma (2013) also reported that detrimental effect of As (at 400 μM and 600 μM) was reflected by poor root and shoot biomass along with an alteration in stelar system (reduced vascular bundles) of spinach plants. When compared in terms of anatomy, roots exhibit higher phytotoxic effects than aboveground parts. Talukdar (2013) observed the development of crystal sand and needle-like deposits in the leaves of *Phaseolus vulgaris* that could be considered as one of the defense mechanisms adopted by the plant to avoid the metalloid stress. Zu et al. (2016) also observed a decline in plant biomass, root to shoot ratio, and relative growth rate of 2-year-old *Panax notoginseng* (Burk.) when subjected to varying concentrations of As (20–260 mg kg^{-1}). Talukdar (2011) observed that roots of *Trigonella foenum-graecum* and *Lathyrus sativus* L. were more sensitive toward As contamination which was indicted by reduced root length and dry weight. Ahmed et al. (2006) documented a decline in plant growth and productivity in terms of root length, leaf number, plant biomass, and pod number plant in *Lens culinaris* irrigated with As-contaminated water. In another study, a decline in height and biomass of hydroponically grown *Boehmeria nivea* L. was observed with the increase in As concentration in the substrate medium (Mubarak et al. 2016). Furthermore, Melo et al. (2009) observed that castor bean grown in As-contaminated nutrient medium (0–5000 μg L^{-1}) accumulated a larger portion of As in roots (468.40 mg kg^{-1}), leading to decline in root and shoot biomass. Garg and Singla (2012) observed a significant reduction in root and shoot biomass in *Pisum sativum* subjected to 0, 30, 60, and 90 mg kg^{-1}As V stress, and the decline could be attributed to its inference with various major plant metabolisms such as chlorophyll content, nutrient uptake, and crop yield. Further, Garg and Kashyap (2017) observed a tremendous decline in plant growth and productivity in pigeon pea plants exposed to varying concentrations of As V and As III because of its lesser ability of roots to explore rhizosphere resulting in reduced aptitude for water and nutrient uptake. As-induced growth inhibition could be associated with marked anomalies in anatomical features, increased root oxidizability, discoloration of roots, and loss of root vigor (Bandaru et al. 2010).

6.2.2 *Effect of Arsenic on Functional Biology*

Processes like photosynthesis, respiration, and photorespiration are intrinsically connected and are indispensable for plants' tolerance under abiotic stresses, being prudently regulated in adverse conditions (Farnese et al. 2017). As disturbs light and dark reactions involved in photosynthesis by disrupting the morphology of thylakoid membranes and by binding to several sensitive sites of photosynthetic apparatus such as PS II (Hasanuzzaman et al. 2015). Numerous studies have validated that As reduced the net photosynthetic rate by inducing degradation of chlorophyll pigment, alteration in chloroplast structure, and transport of photosynthetic electrons, thus displaying visible symptoms of chlorosis and necrosis (Finnegan and Chen 2012). As has been reported to disintegrate photosynthetic apparatus in rice plants (leaves) subjected to As stresses by altering the shape of chloroplast, shortening the longitudinal axis of plants, concaving membranes, and restricting the accumulation and flow of assimilates which further decreased the chlorophyll content (Miteva and Merakchiyska 2002; Rahman et al. 2007). Furthermore, Rahman et al. (2007) observed the downregulation of transcriptional and posttranscriptional genes (29 kDa ribonucleoprotein) responsible for proper functioning of chloroplast which damages chloroplast ultrastructure, chlorophyll pigments, and eventually photosynthesis, therefore resulting into reduced plant growth and productivity. Disruption in ATP generation was observed in *Microcystis aeruginosa* cells when subjected to As III stress at 10 mg L^{-1} leading to reduction in cell number and yellowing of cells (Wang et al. 2012). In another study, Zu et al. (2016) observed that *P. notoginseng* when grown in soil supplemented with 20, 80, 140, 200, and 260 mg kg^{-1} As ($Na_3AsO_4 \bullet 12H_2O$) increase total biomass, chlorophyll content (Chl), Chl A, and Chl B at low concentrations (20 mg kg^{-1}) while decline was found at higher As concentrations. In another case, As accumulation in *S. densiflora* incited adverse effect on the photosynthetic pigments leading to the disintegration of photochemical apparatus which reduced plant growth (Mateos-Naranjo et al. 2012). Similarly, As-induced decline in photosynthesis was observed in numerous crop plants such as maize (Silva et al. 2015), rice (Rahman et al. 2007), wheat (Mahdieh et al. 2013), *C. arietinum* (Mondal et al. 2016), *P. sativum* (Garg and Singla 2012), lettuce (Gusman et al. 2013), mung bean (Upadhyaya et al. 2014), and pigeon pea (Garg and Kashyap 2017). Numerous studies have reported that As species (As V, As III) induce reduction in chlorophyll content and inhibit PS II activity which consequently lead to reduction in net photosynthetic efficiency (Marin et al. 1993; Stoeva and Bineva 2003; Rahman et al. 2007; Duman et al. 2010; Finnegan and Chen 2012). Dutta and Mondal (2014) observed adverse relative effects in photosynthetic pigments, net photosynthetic rate, intrinsic water-use efficiency (IWUE), stomatal conductance, and transpiration rate in cowpea under varying concentrations of As V and As III. The authors observed that As III exhibited a higher reduction in chlorophyll content, transpiration, and stomatal conductance, while net photosynthetic rate and IWUE were inhibited by As V. As-induced reduction in photosynthesis could be attributed to impairment in chloroplast membrane, consequently leading to

plasmolysis and necrosis (Li et al. 2006). In addition, decreased photosynthetic efficiency under As stress could be associated with reduced stomatal conductance (through lessening CO_2 uptake) and thylakoid (through photosynthetic electron transport, ATP), thus consequently damaging the plant growth and crop productivity. Various studies have reported that As-induced depression in net photosynthetic electron transport across the thylakoid membrane led to its reduced potential to make ATP and NADPH, both of which serve as a fuel to the carbon fixation reactions (Rahman et al. 2007; Duman et al. 2010; Finnegan and Chen 2012). As has also been reported to disrupt the chlorophyll biosynthesis through replacement of Mg and Fe by interfering with chlorophyll synthase (the enzyme responsible for Chl synthesis) (Li et al. 2008). Besides this, As have also been reported to decrease the enzyme activity of ribulose-1,5-biscarboxylase (RuBisCO) which is an important ingredient required during carbon fixation process (Rai et al. 2014). Ahsan et al. (2010) observed downregulation of RuBisCO activity in leaves of rice plants treated with As V suggesting interference of As with gene expression of chloroplast DNA. Therefore, the decline in plant growth and productivity could be greatly attributed to decreased chlorophyll content and decreased photosynthetic rate (Karimi et al. 2008).

Saha et al. (2017) observed decrement in dehydrogenase enzyme activities such as pyruvate dehydrogenase, isocitrate dehydrogenase, malate dehyLrogenase, and succinate dehydrogenase in TCA cycle due to conformational changes induced by As V, therefore, resulting into inhibition of major processes involved in plant respiration (Duporque and Kun 1969; Saha et al. 2017. Alteration in these enzyme activities disturbs the proper functioning of the citric acid cycle, ETC in the inner mitochondrial membrane (Dixit et al. 2002; Mukherjee et al. 2010). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a housekeeping enzyme which plays an important role in photosynthesis as well as in glycolysis, was downregulated by As treatments in rice plants, thus inhibiting prime step toward respiration (Finnegan and Chen 2012). Reduced activity of GAPDH was also reported in *Arabidopsis* (Hancock et al. 2005) and non-As hyperaccumulator *Pteris* species (Wang et al. 2012) when exposed to As V stress (Ahsan et al. 2010). The decline in GADPH activity might be due to its involvement in As detoxification by serving a function of arsenate reductase (AR) besides glycolysis (Hancock et al. 2005). According to Abercrombie et al. (2008), alcohol dehydrogenase activity increased in As V-treated *Arabidopsis* suggesting that As in the form of As V limit the carbon flow from pyruvate into the citric acid cycle by increasing fermentation (pyruvate to ethanol). In addition to this, dicarboxylate carrier 2 (DIC2), localized in the inner mitochondrial membrane, catalyzes the transport of Pi, As V, malate, oxaloacetate, succinate, or sulfate across the membrane and plays an important role in maintaining redox equilibrium between the exchange of malate and oxaloacetate. However, DIC2, when interacts with As V, negatively affects redox equilibrium between the mitochondrial matrix and the cytosol by hindering effective malate/oxaloacetate trade (Palmieri et al. 2008), thus inhibiting mitochondrial respiration and other metabolic processes. Chakrabarty et al. (2009) observed upregulation of triose phosphate/phosphate translocator gene in rice seedlings subjected to As stress which might probably

result into the transport of arsenate instead of triose phosphate across the plastid membrane. Chen et al. (2014) reported that mutated mitochondrial lipoamide dehydrogenase, a component of pyruvate dehydrogenase complex (PDC), induced sensitivity in *A. thaliana* under As III stress due to the presence of dithiol group. LPD activity was reportedly inhibited by As III not by As V because of the tendency of As III to bind the dithiol group present in LPD. Thus, inhibition of PDC by As III consequently lowers the formation of the end product of this enzyme, i.e., acetyl coenzymes, required for the citric acid cycle (Requejo and Tena 2005). Contrary to this, intermediate organic acids (pyruvate, citrate, malate, etc.) along with citrate synthase activity increased with increasing As V stress conditions indicating the adaptive tolerance mechanisms in plants.

6.2.3 *Effect of Arsenic on Membrane Stability and Nutrient Acquisition*

Higher concentration of As in the environment obstructs plant metabolic activities and hinders water as well as nutrient uptake. As competes with macro- and micronutrients for uptake either directly due to similar transport mechanisms or indirectly by altering the metabolic processes (Tu and Ma 2005; Mokgalaka-Matlala et al. 2008; Garg and Singla 2012). Stoeva et al. (2005) observed a reduction in transpiration and relative leaf water content in As-treated *P. vulgaris* cultivars. Siddiqui et al. (2015a, b) demonstrated that the water status of *W. somnifera* declined with increasing concentrations of As V and As III in the soil and the higher decline was reported in As III-treated plants. The decrement in relative leaf water content in *W. somnifera* could be attributed to impairment in working of aquaporins of plasma membrane intrinsic proteins (PIPs) which are significant channels for water and As III uptake (Srivastava et al. 2013; Siddiqui et al. 2015a, b). Contrarily, Sridhar et al. (2011) observed no effect on RWC in As-treated brake fern indicating its tendency to tolerate As stress.

In the presence of toxic elements, plant metabolism is affected directly due to deficiency of essential elements by substantial competition for uptake as well as membrane disintegration. As damages, the root membrane restricts the uptake and transport of mineral elements from soil to roots than to aboveground parts (Sachs and Michaels 1971; Reed et al. 2015). As in soil had a drastic effect on Ca, Mg, and K concentration in plants (Reed et al. 2015), thus disturbing the photosynthetic efficiency, stomatal conductance, and membrane stability. Carbonell-Barrachina et al. (1997) observed a decrease in both macro- and micronutrients in tomato plants under As stress conditions. According to Shaibur et al. (2008), nutrients such as P, K, Cu, Ca, and Mg were observed to decrease in both roots and leaves of *H. vulgare* under increasing As concentration, whereas Fe concentration was reported to increase in roots and decrease in leaves. Mateos-Naranjo et al. (2012) observed decrement in Na, K, P, and Mg concentrations in the leaves and roots of *Spartina*

densiflora with increasing As concentration. However, in the same study, Ca, Fe, and Cu concentrations were witnessed to increase with increasing As concentration in the roots, while the decline was observed in leaves. In another study, Roy et al. (2012) examined the impact of As on nutrients in red and green *Amaranthus* species and observed positive correlation of As with Ca and Mg in green *Amaranthus*, while negative correlation was reported for red *Amaranthus* depicting their variable tendency to take up nutrients under stressed conditions. Quanji et al. (2008) found the increased concentration of Mg and Ca in shoots under As stress in *T. aestivum* L., but K, N, and P concentrations decreased in both shoots and roots. Increased Ca and Mg status under As stress could be one of the defense mechanisms adopted by the plants to withstand metalloid by strengthening their cell structure and inducing cell stability as both of them are an important constituent of the cell wall. Furthermore, Dwivedi et al. (2010) recorded higher nutrient acquisition under low As V concentration (4 and 8 mg L^{-1} As); however, under higher As concentrations (12 mg L^{-1} As), the nutrient uptake was limited. Mateos-Naranjo et al. (2012) observed phytotoxic effects of As on *S. densiflora* due to lesser absorption of nutrients, thus reduced photosynthesis and plant growth. Carbonell-Barrachina et al. (1998) observed a reduction in K, Ca, Mg, B, Cu, Mn, and Zn in *Lycopersicum esculentum* Mill. under As stress. Tu and Ma (2005) examined the concentrations of macro- and micronutrients in fronds of hyperaccumulator and non-hyperaccumulator *Pteris vittata* species and found that nutritional status was within the normal range in non-hyperaccumulators while, in hyperaccumulators, P and K were reported to enhance under stressed conditions. As-mediated increased K uptake could be mechanisms adopted by hyperaccumulators to counter extra balance anions produced by As accumulation in fronds. Similarly, Gomes et al. (2012) observed enhancement in macronutrients (P, K, Ca, Mg, N, and S), while micronutrient concentrations were noticed to reduce (Fe, Mn, Cu, Zn, B, and Mo) in both roots and shoots of *Anadenanthera peregrina* under varying concentrations of As. This increase in macronutrients under high As stress might be a defense strategy adopted by the plants to cope up with the stress as these elements are the main constituents of secondary metabolites and enzymatic and nonenzymatic antioxidants required by the plants under unfavorable conditions. In addition to this, Farnese et al. (2014) measured mineral (P, K, Ca, Mg, Fe, Mn, Cu, and Zn) concentration *via* inductively coupled plasma spectrophotometer in *P. stratiotes* under As stress where it was reported to decrease Cu, Fe, and Mn uptake in the plants with no effect on K, Mg, and Ca uptake. However, Klei et al. (1997) documented an increase in nitrogen, phosphorus, potassium, calcium, and magnesium concentrations in shoots of *P. vulgaris* L. Garg et al. (2015) observed deterioration in nutritional status especially N, P, and K in roots and leaves of the *P. sativum* under As toxicity under As stress. In another study, Garg and Kashyap (2017) also observed substantial decline in macro- and micronutrients in *Cajanus cajan* plants subjected to As (As V and As III) stress. Thus, it could be inferred on the basis of numerous studies that this differential response of As toward nutrients is inconsistent and highly depends upon the plant species as well as metalloid species.

6.2.4 Oxidative Stress

Oxidative stress is the chemical and physiological process which is associated with abiotic stresses in plants and develops as a result of overproduction of reactive oxygen species (ROS). It is considered as an indicator of stress and triggers signaling process and defense mechanisms of the plants (Demidchik 2015). Oxygen (O_2) can be seen as a double-edged sword where on the one hand it is necessary for aerobic metabolism, normal growth, and development and, on the other hand, its incomplete reduction often leads to formation of ROS such as singlet oxygen ($^1O_2$), superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\cdot}$) (Gratão et al. 2005; Halliwell 2006; Garg and Manchanda 2009). ROS is generally produced in chloroplast and mitochondria during electron transport at photosystem I (PS I) and photosystem II (PS II); however, during stressed conditions, absorption of light energy exceeds the capacity of photosynthetic electron transport (Sewelam et al. 2016), generates $^1O_2$ at PS II and $O^{2-\bullet}$ as by-products at PS I and PS II (Asada 2006; Schmitt et al. 2014), and affects the plastoquinone pool redox balance. Superoxide radicals generated in chloroplast as a result of NADPH reoxidation and O_2 reduction at PS I are dismutated into H_2O_2 by superoxide dismutase (SOD). Superoxide radical, which is unable to diffuse out of cell membrane, reacts with H_2O_2 to form a highly reactive substance, i.e., hydroxyl radicals (OH•) (Bowler et al. 1992). Besides this, enzymes such as amine oxidase, oxalate oxidase, and xanthine oxidase are considered as a major source of H_2O_2 (Mittler 2002), while NADPH oxidase is the major source of $O_2^{\cdot}$ in plant cells and cell wall-bound peroxidase activities (Torres et al. 2005).

Numerous studies have advocated that plants exposed to As (organic as well as inorganic) result into the production of ROS, thus leading to reduced photosynthesis, respiration, membrane integrity, and redox balance (Meharg and Hartley-Whitaker 2002; Mishra et al. 2011). Mylona et al. (1998) suggested that intra-conversion of one ionic form of As to the other may restrain mitochondrial electron transport chain which may be involved in the generation of oxidative radicles in plants (Meharg and Hartley-Whitaker 2002) inducing oxidative damage to protein, lipid, and nucleic acid (Tripathi et al. 2007). Similarly, in another study, cytochrome/COD have been reported to induce reduction of As V to As III where oxygen acts as last electron acceptor in mitochondria and chloroplast (Tamaki and Frankenberger 1992). Furthermore, Singh et al. (2007) observed that cytochrome oxidase reacts with oxygen and increases root oxidizability as well as H_2O_2 content in the plants exposed to As, thereby resulting into peroxidation of membranes. Plants exposed to different forms of As disrupt the equilibrium between ROS generation and antioxidants that interfere with cellular function as well as metabolism (Anjum et al. 2016). Plants under stressed conditions tend to stimulate lipid peroxidation by inducing the peroxidation of polyunsaturated fatty acids, hence increasing electrolyte leakage and thiobarbituric acid-reacting substance (TBARS) content (Singh et al. 2007). Peroxidation of membranes has also been reported in *P. vulgaris* (Talukdar 2013), *Ocimum tenuiflorum* (Siddiqui et al. 2015a, b), maize (Anjum et al. 2016), and *Artemisia annua* (Kumari et al. 2017) under As stress, which results

into loss of structural integrity as well as physiological activities (Ayala et al. 2014), thereby reducing ultimate crop yield. Hartley-Whitaker et al. (2001) witnessed differential response of four *H. lanatus* L. clones in terms of lipid peroxidation with no significant change in As V-tolerant clone while an increase significantly in non-tolerant clones. Mishra et al. (2008) observed that *C. demersum* did not experience oxidative stress under low As concentration (As V 50 μM) till 4 days; however, further increase in As stress (As V 250 μM) and duration (7 days) led to an escalation of oxidative stress and hampered plant growth. In addition, As-induced oxidative stress was also reported in *P. sativum* (Garg and Singla 2012), *V. radiata* (Upadhyaya et al. 2014), *P. vittata* L. and *P. ensiformis (*Singh et al. 2006), *H. lanatus* L. (Hartley-Whitaker et al. 2001), chickpea (Gunes et al. 2009), *Phaseolus aureus Roxb.* (Singh et al. 2007), rice (Shri et al. 2009), and *T. aestivum* L. (Hasanuzzaman and Fujita 2013). Besides destructive nature of ROS, they are also believed to regulate signaling pathway under stressed conditions and trigger defense mechanism (Meharg and Hartley-Whitaker 2002; Catarecha et al. 2007).

6.2.5 Nitrogen Metabolism

Ammonium and nitrates are the accessible forms of nitrogen absorbed by the plant's roots. BNF is the process of conversion of atmospheric N_2 into plant-usable form, i.e., NH_3 by nitrogen-fixing bacteria encoding a nitrogen-fixing enzyme known as nitrogenase (Franche et al. 2009; Santi et al. 2013). Rhizobia often experience different stresses that influence their development, initial steps of symbiotic interactions, and their ability of nitrogen fixation (Niste et al. 2013). Pandey et al. (2012) observed a decline in growth and nitrogenase activity in *Anabaena* sp. PCC7120 after 1 day and recuperation following 15 days (15 d) of As introduction to the BG-11 medium. Sultana et al. (2012) contemplated pot experiments to study the impacts of As (present in irrigation water) on nodules in *V. radiata* in Sara soil (non-calcareous and non-saline) and Barisal soil (calcareous and marginally saline) where an increase in nodule number was recorded till 2 ppm As concentration, while beyond this nodule number declined. Mandal et al. (2011) observed a significant delay in nodule formation and development along with a twofold decline in nitrogenase activity in the *V. mungo* plants grown in As-treated composite. Pajuelo et al. (2007) observed 75% decline in nodule number and 90% reduction in infection sites of *Rhizobium* (*Sinorhizobium* sp. strain MA11) under As stress (25–35 mM arsenite) in *Medicago sativa*. Singh et al. (2014) performed RT and qRT to articulate the expression of nifH1 gene under As, butachlor, Cd, Cu, and NaCl in *Anabaena*. The study validated that nifH1 gene expression decreased when *Anabaena* sp. (PCC7120) were grown in nitrogen-deficient BG-11 liquid medium at 27 °C, pH 7.5, under As, Cd, Cu, and NaCl. No corresponding bands were found in RT-PCR under As and Cd, suggesting the high sensitivity of both the stresses toward nifH1 gene, i.e., nitrogenase activity. Lafuente et al. (2010) examined the molecular mechanisms to analyze different events leading to nodule formation by

reverse transcription-polymerase chain reaction (RT-PCR) and real-time RT-PCR. The outcome from RT-PCR recorded a significant decline in gene expression of early nodulin genes such as nork (nodulation receptor kinase)- gene encoding Nod factor receptor, NIN (for knob commencement), N6 (a marker for the continuation of infection), and Enod2 (nodule organogenesis). The authors also documented that As could negatively affect nodulation at early stages and affect the expression of genes in the epidermis and outer cortical cells; however, genes involved in the process present in inner cortical cells are less affected, indicating higher toxicity during establishment. Jha and Dubey (2004) in another study demonstrated that As hindered the activities of enzymes involved in nitrate assimilation such as NR (nitrate reductase), NiR (nitrite reductase), and GS (glutamate synthetase), thus resulting into limited reduction of NO^{3-} in rice seedlings during germination. Furthermore, Singh et al. (2009) also found a decrease in nitrate and nitrite reductase activities in roots, rhizome, and fronds of *P. ensiformis* and *P. vittata* grown hydroponically under 150 or 300 μM of As stress (Na_2HAsO_4). Similarly, in another study, a sharp decline in nitrate assimilatory enzymes was reported in rice seedlings grown in sandy soils, suggesting decreased affinity of nitrogen assimilatory enzyme toward their substrate (Jha and Dubey 2004). Bianucci et al. (2017) also found significant decline in nodule number, nodule dry weight, and nitrogen content in alfalfa plants exposed to As V stress. Lafuente et al. (2015) studied the impact of As III on genetic regulation of nodulation in *Medicago-Ensifer* and observed the inhibitory effect on nodulation potential. Furthermore, the results suggested that chalcone synthase, the enzymes involved in the first step of the legume-rhizobia cross talk, enhanced, while other genes involved in infection, thread formation, and nodule organogenesis were suppressed. Therefore, the decrease in nodulation potential and efficiency under As stress could be due to root hair damage, thus shorter root zone and resultant less nutrient acquisition.

6.3 Adaptive Tolerance Mechanisms

Variability among the plants to tolerate and take up As provides a potential to develop agronomic species more suitable to As-enriched soils. Cultivars should be developed that have lesser ability to uptake As or have a tendency to restrict As translocation to the aboveground parts, so that dietary exposure of animals/humans to As is reduced. The plants that inhabit in metal-contaminated areas are evolved inherently to tolerate metal stress (Garg and Singla 2012) and can even flourish on contaminated soils (Tlustos et al. 2006). Plants can be categorized into following categories depending upon their capability to tolerate As stress.

Indicators: These are the plant that tends to accrue metals in the aboveground parts, and the number of metals in these tissues reveal the metal levels in the soil.

Avoiders: One of the tolerance mechanisms adopted by the plants growing in As-contaminated soils includes reduced As uptake. Plants such as *H. lanatus* and *Cytisus striatus* generally inhabit in As-enriched soils and are adapted to grow in

such environment by constitutive suppression of high-affinity phosphate transporters (which also mediate As uptake) (Meharg and Macnair 1992; Bleeker et al. 2003), which lead to reduce As V uptake. Various studies have found that overexpression of high-affinity P transporters (PHT1) in roots and leaves of rice plants increased As V and P uptake as well as translocation (Jia et al. 2011; Wu et al. 2011).

Excluders: These are the plants that efficiently accumulate a large number of metalloids in their roots and prevent further translocation into aerial parts. Exclusion and volatilization of metalloids occur in all living organisms to reduce total accumulation and tolerate As stress. One of the basic mechanisms of detoxification in plants involves the reduction of As V to As III, which is then effluxed out of the cells through specific arsenite transporters (ArsB or ACR3) (Yang et al. 2012; Saunders and Rocap 2016). However, several bacteria, yeast, and mammals have the capability to methylate As III into gaseous trimethylarsine (TMA) which is less toxic than inorganic As species *(*Messens and Silver 2006).

Accumulators: These are the plant species that have the capacity to accumulate metal(loid)s in the aboveground parts, to the levels so far surpassing those present in the soil. Despite the high concentration of metals in the cytosol of the cells, they can survive and can carry out biochemical and physiological activities proficiently.

Some fundamental detoxification mechanisms adopted by the plants to withstand As stress (Fig. 6.2) are as follows.

6.3.1 Reduction of Arsenate to Arsenite

Arsenic speciation is a significant determinant of its uptake, transport, and detoxification in the plants. Numerous studies have documented that the plants have an inherent aptitude to reduce As V into As III with the help of an enzyme arsenate reductase (AR) (Dhankher et al. 2006; Xu et al. 2007). This conversion of As V to As III is considered as the first step involved in detoxification in prokaryotes as well as eukaryotes either by the exclusion of As III from the cell back to the soil or by sequestration in vacuoles (Shi et al. 2016). AR has been reported to evolve at least thrice by the process of convergent evolution (Mukhopadhyay et al. 2002) where two families of AR (ArsC) have been identified in bacteria and one family (ARC) discovered in eukaryotes. AR genes have been identified in many plants such as *P. vittata (PvACR2*), rice (*OsACR2; OsACR2*), *A. thaliana (*ATQ1 and HAC1), and *H. lanatus* (HlAsr) by using sequence homology with ACR2, the arsenate reductase gene found in *S. cerevisiae*. Dhankher et al. (2006) observed that knocking out ACR2 gene in *A. thaliana* led to higher As accumulation in aboveground parts and therefore more susceptibility toward As V stress. Duan et al. (2007) observed that increased expression of *OsACR2;1* and *OsACR2*;2 in the rice roots and shoots had higher resistance for As, when the plants were exposed to As V stress, indicating

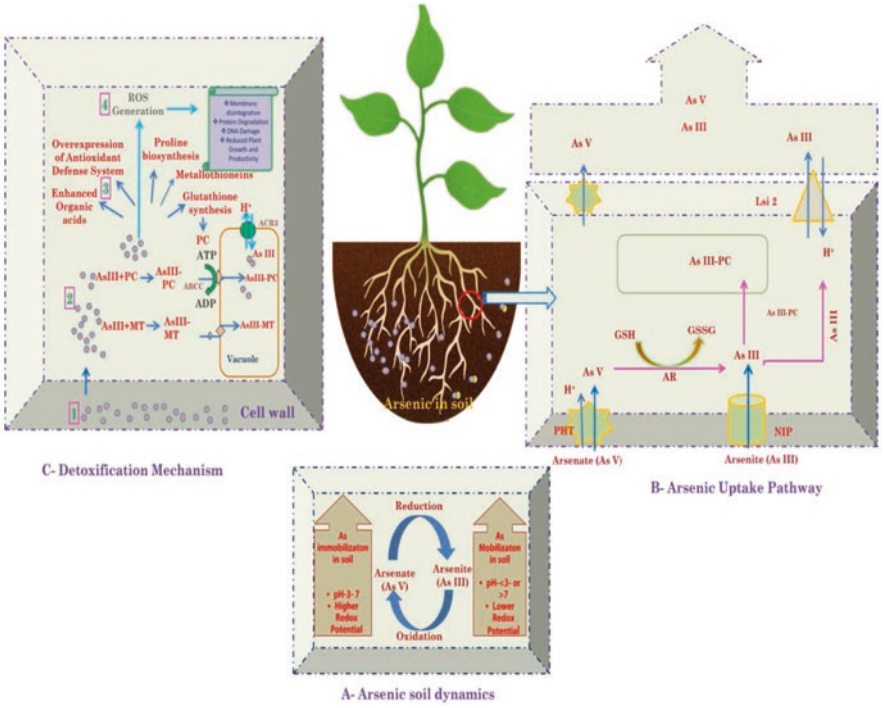

Fig. 6.2 Arsenic uptake pathways and detoxification mechanisms in plants. (**a**) Dynamics of arsenic in soil; (**b**) arsenate (As V) and arsenite (As III) are taken up by the plant roots via PHT (phosphate transporters) and NIP (nodulin 26-like intrinsic protein), respectively. Inside the plant cells, As V gets reduced to As III by AR (arsenate reductase) in the presence of GSH (glutathione acting as reductant) which forms complexation with PC (phytochelatin) and gets sequestered in the vacuoles; (**c**) arsenic detoxification mechanism (1) immobilization of metalloid in the soil or to the cell wall to avoid As uptake, (2) binding of metalloid to PC and sequestration into the vacuoles with the help of ABCC transporter, (3) activation of various biochemical processes, (4) free metalloid in the cytosol-induced ROS generation and consequently inhibit cellular functions

their role in detoxifying As V. Two AR genes, namely, ATQ1 (arsenate tolerance QTL) (Sánchez-Bermejo et al. 2014) and HAC1 (high As content) (Chao et al. 2014), were identified in *A. thaliana* and were reported to have functional role in As tolerance in plants. Furthermore, Shi et al. (2016) also identified OsHAC1;1 (epidermis, root hairs, and pericycle cells) and OsHAC1;2 (epidermis, outer layers of cortex, and endodermis cells) in the roots of *Arabidopsis* exposed to As V where knocking out of OsHAC1;1 or OsHAC1;2 gene reduced the conversion of As V to As III, thus lessened the exclusion of As III to the external medium. However, when these two genes were overexpressed, As III efflux increased, thereby, reducing As accumulation and hence enhanced As tolerance. Similar to OsHAC1;1 and OsHAC1;2, a mutation in OsHAC4 (maturation and elongation zone of the rice roots) led to the decreased exclusion of As III out of the cell which in turn increased As accumulation, therefore higher As sensitivity (Xu et al. 2017). Thus, it could be

inferred that AR could restrict the translocation of As V to the aboveground by chemically reducing As V to As III parts of the plants in the outer layer of the cell and reducing the cellular As burden by efficiently effluxing As III out of the cells.

6.3.2 Chelation and Sequestration

Plants are inherently equipped with an efficient defense system to withstand the toxic effects of metalloids that penetrate into the cytosol (Lyubenova and Schröder 2010). Besides reduction of As V, another important strategy adopted by the plants to detoxify this nonessential metalloid is by biosynthesis/stimulation of low molecular weight proteins such as glutathione, phytochelatins (PCs), and metallothioneins (MTs) that form stable complexes with this metalloid and enable its transport to the vacuoles (Verbruggen et al. 2009). PCs are considered as the early indicators of metalloid stress in the plants which form a metalloid-phytochelatin complex (As III-PC) and are then shipped into the vacuoles (Song et al. 2014). GSH, which is the precursor for biosynthesis of PCs, also plays a vital role in imparting tolerance to plants against As stress. Since PCs are constitutively expressed in plants, their activity is highly dependent on the presence of metalloid in the cytosol (Gasic and Korban 2007). Chelation of As III with PCs is one of the critical mechanisms adopted by the plants to detoxify metalloid in hyperaccumulator or non-accumulators (Zhao et al. 2003; Duan et al. 2013). The chelation of As with glutathione and phytochelatins has been reported in various plant species, such as *C. arietinum*, *Rauvolfia serpentina*, *H. lanatus*, *P. cretica*, *H. annuus*, and *Brassica juncea* (Tripathi et al. 2007; Gupta et al. 2008). Kumari et al. (2017) observed that the expression of PCs increased by 2.4-fold and 1.6-folds in leaves and roots of *Artemisia* grown under As stress. Furthermore, Batista et al. (2014) observed upregulation of gene responsible for PC biosynthesis (ECS gene (γ-glutamylcysteine synthetase) and AtPCS1 (PC synthase) in *Arabidopsis* plants subjected to As V stress, thus increasing tolerance against the stress. Kamiya and Fujiraw (2011) observed that after mutation in ATPCS1 genes (mutation in Glu^{52}), *Arabidopsis* became sensitive for As V stress; however, with the introduction of AtPCS1 gene from wild type, the function of PC was restored, thereby validating the role of PCs in detoxifying metalloid. Genes responsible for phytochelatin synthase in *C. demersum (CdPCS1)* were introduced into tobacco (Shukla et al. 2012) and *Arabidopsis* (Shukla et al. 2013), and the resultant transgenic plants displayed higher metal accumulation without exhibiting negative effects on its growth and metabolism. Besides PCs, metallothioneins are cysteine-rich metalloid chelating proteins which also perform a function similar to that of PC, i.e., immobilization and sequestration of metalloid into the vacuoles (Capdevila and Atrian 2011). Malik et al. (2012) observed 74% increase in metallothioneins in mung bean seedlings subjected to As stress. The detoxification mechanism is incomplete without stabilization and sequestration of the nonessential metalloid into the vacuoles (Furini 2012) via specific energy-driven transporters (V-ATPase and V-PPase) (Sharma et al. 2016). Metalloids in the cytosol, when

complexed with thiol peptide groups, are then sequestered into the vacuoles to protect the plants from damaging effects of these toxic ions. Song et al. (2010) identified two ATP-binding cascade (ABC) transporters in the vacuoles of *Arabidopsis*, namely, AtABCC1 and AtABCC2, which sequestered As III-PC complexes into the vacuoles. In the similar study, authors observed that double-mutant atabcc1 and atabcc2 were highly sensitive to As stress than their wild types. However, As resistance was provided to *Arabidopsis* when these two ABCC transporters were expressed in *S. cerevisiae* strain expressing phytochelatin synthase gene, thus indicating interdependence of PC biosynthetic gene and PC transporter for mediating tolerance. However, overexpression of OsABCC1 in yeast or *Arabidopsis* led to enhanced As resistance than their wild type by vacuolar sequestration of the metalloid. In addition, some As transporters involved in tonoplast transport and vacuole sequestration have been characterized in *P. vittata* (PvACR3). PvACR3, As(III) efflux protein, is located in the tonoplast which sequesters As III into the vacuoles. Overexpression of PvACR3 in transgenic *A. thaliana* had higher tendency to sequester As into the vacuoles (Indriolo et al. 2010). In another study, Song et al. (2014) observed ABCC1 transporters in the roots, leaves, nodes, peduncle, and rachis of rice plants. Knockout of this gene in the nodes of rice resulted into 13–18-fold increased allocation of As V to the rice grains and flag leaves. Furthermore, Moore et al. (2014) found OsABCC1 transporter in the companion cells of phloem in nodes which prevent As translocation into the grains by shipping PC-As III complexes into vacuoles (in nodes). Taken together, it could be inferred that for better As resilience, plants require both PCs and the PC transporter (Song et al. 2010).

6.3.3 Upregulation of Antioxidative Enzymes

Primary mechanism adopted by the plants in detoxifying metal(loid)s is by the generation of metabolites like glutathione, metallothioneins, and phytochelatins that can bind the xenobiotics in the cytosol, subsequently compartmentalizing them into the cell vacuoles (Gupta et al. 2011). However, in case of inability of these defense mechanisms to compartmentalize the toxic ion, various biochemical processes get turned on due to overproduction of ROS and activate other defense mechanisms (Srivastava et al. 2005). To neutralize the impacts of oxidative stress, endogenous defense mechanisms such as enzymatic and nonenzymatic antioxidants get activated (Gusman et al. 2013). The enzymatic defense antioxidants include superoxide dismutase (SOD), guaiacol peroxidase (GPOX), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPx), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR); and nonenzymatic antioxidants are a-tocopherol, ascorbic acid (AA), carotenoids, polyamines, anthocyanins, and flavonoids (Panda et al. 2003). Upregulation of antioxidative defense enzymes was reported in *H. lanatus* (Hartley-Whitaker et al. 2001), *Z. mays* (Requejo and Tena 2005), *C. demersum* L. (Mishra et al. 2008), *Hydrilla* (Srivastava and D'Souza 2010), *O. sativa* (Dave et al. 2013; Tripathi et al. 2013), *P. sativum* (Garg and Singla

2012), jute (Islamit et al. 2014), and *Z. mays* (Yadav and Srivastava 2015) and *O. tenuiflorum* (Siddiqui et al. 2015a, b). A 46–61% higher SOD activity was observed in the fronds of *P. vittata* which was linearly in line with the As accumulation in the fronds (Singh et al. 2010). Zu et al. (2016) observed a significant increase in SOD, CAT, and POD activities in *P. notoginseng* grown under As stress. Shri et al. (2009) also documented an upregulation in activities in SOD, POD APX, and GR isozymes in rice seedlings grown under As stress. Rai et al. (2011) observed a genotypic difference among the rice cultivars where As-tolerant rice cultivars (Triguna, IR-36) had elevated antioxidative enzyme activities, while a decline was observed in susceptible cultivar (PNR-519) grown under As stress.

6.4 Remedial Strategies

Recently, some exogenous measures such as the use of silicon (Si) and arbuscular mycorrhiza (AM) fungi have been widely used as important strategies for remediation of As-contaminated soils.

6.4.1 Arbuscular Mycorrhiza

Arbuscular mycorrhizae (AM) are obligate fungi, belonging to the Glomeromycota (Schüβler 2014), with a tendency to form a symbiotic association with more than 80% of plant species (Smith and Read 2008). This association is beneficial for both symbionts as the host plant supplies the fungi with carbon and AM fungi colonize the root cortex of plants and develop extrametrical hyphal network that can absorb nutrients especially P, N, etc. from the soil (Bhushan et al. 2014). In this symbiosis, plants provide the fungal partner with the carbohydrates, while AM benefits the host plants by improving nutrient uptake, increasing growth hormones in plants, and enhancing tolerance to different abiotic stresses (Upadhyaya et al. 2010). Twenty-three AM species belonging to different genera *Paraglomus* (1 species), *Gigaspora* (1 species), *Racocetra* (3 species), *Glomus* (4 species), *Scutellospora* (4 species), and *Acaulospora* (10 species) were found naturally occurring in the highly As-contaminated areas of Brazil (Schneider et al. 2013); *Glomus mosseae* and *G. caledonium* in mine spoil soil in England (Gonzalez-Chavez et al. 2002). The occurrence of arbuscular mycorrhizal species in As-contaminated soils (Smith et al. 2010) indicated their inherent capacity to withstand and proliferate under stressed conditions. Mycorrhizal inoculations have been documented to increase As tolerance of tomato (Liu et al. 2005a, b), *H. lanatus* (Gonzalez-Chavez et al. 2002), maize (Bai et al. 2008; Yu et al. 2010), white clover (Dong *et al.* 2008), *Eucalyptus globulus* (Arriagada et al. 2009), *Lactuca sativa* (Cozzolino et al. 2010), pea (Garg and Singla 2012), Chinese brake fern *(Bona et al.* 2010*;* Leung *et al*. 2013), soya bean (Spagnoletti and Lavado 2015), *T. aestivum* L. (Sharma et al. 2017), and *C. cajan*

(Garg and Kashyap 2017). AM has been reported to confer resistance to plants through extracellular chelation of metal(loids) by plant root exudates (histidine, citric, malic acid, etc.), sequestration of metal ions through glomalin (glycoprotein) (Gonzalez-Chavez et al. 2004), and binding of metal(loids) to the fungal cell wall due to presence of free amino, hydroxyl, and carboxyl groups (Upadhyaya et al. 2010), thus reducing metalloid uptake in the plants. Symbiotic association between mycorrhiza and host plant roots has been reported to increase root rhizosphere which modifies the chemical structure of root exudates and provides a friendly environment to the indigenous microflora (Ultra et al. 2007). This stimulates As co-metabolism between plant and mycorrhizal fungi and paves the way for biomethylation to transform inorganic As to organic form (Barea et al. 2005). Similarly, Mukhopadhyay et al. (2002) observed activation of microbial activities in the vicinity of mycorrhizal roots and further biomethylation of As by microbes due to release of organic acids in the substratum. In addition, various reports indicate an important role of AM in the alleviation of metal(loid) toxicity to host plants by acting as a barrier for its uptake by discriminating ions during fungal uptake of nutrients from the soil or during transfer to plant host (Sharples et al. 2000). The increased nutrient acquisition has been recorded in *M. truncatula (*Zhang et al. 2015*)*, *P. sativum* (Garg and Singla 2012), *C. cajan (*Garg and Kashyap 2017), and *Glycine max* (Spagnoletti et al. 2016). AM has been observed to reduce As V uptake by suppression of high-affinity PO_4^- transporter (mainly responsible for As V uptake) in the plant roots, and P uptake is mediated by mycorrhizal PO_4^- transporters (Gonzalez-Chavez et al. 2002). AM fungal-mediated reduced As uptake has also been observed in barley plants where downregulation of HvPht1;1 and HvPht1;2 (high-affinity P transporters) was observed, with higher expression of HvPht1;8 which transported little or no As to the plants (Christophersen et al. 2009). Rice plants inoculated with *G. intraradices* AH01 had increased expression of OsPT11-phosphate transporters and reduced expression of OsPT2 under As stress resulting into reduced As concentration inside the plants (Chen et al. 2013). In addition, stimulation of phosphate transporters such as GiPT (González-Chávez et al. 2011) and RiPT (Spagnoletti et al. 2016) was also reported under stress, indicating its functional role in improving P acquisition. Due to a higher proportion of As III than As V in mycorrhizal plants, it is hypothesized that AM might play crucial role in reducing As V to As III. However, direct evidence for AM-induced reduction of As V to As III is yet to be confirmed. Induction of RiArsA (Spagnoletti et al. 2016) and GiArsA (González-Chávez et al. 2011), gene expressions responsible for As III efflux, was found in the mycelia of *G. intraradices (Rhizophagus irregularis)* indicating a role of mycorrhiza in keeping a minimum level of As in plants. Furthermore, Chen et al. (2012) observed downregulation of Lsi1 and Lsi2 expression (genes responsible for As III uptake and exclusion, respectively) in mycorrhizal inoculated rice plants, therefore ensuing reduced As III accumulation in the plants. Therefore, AM imparts tolerance to the plants by influencing the synchronization of multiple genes for permitting sufficient nutrient uptake along with limiting As entry. In addition, involvement of AM has also been reported in complexation of metal(loid)s with cytosolic polypeptides (glutathione, phytochelatins, metallothioneins) (Javaid 2011), followed by

compartmentalization of metal(loid)s into vacuoles in plants as well as in the fungal tissues (Cobbett and Goldsbrough, 2002). Genes encoding metallothioneins have been identified in *Gigaspora rosea* (GrosMT1), *G. margarita* (GmarMT1), and *G. intraradices* (GintMT1) under stressed as well as unstressed conditions, signifying the role of AM in compartmentalizing As into vacuoles (as reviewed by Garg et al. 2017). Sharma et al. (2017) observed enhanced concentrations of cysteine, GSH, PCs, and GSTs in *T. aestivum* grown under As stress and inoculated with mycorrhiza (*R. intraradices* and *G. etunicatum*), thus, facilitating the sequestration of metalloid into the vacuoles. Lastly, the dilution effects of toxic ions through improved plant root biomass, water-use efficiency (Garg and Chandel 2011; Garg et al. 2015), and synthesis of antioxidants are other defense mechanisms that reduce oxidative stress.

6.4.2 Silicon

Silicon is a quasi-essential element which is ubiquitously present on the earth's crust, with its concentration ranging from 0.1% to 10% of dry weight depending upon the plant species (Epstein, 1999; Garg and Bhandari 2016; Garg and Singh 2017; Garg and Kashyap 2017). However, variability among different plant species has been observed concerning Si uptake. High Si accumulation has been reported in monocots such as Gramineae and Cyperaceae and low to intermediate in dicots like Cucurbitales, Urticales, Commelinaceae, and Fabaceae (Thiagalingam et al. 1977). Si is taken up by plants in the form of silicic acid in the roots through NIP transporters (similar to that of As III) and gets translocated to the shoot and subsequently deposited on the surface of leaves and stems as silica gel (Ma et al. 2001). High accumulation of Si in monocots has been attributed to active Si transporters, i.e., Lsi1 and Lsi2 which are present at the different location at the plasma membrane of exodermis and endodermis cells (Ma et al. 2006). Numerous studies have reported the beneficial role of Si in imparting metal(loid) stress in various crop plants, e.g., rice (Ma et al. 2008; Fleck et al. 2013; Song et al. 2014; Raza et al. 2016; Chen et al. 2017; maize (Silva et al. 2015; Tripathi et al. 2017), tomato (Marmiroli et al. 2014), and pigeon pea (Garg and Kashyap 2017). Si has been reported to reduce metalloid translocation from soil to roots and then to shoots via competitive inhibition. As concentration in rice roots and shoots has been reported to decrease significantly in the presence of Si in a nutrient medium containing varying concentrations of As (Guo et al. 2005, 2009). Similarly, a considerable decline in As concentration was observed in straw and husk of rice plants, signifying negative correlation between Si and As (Li et al. 2009; Fleck et al. 2013). In addition, Si imparts metal(loid) tolerance through deposition of silica in the apoplast of the roots and beneath the cuticle, thereby acting as a barrier for the apoplastic flow of toxic ions and transpiration flux (Jaiswal et al. 2017). Silicon has been reported to retain water status in the plant tissues by mechanically strengthening the cell wall by forming an association with pectin and calcium ions (Ma 2003). Furthermore, Si has also been reported improve water influx, mineral acquisition, and translocation through cell wall hardening of

the stele and endodermal tissues of roots (Hattori et al. 2003). Si-mediated nutrient use efficiency was observed in wheat plants, thereby leading to higher biomass accumulation (Neu et al. 2017). Furthermore, Ju et al. (2017) reported that concentration of macro- and micronutrients increased in plants supplemented with exogenous Si supplementation. In addition, Si also mitigates As stress by reducing the oxidative stress and inducing antioxidant defense systems in plants (Shi et al. 2005; Song et al. 2009; Tripathi et al. 2012). Tripathi et al. (2013) observed a significant increase in cysteine levels as well as antioxidative enzyme activities in rice plants supplemented with Si, thereby reducing oxidative stress.

6.5 Conclusion and Future Perspectives

Increasing levels of As in soil and the resultant toxic impacts on plant and animal systems have generated a lot of awareness and interest in the minds of scientific community worldwide. The information gathered here from the available literature comprehends the various geological, physicochemical, and biological processes which are responsible for the uptake of As by the plants and their subsequent entry into the food chain. Recent researches have been focused to understand molecular mechanisms responsible for modulating As a transport, speciation, and detoxification, highly dependent on the type of species and genotype. Application of bioinoculants such as arbuscular mycorrhizal fungi as well as fortification of soils with nutrients like Si has gained importance recently in reducing As uptake in plants due to their ability to adsorb/chelate metal(loid)s, thereby improving growth and productivity in stressed soils. However, large-scale multi-location field trials need to be carried out to understand the role of these amendments in imparting abiotic stress tolerance in various agriculturally important plant species. Moreover, the exact mechanisms adopted by these two approaches need to be well understood at genetic and molecular levels before implementation in agricultural systems.

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Chapter 7
Plant's Adaptive Mechanisms under Arsenic Pollution

Rabia Amir, Momina Hayat, Irum Nauman, Marrium Khan, and Faiza Munir

Abstract Metals/metalloids such as arsenic (As), cadmium, lead, and mercury are nonessential elements. High tissue concentrations of all these metals constitute stress and are proven to be toxic to plants. When the metal concentration in plant tissues exceeds tolerant levels, several vital plant processes such as photosynthesis, transpiration, nitrogen fixation (in leguminous plants), and carbohydrate metabolism are disrupted. Other metabolic disturbances include disruption of crucial biomolecules such as enzymes, damage to the cytoplasmic membrane, hindering functional groups of important cellular molecules, and the disruption of plant homeostasis by producing reactive oxygen species (ROS). Arsenite and arsenate produce ROS such as superoxides or peroxides in plants on exposure. ROS can damage amino acids, nucleic acids, proteins, or lipids which compromise the cellular function and can even cause cell death in plants. Moreover, As consumption is proven to be highly toxic to both animals and humans. Many plants are used as food sources around the globe. Hence, it is imperative to develop remediation measures for combating metal toxicity. Research studies have unraveled the physiology of how plants adapt when presented with elevated levels of As. The plants are highly susceptible to accumulate, uptake, and transport As to the higher levels which become toxic for the plants. In this chapter plants' tolerance mechanisms particular to As stress will be discussed. These mechanisms include metal hyperaccumulation, modification of the uptake system, and adaptation via other organisms, chelation, and precipitation.

Keywords Arsenic toxicity · ROS · HSP · Metallothioneins · Metal detoxification

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7.1 Introduction

Arsenic (As) is a class 1 carcinogen classified by IARC (International Agency for Research on Cancer) that has toxic effects on animals, plants, and human beings based on the type of the exposure (Lyon 1994). As toxicity received attention when a high level of As was reported in drinking water in Southeast Asian countries causing possible health hazards and causing serious toxicological effects on human health. As contamination is not just limited to water sources, but it is also affecting soil posing a great threat to environment and agricultural fields (Farooq et al. 2016). Due to soil contamination or As contamination in irrigation water, As tends to be taken up by the plants and accumulates in their edible parts which when consumed by human introduce As toxicity in them (Finnegan and Chen 2012). Increased As levels in plants also induce deleterious effects in plants such as chlorosis, necrosis, growth inhibition, and plant death. But response toward As contamination is different in different species like tomatoes which are more resistant to As contamination and do not accumulate it, but facts based on extended research reveal that rice is an efficient accumulator of As. This difference in behavior of plants toward As contamination is important in relation to accumulation and plants' ability to uptake, transport, and tolerate the contamination (Hettick et al. 2015).

Different types of effects are reported in plants due to As contamination, like plants contaminated due to the high concentration of As in irrigation water, have decreased height. A similar trend has been reported for leaf number, biomass, and root length in affected plants (Ahmed et al. 2006). Plant metabolic processes get affected due to an elevated level of As contamination. That leads to decrease in fresh and dry biomass of roots and shoots, low yield and fruit production, and changes in plants morphology (Garg and Singla 2011).

Two types of As are present in nature, that is, organic and inorganic As; out of these two, inorganic As is highly toxic to plants. Inorganic As includes two species arsenate and arsenite. Arsenate mimics phosphate and enters the plasma membrane phosphate transport system. Once it enters the cytoplasm, it comes in competition with phosphate and starts replacing phosphate in ATP and forms unstable ADP-As and interrupts the cell's energy flow (Ullrich-Eberius et al. 1989). Arsenite, on the other hand, is also very toxic as it interacts with the sulfhydryl group of tissue proteins and enzymes causing inhibition of cellular functions and finally death. Inorganic species are also involved in the production of reactive oxygen species (ROS). These ROS are produced in plants as the unnecessary by-product of the metabolic pathways going on in the mitochondria and chloroplast (Navrot et al. 2007). These oxygen derivatives which are partially active or reduced are highly toxic and reactive and cause the oxidative destruction of cells by damaging lipids, proteins, and DNA (Meharg and Hartley-Whitaker 2002). Due to change in the valency of As, it transforms into its highly toxic form which also helps in the production of ROS in plants (Flora 1999). This conversion is probably of arsenate to arsenite which is a more toxic form of As and produces enzymatic (catalase (CAT),

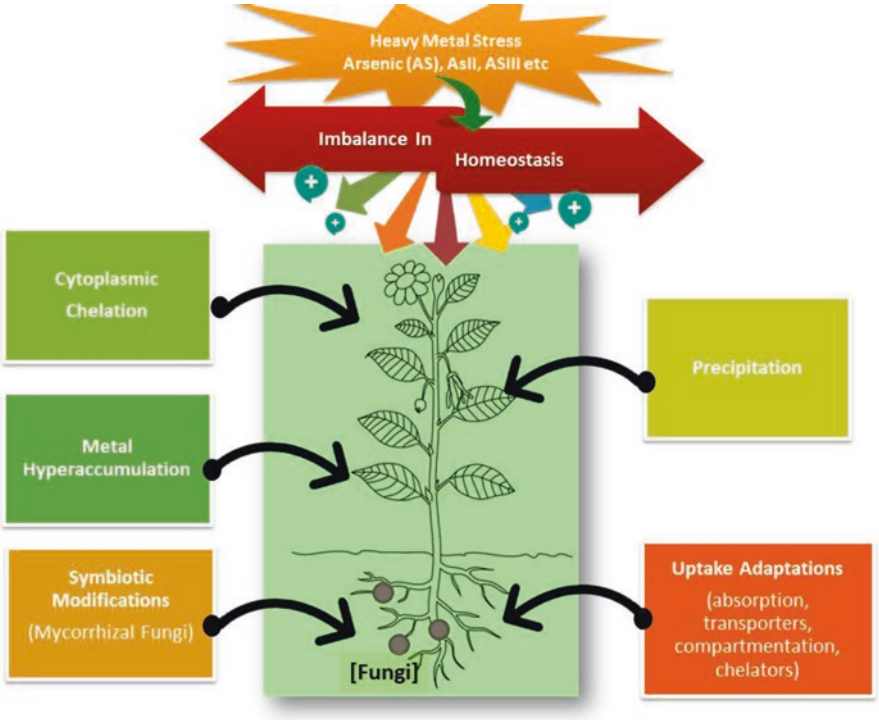

Fig. 7.1 Arsenic stress imposes an imbalance in the internal homeostasis of the plant. This results in the plant combating this stress with multiple coping mechanisms such as chelation, precipitation of the metal of plant leaves, hyperaccumulation, symbiotic modifications, etc.

superoxide dismutase (SOD), glutathione *S*-transferase) and nonenzymatic oxidants (ascorbate, AsA, and glutathione, GSH) in plants. With this reduction of arsenate to arsenite, As can be further metabolized and form methylated species increasing oxidative stress in plants and leads to the production of ROS (Zaman and Pardini 1996). In the current chapter, different adaptive mechanisms used by the plants against As stress are discussed in Fig. 7.1.

7.2 As Uptake, Accumulation, and Transport in Plants

As is a nonessential metal that is present in the environment which contaminates soil and toxic for plants. Roots of the plants are the first tissue that encounters the toxic metal and through which it enters the plant. Further, it accumulates in the biomass, and with the help of different transport pathways, it travels in the plant and negatively affects many physiological processes of the plants.

7.2.1 As Uptake and Accumulation

As exists in its organic and inorganic forms in the environment. These chemical species of As have the ability to enter the plant and accumulate in higher and lower concentrations inside the plant. Many plants can accumulate a large amount of As due to specialized cells present in the plants which take up the As from the roots to the shoots of the plants. Those plants that have less accumulation tolerance for As keeping it in the root system and do not transfer it to the shoots (Finnegan and Chen 2012). Metal hyperaccumulation can be defined as uptake and sequestration of unusually high amounts of metallic elements in the aerial biomass of a plant under field conditions (Pollard 2000). Such an example of exceptional tolerance to heavy metals is seen in certain plant species. These plant species are known as hyperaccumulators. Jaffre et al. in 1976 first used this term while studying the plant *Sebertia acumunita* of the French territory New Caledonian (Jaffré et al. 1976). The studies revealed that this plant accumulates hostile concentration of nickel (Ni) in its aboveground parts.

Many plants have the ability to accumulate a large amount of As, but they cannot be classified as hyperaccumulators since most of the uptake by these plants is slow and extended over a long period. In another study, Ma et al. (2001) reported the fast-growing Chinese brake (*Pteris vittata*) fern, as the first identified As hyperaccumulator. It was also reported that the Chinese brake fern was able to tolerate high levels of As, as well as accumulate incredible concentration of the heavy metal in its fronds.

The distinct characteristics of hyperaccumulator species are owed to their extraordinary ability of uptaking high levels of metal ions, the root to shoot translocation of these heavy metal ions, and, lastly, their sequestration in the cellular vacuole (Kanoun-Boulé et al. 2009). Plants constitute different routes for uptake of various forms of As. The two most toxic inorganic forms of As taken up by plants are arsenate (As V) and the reduced form arsenite (As III). The uptake of both these species varies. As (V) is taken up through high-affinity phosphate transporters (Meharg and Macnair 1991), whereas As (III), in the reducing conditions, is known to be up taken via aquaporins of the nodulin26-like intrinsic protein (NIP) subfamily (Ali et al. 2009). As As (V) is uptaken, it is readily reduced to As (III). This is followed by the root to shoot translocation with the final step being vacuolar sequestration.

7.2.2 As Transport Via Aquaporin Channels

The development and growth of a plant rely heavily on the essential processes carried out in its leaves. And leaves require an adequate water supply and balance to function properly and carry out these processes. The movement of water through

plants' cell membranes is facilitated by water channels, called aquaporins (AQPs). They are the proteins that belong to the major intrinsic protein (MIP) family. Members of the MIP family are found in almost all living organisms.

Competition experiments by Meharg and Jardine (2003) indicate the involvement of these aquaporin channels in As transport (Meharg and Jardine 2003). Plant aquaporins are permeable to As (III). AQPs are expressed in high concentrations in the plasma membrane of plant roots such as in *Oryza sativa* (rice) and *Arabidopsis thaliana*. Some subgroups of the nodulin26-like intrinsic proteins (NIPs), a subfamily of MIPs, have also been reported to regulate the As efflux as well as its uptake mechanisms (Dordas and Brown 2001). The two controlling factors of substrate selectivity in these channels are asparagine-proline-alanine known as NPA boxes and the aromatic/arginine selectivity filter. Although the selectivity filter does not have a defined role in As efflux, NPA boxes seem to control the transport of As into the external medium. Arsenate efflux is directly proportional to its uptake as reported in *Holcus lanatus* (Logoteta et al. 2009).

7.3 Plants' Adaptive Mechanisms for As Toxicity

Due to elevated levels of As in the environment and increased risk factors of As toxicity to the plants, plants have adapted various mechanism to combat the harmful effects. Different plants follow different mechanism to tolerate heavy metal stress (Table 7.1).

Table 7.1 General mechanisms adopted by various species to confer arsenic tolerance

Species	Tolerance mechanism	References
Pteris vittata	Hyperaccumulation	Ma et al. (2001)
Oryza sativa	Periphyton association	Shi et al. (2017)
	Efflux via Si transporters	Ma and Yamaji (2006)
Holcus lanatus	Alteration of the Pi uptake system	Macnair and Cumbes (1987)
Salvinia molesta	Glutathione-mediated detoxification	Silva et al. (2017)
Silene vulgaris	Chelation	Sneller et al. (1999)
Arabidopsis thaliana		Song et al. (2010)
Brassica juncea	Signaling via *MAP kinases*	Gupta et al. (2009)
Zea mays	Symbiotic association with mycorrhizal fungi	Ramírez-Flores et al. (2017)
Lactuca sativa		Cozzolino et al. (2010)
Eucalyptus globulus		Arriagada et al. (2009)

7.3.1 Antioxidant Defense System

Plants have complex antioxidant defense systems including an array of nonenzymatic and enzymatic processes that are able to control the unnecessary oxidation in plant cells.

7.3.1.1 Nonenzymatic Antioxidants

Toxic levels of metals are known to be responsible for disrupting the redox homeostasis in plants owing to the formation of several reactive oxygen species (ROS) (Sharma and Dietz 2009). Apart from ROS, another harmful cytotoxic compound methylglyoxal (MG) has been identified which increases as a response to heavy metals (Singla-Pareek et al. 2006). Another mechanism of heavy metal detoxification is through compounds that exhibit antioxidant activity. Plants possess a well-established antioxidant defense system. This system consists of various antioxidant enzymes and metabolites. One such component of the antioxidant defense system is the compound "glutathione."

Glutathione (GSH) is one of the major nonprotein sources of thiols in the most plant. It is an ideal biochemical that can be used to protect plants from heavy metals and related oxidative stress. GSH has been reported as part of the antioxidant barrier as it is oxidized by ROS and prevents excessive oxidation of sensitive cellular entities. It can be found in two forms, either as reduced GSH or in an oxidized form as GSSG (Yadav 2010). GSH has been known to take part in regulating hydrogen peroxide (H_2O_2) levels in plant cells. During degradation of hydrogen peroxide, the ratio of reduced GSH changes to the oxidized GSSG from.

The ratio of GSH/GSSG may be involved in ROS recognition, therefore serving as an indication of cellular redox balance. The reduced form of glutathione is directly involved in the reduction of stress generated ROS. Glutathione is also a component of AsA-GSH pathway, which serves as plant's defense weapon to scavenge ROS (Noctor and Foyer 1998). It also acts as a precursor for the synthesis of heavy-metal-binding peptides known as phytochelatins (PCs). Thus, GSH is extensively involved in the maintenance of the cellular ionic homeostasis and detoxification mechanisms of heavy metals (Yadav 2010).

7.3.1.2 Enzymatic Method

Apart from nonenzymatic metal detoxification methods, plants also adapt some enzymatic methods to deal with metal stress. Different enzymes like superoxide dismutase (SOD), catalase, ascorbate peroxidase, and glutathione reductase are involved in As toxicity to the plants. ROS species are solely responsible for the oxidative damage faced by plants as many metabolic processes produce ROS. As far

as As toxicity is concerned, inorganic As forms are highly involved in ROS generation in plants. Plants utilize their antioxidant enzymes to terminate the chains of reaction initiated by free radicals of oxidation reactions. Most of the time, enzymes are successful, but in some cases, the production of ROS is over the limits which become a problem for the plants, and they undergo oxidative stress.

Pathways involved in ROS scavenging in plants majorly include SOD and glutathione-ascorbate cycle which are found mostly in all cellular compartments, ascorbate peroxidase, and catalase (CAT) present in peroxisomes. Apart from all enzymes, SOD and APX show high affinity for H_2O_2 which suggests that these enzymes play a very important role in scavenging and controlling ROS levels during heavy metal stress. On the other hand, CAT is only present in peroxisomes, but it also has a very crucial role in detoxification of stress under high levels of ROS (Bhaduri and Fulekar 2012).

Under As stress, the first enzyme that initiates the antioxidant defense response is SOD, by reducing the superoxide free radicals into H_2O_2 and O_2. H_2O_2 is the by-product of the SOD activity, and it is further needed to be neutralized as H_2O_2 is toxic for plant health. The further detoxification is carried out by ascorbate-glutathione cycle and catalase (CAT). Ascorbate-glutathione cycle converts H_2O_2 into H_2O and O_2 (Dietz et al. 1999). This cycle involves two enzymes APX and GR. Glutathione and ascorbate both act as oxi-reductants and H_2O_2 as an electron acceptor and NADPH as a proton donor (Asada 1992).

A great amount of work is present on the effect of As-induced oxidative stress in rice seedlings. According to one study, the levels of SOD, APX, and GR increased with increase in As levels in the rice seedlings displaying adaptation of plants for oxidative stress. Based on research from few studies, it is concluded that GR (glutathione reductase) levels are high in plants under As stress especially in rice seedlings (Shri et al. 2009). According to a study related to effect of As on Indian mustard, it was revealed that SOD acted as the first line of defense, and for the H_2O_2 detoxification, APX played a very important role, and its levels were upregulated with increase in As stress, but CAT was not much involved in H_2O_2 detoxification (Khan et al. 2009). *Zea mays* plants are also investigated for their response to As oxidative stress, and it was revealed that SOD, APX, and peroxidases levels were increased, and efficient defense response was observed. To keep the free radical levels under control in plants which are increased during As stress in plants, balanced SOD, APX, and CAT activity is required.

7.3.2 Chelation Mechanisms

Heavy metal chelation is a very important mechanism which helps in the detoxification of heavy metals. The mechanism used under this process is that the heavy metals are chelated in the cytosol by ligands. Two types of ligands are present these are phytochelatins and metallothioneins.

7.3.2.1 Phytochelatins

The anions, arsenite (As III) and arsenate (As V), induce biosynthesis of compounds known as PCs (Grill et al. 1985). PCs have the general structure of (gamma-glutamyl-cysteinyl)$_n$-glycine (n = 2–11), with PC_2 and PC_3 being the most common forms. They are derived from glutathione (GSH), in the presence of the enzyme PC synthase (Inouhe 2005). PCs are thiol (-SH)-rich peptides that are characterized as heavy-metal-binding ligands, binding heavy metals to their -SH groups. As the immobilized ions are less toxic than free ions, the phytochelatins are considered as a major component for detoxification in higher plants (Schmöger et al. 2000). It is reported that, As-phytochelatin complexes are transported inside the vacuole, where they might remain stable and prevent reoxidation of arsenite to arsenate due to the acidic pH of the vacuole. Therefore, allowing the accumulation of high concentrations of As phytochelatin complexes. Plant vacuole serves as the destination of the detoxification mechanisms of heavy metal species (Hartley-Whitaker et al. 2001).

The transporters involved in As (III) PC complex transport inside the vacuole are suggested to be ABC transporters. For instance, in *Saccharomyces cerevisiae*, Ycf1p, a member of the ABC transporter superfamily, regulates the energy-driven As (III) uptake into the vacuole, conferring arsenite resistance through accumulation (Ghosh et al. 1999). In a study carried out on *Arabidopsis thaliana,* it was depicted that members of the ABCC subfamily of ABC transporter family play a vital role in As detoxification in the plant (Song et al. 2010). Another vacuolar transporter named as PcACR3 has been reported to be associated with the As tolerance in the hyperaccumulator *P. vittata*. When the transcripts of ACR3 were suppressed in the gametophyte, it resulted in enhanced sensitivity toward arsenite. This study concluded the role of the ACR3 transporter in the vacuolar sequestration of arsenite that leads to the tolerance of As in the hyperaccumulator species of *P. vittata*. The study reveals that the plants adapt to the heavy metal particularly the As stress through the modulation of the transcription pathways, phosphorylation/dephosphorylation, as well as various biochemicals such as hormones that control the transporters and their activities (Sharma et al. 2016).

Evolutionary developments have resulted in plant living systems combating reactive heavy metal ions present in their immediate environment as well as utilizing them for metabolic processes as essential components. This ability of plants was understood when the relationship between biologically available metals and their transport through cells, with specific metal-binding macromolecules, was established (Kägi and Vallee 1960).

7.3.2.2 Metallothioneins

In higher plants, a type of metal-binding macromolecule or peptide is the cysteine-rich “metallothionein” (MT) protein (Hall 2002). These compounds contain groups of d^{10} transition metal ions that cluster in accordance with particular coordination to the arrays of adjacent Cys thiolate groups (Schaffer and Andreas 1988). MTs are

named as such because they contain extremely high content of both metal and sulfur that vary according to the metal species present (Schaffer and Andreas 1988). The high sulfur content is a direct result of the presence of sulfur-containing cysteine groups. The discovery of MTs was made by Margoshes and Vallee in 1957, when they identified a cadmium-binding protein responsible for the natural accumulation of cadmium in the tissue of kidney cortex (Kägi and Vallee 1960).

Members of the MT family are found to be universal across multiple species from bacteria, yeast, plants, and mammals. MTs possess the ability to bind heavy metals of the physiological nature such as copper, zinc, and selenium that are present and needed by living systems, as well as xenobiotic ones such as mercury, silver, cadmium, and As which are foreign and often toxic. The mechanism is carried out via thiol groups in the cysteine residues that constitute about 30% of its amino acid residues (Sigel et al. 2009).

Throughout the kingdoms, MTs are classified as class I or II, based on the arrangement of Cys residues; all plant MTs belong to the latter. Plant MTs have been classified into four father types (1, 2, 3, and 4) determined by the distribution of (a) Cys residues and (b) Cys-devoid regions commonly known as "spacers" (Cobbett and Goldsbrough 2002). Various cellular functions of MTs have been studied. These include metabolism and trafficking of essential metals, sequestration of toxic metal, and their action as a free radical scavenger (Sigel et al. 2009). MTs exhibit extreme diversity in the plant kingdom, but specific studies on their role as a hyperaccumulator are limited. To understand the adaptive effects by downstream processes, more studies need to be carried out, especially considering the number of metals in plant colonizing soils of resistant plants (Roosens 2004).

7.3.3 Vacuolar Sequestration: An Adaptation to Regulate Hyperaccumulation

Vacuolar sequestration is one of the major mechanisms of detoxification of As in most hyperaccumulator species (Zhao et al. 2003). It is integral to the maintenance of heavy metal homeostasis in plants. There are two vacuolar pumps, namely, vacuolar proton ATPase (V-ATPase) and vacuolar proton pyrophosphatase (V-Ppase), that govern the process in coordination with a set of tonoplast-located transporter families such as ABC. Metal tolerance protein (MTP), also known as the CDA transporter family, functions to transport metallic ions such as Cd^{2+} and Ni^{2+} to the vacuole. The hyperaccumulators isolate heavy metals in the vacuoles of leaf cells followed by the long-distance translocation. These pumps require being adjusted both structurally and functionally to the needs of the cell for adapting to prevailing circumstances. Hyperaccumulation property of heavy-metal-tolerant species can be utilized in various emerging novel technologies and most importantly phytoremediation.

7.3.4 Modified Phosphate Uptake System

There are certain mechanisms in which phosphate uptake is involved in generating As tolerance in plants. Arsenate or As (V) is chemically analogous to inorganic phosphate (Pi), and both of them are taken up by the roots using the same uptake system (Asher and Reay 1979; Meharg and Macnair 1991). As (V) competes with Pi within the cytoplasm to confer toxicity. Hence, for normal cellular functions, it is vital that the concentration of As (V) (and its derivative As (III)) be kept low (Meharg 1994).

Increased exudation of carboxylic acids that includes malic, citric, and oxalic acids that has been reported in phosphorus-deficit plants can lead to As accumulation in these plants resulting in the decreased external amount of As. The exudation changes the pH of the soil, displacing P from the absorption site leading to enhanced phosphate availability. This phenomenon could, therefore, deploy the As in rhizosphere and ultimately increases its uptake leading to accumulation. Another mechanism of alleviating As toxicity in this regard is by increasing phosphate nutrition. A study conducted by Macnair and Cumbes in 1987 proves these findings for *H. lanatus* L. (velvet grass). Studies have revealed that the phosphate/arsenate transporter has a higher affinity for phosphate than its arsenate analogue (Macnair and Cumbes 1987). In case of high amounts of external P, it will be taken up more effectively compared to arsenate (Meharg and Macnair 1994), thus reducing arsenate influx. In other words, As causes higher oxidative stress in plants grown in soils with low P content.

7.3.5 Defense Adaptation Through Cellular Signaling

In cell signaling, cells coordinate and communicate with each other to direct and harmonize crucial actions to perform multiple functions. Different processes related to growth and development and stress resistance are carried out by the help of cell signaling pathways. Cellular signaling also plays a significant role in tolerance to metals stress in plants. Prominent examples include MAP kinases pathways, chromatin remodeling factors, and heat shock proteins.

7.3.5.1 Role of MAP Kinases

Several molecular processes are induced as a result of a buildup of high concentrations of toxic arsenite. One such molecular response is the activation of the "mitogen-activated protein kinases" (MAPKs). MAPKs have already been reported to be induced by other heavy metals in higher plants (Jonak et al. 2004; Liu et al. 2010b). MAPKs are a group of proteins kinases that take part in a relay system, passing on signals generated by both exogenous and endogenous stimuli to the cell's interior by means of phosphorylation cascades of proteins involved in the

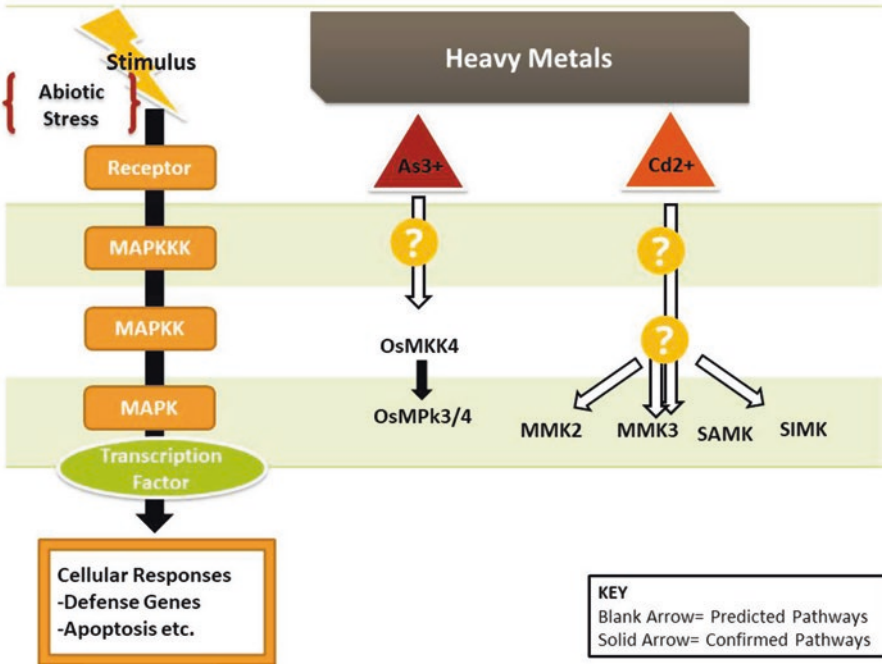

Fig. 7.2 The left side displays a general flowchart of the MAP kinase signaling pathway, while the right displays the corresponding components to this pathway. The signaling molecules are the heavy metals, As^{3+} and Cd^{2+}. This triggers OsMKK4 and OsMPK3 and OsMPK4 MKK and MKKK, respectively, in rice. The question mark denotes those components of the pathway that are yet to be identified. Adapted from (Sinha et al. 2011)

pathway (Leonard et al. 2004). These cascades are universal signal transduction modules that participate in numerous biotic and abiotic stress signaling mechanisms (Taj et al. 2010).

Metals exhibit toxic effects either by direct protein-metal interactions or indirectly via the induction of reactive oxygen species (ROS) (Leonard et al. 2004). The ROS are involved in activating the MAP kinases in plants such as *Medicago sativa* commonly known as "alfalfa" (Jonak et al. 2004; Rao et al. 2011). As stress demonstrated active MAPK in roots and leaves of rice seedlings; OsMPK3 and OsMPK4 transcripts were observed in the roots and OsMPK3 was observed in leaves. These transcripts displayed maximum induction by arsenite stress in a dose-dependent manner (Rao et al. 2011). MAPK activation in response to As(III) indicates a role of this important cascade in stimulating As(III) mediated signals as demonstrated in Fig. 7.2, the signaling mechanism of MAP kinases (Sinha et al. 2011).

Brassica juncea L., a potential model plant for phytoremediation of multiple heavy metals (Gupta et al. 2009), also exhibited activation of MAPK by arsenite. The kinetic analysis of increasing concentration of arsenite also increased the activity MAPK, in both roots and leaves, demonstrating the relation between concentrations versus the activity. This strengthens the involvement of the MAPK cascade in stress signaling of arsenite (Gupta et al. 2009).

7.3.5.2 Role of Chromatin Remodeling Factors

The genome of eukaryotic organisms is organized and packaged by a multitude of proteins forming a superstructure known as the "chromatin." It is crucial for the chromatin structure to be accurately duplicated during the replication of DNA, to maintain gene-expression patterns and domains (Varga-Weisz 2005). Key components involved in the maintenance of this process are known as "chromatin remodeling factors." These factors consist of histone-modifying enzymes, histone chaperones, and ATP-dependent chromatin remodeling complexes. Chromatin remodeling complexes harbor the genome in such a way as to prevent loss of access to the compact coiled DNA molecule while fitting it into the nucleus As demonstrated in Fig. 7.3 (Chromatin remodeling factor, types, and induced responses due to As stress) (Varga-Weisz 2005).

As (either as As(V) or As (III)) and cadmium (Cd^{2+}) are both mutagenic, with As (III) and Cd^{2+} sharing a similarity in their toxicology and sequestration machinery (Verbruggen et al. 2009). They inactivate DNA mismatch repair in human cells and yeast; a similar mechanism may act in plants (Verbruggen et al. 2009). Oxidative

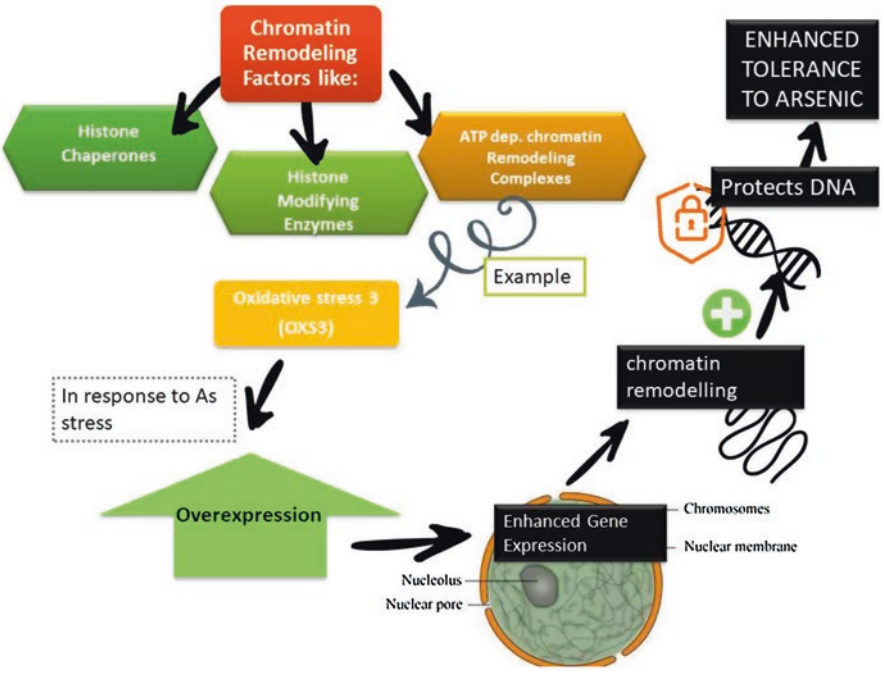

Fig. 7.3 Oxidative stress 3 (OXS3) is a type of chromatin remodeling complex. As a result of arsenic stress, DNA is damaged. As a protective mechanism, an overexpression of this chromatin remodeling factor occurs. This results in enhanced gene expression which stimulates chromatin remodeling, hence protecting DNA by allowing proper folding and packaging into the nucleus. This ultimately provides a boosted protection from arsenic or heavy metal poisoning

stress 3 (OXS3), a putative chromatin remodeling factor, was recently identified in a study of Cd tolerance of a *B. juncea* cDNA library in *Schizosaccharomyces pombe.* A Cd hypersensitive OXS3 mutants' overexpression improved Cd tolerance hence suggesting that OXS3 functions to protect the DNA. In addition, OXS3 overexpression enhanced tolerance to other metals, including As (Verbruggen et al. 2009). It is also proposed that OXS3 and other members of the family, putative N-acetyltransferases might trigger specific gene expression in response to stress through chromatin remodeling.

By an independent mode of action, the putative MDB domain might give OXS3 properties like scavenging that aids the cell in tolerating heavy metals such as As. Further research is yet required in order to establish the precise role of these proteins to strengthen the hypothesis that OXS3 and other family members are chromatin remodeling factors (Blanvillain et al. 2008).

7.3.5.3 Role of Heat Shock Proteins

Heat shock proteins (HSPs) are a family of proteins known to be present in all types of life forms animals, plants, fungi, bacteria, etc. being nestled deep within their cells. They are also called "stress proteins" due to their production as a response to multiple stress conditions (Li and Srivastava 2004), although being initially named "heat" shock. This is because they were first characterized by their expression induced by heat stress in *Drosophila* (Ritossa 1962). Heat shock proteins are also expressed under stresses other than heat such as UV light (Murakami et al. 2004), wound healing, cold (Matz et al. 1995), and heavy metal stress (Lewis et al. 1999).

Their nomenclature is based on their molecular weight (Vierling 1991), for example, HSP60, HSP70, and HSP80 have molecular weights of 60, 70, and 80 kilodalton, respectively. They function primarily as molecular chaperones of normal protein folding and assemblage but have also displayed protective repairing and damage control of proteins under stressed conditions (Tseng et al. 1993). Such protective effects include ensuring a protein's proper conformation, refolding of those proteins that became untangled due to denaturation as a result of stress, preventing clumping of non-native proteins, and even eliminating nonfunctional but potentially destructive polypeptides. Thus, once triggered, HSPs play a crucial role in sustaining homeostasis and aiding an organism's cells to revert to an equilibrium state (Timperio et al. 2008).

Several reports indicate a rise in heat shock protein expression in plants as a response to heavy metal stress (Tseng et al. 1993). Studies on the effect of toxic metals As and Cd as well as heat, on rice, showed an increased expression of low molecular weight HSP mRNAs (16–20 kDa), with As exhibiting a greater potency (Tseng et al. 1993; Goswami et al. 2010). When rice seedlings were exposed to heat, and As stress, a cellular chaperone gene, *HSP70*, was overexpressed at both the mRNA and protein levels. A cumulative effect was observed that was elevated with the extended duration of the stress of 3 h. Those plants that were previously exposed to As expressed a substantially higher level of the HSP70 chaperone during the heat

shock recovery period (Goswami et al. 2010). Furthermore, when the plants were treated with As along with heat shock, day 1 results displayed a considerably elevated HSP70 expression than in plants treated with heat shock as the sole stress factor (Goswami et al. 2010).

Plants have several organizational and temporal levels of adaptations to external environmental stimulus. Usually, the responses are of reversible nature, such as modification of ion fluxes and networks of signal transduction which may proceed to change at the genetic level for long-term adaptations. Such changes, including the effect of HSPs, may be tuned by the ongoing presence of the environmental signal (Trewavas 2003). Hydrophobic residues unveiled during stress conditions are often a transient binding site for HSPs like HSP70 to save partly denatured proteins from aggregating, chaperoning them to refold back into their proper conformation. The aforementioned studies (Goswami et al. 2010; Tseng et al. 1993) established this phenomenon of the time-dependent cumulating HSPs in response to heavy metal stress, particularly As, indicating an impact of stress imprint and its role as an adaptive response.

This correlation clues to the possibility that the signaling network downstream of As (and heat stress) and that upstream of overexpression of HSP70 have certain common elements linking the cause-effect response (Goswami et al. 2010). HSP70 was hence proposed to be used as an environmental, biological marker for levels of toxic metal, including As toxicity.

A study is analyzing the effect of As on genome-wide expression was carried out on rice. A total of 11 shock proteins (HSPs: Os03g16030, Os03g16920, Os02g52150, Os04g01740, Os03g16020, Os04g36750, Os02g54140, Os04g45480, Os01g08860, Os03g14180, and Os04g01740) were upregulated in response to As stress, out of which only 3 were observed to be upregulated in As (III) stress.

7.3.6 Defense Adaptations through Beneficial Living Association

7.3.6.1 Role of Periphyton

Periphyton constitutes a wide range of autotrophic and heterotrophic organisms that grow on submerged surfaces in aquatic ecosystems. It is an important aquatic food resource for some fish and invertebrates. It is also extremely sensitive to environmental stress stimuli. Similarly, periphyton can also act as an accumulator of heavy metals (Tang et al. 2014). Periphyton is reported to be located ubiquitously in rice paddy fields. A study was conducted to analyze the effect on heavy metal concentration in rice seedlings in the presence of periphyton. The results revealed that periphyton presence aided in enhanced plant growth as it effectively regulated As and Cd concentration. The mechanism involved leads to a decrease in Cd concentration in roots and shoots while As was hyperaccumulated in the plant organs. Both of these occurrences had a positive impact on the growth of rice plant (Shi et al. 2017).

7.3.6.2 Role of Symbiotic Mycorrhiza

Fungi influence decomposition, soil fertility, nutrient uptake, and overall plants' health (Pichardo et al. 2012). A majority of higher plants have a capability to form a symbiotic relationship with fungi. In this symbiosis, the fungi provide the host plant with water and nutrients in exchange for carbon. Mycorrhizal plants have greater tolerance to stress conditions including heavy metal toxicity, water stress, salinity, oxidative stress, high soil temperature, and effects of soil acidification. The dominant type of fungi for symbiosis in plants is found to be the arbuscular mycorrhizal (AM) fungi. Such a symbiosis occurs in almost all habitats and climates including the heavy metal contaminated soils. Various studies have reported that plants grown in the presence of AM fungi have developed a variety of effective mechanisms that help in reducing the metal uptake, increasing the nutrient contents, and forming an efficient symbiotic relationship by inducing several mycorrhizal structures inside plant roots such as arbuscules or vesicles (Göhre and Paszkowski 2006). One such heavy metal remediation mechanism by AM fungi is the formation of an insoluble glycoprotein "glomalin." Glomalin can sequester trace elements in the fungi's arbuscules, hyphae, and vesicles, hence preventing their translocation to aboveground aerial parts of the plant (Sharma et al. 2017).

The AM fungi also have a well-documented role in the uptake of P (Bolan 1991). A common hyperaccumulator of As, *P. vittata* L. (Chinese brake fern), is known to be colonized by AM fungi. These fungi are said to be involved in conferring As tolerance in host plants. They do so by channelizing P uptake through their mycorrhizal pathway, hence suppressing the AsV-P uptake system and consequently, reducing As uptake (Sharma et al. 2017). According to various studies, it was suggested that AM fungi helped in enhancing As tolerance and P nutrition in both shoots and roots of *Z. mays*, *Lactuca sativa*, and *Eucalyptus globulus*. Furthermore, it was indicated that AM colonization has the ability to inhibit As reduction from As (V) to As (III), thus alleviating As toxicity. This was done through the effective activity of certain enzymes such as peroxidase, superoxide dismutase, and As (V) reductase which were suppressed by *Glomus mosseae/Funneliformis mosseae* (He and Lilleskov 2014). Another study revealed that AM fungi have a significant role in As hyperaccumulation by the Chinese brake fern (Al Agely et al. 2005). Additionally, it was exhibited that the higher selectivity of membrane transporters with respect to P than As (V), resulted in competitive inhibition of As uptake (Panuccio et al. 2012).

7.4 As Efflux Mechanisms

As efflux from the cells is prominent adaptation employed by plants to fight As stress. Efflux is an effective method to reduce metal toxicity. Studies conducted on *A. thaliana* suggested that As (V) taken up by the roots is removed from the roots as an As (III) species within a day of the uptake (Liu et al. 2010a). Additionally, it has also

been reported that As (III) efflux from As hyperaccumulators occurs at a lower rate as compared to non-hyperaccumulators such as *A. thaliana,* hence justifying high accumulation in the former species (Huang et al. 2011). As efflux mechanism has also been reported in several other plant species such as in tomato (Xu et al. 2007).

In some plants, such as rice, silicon (Si) and As share the same transport channels. Two silicon transporters Lsi1 and Lsi2 are predominantly involved in As uptake and efflux, respectively. The Lsi2 protein is located on the proximal side of the plasma membrane of endodermis as well as exodermis cells, where casparian strips are also present. During the prolonged period of growth, this transporter protein mediates the arsenite efflux toward xylem, along with Si efflux toward the stele, and As reuptake is then competitively inhibited by Si (Ma and Yamaji 2006). This process controls the As accumulation in shoots and roots of the plants, preventing phytotoxicity under As pollution.

A significant mechanism for As efflux is through iron plaques formation that has a greater role in As sequestration and reduced uptake of As by the plants (Wu et al. 2012). In this regard, pH is a major controlling factor of arsenate removal via iron precipitation.

This efflux mechanism starts by the movement of oxygen from aerenchyma cells to the soil and forms a barrier to radial oxygen loss (ROL) leading to the conversion of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) by oxidation on the roots of plants. This facilitates the formation of Fe (III) oxide coating on the roots, eventually forming large plaques. These iron plaques increase the absorption capacity for As, ultimately displaying the ability to alleviate the As toxicity by As sequestration with absorbed As (Liu et al. 2005).

The ROL rate is directly proportional to As removal. Higher levels of ROL increases plaque formation and consequently increases sequestration sites (Chen et al. 2017). Increased sites boost up the chances of sequestration of absorbed As, and hence efflux of As is mediated by Fe (III) precipitates on the roots.

A recent study analyzed the potential of amorphous iron hydroxide (FeOH) amendments to mitigate the As toxicity in rice paddy fields. Results of the study suggested that the amount of Fe plaque increased when the amount of amorphous FeOH added to the soil was increased. This affected the uptake and transport of As by the rice plants. As iron hydroxide and oxides have a high affinity for As, they can sequester As and consequently reduce the translocation of As from the roots to the shoots. (ULTRA et al. 2009).

7.5 Conclusion

Arsenic is a highly toxic metal and also listed as class 1 carcinogen. As is transported into the plants through soil and irrigation water and becomes toxic when it starts to accumulate in various parts of the plants including edible parts like fruits. Through this mode, As enters the food chain and ultimately disrupts human health. As toxicity brings many physiological, morphological, and biochemical damages in

plants like a disruption in photosynthesis, transpiration, and nitrogen fixation (in leguminous plants). Additionally, numerous metabolic processes also get affected like carbohydrate metabolism. To combat those detrimental effects, plants initiate defense mechanism including enzymatic and nonenzymatic defense mechanism, chelation, precipitation of the metal of plant leaves, hyperaccumulation regulation, and symbiotic modifications. Literature suggests that these mechanisms are effective in overcoming As stress and extended research is needed to understand the underlying factors that are related to As tolerance mechanisms in plants. This will help in developing a better understanding of modifying plants with high productivity under stressed conditions.

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Chapter 8
Mitigating Arsenic Toxicity in Plants: Role of Microbiota

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Abstract Arsenic (As) pollution, particularly in soil and water, is a very prominent environmental issue which seriously threatens plant growth, development, and productivity. Since As is ubiquitous in the natural environment, microorganisms have developed mechanisms to resist the toxic effects of this metalloid. A large number of microorganisms, viz. *Acinetobacter*, *Aeromonas*, *Bacillus*, *Exiguobacterium*, and *Pseudomonas*, are capable of growing in the presence of high concentrations of As. But relatively less information is available on accumulation, mobilization, distribution, and speciation of As by rhizospheric microbiota and their impact on plant growth and development. The use of As-resistant and plant growth promoting microorganisms (PGPMs) for the restoration of plants growing on contaminated soils is the need of the time. The use of PGPM occupies a small but growing niche in the development of organic agriculture and has attracted attention during the last decade only. There are several reports revealing the multifarious role of soil microbiota in amelioration of As toxicity and improving metal tolerance in plants. Colonization of PGPMs helps the host plant to overcome As-induced phosphate (P) deficiency and consequently maintain favorable P:As ratio. Further, they also improve nutritional status and reduce As uptake and translocation in plants. Inoculation of bacteria/fungi can exert protective effects on vascular plants under As contamination by transforming more toxic inorganic forms into less toxic organic forms or via reducing the concentration of As by enhancing plant biomass. The PGPMs also result in higher activities of the antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase, and guaiacol peroxidase) and accumulation of nonenzymatic antioxidants (carotenoids, ascorbic acid, proline, and α-tocopherol). Increased concentrations of cysteine, glutathione, and non-protein thiols, and activity of glutathione *S*-transferase have also been reported that facilitate sequestration of As into nontoxic complexes. Thus, application of As-resistant PGPMs could pro-

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vide a low cost and eco-friendly mitigation approach to diminish As accumulation in plants, thereby promoting higher growth, development, and yield responses. There is also a need to improve our understanding of the mechanisms involved in extenuating toxic effects of As by rhizospheric microbiota and to improve the stabilization of plants in contaminated sites.

Keywords Phytoremediation · Plant-microbe interactions · Plant growth · Rhizosphere · Toxic metalloids

8.1 Introduction

Arsenic (As) is a toxic metalloid of global concern, as it cannot be degraded to harmless products instead persists indefinitely in the environment. It occurs in trace quantities in rocks, soil, water, and air but can be intensified by anthropogenic activities (Garelick et al. 2008). Consequently, elevated levels of As have been reported in soils and groundwater worldwide. The amount of As in a plant depends on the amount of As it is exposed to. Terrestrial plants may accumulate As by root uptake from the soil (Li et al. 2015) or by adsorption of airborne As deposited on the leaves. Background As concentrations in terrestrial biota are usually less than 1 mg kg^{-1}. The highest As concentration occurs in the roots of plants (Álvarez-Ayuso et al. 2015).

Arsenic is a nonessential element for plant and is expected to negatively affect growth responses. Plants cope with As toxicity by employing various detoxification mechanisms such as accumulation, sequestration, and compartmentalization. Nevertheless, failure of any once of these mechanisms leads to symptoms of phytotoxicity (Moreno-Jiménez et al. 2012). Alteration in plant growth usually occurs at higher levels of As application leading to different physiological and chemical changes in plants, such as necrosis and wilting, inhibition of seed germination, decrease in plant growth, restricted root and shoot length, lower fruit and grain yield, reduced enzymatic activity, and replacement of As with phosphorus in reactions (Zhao et al. 2009; Chandrakar et al. 2016). Bioavailability, uptake, and phytotoxicity of As in plants are influenced by factors like concentration and speciation of As, plant species, soil properties such as pH, redox potential, and soil phosphorus content (Fitz and Wenzel 2002).

Remediation of As-contaminated soils and groundwater is necessary for limiting the entry of this toxic metalloid in the food chain. The use of microorganisms in the bioremediation of contaminated ecosystems showed a great prospective for future developments due to its environmental compatibility and possible cost-effectiveness. Microorganisms have evolved dynamic mechanisms for facing the toxicity of As which enable them to survive in As-rich environments. The strategies developed by microbes to counteract As toxicity include the active exclusion of it from cells by establishing permeability barrier, intra- and extracellular sequestration, active efflux pumps, and microbial bioaccumulation (Tsai et al. 2009; Wang et al. 2013). Microorganisms are also known to survive As exposure by transforming it through oxidation-reduction reactions and methylation-demethylation processes into less toxic forms (Cai et al. 2009; Liao et al. 2011; Kuramata et al. 2015). A variety of

microbes are known today which utilize As in their metabolism, using either arsenate [As(V)] as a terminal electron acceptor in anaerobic respiration or generating energy by oxidation of arsenite [As(III)] during chemolithoautotrophic growth (Kruger et al. 2013).

The application of metal-resistant and plant growth promoting soil microbes as bioinoculants for supplying nutrients and/or stimulating plant growth are gaining momentum. These microbes have an extensive range of growth modes and are capable of growing, utilizing a variety of organic substrates. These properties make them ideal for the treatment of contaminated soils, assisting in possible remediation. Several rhizospheric metal-resistant microbes are also known to have plant growth promoting abilities, viz. nitrogen fixation, phosphate solubilization, and production of siderophores and phytohormones, suggesting their substantial role in As-biogeochemistry and bioremediation (Beneduzi et al. 2012; Bhattacharyya and Jha 2012; Gupta et al. 2015; Batool et al. 2017).

8.2 Microbial Diversity in Arsenic-Contaminated Ecosystems

Diverse microbial community exists in As-contaminated sites that can uptake and transform As in the environment (Banerjee et al. 2011; Sarkar et al. 2013; Ao et al. 2014). Although As is toxic to microbes, certain bacteria and fungi survive its exposure by developing As detoxification mechanisms that permit the cell to neutralize its toxic effects (Srivastava et al. 2011; Kruger et al. 2013). Countless native, As-resistant, phylogenetically diverse, and metabolically versatile bacteria and fungi have been isolated from varied habitats, including soil, water, and sediments, contaminated with high levels of As (Tables 8.1 and 8.2). Presence of As-resistant microbial species in the As-rich environment is highly expected since high levels of metallic species are likely to exert a strong selective pressure thereby reducing the overall growth of sensitive microbial population (Sarkar et al. 2013). This favors the survival of indigenous As-resistant microorganisms in the As-rich habitats (Pepi et al. 2007). Long-term association of microorganisms with toxic metal within metal-rich sites often promotes adaptation of the microbes in such environmental conditions (Banerjee et al. 2013; Majumder et al. 2013).

Microbial activities account direct effect on the bioavailability of As in soil, water, and sediments (Sarkar et al. 2013). Studies on the existence and dispersal of innate metal-tolerant microbial flora bear tremendous applicability in microbial bionetwork to understand the degree of metal pollution of the ecosystem. Such potential microbes may be channelized not only in detecting environmental alterations but also in treating toxic substances. Development of advanced technologies involving complex microbial reactions is utmost important for the enhanced bioremediation of As-contaminated milieus (Kruger et al. 2013).

Table 8.1 Arsenic-resistant bacteria reported from As-contaminated ecosystems

Bacterial strain	Source	Contaminated site	References
Pseudomonas sp.	Soil	Karamy, Xinjiang, China	Karn et al. (2017)
Pseudomonas sp. HN2	Soil	Hunan, China	Zhang et al. (2016)
Exiguobacterium, Bacillus	Soil	Kaudikasa village, Chhattisgarh, India	Pandey and Bhatt (2015)
Acinetobacter, Pseudomonas, Psychrobacter, Alishewanella	Groundwater	Hetao Basin, Inner Mongolia, China	Li et al. (2015)
Pseudomonas, Flavobacterium, Brevundimonas, Polaromonas, Rhodococcus, Methyloversatilis, Methylotenera	Groundwater	Barasat and Chakdaha of Parganas and Nadia Districts, West Bengal, India	Paul et al. (2015)
Actinobacteria, Rhizobium Microbacterium, Pseudomonas	Groundwater	Barasat and Chakdaha areas of Parganas, West Bengal, India	Paul et al. (2014)
Lysinibacillus	Soil	Chuadanga District, Bangladesh	Rahman et al. (2014)
Brevundimonas, Acidovorax, Acinetobacter, Pseudomonas, Undibacterium, Bosea, Herbaspirillum, Bacillus, Rhodococcus, Ralstonia, Staphylococcus, Caulobacter, Rhizobiales	Groundwater	Assam, India	Ghosh and Sar (2013)
Pseudomonas	Sediment	Taiwan	Kao et al. (2013)
Actinobacteria, Agrobacterium Ochrobactrum, Rhizobium Brevundimonas	Groundwater	Kolsure village, West Bengal, India	Sarkar et al. (2013)
Micrococcus, Pseudomonas, Vibrio Bacillus	Water	Rautahat District, Nepal	Shakya et al. (2012)
Geobacillus kaustophilus strain A1	Soil	Metallifere Hills, Italy	Cuebas et al. (2011)
Bacillus idriensis, Sphingomonas desiccabilis	Soil	Beijing, China	Liu et al. (2011)
Acinetobacter, Agrobacterium, Arthrobacter, Comamonas, Rhodococcus, Stenotrophomonas, Pseudomonas	Soil	Tieshan District, Huangshi City, Hubei Province, Central China	Cai et al. (2009)

8.3 Mechanisms of Arsenic Resistance in Microorganisms

8.3.1 Bacteria

It is well established that a number of microorganisms possess metabolic mechanisms to resist the disruptive effects of As and to survive in its presence (Rosen 2002; Li et al. 2010). The greater part of As resistance and detoxification studies have been performed in bacteria, and their molecular mechanism has been elucidated precisely (Rahman and Hassler 2014). The strategies to manage or neutralize

Table 8.2 Arsenic-resistant fungi reported from arsenic-contaminated ecosystems

Microorganisms	Source	Contaminated site	References
Aspergillus oryzae, Fusarium sp., *A. nidulans, Rhizomucor variabilis, Emericella* sp.	Soil	Middle Indo-Gangetic Plains, India	Singh et al. (2015)
Penicillium coffeae	Soil	India	Bhargavi and Savitha (2014)
Aspergillus, Trichoderma Neocosmospora, Sordaria, Rhizopus, Penicillium	Soil	West Bengal, India	Srivastava et al. (2011)
Trichoderma asperellum SM-12F1, *Penicillium janthinellum* SM-12F4, *Fusarium oxysporum* CZ-8F1	Soil	Beijing, China	Su et al. (2011)
Aspergillus niger, A. clavatus, A. fischeri	Soil	Pezinok, Slovakia	Cernanský et al. (2009)
Neosartorya fischeri, *Talaromyces wortmannii*, *Talaromyces flavus*, *Eupenicillium cinnamopurpureum*	Sediment	Landsberg, Germany	Heinrich (2007)

As toxicity mainly include accumulation (Wang et al. 2013; Rahman et al. 2014), active extrusion of As, extracellular precipitation, complexation into peptides (Kruger et al. 2013; Kuramata et al. 2015), and As transformation including methylation, demethylation (Bentley and Chasteen 2002), oxidation, and reduction (Liao et al. 2011). Some bacteria even use As compounds to fuel their energy metabolism, either as an electron donor or as an electron acceptor (Silver and Phung 2005).

Of all the processes, enzymatic reduction of As(V) to As(III) mediated by the gene products of the widespread *ars* operon followed by efflux of As(III) from the cell is the most common one and is found in both gram positive and gram negative bacteria (Silver and Phung 2005; Vishnoi and Singh 2014). The As resistance gene systems which are responsible for the metabolism and detoxification of As may be found either in plasmids (Drewniak et al. 2013) or chromosomes (Bhat et al. 2011).

8.3.1.1 Arsenic Uptake Systems

Pathways for uptake of As into bacterial cells have only recently been discovered. The most common and abundantly existing forms of As oxyanions, arsenate and arsenite, use uptake systems that transport these compounds into cells (Fig. 8.1). Arsenate is a substrate analogue of phosphate and is taken up via the phosphate transporters by most of the organisms (Rosen 2002). Two phosphate transporters, *Pit* and *Pst*, have been identified in different bacterial groups, both of which catalyze As(V) uptake. Of the two transporters, *Pit* is the major transport system (Tsai et al. 2009).

Arsenite shows very strong similarity with the conformation of glycerol and is adventitiously taken up by a trivalent metalloid transporter identified as *GlpF* (Rosen and Liu 2009; Tsai et al. 2009) (Fig. 8.1). Transporter GlpF is an aquaglyceroporin and a member of the major intrinsic protein (MIP) superfamily that allows the transport of water and small solutes such as glycerol and urea by an energy-independent mechanism (Rosen 2002).

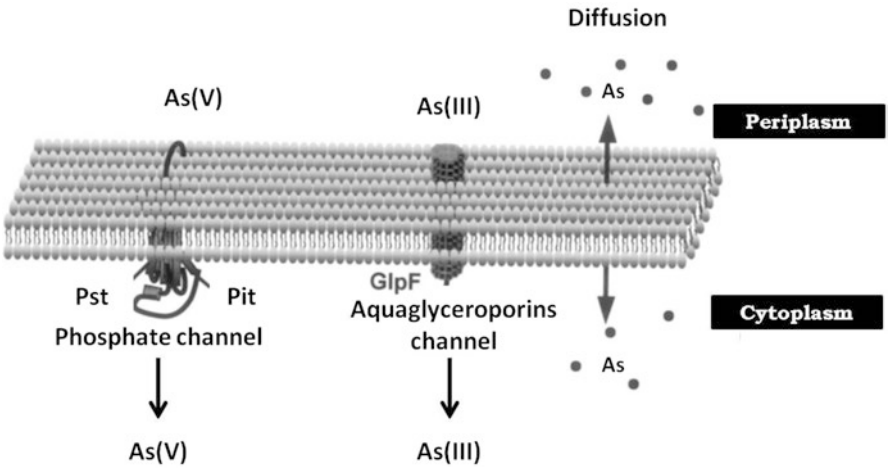

Fig. 8.1 Transporters for arsenic uptake in bacteria

8.3.1.2 Arsenate Reduction: Detoxification

A wide range of microorganisms have evolved As resistance mechanisms to survive in As-contaminated environments (Tsai et al. 2009; Nagvenkar and Ramaiah 2010). One such process involves the reduction of As(V), which has the purpose of detoxification by converting As(V) to As(III) (Páez-Espino et al. 2009). Microorganisms combat the importation of As(V) by a two-step mechanism in which this compound is first reduced to As(III) by a cytoplasmic As(V) reductase (Rosen 2002). Although As(III) is a more potent toxicant than As(V), the former is selectively expelled from the interior of the cell via membrane efflux pump (Mateos et al. 2006).

8.3.1.3 Arsenate Reduction: Energy Generation

In addition to the detoxification of As(V), certain bacteria can reduce As(V) as the terminal electron acceptor during anaerobic respiration for generating energy (Hudson-Edwards and Santini 2013; Kruger et al. 2013). These are defined as dissimilatory As(V)-respiring bacteria (Oremland and Stolz 2005), in which respiration is recognized to be mediated by a specific enzyme or respiratory chains (Silver and Phung 2005). Arsenic respiration can influence both the speciation of As and its mobility. Dissimilatory As(V)-respiring bacteria are able to reduce both sorbed and dissolved As(V) to As(III) coupled to As(III) efflux systems, presumably because the enzymes responsible for As(V) reduction are located in the periplasmic membrane (Silver and Phung 2005).

8.3.1.4 Arsenite Oxidation: Detoxification and Energy Generation

Bacterial oxidation of As(III) to As(V) is very well documented, particularly with isolates from As-impacted environments (Bahar et al. 2012). Many heterotrophic bacteria oxidize As(III) to As(V) to speedily detoxify their surroundings, while others use As(III) as an electron donor. Over 30 strains representing at least 9 genera of As-oxidizing prokaryotes have been reported (Stolz et al. 2002). The transformation reaction involving the energy gain by conversion of more toxic As(III) into less toxic As(V) has increased the ecological significance of these bacteria over other microorganisms. Since As(V) is less soluble and is much more effectively removed by physicochemical methods, oxidation of As(III) can be important for As removal (Tsai et al. 2009).

8.3.1.5 Methylation

Methylation was formerly thought to be a detoxification step; however, recent information suggests that not all methylated As products are less toxic (Páez-Espino et al. 2009). Methylation process produces both volatile and nonvolatile methylated compounds of As (Qin et al. 2006). The major volatile methylated As compounds are mono-, di-, and trimethylarsine, while the major nonvolatile compounds are methylarsonate and dimethylarsinate (Fig. 8.2).

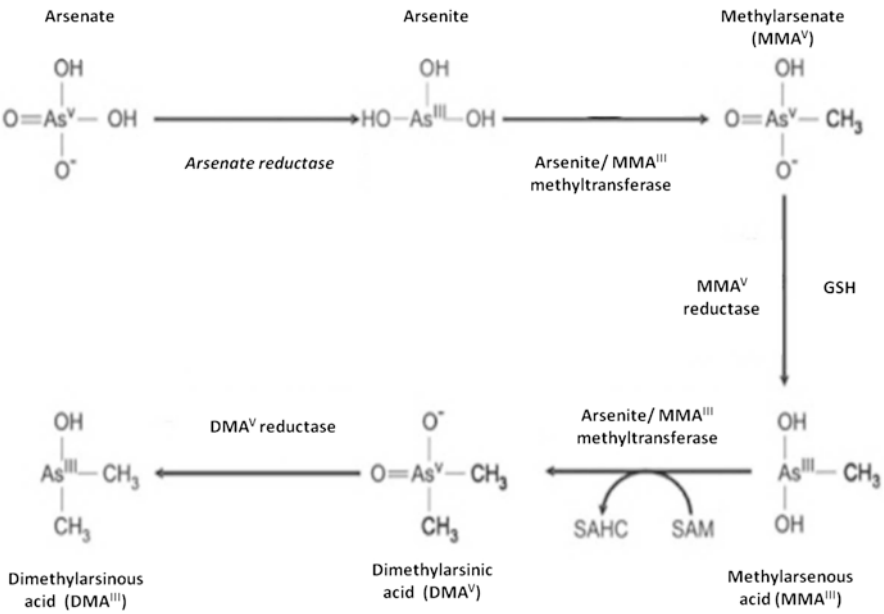

Fig. 8.2 Bacterial methylation of arsenic

The methylation reaction follows the reduction of As(V) and subsequent oxidative addition of methyl groups, thus generating arsines and methyl arsenicals (Páez-Espino et al. 2009; Kruger et al. 2013). The volatile methylated forms of As are readily released into the environment where oxidation might convert them back to the oxidized form, As(V). A variety of microorganisms have been implicated as the major contributors of As methylation (Wang et al. 2015) and are included in the genera: *E. Coli* (Yuan et al. 2008), *Pseudomonas* (Chen et al. 2014), *Streptomyces* (Kuramata et al. 2015), and *Clostridium* (Wang et al. 2015). Understanding of these mechanisms will not only shed light on the As mobilization but may also open up new horizons in metabolic pathway engineering to exploit those for As remediation (Huang et al. 2015).

8.3.2 Yeast and Fungi

Arsenate [As(V)/$H_2AsO_4^-$] is a chemical analogue of phosphate and hence gets transported by high-affinity phosphate uptake system Pho87. Moreover, As(III) (H_3AsO_3) is not a phosphate analog and therefore is transported into the cell either through *GlpF* or passive diffusion (Sharples et al. 2000). Although the mechanisms of As detoxification remain unstudied in fungi, several pathways mediating detoxification of As have been elucidated in the yeast *Saccharomyces cerevisiae*. It is now an established fact that the most common mechanism of resistance in yeast is mediated by the products of three adjacent genes *ACR1*, *ACR2*, and *ACR3*. The putative product of the *ACR1* gene showed similarity with the yeast transcriptional regulatory proteins, encoded by *YAP1* and *YAP2* genes. Gene *ACR2* encodes arsenate reductase enzyme which is known to mediate a rapid internal reduction of As(V) to As(III), which then initiates efflux of As(III) from the hyphae (Mukhopadhyay et al. 2000). Reduction of As(V) to As(III) is also mediated by *P37* in species like *Aspergillus*; however, it does not methylate As to any significant extent (Ca'novas et al. 2004).

Arsenite efflux has As(V) resistance in *S. cerevisiae* and many other fungi and is mediated by an As(III) transporter gene *ACR3*. Likewise, *ACR3* encodes a putative plasma membrane As(III) efflux transporter protein that pumps As(III) out of the cell, whose expression is strongly induced by the presence of both As(III) and As(V) (Wysocki et al. 1997). In addition, conjugation of As(III) with glutathione by *GstA* and its subsequent transport into vacuoles by an *ABC* metal ion transporter is also one of the methods of detoxification. Moreover, biovolatilization via methylation by *Cyt19* for detoxification purposes has been further proposed to increase As resistance in fungi (Ghosh et al. 1999; Choe et al. 2012).

8.4 Influence of Microorganisms on the Speciation and Bioavailability of Arsenic

The accumulation of different As species in various ecosystem compartments is regulated by different microbial processes. Microorganisms play an important role in the speciation and cycling of environmental As with a variety of mechanisms affecting As transformation between soluble and insoluble forms. Although As is highly toxic to most of the life forms, some microorganisms have developed As resistance mechanisms while some even thrive on the metalloid, using it as a source of energy for growth (Wang et al. 2011; Kruger et al. 2013). The actual state of As strongly depends on microbial transformations in soils, including reduction, oxidation, and methylation processes. Microbial enzymatic activities catalyze the conversion of the As species into forms having a different solubility, mobility, bioavailability, and toxicity (Lièvremont et al. 2009). The inorganic As forms, especially As(V) and As(III), are subjected to microbiologically mediated oxidation-reduction reactions. Microbes could even derive metabolic energy by the oxidation of As(III) or could use it as the sole energy source. Arsenate, on the other hand, is reduced by dissimilatory reduction and utilized as a terminal electron acceptor for anaerobic respiration. In addition, microorganisms may possess As(V) reduction mechanisms that are not coupled to respiration but instead are thought to impart As resistance. The detoxifying reductases present in the cytoplasm reduce As(V) to As(III) for its rapid extrusion from the cell. Even microbes capable of both As(III) oxidation and As(V) reduction are recognized (Pandey and Bhatt 2016). Soil microbes are also able to biomethylate inorganic arsenicals to monomethyl arsenic acid (MMAA) and dimethylarsenic acid (DMAA), and to other organic arsenicals (e.g., arsenocholine, arsenobetaine, arsenosugars, arsenolipids). The organo arsenicals are further metabolized to complete the As cycle (Dhuldhaj et al. 2013). In contrast, demethylating microorganisms carry out the conversion of methylated As species back to inorganic forms. Further, microbial activity can also methylate As(III) resulting in volatilization of As to gaseous arsines, viz. trimethylarsine gas, thereby releasing As into the atmosphere (Islam et al. 2007). Arsines are toxins which may travel in air for indefinite time period, or they may be rapidly oxidized subject to environmental conditions. Oxidation again yields inorganic As species, As(V) or As(III), or organic forms, MMAA and DMAA, which are deposited back to the soil by rain or by dry deposition, thereby completing the cycle of As.

8.5 Plant Growth Promoting Microorganisms

The plant health and soil fertility are the determinants of beneficial plant-microbe interactions in the rhizosphere which play a pivotal role in transformation, mobilization, solubilization, and subsequent uptake of essential nutrients by the plants (Dey et al. 2004). Despite the long history of interest in As-resistant plants and microbes, the attention of microbiologists toward plant-associated bacteria from

heavy metal enriched habitats is more recent (Sessitsch et al. 2013). It has been well established that the soil hosts a large number of microbes, including bacteria and fungi. The microbial count of different soils is subjective to the soil conditions including temperature, pH, moisture, salinity, and presence of other chemicals, as well as by the type of vegetation of that area. There is a huge diversity of soil microbial communities which is exceptionally rich and has developed diverse As resistance and detoxification mechanisms thereby redistributing As in soil (Sheik et al. 2012). Soil microorganisms affect As mobility and availability to the plant; they produce iron chelators and siderophores, solubilize metal phosphates, and produce growth hormones (Cavalca et al. 2010). Numerous metal-resistant microbes could transform As compounds by oxidation, reduction, methylation, and demethylation (Stolz et al. 2002) and are known to promote plant growth by direct and/or indirect mechanisms (Rajkumar et al. 2012). They act as a barrier and limit the transfer of As into plant tissues, thereby improving growth of the host plant (Fitz and Wenzel 2002). These microbes may prove beneficial for the revegetation and phytostabilization of As polluted sites. Thus, the need of the hour is to enhance the efficiency of the beneficial microbes for sustainable agricultural production (Khan 2005).

8.5.1 Rhizobacteria

The beneficial, free-living bacteria that colonize roots and establish a symbiotic relationship with plants to promote growth are referred to as plant growth-promoting rhizobacteria (PGPR). The abundance of bacteria in the rhizosphere is because of the presence of nutrients including sugars, amino acids, organic acids, and other small molecules released from plant root exudates (Gupta et al. 2015). While only 1–2% of bacteria promote plant growth in the rhizosphere, even lesser is known about the heavy metal–resistant PGPR. Additionally, it is also recognized that PGPR unwilling As are widespread in nature and are phylogenetically diverse (Table 8.3). They can have the advantages of oxidizing/reducing As and promoting plant growth in As stressed soil (Das et al. 2016). Moreover, it is now an accepted fact that the bacterial inoculation significantly reduces As uptake and its accumulation in shoot and grains of many plant species. Owing to its wide action spectrum, the As-resistant PGPR could serve as a potential bioinoculant for mitigation of As in different plants, thereby contributing to sustainable crop production in As-contaminated areas (Bhattacharyya and Jha 2012).

Plant growth promoting rhizobacteria are broadly categorized into extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR) (Viveros et al. 2010). These rhizobacteria effect plant growth either directly or indirectly, even in As stressed soils. The direct effect on plant growth is facilitated by the production of phytohormones (e.g., IAA), antibiotics, inorganic phosphate solubilization, increased production of iron-chelating siderophores, ammonia, and exopolysaccharides, thereby stimulating many important pathways (Fig. 8.3). However, their indirect effects can occur by antibiosis, synthesis of hydrolytic enzymes, struggle for the availability of nutrients with pathogenic bacteria

Table 8.3 Arsenic-resistant and plant growth promoting rhizobacteria

Bacterial class	Beneficiary plant	Mechanism	References
Rhodopseudomonas palustris CS2, *Rhodopseudomonas faecalis* SS5	*Vigna mungo*	As transformation and plant growth promotion	Batool et al. (2017)
Klebsiella pneumoniae T22, *Klebsiella oxytoca* N53, *Bacillus subtilis* T23, *Acinetobacter lwoffii* T24, and *Citrobacter freundii* N52	*Triticum aestivum*	As(III) oxidation and plant growth promotion	Qamar et al. (2017)
Rhizobium radiobacter strain VBCK1062	*V. radiata*	Production of exopolysaccharide (EPS) and As(V) sequestration	Deepika et al. (2016)
Brevundimonas diminuta	*Oryza sativa*	Phytostabilization of As	Singh et al. (2016)
Bacillus flexus ASO6	*O. sativa*	As oxidation and plant growth promotion	Das et al. (2016)
Streptomyces sp.	*O. sativa*	As methylation	Kuramata et al. (2015)
Pseudomonas sp. P1III2, *Delftia* sp. P2III5, *Bacillus* sp. MPV12, *Variovorax* sp. P4III4, *Pseudoxanthomonas* sp. P4V6	*Pteris vittata*	As removal and production of siderophore and indoleacetic acid (IAA)	Lampis et al. (2015)
Pantoea sp. strain (EA106)	*O. sativa*	As oxidation and plant growth promotion	Lakshmanan et al. (2015)
Bacillus flexus ASO-6	*O. sativa*	Production of siderophore, IAA, 1-aminocyclopropane-1 carboxylic acid (ACC)-deaminase and solubilization of phosphate, improved seed germination, and reduced As accumulation in grains	Das et al. (2015)
Pseudomonas fluorescens	*Solanum lycopersicum*	Phosphate solubilization and siderophore production	Ghosh et al. (2015)
Brevibacterium, Rahnella, Pseudomonas, Rhodococcus, Microbacterium, Paenibacillus	*Zea mays*	As oxidation, production of siderophore, IAA, ACC-deaminase, and solubilization of phosphate	Shagol et al. (2014)
Alcaligenes sp.	*Helianthus annuus*	Production of ACC- deaminase, siderophore, and IAA	Cavalca et al. (2013)
Staphylococcus arlettae strain NBRIEAG-6	*Brassica juncea* (L.) Czern. Var. R-46	Production of IAA, siderophores, and ACC- deaminase	Srivastava et al. (2013)

(continued)

Table 8.3 (continued)

Bacterial class	Beneficiary plant	Mechanism	References
Pseudomonas, Comamonas, Stenotrophomonas	*Pteris vittata*	As solubilization	Ghosh et al. (2011)
Bacillus, Achromobacter, Brevundimonas, Microbacterium, Ochrobactrum	*Cirsium arvense* (L.)	As oxidation and plant growth promotion	Cavalca et al. (2010)

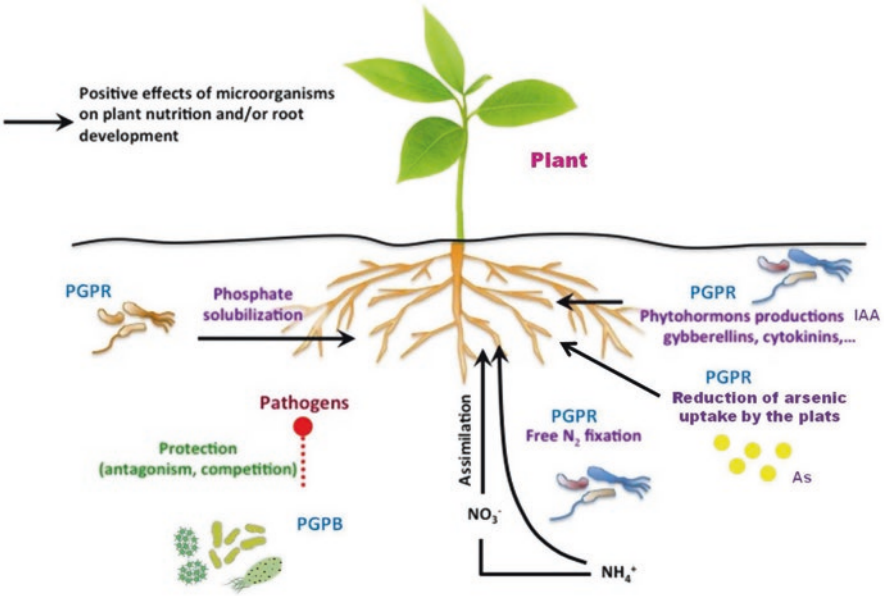

Fig. 8.3 Beneficial effects of metal-resistant and plant growth promoting bacteria

in soil, or by inducing systemic resistance (ISR) against wide-ranging root pathogens in the rhizosphere. Out of the many resistance mechanisms acquired by rhizobacteria, salicylic acid-dependent SAR pathway or jasmonic acid and ethylene perception from the plant are the most evident ones (Beneduzi et al. 2012). Arsenic-resistant PGPR's might be useful in framing new amalgamations, leading to a more efficient use for phytostabilization and improvement of cropping systems (Gupta et al. 2015).

8.5.2 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in nature and establish important symbiotic relationships with most terrestrial plants even in heavy metal–contaminated environments (Sun et al. 2016). Moreover, plant compatibility with

mycorrhizal fungi is a generalized and primordial phenomenon. Arbuscular mycorrhizas are characterized by the formation of highly branched and unique structures called arbuscules, which help plants to capture nutrients such as phosphorus, carbon, sulfur, nitrogen, and other micronutrients from soil (Gianinazzi-Pearson 1996).

However, AMF are widespread in different regions of the world; they are influenced by As concentrations and seasonal variations. A variety of species have been identified, belonging to the genus *Acaulospora*, *Glomus*, *Gigaspora*, *Westerdykella*, *Trichoderma*, *Rhizopus*, *Lasiodiplodia*, *Paraglomus*, *Rhizophagus*, etc. (Table 8.4). Arbuscular mycorrhizal fungi belonging to the phylum *Glomeromycota* form a symbiotic association with more than 80% of terrestrial plants. The predominance

Table 8.4 Arsenic-resistant and plant growth promoting fungi

Fungi	Beneficiary plant	Mechanism	References
Acaulospora morrowiae, Rhizophagus clarus, Gigaspora albida, Paraglomus occultum	*Leucaena leucocephala*	As phytostabilization	Schneider et al. (2017)
Rhizoglomus intraradices and *Glomus etunicatum*	*T. aestivum*	Reduced translocation of As to grains, and higher activities of the antioxidant enzymes	Sharma et al. (2017)
Aspergillus oryzae, Fusarium sp., *Aspergillus nidulans, Rhizomucor variabilis, Emericella* sp.	*Calendula, Withania,* and *Avena sativa* plants	As bioaccumulation and biovolatilization	Singh et al. (2015)
Glomus etunicatum, Acaulospora morrowiae, Gigaspora gigantea, Acaulospora sp.	*Acacia mangium, Crotalaria juncea, Enterolobium contortisiliquum, Stizolobium aterrimum*	Phytoprotective effect and phytoremediation of As contaminated soil	de Melo et al. (2014)
Acaulospora morrowiae, Glomus clarum, Gigaspora albida	*L. leucocephala*	As removal from the soil and phytoextraction	Schneider et al. (2013b)
Westerdykella, Trichoderma, Rhizopus, Lasiodiplodia	*O. sativa* and *Pisum sativum*	Improved soil nutrient content and enhanced plant growth	Srivastava et al. (2012)
Glomus mosseae, Glomus intraradices, Glomus etunicatum	*Pityrogramma calomelanos, Tagetes erecta, Melastoma malabathricum*	Improved growth and As accumulation	Jankong and Visoottiviseth (2008)
Glomus mosseae	*S. lycopersicum, Medicago sativa Linn., Z. mays* L., *M. truncatula*	Enhancing phosphorus nutrition and restricting root As uptake	Liu et al. (2005); Chen et al. (2007); Xia et al. (2007); Xu et al. (2008)

of these species indicates their high tolerance to excess As; nevertheless, As contamination adversely affects the diversity and function of AMF (Schneider et al. 2013a; Sun et al. 2016). Dominant plant species present in As-contaminated sites are habitually colonized by AMF, which is indicative of their central role in mitigating heavy metal stress in plants. Arbuscular mycorrhizal fungi improve the tolerance of host plants to As in contaminated soils by performing two different roles in phytoremediation: one being phytoextraction and another phytostabilization (de Melo et al. 2014; Krishnamoorthy et al. 2015).

8.6 Plant Growth Promoting Microorganisms in Extenuating Arsenic Toxicity

8.6.1 Precipitation, Binding, or Chelation of Arsenic in the Rhizosphere

The biochemical and molecular mechanisms of plant-microbe interactions in As stressed soils can change metal bioavailability in soil through various mechanisms such as acidification, precipitation, chelation, complexation, and redox reactions. The microbial activities are known to enhance the mobility of metals by forming metal/mineral complexes, intracellular binding, electron transfer by enzymes in the rhizosphere (redox reactions), etc. The microbes present in the As-contaminated soils have evolved resistance strategies to tolerate considerably high concentrations of metals. The extensive research on the metal-resistant PGPM have shown many possibilities to improve plant growth by alleviating metal toxicity, and improving restoration by mobilization/transformation of metals in soil (Rajkumar et al. 2012; Tak et al. 2013; Ma et al. 2016).

8.6.2 Root Colonization

Rhizobacteria provide nutrients to the plant by habituating the soil surrounding the roots of the beneficiary plant. In turn, the plant creates and maintains root nodules for rhizobacteria, thereby providing a proper place and conditions for the bacteria to be alive and active. This colonization makes the soil, surrounding the plant, more rich in terms of nutrients. The effectiveness of PGPR has often been attributed to their ability to colonize plant roots. Bacterial colonization into plant roots is a series of a process involving various steps. It defines the ability of the bacteria to survive and multiply in the region surrounding the roots in response to the plant exudates, to attach with the root surface, and to colonize developing a root system. There could be multifactorial phenomenon contributing the process of colonization, viz. chemotaxis to root exudates, cell motility, production of pili or fimbriae, quorum sensing, etc. (Ma et al. 2016).

In case of AMF, the process of colonization takes place in a way different to that in bacteria. The plant-microbe interaction starts with the signal exchange and recognition between host plants and AMF before they come into physical contact. Signal recognition has been proposed to help in cellular adaptation in fungi required for the functional compatibility between the plant and fungal cells. This finely tuned recognition processes help in establishing molecular coordination between the two. The recognition signal from the host plant helps in inducing hyphal branching followed by the formation of appressoria at the root surface during the early hours of contact. Metabolites exuded by plant roots specifically enhance spore germination and fungal growth which helps the fungus to invade the root. Fungal development is limited to the outer root tissues, while hyphae quickly enter the inner cortical cells of host plant where they differentiate into the highly ramified arbuscules (Gianinazzi-Pearson 1996).

This proves that the successful colonization forms an important part of the plant-microbe relationship. It not only helps in the exchange of nutrients but also limits the uptake of harmful chemicals and heavy metals like As to the plant tissues and prevents the rate and extent of pathogen colonization in roots, thereby helping the plants to grow and propagate at high concentrations of As (Jog et al. 2014; Mallick et al. 2014).

8.6.3 Facilitating Resource Acquisition

8.6.3.1 Nitrogen Fixation

Nitrogen is an imperative nutrient to all life forms; however, gaseous nitrogen remains unavailable to the plants due to the high energy required to break the triple bonds present between the two atoms. Rhizobacteria, through nitrogen fixation, are able to convert gaseous nitrogen to ammonia, and making it available to the host plant (Ullah and Bano 2015). This is one of the most mutualistic relationships between a microbe and the plant taking place in soil, where the host plant provides the bacteria with amino acids for the production of ammonia. The enzyme involved in the process of nitrogen fixation is called nitrogenase, while the oxygen is provided by a protein called leghemoglobin, which is produced within the nodules (Vejan et al. 2016). A wide range of PGPR is present in nature and belongs to the genera: *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Bradyrhizobium*, and *Frankia* (Masson-Boivin et al. 2009). Although the presence of As adversely affects the process of biological nitrogen fixation, the As-resistant microbes have all managed to cope up with such extreme conditions, hence providing the plants with the requited nitrogen even in the presence of As.

8.6.3.2 Phosphate Solubilization

Plant growth promoting rhizobacteria solubilize insoluble phosphorus in the soil and increase the accessibility of unavailable nutrients to plants. Phosphorus, a key element required for the nutrition of plants, is abundantly present in soil, but its insoluble nature makes it inaccessible to plants. The two soluble forms of

phosphate, i.e., monobasic ($H_2PO_4^-$) and diabasic (HPO_4^-) ions, are readily absorbed by the plants. Rhizobacteria may employ one of the following mechanisms to facilitate the conversion of insoluble forms of phosphorus to soluble forms: release of compounds, for example, organic acid anions to dissolve minerals; production of extracellular enzymes like phosphatases for phosphate solubilization; release of phosphate during substrate degradation, etc. (Kumar et al. 2010). Phosphate solubilizing rhizobacteria are included in the *genera Rhodococcus*, *Bacillus*, *Serratia*, *Erwinia*, *Flavobacterium*, *Arthrobacter*, *Pseudomonas*, *Beijerinckia*, *Burkholderia*, *Microbacterium*, *Rhizobium*, and *Enterobacter*, and are extensively studied to improve plant growth and yield responses. Further, the application of phosphate solubilizing bacteria has also been reported to show many beneficial effects when employed with other metal-resistant rhizospheric microbes for the increased productivity and stability of plants in contaminated soils (Tian et al. 2014).

8.6.3.3 Siderophore Production

The plant's nutritional requirements of iron are satisfied by specific microorganisms which have evolved precise pathways that employ low molecular weight iron chelators termed siderophores. Siderophores are small peptidic molecules secreted to solubilize iron from their surrounding environments, forming a complex called ferric-siderophore that can move by diffusion (Glick 2012). Siderophores can chelate ferric ion with high affinity, allowing its solubilization and extraction from most mineral or organic complexes. In soil, siderophore production activity plays a central role in determining the ability of different microorganisms to improve plant development (Arora et al. 2013). Microbial siderophores enhance iron uptake by plants that are able to recognize the bacterial ferric-siderophore complex. The highly competitive conditions in the root environment help the plants to selectively use iron for their growth. Siderophore-forming microbes also selectively inhibit the growth of other soil-borne fungal and bacterial pathogens through the release of iron-chelating substances (Dwivedi and Johri 2003). Siderophore-producing bacteria have been extensively studied belonging to the genera *Bradyrhizobium*, *Pseudomonas*, *Rhizobium*, *Serratia*, and *Streptomyces* (Sujatha and Ammani 2013). Siderophore-producing metal-resistant microbes are also identified which serve a dual role in soil; they are actively engaged in providing nutrients and helping the plants to survive in heavy metal–contaminated soil (Wani and Khan 2013).

8.6.4 Modulating Phytohormones

A wide range of microorganisms has been found in the rhizosphere which is capable of producing plant growth regulating substances. Plant growth promoting microorganisms produce phytohormones such as auxin(s), cytokinin(s), gibberellin(s), and ethylene, which affect cell proliferation with a subsequent increase in nutrient and water uptake.

8.6.4.1 Cytokinin

Several PGPMs of the genera *Azotobacter*, *Rhizobium*, *Rhodospirillum*, *Pseudomonas*, *Bacillus*, and *Paenibacillus* are known which can produce cytokinins (Glick 2012). However, a detailed understanding of the role of bacterial-synthesized hormones is still unclear; it is believed to provide exogenous and purified hormones to growing plants.

8.6.4.2 Gibberellin

Gibberellin is another hormone actively engaged in regulating plant growth and influencing various developmental processes including stem elongation, germination, dormancy breaking, flowering, sex expression, enzyme induction, and leaf and fruit senescence. Many bacterial species have been identified contributing to the production of this hormone in the soil, thereby promoting plant growth (Glick 2012).

8.6.4.3 Auxin

Although several naturally occurring plant growth regulators, indole-3-acetic acid (IAA) is by far the most common as well as the most studied auxin and much of the scientific concern, exerting a positive effect on root growth. It is evident that up to 80% of root colonizing rhizobacteria can synthesize IAA to stimulate cell proliferation and enhance the uptake of minerals and nutrients from the soil (Gupta et al. 2015). Indole-3-acetic acid not only affects plant cell division, extension, and differentiation but also provides resistance to stressful conditions. An array of metabolically active PGPRs like *Pseudomonas*, *Rhizobium*, *Bradyrhizobium*, *Agrobacterium*, *Enterobacter*, and *Klebsiella* are known to promote root growth in various plant species (Shilev 2013).

8.6.4.4 Ethylene

Ethylene is a key phytohormone having a wide range of biological activities. It affects plant growth and development by promoting root initiation, fruit ripening, seed germination, leaf abscission, and synthesis of other plant hormones, while inhibiting root elongation and wilting (Glick et al. 2007). The enzyme ACC is a prerequisite for ethylene production which is reported to improve dry weight, grain yield, straw production, and nitrogen content in different plant species (Glick 2012; Gupta et al. 2015). Currently, bacterial strains exhibiting ACC deaminase activity have been identified in a wide range of genera such as *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia*, and *Rhizobium* (Kang et al. 2010).

8.6.5 Reducing Oxidative Stress/ Improving Antioxidant Potential

Arsenic induces toxicity in plants that has been linked to the production of reactive oxygen species (ROS), thereby inducing lipid peroxidation and damage to nucleic acids and proteins (Aksakal and Esim 2015; Chandrakar et al. 2016; Chandrakar et al. 2017). Production of ROS in plants is regulated by antioxidative enzymes which remove free radicals thereby protecting the cells from oxidative damage. In the presence of metal-resistant PGPM, the oxidative stress decreases to a considerable level, owing to the reduced uptake of As, thereby balancing the levels of antioxidative enzymes required in the cell (Mishra et al. 2013; Talukdar and Talukdar 2013).

8.6.6 Induced Systemic Resistance

Plant growth promoting microorganisms are capable of controlling diseases that are caused by inhabiting pathogenic bacteria and fungi. The disease is suppressed through Induced Systemic Resistance (ISR) against a broad spectrum of plant pathogens and the production of antibacterial/antifungal metabolites. The production of antibiotics is considered to be one of the most powerful and studied biocontrol mechanisms of PGPR against phytopathogens. The microbial metabolites produced in the rhizosphere help in the reduction of diseases of fungal, bacterial, and/or viral origin, and in some instances even damage caused by insects and nematodes (Choudhary et al. 2007). Beneficial microbes also induce signaling pathways within the plant to stimulate the production of hormones involved in plants defense responses against a variety of pathogens (Haas et al. 2002). A number of bacterial components like lipopolysaccharides (LPS), siderophores, cyclic lipopeptides, and some volatiles are known to encourage ISR, which have now been commercialized to increase the resistance of host plant (Ongena et al. 2004; Ryu et al. 2004). This has led to the genetic modification of many bacterial strains to develop as a bioinoculant to improve plant growth and disease resistance of agricultural crops.

8.6.7 Reducing Arsenic Absorption and Translocation

Metal-resistant PGPR and AMF, particularly those isolated from metalliferous sites, are capable of boosting plant growth by reducing As absorption, changing available As through alteration of soil pH, and by affecting As translocation (Rajkumar et al. 2012). Moreover, As accumulation in plant tissues is more likely to be associated with the availability of As in the soil. The presence of microbial population is known to significantly decrease the uptake and accumulation of As in plant tissues even in the As rich soil, may be due to the microbial uptake of a metalloid. It is well established that the presence of the beneficial bacteria/fungi not only colonizes the roots

of the plants but also decreases the uptake of As in tissues, thereby helping the plants to grow and propagate at high concentrations of As (Wani and Khan 2013; Mallick et al. 2014; Selamat et al. 2014).

8.6.8 Enhancing Plant Growth and Biomass

Certain bacterial strains synthesize a wide spectrum of multifunctional exopolysaccharide (EPS) in response to the heavy metals. Exopolysaccharide is a mode of protecting bacteria from desiccation, environmental stress, and plant defense responses in plant-microbe interactions (Limoli et al. 2015). This class of polysaccharide also helps plants to flourish in As stressed soil in many different ways. Production of EPS is known to assist in biofilm formation, attachment to plant surface, root colonization, holding free phosphorus in soils, cation binding including Na^+, circulating essential nutrients to the plant for proper growth and development, and protecting it from the attack of foreign pathogens (Vu et al. 2009; Gupta et al. 2015). This indicates the importance of PGPR producing EPS in promoting plant growth and biomass production by establishing beneficial interactions, satisfying environmental stress. and providing defense response during the infection process.

8.6.9 Improving Plant Establishment in Contaminated Soils

The association of As-resistant PGPMs with the roots opens new ways in securing the host plant not only against As but also from the deleterious effects of other cross contaminating heavy metals. The metal-resistant systems in bacteria/fungi enable them to limit the availability of metals to plants by producing and transporting inorganic phosphate and other growth-promoting substances from the soil. Further, the efflux of As from the host plant is absorbed by the microbes residing in the roots, thereby ensures reabsorption of As decreasing the overall metal concentration of the soil. The AMF may also act as a filter to maintain low plant As levels while maintaining an adequate supply of phosphorus to the host (Liu et al. 2005; Chen et al. 2007; Xia et al. 2007; Xu et al. 2008).

8.7 Microbial Technologies for the Alleviation of Arsenic Effects in Plants

8.7.1 Enzyme Technology

Among biological agents, enzymes have a great potential to effectively transform and detoxify environmental pollutants (Rao et al. 2010). Biocatalysts may serve as an alternate method for As remediation, and recently, there has been increasing

interest in their potential applications. Enzymes could provide a more specific and clean way of dealing with the toxic metalloid, As (Dhankher et al. 2002; Bahar et al. 2012). Many microorganisms are known to produce enzymes involved in As detoxification, which could be successfully extracted and used for the remediation purposes. Enzymes in their immobilized forms are drawing significant attention for potential applications in As removal due to the reducing operational expenses and the increased process utilization of the enzymes (Talat et al. 2009). Typically, immobilized enzymes have greater thermal and operational stability at various pH values, ionic strengths and are more resistant to denaturation than the soluble native form of the enzyme (Bayramoglu et al. 2013). Similarly, immobilized biocatalyst displays the property of recycling and offer continued activity and stability for being reused many times (Eş et al. 2015).

However, the use of nanoparticle as supporting material for immobilized enzymes of microbial origin is drawing great attention to other materials. It provides many advantages such as the higher surface area that allows greater enzyme loading, higher stability and lower mass transfer resistance (Ho et al. 2008; Ansari and Husain 2012). The selectivity and increased activity of the immobilized enzymes could be utilized on a large scale for the improved As removal from soil and treatment process.

8.7.2 Genetic Engineering

The severity of the As contamination had raised an alarming situation demanding immediate attention and novel methods of remediation. The method of genetic engineering could be a potential strategy to overcome the drawbacks of this toxic metalloid. Many studies have been undertaken for the improvement of As methylation and detoxification abilities of the microbes by successful expression of the target gene (Qin et al. 2006; Yuan et al. 2008; Chen et al. 2014; Huang et al. 2015). These recombinant bacteria had 10-fold increase in response and resistance toward As and showed the successful transformation of inorganic As into less toxic organoarsenicals when compared to wild-type strains (Liu et al. 2011). This demonstrates the potential application of genetically engineered microorganisms as an efficient strategy for As bioremediation from contaminated soil (Liu et al. 2011), thereby reducing its translocation and accumulation in food stuff (Chen et al. 2013).

8.8 Conclusions and Prospects

The uniqueness of microorganisms and their often unpredictable nature and biosynthetic competences have made them promising candidates for solving As stress in different plant species. The conscientious use of indigenous microorganisms with the advanced technologies and modern techniques would attract economic, social,

and environmental benefits which would provide an efficient way to protect the ecosystem. Microbial populations are known to affect As mobility and availability to the plant and therefore have potential to enhance phytoremediation processes. The advances in exploring and utilizing the significance of As-resistant rhizobacteria and AMF would be the simplest phenomenon to protect crops from As, as they may prevent the transport of As from root to shoot, thereby decreasing its uptake by plants. There is an urgent need to improve our understanding of the mechanisms involved in the transfer and mobilization of As by PGPRs and AMF, and to conduct research on the selection of microbial isolates from the rhizosphere of plants growing on As-contaminated soils for specific restoration programs.

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Chapter 9
Role of Plant-Microorganism Interactions in Plant Tolerance to Arsenic

Anna Kowalczyk and Dariusz Latowski

Abstract In this chapter, the review of the newest reports on plant-microbe interactions in plant tolerance to arsenic (As) is presented in two aspects. One is the bacteria effect on regulation of As availability in growth environment of the plant, and the second is direct influence of them on plant organism tolerance to As. The role of As oxidization or reduction mechanisms which were developed by microbes colonizing soil or water in plant tolerance to As is discussed. The meaning of rhizospheric bacteria contribution to bioavailability of elements such as phosphorus, iron, silicon or As, by mineral solubilization, as well as the significance of the bacteria siderophores in plant As tolerance is also explained. As and Fe released from iron(III) arsenate by symbiotic bacteria of As-hyperaccumulator fern, *Pteris vittata*, are not omitted. The role of As-resistant representatives of plant growth-promoting bacteria (PGPB) group in the reduction of As uptake by plants from contaminated soil is also described. Considering novel aspects of plant-microbe interactions under As stress, the content of this chapter refines previous knowledge about plant physiology in terms of As tolerance and in the field of As-resistant plant-microbe model application in environment remediation.

Keywords Metalloids · Phytoremediation · Plant-microbe interaction · *Pteris vittata* · Soil pollution

9.1 Introduction

In the environment, besides herbivores, plants are exposed to different biotic factors, both beneficial and pathogenic such as insects, nematodes, fungi, bacteria, archaea or viruses (Coats and Rumpho 2014; Müller et al. 2016; Busby et al. 2017). They interact with plant affecting its metabolism and development (Martin et al.

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2016; Martin et al. 2017). There are long-term symbiotic interactions, bringing bidirectional benefits (mutualism), exploiting one side of the relation (parasitism) or mutualistic and detrimental for both sides of interrelationship at the same time (Martin et al. 2017). Since plants colonized terrestrial environment, they have evolved a variety of interrelationships with actively selected microorganisms, what seemed to be the most beneficial way to provide poorly soluble nutrients and protect from toxins in environment deficient in water, main solvent and transporter, comparing to previous habitat (Bulgarelli et al. 2013; Zgadzaj et al. 2016; Martin et al. 2017). The common pathway of symbiotic signalling is shared by all plants of endosymbiotic interrelationship with fungi and bacteria, including *Actinobacteria,* from rhizosphere (Martin et al. 2017). Chitin-based signalling molecules secreted by symbiotic fungi and rhizobia, which are detected by receptor-like kinases, activate the signalling pathway of plant host, and this mechanism is common regardless of plant species, genus or family (Martin et al. 2017). That process, although similar to an infection, leads to bidirectional mutualistic benefits and also modifications of hormone activity and such organs as root development towards colonization by determined microorganisms (Martin et al. 2017). Establishing mutualistic interrelationships, such as mycorrhizae or bacteria-host plant relation, can be crucial in case of exposition to different environmental biotic and abiotic stress factors, enhancing plant resistance to metals and metalloids by sequestering them into roots and by protecting these other organs from translocation of uptaken toxic ions or molecules (Garg et al. 2015), on the other hand. It is worth to emphasize, that recently microbial symbionts interacting with plants were distinguished into two groups and are investigated as endophytes or rhizospheric microorganisms.

9.2 Microbial-Mediated Arsenic Transformations in Plant Symbionts

Amongst potentially harmful substances, arsenic (As) contamination is one of the major environmental problems affecting plants (Zhao et al. 2009; Gupta and Khan 2015; Latowski and Kowalczyk 2016). Its uptake by the plants causes damage to natural plant ecosystems and crops as well, ceasing by this continuity of agriculture production. Arsenic causes a variety of damages to nucleic acids, proteins, membranes and other cell compounds (Gupta and Khan 2015). In environment, there are different speciations of this element existing, including organic and inorganic compounds, and it is commonly known that those inorganic, such as arsenite and arsenate, are more toxic to living organisms, including plants (Gupta and Khan 2015; Latowski and Kowalczyk 2016). The toxic effect also depends on valency state of As in given compound (III state of oxidation in arsenite or V state of oxidation in arsenate). Arsenic toxicity effects to plants are mainly due to As(III) because As(V) is immediately reduced to As(III) by arsenate reductase when absorbed into the cell

(Hu et al. 2013; Jia et al. 2014). On the other hand, in the external environment, which is rhizosphere, arsenite is oxidized to arsenate either by arsenite-oxidizing microbes (Hu et al. 2013). It was confirmed by several studies that As processing is strongly influenced by microbial activity in addition to chemical transformations in soils or water (Xu et al. 2007; Wang et al. 2011a, b; Hu et al. 2013; Jia et al. 2014; Han et al. 2017b). On the other hand, little is known about interactions between As-resistant plant endosymbionts and root and rhizosphere microbes, particularly on As oxidation and reduction in terms of plant uptake, acquisition, transformation and tolerance to As.

Arsenic transport into plant cells is facilitated, as it is recognized and bound by phosphate or silicon transporters. So far, there are no results explaining what are interactive effects of As and Si to plants (Hu et al. 2013; Schneider et al. 2013); however it was reported that presence of silicon facilitates forming iron plaque on root surface of rice and other wetland plants, thus building barrier for As uptake or space for transformations of As (oxidation/reduction reactions) (Awasthi et al. 2017). In plant roots, arsenate is quickly reduced to arsenite and can outflow back to the soil (Xu et al. 2007; Hu et al. 2013) where under aerobic conditions, it is oxidized rapidly again to arsenate by arsenite-oxidizing microbes. It is worth to notice that As(V) is much stronger bound with soil particles such as ferrihydrite, aluminosilicates or aluminium hydroxides than As(III), and thus oxidation of As(III) to As_{in}(V) is one of the most significant factors decreasing As bioavailability to plants. It is also known that iron plaque can bind As(V) even four times easier than As(III) (Blute et al. 2004). Studies on rice (*Oryza sativa*), grown on submerged areas deficient in oxygen, revealed that As uptake by roots is strongly dependent on microbial oxidization of As(III) to As(V) in combination with root radial oxygen loss (ROL) and availability of organic matter, such as addition of extra rice straw (Jia et al. 2014). Rice cultivars with higher ROL demonstrated lower As uptake than those with lower ROL. The enhancement of this rhizospheric effect on the abundance of the As(III) oxidase gene (aroA-like) was greater than on the As(V) reductase gene (arsC) and As(V) respiratory reductase gene (arrA). The direct effect was As(III) oxidation and sequestration in the rhizosphere, protecting the plant from the uptake of As(III), which intoxicate plant much easier than As(V). These rhizospheric processes, together with the addition of rice straw and growth stage dependently, influenced the rhizospheric population of bacteria, which therefore was dominated by α,- β- and γ-*Proteobacteria*. Mentioned proteobacteria inhabiting rhizosphere are both As(III)-oxidizing, with *arsC* and *arrA* gene representatives including *Enterobacteriales*, *Rhizobiales*, *Sphingomonadales*, *Burkholderiales* and *Xanthomonadales*, and also As(V)-reducing with *aroA*-like sequences, such as *Phyllobacteriaceae*, *Bradyrhizobiaceae*, *Methylobacteriaceae*, *Rhizobiaceae*, *Bulkholderiaceae*, *Comamonadaceae* and also seven unidentified mainly rhizospheric clusters (Jia et al. 2014). Recent studies on As hyperaccumulator fern, *Pteris vittata*, demonstrated that about 50% of the tissue microbiota consists of mentioned above α-, β- and γ-*Proteobacteria* species, but the half is dominated

by *Bacteroidetes* and *Firmicutes* (Han et al. 2017a). The important role of the plant organism microbiota was proved e.g. by experiments performed by Mathews et al. in 2010, when under aerobic conditions after 24 h of incubation of 0.1 mM As(III) solution with unsterilized plant samples, considerable amount of the arsenite was oxidized to As(V), while oxidization was not observed in control without plants or with plants sterilized (Mathews et al. 2010).

9.3 Element Availability and Role of Siderophores

Plants and microorganisms involved in biogeochemical element cycles have to deal with reduced access to sufficient nutrients bound in forms of insoluble minerals (Sharma and Sohn 2009; Han et al. 2017b). It is commonly known that mycorrhizal interaction of fungi with plant brings to plant increase of water and nutrient uptake, which is significant concerning such macroelements like P or N, especially in low-fertility soils (Bais et al. 2006; Schneider et al. 2013; Garg et al. 2015).

In terrestrial environments P and As(V), which are chemically P homologue, often bind with Fe. Another factor limiting element availability to plants is pH; the more alkaline, the less mobile are metals and metalloids. *P. vittata* fern, a model plant organism which Han with the team broadly researches currently (Chen et al. 2016; Han et al. 2016a; Han et al. 2016b; Han et al. 2017a; Han et al. 2017b), as a hyperaccumulator of As had to evolve mechanisms to mobilize nutrients, and As as well, from insoluble minerals in rhizosphere soils and also translocation and detoxification pathways (Chen et al. 2016; Han et al. 2016b; Han et al. 2017b). Obtaining nutrients such as P or Fe by plants is possible, e.g. by excretion to rhizosphere substances such as organic acids, decreasing pH of the microenvironment and thus increasing the concentration of soluble P compounds (Han et al. 2017b; Liu et al. 2017a). Besides plant exudates, also microorganisms play a significant role in mineral solubilization (Han et al. 2017b). In soil different elements, including Fe and As, coexist. Under nutrient deficiency bacteria developed an efficient pathway of their harvest by producing siderophores, low-molecular-mass molecules enhancing Fe uptake (Liu et al. 2015; Han et al. 2017b; Liu et al. 2017a; Liu et al. 2017b) divided by chemical structure into three groups: catecholates, hydroxamates and carboxylates (Liu et al. 2016). The ability of elements such as phosphorus and metals or metalloid uptake and transformation is species- and genotype-specific, and this concerns microorganisms (including bacteria and fungi) and plants as well (Garg and Aggarwal 2012; Garg and Bhandari 2012). Siderophores besides other ions such as Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Mn^{3+}, Co^{3+}, Al^{3+}, Th^{4+}, U^{4+} and Pu^{4+} (Ahmed and Holmström 2014) release Fe^{3+}, As^{5+} and P^{5+} from minerals, thus making them available and facilitating their uptake also to plant roots (Azeem et al. 2014; Han et al. 2016a). However, microbial activity within rhizome space and on the root surface can also determine species of As in iron plaque coating roots, i.e. in the direct vicinity of the plant tissues. *Acidovorax* and *Hydrogenophaga* genera colonizing iron plaque were reported to be involved in oxidation of As(III) bound within,

decreasing uptake, hence total As concentration in the rice roots (from 30 mg kg^{-1} to about 1 mg kg^{-1}) straw (from 8 mg kg^{-1} to 1 mg kg^{-1}) and grain (from 23 mg kg^{-1}, to 10 mg kg^{-1}) (Hu et al. 2015). Ghosh with the team (Ghosh et al. 2011; Ghosh et al. 2015) proved that release of ions from iron(III) arsenate ($FeAsO_4$) is observed, when As-resistant bacteria producing siderophores and isolated from *P. vittata* rhizosphere are present in the direct environment. What is more, some strains, like *Pseudomonas* PG12 isolated from studied fern and producing catecholate type of siderophores, can thus enhance biomass growth and are more efficient than fungal siderophores. Research performed by Liu et al. (2016) indicated that *Pseudomonas* PG012 siderophore was more effective in promoting $FeAsO_4$ dissolution and Fe and As plant uptake, than fungal-siderophore desferrioxamine B (DFOB). Assays performed on *P. vittata* with DFOB demonstrated that DFOB treatment caused uptake and accumulation of mainly As(V) in roots inhibiting its reduction and transport to other organs, whereas bacterial PG12 siderophore treatment resulted in more efficient uptake of As(V) from soil and then its reduction to As(III) and translocation to rhizome and fronds (Liu et al. 2016). Accumulation of As(III) is typical for this fern (Wang et al. 2011a) and beneficial for potential phytoremediation, as above-ground organs are easy to remove from the ground surface. What is interesting, in pot experiments performed on pigeon pea (*Cajanus cajan*) and pea (*Pisum sativum*) under As stress (30 or 60 mg kg^{-1} dry soil), in which arbuscular mycorrhiza with *Funneliformis mosseae* was investigated in terms of protective for plant role towards As toxicity, demonstrated two important aspects of plant-microbial interactions. Besides beneficial effects of inoculation with mycorrhizal *F. mosseae* enhancing As tolerance of tested plants, the important role played rhizospheric bacteria *Sinorhizobium fredii* AR-4 (inoculated to pigeon pea) and *Rhizobium leguminosarum bv. viciae* strain PRH-1 (inoculated to pea). Those endophytic bacterial strains are reported to fix nitrogen and nodulate legumes (Mora et al. 2014). Pretreatment of sterilized seeds with mentioned strains facilitated fertilization of plants by fixing nitrogen from rhizosphere under stress conditions (As contamination). Interaction of inoculates with germinating seeds and forming organs resulted in increase of P, N and K uptake and, on the other hand, decrease of As uptake, thus diminishing its deleterious effect towards seedlings, as concentration of As after 75 days after sowing was up to about 22% lower in leaves and roots of As-treated plants comparing to uninoculated ones (Garg et al. 2015).

9.4 Plant Growth-Promoting Bacteria Under Arsenic Stress

It is commonly known that community of microbes inhabiting rhizosphere influence coexisting plants. There is a variety of phenomena and processes ranging from biochemical to ecological level, which indirectly or directly cause-effect to plant organisms. Recently research focus on beneficial aspects of them, as so far microorganisms were supposed to be mainly pathogens.

One of the protective roles of microorganisms colonizing rhizosphere is just occupying an environmental niche, potential habitat for pathogens. A consequence is competing and limiting indispensable nutrients, such as discussed P or Fe, for pathogen growth. Direct important benefit for plant organism is mitigation or even elimination of additional stress factor, what can be crucial to survive and develop in the environment with overlapping endangerments exposure. Rhizospheric bacteria can limit pathogen reproduction by synthesizing signal components, lytic enzymes, antibiotics or other toxins for potential pathogens and alter plant defence or induce mechanisms of resistance (Bais et al. 2006; Coats and Rumpho 2014). Several research proved that metal(loid)-resistant microorganisms colonizing rhizosphere and/or becoming plant endosymbionts, besides chemical transformation of molecules or ions thus being "alive targets", can promote growth of plant (plant growth-promoting bacteria, PGPB) (Wang et al. 2011a, b; Liu et al. 2015; Han et al. 2016b; Liu et al. 2017b). Experimental studies on poplar (*Populus deltoides*), rice (*O. sativa*) and ferns *P. vittata* or *Vigna radiata* demonstrated that presence of symbiotic bacteria in rhizosphere or within plant tissues not only induces plant growth-promoting effect but also conduces As transformation and detoxification (Mathews et al. 2010; Liu et al. 2015; Han et al. 2016a; Singh et al. 2016; Batool et al. 2017; Liu et al. 2017b; Das and Sarkar 2018). Such parameters as germination percentage, biomass growth, chlorophyll, carotenoid and soluble protein or sugar content, indole-3-acetic acid (IAA) synthesis, the activities of ACC deaminase and oxidative stress enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and the malondialdehyde (MDA) concentrations were controlled to determine As stress of plant organisms treated or untreated with different microbial isolates (Mathews et al. 2010; Zhu et al. 2014; Hu et al. 2015; Liu et al. 2015; Han et al. 2016a; Singh et al. 2016; Batool et al. 2017; Liu et al. 2017b).

Inoculation of poplar cuttings pot cultures with isolate *Agrobacterium radiobacter* D14 strain isolated from *P. vittata* rhizosphere contaminated with As resulted in an increase of soluble sugar content in leaves of plants from As-treated cultures in comparison with control assays without As although both types of plants were treated with tested bacterium strain. What is interesting, without inoculation with the D14 strain, the sugar content increased only at a low concentration of As (150 mg kg^{-1}soil), whereas the activity of D14 in inoculated assays resulted in an increase of sugar content also at a high concentration of As (300 mg kg^{-1}). Arsenic contamination caused a decrease of chlorophyll content, but in the presence of microorganisms, chlorophyll contents were higher compared with samples uninoculated (34). Both experiments on rice and poplar plants (Wang et al. 2011b; Pandey et al. 2013) demonstrated increased activity of investigated enzymes, except for POD in poplar tissues. POD activity in poplar was decreased along with the increasing concentration of As, despite inoculation with D14 (Wang et al. 2011b). In experiments on rice (*O. sativa*), inoculation of plants with As-resistant AsSP9 strain increased amylase and protease activity (from 0.001 mg/g d.w./min up to 0.002 for

amylase and 0.002 to 0.0025 mg/g d.w./min for protease, respectively) (Pandey et al. 2013). In lower concentration of As, i.e. 150 mg kg^{-1} without D14 inoculation, SOD activity was increased in response to stress factor, but the higher the concentration of As in the environment, the higher the enzyme activity inhibition observed. However, inoculation with bacteria diminished the effect of As toxicity and facilitates SOD activity even under high concentration of As, i.e. 300 mg kg^{-1}, whereas without bacteria under such condition, SOD was strongly inhibited (Wang et al. 2011b). What is important, the increased activity of enzymes was correlated with the increased percentage of germination and relative root elongation in presence of As. Moreover, biomass amount, i.e. dry mass weight and plant height in presence of endosymbiotic bacteria, was significantly increasing and was comparable with respective parameters measured in control plants grown without As. The most putative explanation of this mechanism is that the effective availability of the As is decreased by bacterial immobilization or exclusion which in a consequence reduces the inhibitory effect of this stress factor. What is more, this mechanism was observed only in the case of bacterial strains which are plant symbionts and As resistant, results obtained from experiments on non-symbiotic As-resistant species or strains did not demonstrate beneficial effect to investigated parameters of treated plants (Pandey et al. 2013).

Production of IAA by bacteria, causes auxin stimulated root cell division, which is an additional factor promoting the growth of a plant (Lehmann et al. 2010). It was reported that isolates of *P. multifida* and *P. vittata* are capable of synthesizing up to 36.5 mg L^{-1} and 18.5 mg L^{-1} of IAA, respectively (Zhu et al. 2014). Soil symbiont of chickpea (*Cicer arietinum* L.), *Acinetobacter* sp. nbri05 strain, produced IAA on level up to 60.93 μg mg^{-1} of fresh cell weight. These results allow concluding that additional content in a toxic environment of a compound stimulating root elongation facilitates plant development and on the other hand can therefore enhance and accelerate As uptake (Srivastava and Singh 2014), which may be useful in terms of application in phytoremediation.

The level of lipid peroxidation, which indicates stress factor exposure, is usually estimated by malondialdehyde (MDA) concentration which is the product of peroxidized polyunsaturated fatty acids of the lipid membrane (Kong et al. 2016). MDA concentration was measured in experiments performed on chickpea, pea, pigeon pea, poplar and rice treated with different concentrations of As(III) and As(V) and inoculated with microbial symbiotic organisms, bacteria and fungi as well (Wang et al. 2011b; Pandey et al. 2013; Srivastava and Singh 2014; Garg et al. 2015). The decrease of measured MDA content was observed in all cases if As-treated plants were inoculated. Each concentration of MDA determined in above-ground parts of plant, i.e. shoot for chickpea, pea and pigeon pea or leaves for poplar as well as in roots of rice, chickpea, pea, pigeon pea and poplar (details: Table 9.1), independently on concentration or state of oxidation of As provided in particular study, was significantly decreased (Wang et al. 2011b; Pandey et al. 2013; Srivastava and Singh 2014; Garg et al. 2015).

Table 9.1 Microorganism-host plant interaction characteristics and growth promoting-like activity review

Source	Host	Activity/property of given As-resistant endosymbiont in terms of potential growth promotion				
		Mineral solubilization	Siderophores	As acquisition	Inoculate effect on biomass	Host pigments content
		Species: *Pseudomonas* sp. (strains PG4, 5, 6, 9, 10, 12, 16), *Comamonas* sp. and				
Pteris vittata (Ghosh et al. 2011;Ghosh et al. 2015)	tomato seedlings	increase bioavailability of Fe^{3+} and As^{5+} from $FeAsO_4$	+, catecholate type (*Pseudomonas*)	As(V) uptake, reduction to As(III), translocation to rhizome and fronds	increase comparing to uninoculated under As stress 1.7 times higher due to P solubilization (PG6 strain) and 44% higher shoot biomass	no data
		Types: *Firmicutes*, *Actinobacteria*, *Proteobacteria*				
P. vittata, *P. multifida* (Zhu et al. 2014)	*P. vittata*, *P. multifida*	no P solubilization	+, synthesized by five strains isolated from *P. multifida*, from three up to five units determined as [(Ar −A As)/Ar] * 100	As(V) uptake, reduction to As(III) by arsenate reductase/As(III) oxidation to As(V) by arsenite oxidase (PV, dependent on concentration of As(III) in environment and correlated with As tolerance; PM, not dependent, not correlated); retain of As(III) in endophytes: PV the higher the outer concentration lower; PM endophytes, the lower the outer conc. higher	no data	no data

Soluble sugars	Enzymes of oxidative stress activity			IAA production	ACC deaminase production	MDA production	other
	SOD	CAT	POD				
Stenotrophomonas sp.							
no data	no data	no data	no data	no data	no data	no data	
no data	no data	no data	no data	+, 0.3–18.5 mg L^{-1} (*P. vittata* isolates) 0.36–36.5 mg L^{-1} (*P. multifida* isolates), higher in *P. multifida*	no data	no data	

(continued)

Table 9.1 (continued)

Source	Host	Activity/property of given As-resistant endosymbiont in terms of potential growth promotion				
		Mineral solubilization	Siderophores	As acquisition	Inoculate effect on biomass	Host pigments content
		Species: *Agrobacterium radiobacter* D14 strain				
P. vittata (Wang et al. 2011b)	*Populus deltoides*			As translocation from roots to shoots, translocation ratio [(stems +leaves)/ roots) 0.8, higher than the uninoculated treatments (0.5). 45% As translocated from roots to the above-ground tissues (19.2% more than uninoculated plants)	increase of roots, stems, leaves inoculated comparing to uninoculated, 150 mg g^{-1} treatment, stems weight comparable with untreated with As; 150 mg g^{-1} and 300 mg g^{-1} treatment, leaves biomass about 26% increase comparing with untreated with As	increase of content in plants inoculated (leaves) comparing to uninoculated (under As 150 mg g^{-1} and 300 mg g^{-1} treatment); inoculation of plants untreated with As does not affect the chlorophyll content
		Species: *Bacillus* sp. AsSP9 strain, *Kocuria flava* AB402 strain, *Bacillus vietnamensis*				
slag disposal site (*Bacillus* sp. AsSP9 strain) *Ceriops decandra* mangrove tree (*Kocuria flava* AB402, *Bacillus vietnamensis* AB403) (Pandey et al. 2013; Mallick et al. 2018)	*Oryza sativa*	no data	+ (*Bacillus* sp. AsSP9 strain)	As retain in biofilm by extracellular polymeric substance (EPS) (*K. flava* AB402, *B. vietnamensis* AB403 strain)	increase 3 times comparing to uninoculated plants and reached about 4/5 mass controls without As and inoculate (*Bacillus* sp. AsSP9 strain); increase of root and shoot length with *K. flava* AB402, *B. vietnamensis* AB403 strain, AB402 effect stronger than AB403 effect	no data (*Bacillus* sp. AsSP9 strain), increase with *K. flava* AB402, *B. vietnamensis* AB403 strain, AB402 effect stronger than AB403, chlorophyll content with AB403 comparable to control (uninoculated, untreated with As)

Soluble sugars	Enzymes of oxidative stress activity			IAA production	ACC deaminase production	MDA production	other
	SOD	CAT	POD				
increase of content in plants inoculated (leaves) comparing to uninoculated (under As 150 mg g^{-1} and 300 mg g^{-1} treatment)	increase after inoculation: up to 31%, roots; up to 51%, leaves	increase after inoculation: up to 98%, roots; 1.42 times, leaves)	decrease: up to 31%, roots; up to 50%, leaves	no data	no data	decrease comparing to uninoculated plants, roots, up to 16%; leaves, up to 30%	soluble proteins: decrease along with increasing As concentration; increase after inoculation comparing to uninoculated plants treated and untreated with AAs, more efficient in leaves comparing to roots
AB403 strain							
no data	no data	+	no data	no data	+(*Bacillus* sp. AsSP9 strain)	decreased up to about 33% (*Bacillus* sp. AsSP9 strain)	AsSP9: increased amylase (from 0.001 up to 0.002m/g d.w./mmin.) and protease (0.002 to 0.0025 m/g d.w./min)

(continued)

Table 9.1 (continued)

Source	Host	Activity/property of given As-resistant endosymbiont in terms of potential growth promotion				
		Mineral solubilization	Siderophores	As acquisition	Inoculate effect on biomass	Host pigments content
		Acinetobacter lwoffii				
soil (Das and Sarkar 2018)	*Vigna radiata*	+, negatively correlated with increasing As concentration	+	arsenic content in the plant differentiation in time, in the first 15 days of As treatment, the highest concentration of As observed in roots; after the next 15 days, arsenic in high concentration in leaves	growth of the plant is inhibited by arsenic presence comparing to control; in bacteria- and arsenic-treated plants, growth is not significantly different from control	decrease of chlorophyll and carotenoid concentration in As-treated plants comparing to control; plants As-treated and inoculated – about two times higher concentration of chlorophyll comparing to control and about 25% higher content of carotenoids than control
	Genus: *Acinetobacter* sp					
soil (Srivastava and Singh 2014)	*Cicer arietinum* chickpea	phosphate solubilization up to 213.88 μg mL^{-1}	+	As uptake in inoculated plants decreased: up to 66% (shoots), 44% (fruit cover), 64% (fruit)	4.3 times increase of inoculated As-treated than uninoculated As-treated plants, shoot length 31%, root length 18% higher comparing to control untreated with inoculum and As	chlorophyll, 20% increase (1.19 mg g^{-1} to 1.43 mg $g^{-1)}$ comparing to uninoculated As-treated plants, comparable to control; carotenoids, 9% decrease comparing to uninoculated As-treated plants

Soluble sugars	Enzymes of oxidative stress activity			IAA production	ACC deaminase production	MDA production	other
	SOD	CAT	POD				
no data	no data	no data	no data	+, increase with concentration of As from 204 µg/ L^{-1} (control) to 325 µg/L (100 mg of As L^{-1}), but at 1000 mg of As L^{-1} decreased to 32 µg L^{-1}	no data	inoculation effected with 13–20% decreased MDA concentration comparing to uninoculated As-treated plants; MDA concentrations in control plants 36%–40% lower than in uninoculated As-treated plants	formation of biofilm observed
+, lactose, sucrose	no data	+	no data	+, 60.93 µ g mg^{-1} (fresh weight)	5.77µ M αKB mg^{-1} h^{-1}	about 70% content decrease in shots and 52% decrease in roots of As-treated and inoculated plants comparing to control without As and inoculate	

(continued)

Table 9.1 (continued)

Source	Host	Activity/property of given As-resistant endosymbiont in terms of potential growth promotion				
		Mineral solubilization	Siderophores	As acquisition	Inoculate effect on biomass	Host pigments content
		Cryptococcus sp., *Rhodotorula* sp., *Exophiala* sp., *Trichosporon* sp., *Cystobasidium* sp.				
Tithonia diversifolia, *Flaveria angustifolia*, *Sphaeralcea angustifolia*, *Prosopis* sp., *Bahia absinthifolia*, *Sphaeralcea* (Ramos-Garza et al. 2015)	*Brassica juncea*	-	+, despite iron abundance synthesized by 8 amongst 31 isolated strains from 1 up to 2.53 units determined as [(Ar − As)/Ar] * 100	arsenate reduction by 51.6% of isolated rhizospheric strains, reduction from 10% up to 40% of As(V) 0.15 mmol L^{-1} present in solution; none capable to oxidize arsenite; no data about influence on uptake by plant	increase of seed germination percentage by *Rhodotorula* sp. and *Cystobasidium* sp. up to 96.6% (all attempts demonstrated >70% germination; conditions without As; increase of seedlings height more than two times comparing to control; conditions without As)	no data

ACC 1-aminocyclopropane-1-carboxylate, *CAT* catalase, *IAA* indole-3-acetic acid, *MDA*
"+", presence of compound observed; "−", no product observed

Soluble sugars	Enzymes of oxidative stress activity			IAA production	ACC deaminase production	MDA production	other
	SOD	CAT	POD				
no data	no data	no data	no data	+, three strains: *Cystobasidium* sp. representative (6.8 μg mL^{-1}), two *Rhodotorula mucilaginosa* strains (9.02 and 9.61 μg mL^{-1}), both capable to produce siderophores)	no data	no data	

malondialdehyde, *POD* peroxidase, *SOD* superoxide dismutase

9.5 Concluding Remarks

Review of recent reports on microorganism-plant interactions showed that the cooperation between microbes and plants particularly under stress condition is beneficial or even indispensable. These interrelationships are intensively explored last years, starting from screening and discovering organisms involved in different interactions, via isolation of microbes, and their characteristics and analyses of their effect on plant physiology, ending with molecular signalling pathways underlying the interrelationship in terms of its beneficial role for both involved sides, however focusing on the plant. Research in this relatively novel topic joins interests of microbiology and plant biochemistry and physiology.

In all discussed cases, experiments comparing physiological and biochemical properties and/or activity of microbial inoculates in treated and in untreated plants exposed to As stress demonstrated, that the presence of As-resistant microorganisms brings irrefutable benefits to colonized plant (Table 9.1). Investigation of such parameters as total biomass of leaves, shoots and roots, as well as fronds or rhizomes (*Pteris* genus) of plant organism interacting with primarily bacteria but also fungi including yeasts under As exposure demonstrated that beyond plant or symbiotic microorganism genus/species, each interrelationship results in increase of physiological potential of studied plant to diminish efficiently deleterious As effect, survive and grow, comparing to plants uninoculated with microorganisms. In several cases studied plants even reached the extent of measured parameters comparable to controls without As and microorganisms.

It was proved that endosymbionts and beneficial rhizosphere bacteria support nutrient uptake by plant releasing such elements as P or Fe from insoluble minerals, thus enabling their sequestration (usually facilitated by siderophores) and transport into root tissue. Another benefit for the plant is that activity of microorganisms efficiently involved in oxidation/reduction of As(III)/As(V) reactions functionally decreases the concentration of As available for plant or contributes its efflux from tissues.

Biochemical analyses of enzyme activity increasing physiologically under stress conditions, such as SOD, CAT and POD, demonstrated that the activity of the enzymes protecting cells from oxidative stress, except for POD activity, increases with the concentration of As(III) or As(V) plant exposure; however when the concentration of As exceeds critical value (depending on species and other factors), inhibition of the activity starts. Colonization of rhizosphere and plant tissues with rhizospheric and endosymbiotic bacteria expands the range of the concentration and decreases the value of the concentration-deactivating enzyme. On the other hand, microorganisms efficiently synthesize compounds such as IAA or ACC deaminase, which support root cell division and nitrogen uptake by the plant, thus directly contributing to plant development. The biodiversity of microorganisms, amongst which there are representatives of bacteria and fungi including yeasts (Ramos-Garza et al. 2015), creating unique and specific for their host microbiomes indicate, that they are responsible for a variety of functions supporting plant organism like another higher organism.

Reviewed results reflect previous knowledge about the role of rhizospheric and endosymbiotic microbes of plants, especially in terms of As uptake, transformation and translocation within tissues and beneficial effect on plant physiology under stress conditions, such as As contamination, therefore changing the significance of those microorganisms towards plant organism, including human interests in terms of potential bio-phytoremediation application.

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Chapter 10
Interaction of Plants and Arbuscular Mycorrhizal Fungi in Responses to Arsenic Stress: A Collaborative Tale Useful to Manage Contaminated Soils

Federico N. Spagnoletti, Raúl S. Lavado, and Romina Giacometti

Abstract Arsenic (As) is a nonessential element, and its uptake and accumulation in plants can produce several negative effects including disturbance in metabolism and physiological disorders or, in extreme cases, cause plant death. However, some microorganisms have the capacity to tolerate those unfavorable effects and to improve plant development under As-enriched environments. Among them, arbuscular mycorrhizal fungi (AMF) are able to alleviate the harmful effects of the metalloid. AMF have been found to occur in contaminated environments, possibly due to several physiological and biochemical mechanisms that fungi display to tolerate As presence. Mycorrhizal plants show more tolerance to As toxicity since (i) AMF inoculation increases plant biomass and promotes a dilution effect in the As concentration in plant; (ii) sequester As in intraradical hyphae, and reducing the metal intake by roots; (iii) mycorrhizal symbiosis immobilizes As, reducing its translocation to different plant tissues; (iv) AMF can reduce arsenic absorption by repressing the arsenate/phosphate transporters; (v) AMF promote the biotransformation of As and (vi) can protect its plant host reducing oxidative damage. This chapter summarizes current knowledge about the effect of As contamination on plants and the role of arbuscular mycorrhizal symbiosis and its contribution to the phytoremediation of polluted soil.

Keywords Arsenic · Arbuscular mycorrhizal fungi · Tolerance mechanisms

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10.1 Introduction

Arsenic (As) is an extremely poisonous element found in environment that has the capacity to be a severe risk to plants and animals, as it is the result of natural activities (rock weathering, volcanic action) and human activities (pesticides, fertilizers, herbicides, mining). Arsenic is found in all rocks of the earth crust, and it is a component of groundwater in several places around the world (Smedley and Kinniburgh 2002). The incidence of high-As groundwater has been detected in China, Hungary, Bangladesh, India, Italy, the United States, Mexico, Chile, Argentina, and other countries (Pigna et al. 2009). Arsenic is a nonessential element; arsenate (As^{V}) and arsenite (As^{III}) are the main forms of As for absorption by plants (Zhao et al. 2009), causing considerable stress that leads to a wide range of toxicity symptoms going from inhibition of seed germination to plant death (Stoeva et al. 2005). Like so, reductions in the chlorophyll content and photosynthesis rate were observed in plants growing in As-enriched environments. These negative effects play a detrimental role in plant morphological parameters, like root and aerial biomass growth, plant height and stem ramifications. Thus, As can decrease plant yields due to reduction of growth, chlorosis, cell necrosis, and even death (Rosas-Castor et al. 2014). Moreover, As can affect food safety negatively due to food chain pollution (Pigna et al. 2009). On the other hand, plants have antioxidant machinery to counter the harmful effect of As. Several enzymatic and nonenzymatic antioxidants act to reduce the oxidative damage produced by As exposure (Farooq et al. 2016; Spagnoletti et al. 2016).

Soils hold several microorganisms; one of the most important groups of fungi is the arbuscular mycorrhizal fungi (AMF) which improve the ability of plants to tolerate a wide range of stresses (Smith and Read 2008). Arbuscular mycorrhizal fungi can enhance plant nutrition, increase efficiency in water uptake, and reduce damages produced by heavy metal contamination (Smith and Read 2008). Furthermore, it was observed that the presence of mycorrhiza in soils improves plant tolerance to As in different environments (Xia et al. 2007; Spagnoletti and Lavado 2015; Spagnoletti et al. 2016).

Nevertheless, there are voids in the understanding of As plant tolerance mechanisms and about the contribution of mycorrhizal fungi in As stress reduction. Hence, this chapter covers current knowledge on As uptake and its detrimental effects in plants, as well as the impact of As stress on abundance and development of AMF.

10.2 Arsenic in Plants

10.2.1 Phytotoxicity of Different Forms of Arsenic

Plants can be differently affected by the concentration of As. For instance, in soybean (*Glycine max* L.) yields are compromised when soil concentration exceeds 35 mg As kg^{-1} (Bustingorri et al. 2015), whereas in rice (*Oryza sativa* L.) soil

concentration of 25 mg As kg^{-1} limits its yield (Das et al. 2004). On the other hand, potatoes (*Solanum tuberosum* L.) did not reduce its yield in soils with 300 mg As kg^{-1} (Codling et al. 2016). Higher reduction in yield by As-enriched soils has been found in rice: 20-100 mg As kg^{-1} in aerial and 1000 mg kg^{-1} in roots biomass (Adriano 2001). Thus, the plant response to As is different among plant species (Chakrabarty et al. 2009; Choudhury et al. 2011). Also it is important to note that in some plants, inorganic forms of As are less toxic than organic, methylated As forms, such as monomethyl As acid (MMA) and dimethylarsinic acid (DMA), are more damaging to DNA than inorganic As forms (Wang and Mulligan 2006). In a similar way, the toxicity of inorganic forms of As may be different between plants. For instance, it has been found that arsenite is more toxic than arsenate to wheat, rice, and lettuce but not in maize (Abbas and Meharg 2008). Hence, to achieve a complete understanding of arsenic toxicity in plants, both organic and inorganic forms should be tested in several plant species.

10.2.2 Arsenic Uptake and Accumulation

In general, arsenate (As^{V}), arsenite (As^{III}), MMA, and DMA are the most ordinary species of As available for plant uptake (Finnegan and Chen 2012). The most common As entryway by plants mainly is through the roots; however, the distribution of As is extremely variable between plant organs. Arsenic is mainly accumulated in roots followed by leaves, shoots, pods, and grains (Lee and Yu 2012). Several researchers have studied the toxicity and accumulation of As in plants analyzing the negative effects on plants biomass (Zhang et al. 2009; Rosas-Castor et al. 2014; Bustingorri et al., 2015; Spagnoletti and Lavado 2015; Spagnoletti et al. 2016). Arsenic is not easily translocated to aerial biomass, though; some plants species accumulate high concentrations (from 5 to 40 mg kg^{-1}) of As (Gulz et al. 2005). Although different As forms exist simultaneously in the soil environment, plants uptake As from the soil with certain degree of preference. Generally, the order is arsenite, arsenate, DMA, and MMA (Finnegan and Chen 2012).

Because Arsenate (As^{V}) has a similar chemical structure to phosphate, it enters to plant root via phosphate transporters (Fig. 10.1). Cotransport of phosphate or arsenate and protons is the involved uptake mechanism in plants (Ullrich-Eberius et al. 1989). This process has been recently studied in rice (Ye et al. 2015). Arsenate has been shown to compete with phosphorus uptake under low phosphate conditions, making plants go under severe phosphorus deficiency symptoms (Catarecha et al. 2007). Phosphate transporters Pht1 and Pht4 have a significant role in As^{V} uptake in *A. thaliana* (Shin et al. 2004). The enzyme arsenate reductase is responsible for intracellular reduction of As from As^{V} to As^{III} (Bleeker et al. 2006), to be then combined with thiol groups and stored in vacuoles (Mukhopadhyay et al. 2000). Arsenite (As^{III}) can enter root cells via nodulin 26-like intrinsic proteins (Pommerrenig et al. 2015). Nodulin-like proteins are members of the aquaporin water channel superfamily (Pommerrenig et al. 2015) and move As from the soil to the roots. While

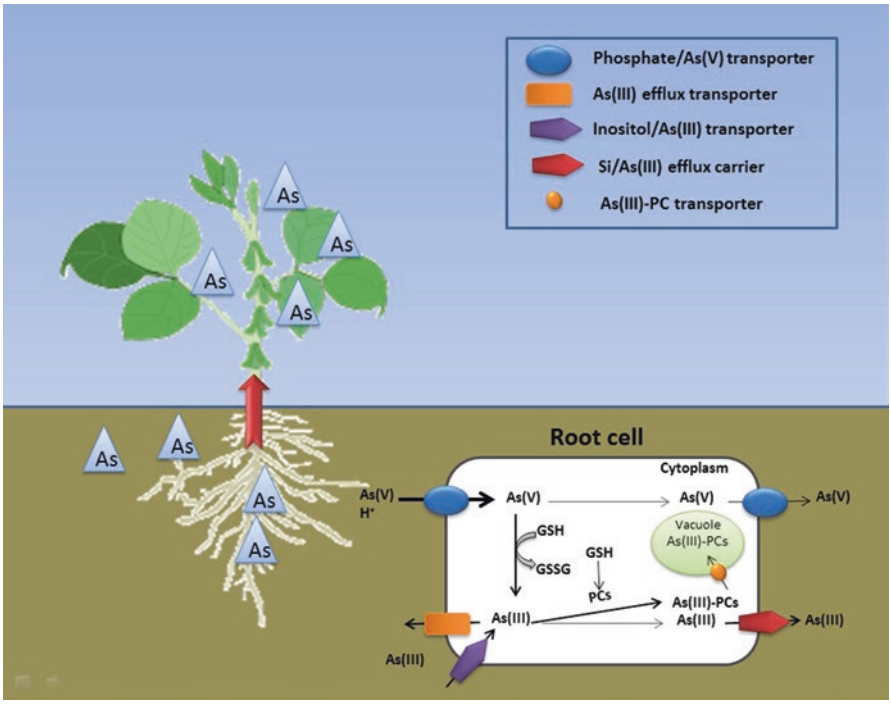

Fig. 10.1 Diagram of arsenic uptake, transport, and metabolism in a root cell. GSH, glutathione; GSSG, oxidized glutathione; PC, phytochelatin

several studies have shown that As^{V} shares its transport pathway with phosphate, As^{III} shares its pathway with silicon (Si) (Li et al. 2009). These authors found in rice that silicic acid transporters Os*Lsi1* and Os*Lsi2* are able to transport arsenite and methylated As species (MMA and DMA). Moreover, MMA and DMA enter to the roots through aquaporin channels (Pommerrenig et al. 2015), and the translocation within the plant is greater than As^{V} or As^{III} (Carey et al. 2011).

10.2.3 Arsenic Effects on Plant Physiology

10.2.3.1 Plant Growth

Presence of As above certain limits in irrigation water or in soil interferes with the physiology of plants. Plants exposed to high As concentrations show reduced germination, wilting, lower biomass, poor root elongation, necrosis, reduction in leaves number, and decrease of leaf area and therefore photosynthesis. Also, As negatively affects the rhizobium-legume symbiosis, stunted growth, and poor yield and may even cause death (Finnegan and Chen 2012). Arsenic has important negative effects

on legumes. An As-induced reduction was observed in the expression of *Medicago sativa* Nod factor receptor (nork), nodule organogenesis (Enod2), markers for infection progression (N6), and the transcription factor (NIN) genes, affecting nodules number (Lafuente et al. 2010). Also, Talano et al. (2013) found that soybean germination and development and even the amount of active nodules were reduced by inorganic forms of As. These authors suggest that As could negatively affect the motility of *Rhizobium* and therefore, lead to a lower nodulation (Talano et al. 2013). On the other hand, other authors found that As effect on growth inhibition and nodule formation of *Medicago truncatula* only appeared in As^{III} subjected roots proving localized toxicity and nonsystemic impact of As on nodulation (Lafuente et al. 2015).

In rice (*Oryza sativa*), organic forms of As induce straighthead disease, leading to reduce the productivity of some rice varieties (Rahman et al. 2008). This disease causes floret sterility and reduces yields. Moreover, sensitivity to metalloids in crops differs significantly between species or even genotypes. For instance, different species of wheat presented different rates of sensitivity to As (Zhu et al. 2006; Pigna et al. 2009).

10.2.3.2 Arsenic Effect on Photosynthesis

Once inside roots, As causes harmful effects on photosynthesis (Stoeva et al. 2004; Gusman et al. 2013). It is known that As can interfere with chlorophyll biosynthesis due to the stimulation of iron insufficiency or by suppressing enzymes involved with chlorophyll synthesis (Gusman et al. 2013). In addition, As affects the heat dissipation and photochemical efficiency, generating variations in gas interchange (Rahman et al. 2007; Gusman et al. 2013). The degree of injury caused by As on CO_2 assimilation is not yet fully determined; nevertheless, it is possible that the metalloid reduces CO_2 fixation or downregulates the gene expression of Rubisco. On the other hand, since As^{V} is an analogue of phosphate, it replaces phosphorus from ATP to form ADP-As, affecting energy generation (Meharg and Hartley-Whitaker 2002). Finally, as described previously by Stoeva et al. (2005), the alterations caused by As exposure result in the manifestation of toxicity symptoms in growth and color of leaves.

10.2.3.3 Cellular Membrane Integrity and Nutrient Uptake

Arsenic causes high damages in cell membranes, therefore interfering in nutrient and water absorption and reducing stomatal conductance. Also, Garg and Singla (2011) suggested that the detrimental effect of As in water uptake is the most important cause of affected transpiration process. Moreover, a work in beans showed that As exposure in plants caused a reduction in transpiration rate and leaf water potential (Stoeva et al. 2004). Thus, as a consequence, As helped plants to survive under severe water stress (Spagnoletti et al., 2015).

10.2.4 Biochemical and Molecular Changes

Arsenic is one metalloid that does not play as a nutrient, but rather as a toxic element that presents harmful effects on many plants (Stoeva et al. 2005; Farooq et al. 2015). As stated previously, the toxicity of As forms vary from species to species. Arsenate is often uptake through the roots using phosphate transporters, while arsenite reacts with sulfhydryl groups (Summers 2009). The metalloid disturbs biological system via two ways. The first way involves the direct inactivation of important enzymes, while the second way is indirect, due to reactive oxygen species (ROS) generation like hydrogen peroxide, hydroxyl radical, and superoxide radical, among others, that result in irreversible damage in plants (Finnegan and Chen 2012). Under stressful conditions, like the presence of As in the environment, the natural balance from plant antioxidant defenses and reactive oxygen species is lost. Scandalios (2002) exposed that a decrease in antioxidants and/or increase in ROS production can be the reason to cause the loss in the mentioned balance. The exposure to As causes high increments in ROS, leading to lipid peroxidation (Bustingorri et al. 2015; Farooq et al. 2016; Spagnoletti et al. 2016). High amount of ROS is negative for plants, though in small concentrations are known to act as signaling molecules (Keshavkant et al. 2012). The conversion from As^{V} to As^{III} produces ROS increments (Talukdar 2013), so this change between inorganic forms of As is the reason for toxic effects in plants, followed by methylation process (Singh et al. 2007). Methylation of As produces different As-species which react with O_2 to form ROS. Moreover, As is able to inhibit protection mechanisms, increasing ROS production (Sharma 2012).

The high levels of ROS produced by the presence of As in soils lead to lipid peroxidation, causing cytotoxicity and affecting the metabolism of plants cells (Farooq et al. 2016). Several authors have shown accumulation of malondialdehyde (MDA) in plants growing in As-enriched soils and determined that the cause was an increment in ROS levels (Singh et al. 2007; Choudhury et al. 2011; Spagnoletti et al. 2016). To fight against oxidative stress detrimental effects, activation of antioxidant molecules (enzymatic and nonenzymatic) have also been described in plants under As exposure. Among enzymatic antioxidants, superoxide dismutase (SOD) is a group of metallo-isozymes that act against ROS. This antioxidant enzyme is associated with metal cofactors. The Cu/Zn-SOD is situated in the cytoplasmic matrix and in peroxisomes, while Fe-SOD is situated in the plastids, and Mn-SOD is located in the mitochondria (Sharma 2012). Increments of SOD activity and decrease in lipid peroxidation have been demonstrated in several plants exposed to As, such as *Holcus lanatus* (Hartley-Whitaker et al. 2001), bean (Singh et al. 2007), rice (Singh et al. 2015), and soybean (Spagnoletti et al. 2016).

Catalase (CAT) scavenges H_2O_2 produced in peroxisomes and break down H_2O_2 into water and O_2 (Karuppanapandian et al. 2011). Higher CAT activity has been found in corn (Mylona et al. 1998), Chinese brake fern (*Pteris vittata*), Boston fern (*Nephrolepis exaltata*) (Shrivastava et al. 2005), rice (Singh et al. 2015) and soybean (Bustingorri et al. 2015; Spagnoletti et al. 2016) during As exposure. However, plants show high disparity in their antioxidant responses to As. For instance, As-induced reduction in CAT activity was found in *Taxithelium nepalense* and bean (Singh et al. 2007).

Peroxidase (POX) participates in plant defense and is responsible for catalyzing lignin biosynthesis and organogenesis via synthesis of ethylene or degradation of auxin phytohormone (Emamverdian et al. 2015). Similarly, ascorbate peroxidase (APX) plays a crucial role in the maintenance of ROS levels by reducing hydrogen peroxide into H_2O (Emamverdian et al. 2015). Like other enzymes, increases in POX activity have been described in corn (Miteva and Peycheva 1999), rice (Shri et al. 2009) pigeonpea and pea (Garg et al. 2015), and soybean subjected to As contamination (Bustingorri et al. 2015; Spagnoletti et al. 2016).

The enzyme glutathione reductase (GR) is responsible to turn oxidized glutathione (GSSG) to reduced glutathione (GSH). Therefore, this enzyme maintains high levels of reduced glutathione (Trivedi et al. 2013). Fewer studies are available regarding the performance of GR in plants growing in As-enriched soils, but Shri et al. (2009) and Goupil et al. (2009) found improvement in the GR activity in rice and tomato (*S. lycopersicum*).

Glutathione (GSH) participates in the As detoxification and maintenance of the redox status of the cell. Moreover, other detox mechanisms imply phytochelatins (PC) and ascorbate-glutathione cycle, which are connected with GSH. GSH is able to combat ROS due to GSH-*S*-transferase, an enzyme detected in rice (Shri et al. 2009) and soybean (Spagnoletti et al., 2016) upon As-exposure. Furthermore, As can establish complexes with glutathione to then be transported inside root cell vacuoles (Lafuente et al. 2015).

The other nonenzymatic antioxidants are carotenoids which are responsible for protecting photosynthetic apparatus by scavenging toxic free radicals (Sharma 2012). Stoeva et al. (2005) found a decrease in the content of these nonenzymatic antioxidants in *Phaseolus vulgaris* subjected to As. This carotenoid reduction could be attributed to the negative effects of As on the thylakoid membranes.

Ascorbate is an antioxidant present in apoplast, chloroplasts, cytoplasmic matrix, and vacuoles (Sharma 2012), which plays a significant function in the generation of carotenoids and α-tocopherol and elimination of hydrogen peroxide (Karuppanapandian et al. 2011). Increases in ascorbate were detected in fronds like *Pteris vittata* (Singh et al. 2006), but results were less consistent in *Trigonella foenum-graecum* L. and cucumber, finding ascorbate increases or decreases in various plant organs of the plants (Czech et al. 2008; Talukdar 2013).

10.3 Arsenic in AMF

Stresses like As contamination result in the deterioration of plant growth and reduce plant yields. However, some beneficial fungi are able to counteract the unfavorable effects and to improve plant development under contaminated soils (Smith and Read 2008). Arbuscular mycorrhizal fungi (AMF) form mutualistic symbioses with the roots of over 80% of plants (Smith and Read 2008). AMF confer beneficial effects to plants, enhancing their capability to tolerate stresses (Smith and Read 2008). Several mechanisms participate in the tolerance of mycorrhizal plants against abiotic stress, such as As contamination, and have been reviewed in depth by some

authors (Lenoir et al. 2016; Miransari 2017). However, scarce information is available about the mechanisms employed by mycorrhizal fungi to cope with the harmful effects promoted by the presence of As in soils.

10.3.1 Effects of Arsenic on AMF Abundance and Biodiversity

In spite of the extensive ubiquity of AMF in numerous environments, few AMF species were studied (Öpik et al. 2013). Only three different taxonomic families (Acaulosporaceae, Gigasporaceae, and Glomeraceae) have been researched in deep. Several authors have shown that the biodiversity of mycorrhizal fungi differs significantly and their presence in different soils is the result of numerous factors such as environmental conditions, agricultural practices, soil type, and host plant (Miransari 2017). The mycorrhizal diversity in noncontaminated soils is normally high (Zangaro et al. 2013) but is usually minor in stressed environments, with a prevalence of Glomeraceae (Lenoir et al. 2016). The family of Glomeraceae has developed features that are beneficial in stressed-environments and has shown an R ecological strategy, since this family spends energy mostly in the generation of a higher number of reproductive structures in a short lapse of time (Sýkorová et al. 2007).

Because arbuscular mycorrhiza symbiosis is the most prevalent in terrestrial ecosystems, AMF species must express physiological and genetic features which allow them to live in dissimilar environments (Miransari 2017). However, AMF are affected by environment (Miransari 2017). Arsenic, like other stressors, may influence in AMF diversity and abundance (Smith et al. 2010; Schneider et al. 2012). It is known that As presence can produce changes in mycorrhizal population, causing an increase of As-tolerant species (Pouyu-Rojas et al. 2006). Gonzalez-Chavez et al. (2002) found that As-contaminated soils in England prevail species of Acaulosporaceae and Glomeraceae, while in As-contaminated soils of Brazil, Schneider et al. (2013) found that the most frequent AMF were the same. Although As-enrichment decreased the number of AMF species, the prevalence of abovementioned species indicates their tolerance to As contamination.

10.3.2 Effect of Arsenic Stress on AMF Development

Various studies have revealed that the essential phases of the AMF development cycle (spore generation and germination, root colonization, and extraradical hypha development) could be inhibited in As-contaminated soils. We have previously found a reduction and in some cases even inhibition of spore germination and hyphal length in As-enriched environments (Spagnoletti et al. 2014; Spagnoletti and Lavado 2015). These results suggest that As harms the first stage of the mycorrhizal development. The negative effects of As in mycorrhizal spores could reduce mycorrhizal colonization (Spagnoletti and Lavado 2015; Spagnoletti et al. 2016). This

could be attributed to the incapability of AMF spores to germinate and to contact a host root disturbing the colonization process (Spagnoletti and Lavado 2015).

Moreover, Shalaby (2003) found that spore germination and hyphal growth were inhibited by toxic elements when AMF strains were isolated from noncontaminated soils, while AMF reproductive structures collected from contaminated soils were tolerant to metals. This natural resistance may be due to a phenotypic plasticity of the spores (Shalaby 2003). Wu et al. (2009) showed that an isolate of *Funneliformis mosseae* from As-enriched soil germinated significantly more in the presence of As. Similarly, Gonzalez-Chavez et al. (2002) reported that *G. caledonium and F. mosseae* strains extracted from As-contaminated soil were As^{V} tolerant in comparison with other strains isolated from noncontaminated soil. These results suggest the existence of inter- and intraspecific variations in As tolerance for AMF.

10.3.3 Arbuscular Mycorrhiza Subjected to Arsenic: The Responses at Biochemical and Molecular Levels

Similarly to plants, the enrichment in toxic elements of the environment has been shown to break down the balance between oxidants and antioxidants in AMF. In *Rhizophagus irregularis*, an increment in the level of membranes damage was detected when spores were subjected to metals (González-Guerrero et al. 2010). Moreover, Calonne et al. (2010) found in *R. irregularis* higher POX activity in the presence of the fungicide propiconazole, proposing that this fungus improves the ROS scavenging systems. Likewise, increases in SOD activity were detected when AMF spores were subjected to high copper levels, suggesting the involvement of SOD in the reduction of ROS (González-Guerrero et al. 2010). Other antioxidants, like glutaredoxins and thioredoxins, were found in AMF. These antioxidant systems are responsible for acting as redox regulators of protein thiols and are involved in the maintenance of the cell redox balance (Lenoir et al. 2016). For instance, the gene *GiGRX1* found in *R. irregularis* is responsible for encoding a protein with antioxidant properties (Benabdellah et al. 2009a). On the other hand, AMF has other antioxidant mechanisms, like GSH and vitamins B6, C, and E (Benabdellah et al. 2009b). These defense systems act as antioxidants and play an important role in ROS homeostasis.

10.4 AMF and Arsenic Tolerance in Plants

Plants are exposed to diverse stresses, like As occurrence. Among microorganisms, AMF can enhance plant performance and development under different impaired environments (Smith and Read 2008). Potentially toxic elements are one of the most significant stresses affecting plant production (Miransari 2017). In most studies, symbiosis with AMF was found to improve As tolerance in host plants.

10.4.1 Ways of Arsenic Alleviation by AMF

10.4.1.1 Inhibition of the Phosphate Transport in Roots

Studies have shown that mycorrhizal colonization can inhibit the transport of phosphorus in roots by reducing As^{V} uptake (Gonzalez-Chavez et al. 2002). More information is available about the beneficial effect of AMF on the nutrition of their host plant. Fundamentally, the enhancement in plant nutrition takes place due to an increase in phosphate uptake from soil, since AMF through its hyphae can improve the nutrient absorption area (Smith and Read, 2008). Since AMF have a significant role in obtaining phosphorus for its hosts, this can be the cause of As^{V} tolerance as the AMF may as well enhance As^{V} uptake. Gonzalez-Chavez et al. (2002) showed that mycorrhizal fungi reduced As^{V} entry in *H. lanatus*. Similarly, Christophersen et al. (2009) found that AMF colonization decreased As^{V} uptake in *Hordeum vulgare* inhibiting the phosphate transports in roots that also uptake arsenate.

10.4.1.2 Effluxing of Arsenic to the External Medium

Arbuscular mycorrhizal fungi can enhance As tolerance in the host plants releasing As from roots to soil. Gonzalez-Chavez et al. (2011) identified in the AMF *G. intraradices* a gene with possible function of Arsenic efflux pump (*GiArsA*). Most recently, we suggested that the low As content in plants that were previously inoculated with a strain of *R. intraradices* could be due to metalloid detoxification mechanism in the AMF, as we found an induction in the expression of a high-affinity phosphate transporter *RiPT* and an As efflux pump *RiArsA* (Spagnoletti et al. 2016). Moreover, As may be absorbed by mycorrhizal hyphae through RiPT proteins. Then As^{V} is reduced to As^{III}, translocated to extra radical hyphae, and released to soil by *RiArsA* (Spagnoletti et al. 2016).

10.4.1.3 Improving Phosphorus Acquisition and Host Nutrition

Arbuscular mycorrhizal symbiosis improves phosphorus acquisition and thereby enhances the phosphorus status of the inoculated plant, improving plant development. This enhancement in the nutritional status causes a dilution effect of As concentrations in the plant organs. These effects were found in *M. sativa* (Chen et al. 2007), corn (Xia et al. 2007), *M. truncatula* (Xu et al. 2008), and soybean (Spagnoletti and Lavado 2015; Spagnoletti et al. 2017). Consequently, mycorrhizal colonization increases the shoot and root P/As content ratio (Chen et al. 2007; Zhao et al. 2009). Therefore, the lower As concentrations found in AMF inoculated plants could be the result of the dilution effect produced by larger biomass (Spagnoletti and Lavado 2015).

10.4.1.4 Transformation of Arsenate into Other Arsenics Forms

Some information is available about soil microorganisms that may methylate As species (Lomax et al. 2012). In this way, AMF releases organic substrates in the rhizosphere soil stimulating organisms that contribute in methylation of As (Mukhopadhyay et al. 2002). For instance, Ultra et al. (2007) detected methylated As forms in soil of *Helianthus annuus* inoculated with *G. aggregatum*, and Li et al. (2016) found the higher amount of DMA in *Oryza sativa* inoculated with *R. intraradices*.

10.4.1.5 Glomalin Production

Arbuscular mycorrhizal fungi can sequester As by biosorption through glomalin-related soil protein (GRSP), produced by AMF. GRSP can immobilize soil heavy metals/metalloids such as Cd, Cu Zn, and Pb (Vodnik et al., 2008). We found higher GRSP content as As concentration increased in the soil (Fig. 10.2; Spagnoletti et al. 2017). These data are in line with Zhou et al. (2009) who found similar results in Zn-contaminated soils. On the other hand, GRSP has high content of iron; thus the possibility of GRSP participation in detoxification by generating As^{III}-Fe^{III} cannot be excluded.

10.4.1.6 Induction of Antioxidant Defenses

Several studies have shown that AMF inoculation reduces H_2O_2 levels and prevents membrane peroxidation (Garg and Singla 2012; Spagnoletti et al. 2016). AMF inoculation may reduce membrane damage reducing ROS production (see Fig. 10.2). Moreover, mycorrhizal colonization activates the antioxidant mechanisms in the plant, increasing SOD, CAT, APX, and guaiacol peroxidase (GPX) activities. Chen et al. (2015) showed that inoculation with the AMF *F. mosseae* improved antioxidant activities (CAT and SOD) in *Populus euphratica* grown in Pb-contaminated soils. Also, Rozpadek et al. (2014) found that inoculation with *R. irregularis* enhanced SOD and CAT activities in *Cichorium intybus* growing in Cd-, Zn-, and Pd-enriched environments. Recently, we showed that SOD, CAT, and GPX activities increased in soybean AMF-inoculated plants growing in As-contaminated soil (Spagnoletti et al. 2016). Conversely, Yu et al. (2009) suggested that ROS generation is reduced in mycorrhizal plants, and thereby, less antioxidant molecules are synthetized.

Glutathione (GSH) is another important antioxidant that was found to increase in the mycorrhizal plants (Bona et al. 2011). For instance, increases in GSH levels under As stress have been reported for *R. intraradices* inoculated soybean plants (Bustingorri et al. 2015; Spagnoletti et al. 2016). Likewise, in AMF inoculated plants, an increase in expression of the gene coding for GR has been observed (Fuentes et al. 2016). These results suggest a recycle of glutathione forms (oxidized

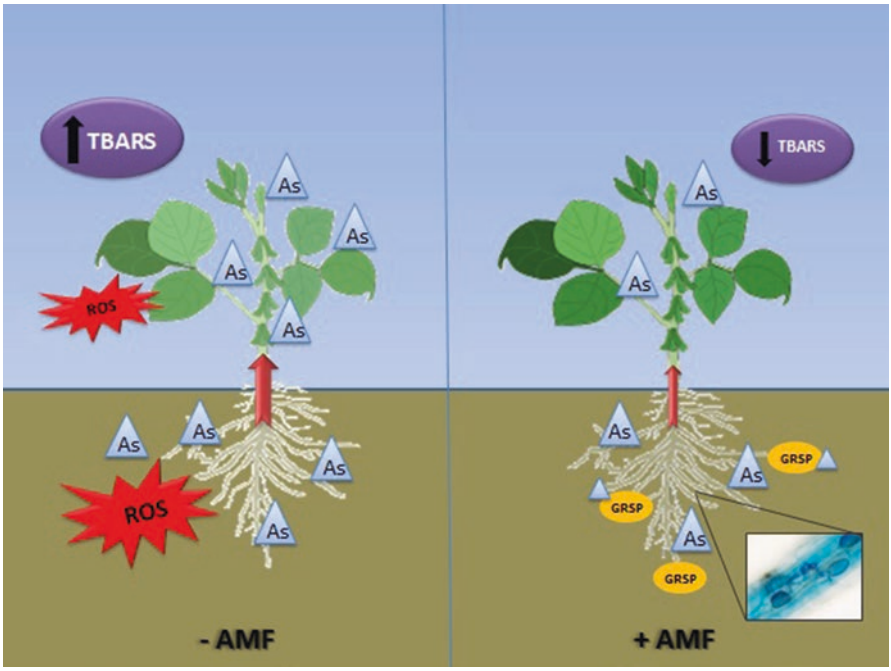

Fig. 10.2 Schemes highlighting major mechanisms underlying arbuscular mycorrhiza fungi (AMF)-mediated plant-tolerance to arsenic stress

or reduced). Furthermore, AMF could generate high quantities of GSH on its own (Schützendübel and Polle 2002), to then translocate this GSH to the plant. Moreover, mycorrhizal colonization could influence the expression of sulfate transporters, increasing glutathione production (Giovannetti et al. 2014).

10.5 Conclusions

Arsenic negatively contributes to the growth and development of AMF and their hosts. However, considerable evidence has been accumulated not only on plants and more recently on AMF survival but also on plant improvement due to AMF effects, in As-contaminated soils. Biological approaches, such as the use of AMF, are among the most environmental-friendly methods to mitigate abiotic stress. In this chapter, we showed that AMF are able to increase the tolerance of As by employing morphological, physiological, and biochemical adaptations. On the other hand, we showed that arbuscular mycorrhizal symbiosis is an efficient way to reduce the damage generated by As. It is evident that the capacity of AMF to uptake As depends on the AMF strain. Consequently, it is necessary to screen As-tolerant strains to increase the efficiency of mutualism in the remediation of arsenic-contaminated environments. However,

information of the biological characteristics of host-AMF-As interaction is still limited. Remediation and management of As-enriched soils is indispensable for food security in the near future. Thus, innovative technologies, like AMF inoculation, are an integral approach, in order to achieve sustainable solutions in As-contaminated soils.

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Chapter 11
Potentials of Aquatic Plants and Algae for Arsenic Accumulation

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Abstract Arsenic (As) is a toxic element which may contaminate water and soil either by geogenic and anthropogenic processes. High concentration of As in groundwater may affect human health through drinking water and contaminate soil through irrigation. High arsenic contents in the groundwater have been reported from various parts of the globe due to the geogenic origin. Different sites have been reported with high arsenic than its maximum permissible limit in the states of West Bengal, Assam, Bihar, and Uttar Pradesh in India. Besides, continuous irrigation with groundwater with the high amount of As contaminating the agricultural soil leads to high As concentration in the soil and plants growing therein. Through the processes of leaching and seepage of agricultural runoff As may mobilize and contaminate freshwater bodies like rivers and lakes. Arsenic contamination and mobilization into the water and soil may lead to food chain contamination and health hazards. Aquatic plants and algae growing in As effected sites concentrate it into their biomass through bioaccumulation process. Various aquatic plants have been identified as an efficient arsenic accumulator like *Eichhornia crassipesm*, *Lemna minor*, and *Spirodela polyrrhiza* which may be exploited in removing arsenic from contaminated water individually and by developing a plant-based treatment system. Similarly, arsenic accumulator algal species may be utilized for biomonitoring, algae-mediated As removal, and amelioration of As toxicity.

Keywords Algae · Aquatic macrophytes · Metalloids · Phytoremediation · Xenobiotics

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11.1 Introduction

The occurrence and mobilization of arsenic (As), a metalloid in the nature, take place through a combination of processes like weathering of rocks, biological activity, volcanic eruption, etc. Geogenic As is the main source of pollution; however, some human activities like mining, electrolytic process, combustion of fossil fuels, urban wastes, medicinal use, sewage sludge, fertilizers, pigments, biocides, etc. also account for its widespread contamination (Smedley and Kinniburgh 2002). Continuous consumption of arsenic-contaminated water and food leads to arsenicosis with different symptoms like hyperkeratosis, palm plantar, hepatic damage, hair loss, damage to the central nervous system, and skin cancer in humans (Litter et al. 2010). High concentration of arsenic in soil and water and its subsequent accumulation in aquatic plants and algae have been reported in India (Chakraborti et al. 2002; Singh et al. 2016; Upadhyay et al. 2016).

11.2 Arsenic Contamination in the Soil and Water

11.2.1 Sources and Fate of Arsenic Contamination

Arsenic is a toxic metalloid which naturally occurs in volcanic ashes, combustion of fossil fuels, pesticides, and fertilizers and also has a geological origin, i.e., weathering of rocks and bioleaching processes (Nordstrom 2002). Arsenic contamination in the soil and water depends on its solubility and mobility under natural conditions. Presence of high amount of As in a geographic region depends on its rocks types, morphology, and land use pattern (Bhattacharya et al. 2007). More than 240 minerals contain As, out of which it strongly interacts with iron, phosphorus, sulfur, and silicon (Bhattacharya et al. 2010) in the form of sodium arsenate and calcium arsenate. Besides naturally occurring As in the environment, human activities also result in the contamination of soil and water and ultimately arsenic gets accumulated in the plants and animals. The active transport of As from groundwater to different parts of the plant is mediated by various processes, for instance, oxidation state of the arsenic, bioavailability and presence of phosphate and iron in the rhizosphere. The Fe plaque has a strong affinity for the adsorption of arsenate on the root surfaces. Due to this strong affinity, the arsenate gets retained on the root surface in which also uptake of some arsenate is possible by the root cell. The Fe plaque was found to have a noteworthy effect on the absorption kinetics of As especially on rice roots, causing a decrease in arsenate uptake but increase in arsenite uptake (Chen et al. 2005).

11.2.2 Arsenic Chemistry

Arsenic exists in the environment in the different oxidation states of arsenate (V), arsenite (III), III(arsine) and 0 (arsenic), of which arsenate and arsenite are the toxic forms and pose a health risk. Arsenate (V) occurs in the aerobic condition, while arsenite (AsIII) dominates under the anaerobic environment (Zhao et al. 2009). Arsenate is key species taken up by the plants growing in aerobic soil, while there are evidences of the presence of arsenite in the rhizosphere (Vetterlein et al. 2007). Occurrence of arsenite is likely to be a result of arsenite efflux from roots (Logoteta et al. 2009). Thus during the absorption of arsenate as well as arsenite, these As species are transferred to other plant parts from the rhizosphere. In plants, arsenate uptake happens through the phosphate transporters, while arsenite and methylated As are transferred through the nodulin 26-like intrinsic (NIP) aquaporin channels. In detoxification mechanism, first arsenate reduces to arsenite in plant and then binds with thiol-rich peptides like phytochelatins and/or undergo for sequestration in the vacuolar.

11.2.3 Arsenic Problem in India

Millions of people in West Bengal, India, and Bangladesh are accessing drinking water contaminated with arsenic (> than 50 mg L^{-1} As), which is above the permissible limit (50 μg L^{-1}) as prescribed by Indian standard of drinking water (Das et al. 1995; Chatterjee et al. 1995; Chakraborti et al. 2002; Upadhyay et al. 2016). Some cases of arsenical dermatitis have been reported from different districts of the West Bengal (Chakraborti et al. 2008). The Ganges-Brahmaputra-Meghna is the most affected plain in the world as arsenic concentration has been reported more than 4000 μg L^{-1} (Rahman et al. 2006). Various districts including North and South 24 Parganas, Murshidabad, Nadia, and Kolkata are reported with high As concentration (Chakraborti et al. 2009). Similarly, some Gangetic plains of Uttar Pradesh, Bihar, and Jharkhand are reported as As-affected areas (Chakraborti et al. 2004).

11.3 Plant Responses to Arsenic

11.3.1 Plants Tolerance to As

Arsenic uptake and accumulation in plants from the soil and water vary differently (Haritonidis and Malea 1999; Haritonidis and Malea 1995). Different aquatic plants have been reported for metal tolerance and commonly used in metal removal due to

their efficiency to accumulate metals in their biomass (Dhir et al. 2009; Rahman and Hasegawa 2011). Many efficient aquatic plants and algae are able to tolerate and remove arsenic from water and may be considered as bioresource for phytoremediation purposes (Knauer and Hemond, 2000; Robinson et al. 2006; Hansen et al. 2006).

11.3.2 Antioxidant Enzymes and As Tolerance

As arsenic is highly toxic in nature, plants adapt various physiological and biochemical responses to cope the toxic effect produced in the form of reactive oxygen species (ROS). In plants, various enzymatic and nonenzymatic antioxidant systems convert the AsV to AsIII followed by further reduction and methylation to nontoxic form (Tripathi et al. 2012). Oxidative damage in plants is scavenged by different antioxidative defense systems, which include low molecular weight antioxidant (glutathione, ascorbate) and enzymatic antioxidants (superoxide dismutase, SOD), catalase, peroxidase (POX), glutathione reductase (GR), and other enzymes of Asada-Halliwell pathways). Glutathione (GSH) is sulfur-containing tripeptide and a precursor of phytochelatins. GSH constituted glutamic acid, cysteine, and glycine (Liu et al. 2015). It is an important antioxidant that plays a vital role in toxicity detoxification. Under stress, the level of GSH increases which counter balance the elevated level of oxidized glutathione and thus protect the plants from injury. Ascorbic acid is also an important antioxidant that protects plants from oxidative stress. The enzymatic antioxidant enzymes include superoxide dismutase (SOD), catalase, guaiacol peroxidase (GPX), and enzymes involved in AsA-GSH cycle (monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR)). SOD acts as the first line of defense against environmental stress and dismutates the superoxide radical into hydrogen peroxide. SOD may exists in different isoforms: Cu/Zn-SOD, Mn-SOD, and Fe-SOD in different localizations such as chloroplast, mitochondria, and peroxisome (Su et al. 2016). SOD activity has been reported in various plants and algae under metal and metalloid stress (Shri et al. 2009; Upadhyay et al. 2016; Kumar et al. 2014; Singh et al. 2018). Catalase is heme-containing enzyme which converts H_2O_2 into water and oxygen during photorespiratory oxidation, β-oxidation of fatty acids, and under oxidative stress in plants and algae. Chen et al. (1997) reported three types of catalase on the basis of occurrence, i.e., CAT I, CAT II, and CAT III expressed in the photosynthetic tissue, vascular tissue, and in seed and young seedlings. The AsA and GSH pathway (Halliwell-Asada pathway) is the recycling pathway converting GSH and ascorbate under stress (Anjum et al. 2010). The AsA-GSH cycle involves redox reaction of AsA, GSH, and NADPH. These reactions are catalyzed by the different enzymes MDHAR, DHAR, and GR.

11.4 Potential Plants for As Accumulation

11.4.1 Hyperaccumulation of As

In the last years, many aquatic plants have been reported as efficient accumulators and bio-indicators of heavy metals (Xing et al. 2013; Bhatia and Goyal 2014; Madera-Parra et al. 2015). The efficient metal accumulator plants could be utilized for biomonitoring and phytoremediation technology to prevent water contamination and maintain natural water quality (Ali et al. 1999). More often the aquatic plants and algae naturally growing in contaminated sites possess potential for metal accumulation (Arica et al. 2005) and well adapt to in terms of survival, growth, and reproduction under metal stress (Yoon et al. 2006). Various aquatic plants have been reported for the accumulation of As which may be utilized for As removal from contaminated sites (Table 11.1).

Plants that can uptake and store 0.1% of the particular element are known as hyperaccumulator (Zhang et al. 2002). Hyperaccumulation of metal in the terrestrial plant is a limited phenomenon. As accumulation was initially discovered in *Pteris vittata* which can accumulate 22,630 mg As kg^{-1} dw (Zhao et al. 2002) in the shoot. As hyperaccumulation property of the plants relies on bioconcentration factor (BCF), high biomass, translocation factor (TF), and efficient mechanism of As transportation to the shoot (Tu et al. 2002). These characteristic features are common in all the plant species having a high potential of As accumulation (Visoottiviseth and Panviroj 2001; Xie et al. 2009; Karimi et al. 2009). The TF is the ratio of the efficiency of a plant in transporting an element from the roots to the shoot, while the BF evaluates the accumulation efficiency of the plant with its background concentration (surrounding environment). Plants with higher values of TF and BCF, show their greater potential for transfering and accumulating the element to the above-ground part of the plant (Rai et al. 2012, 2015; Upadhyay et al. 2017). The TF and CF value greater than 1 signifies their hyperaccumulation trait (Zhao et al. 2002). Kabata-Pendias (2010) reported that plants could tolerate As concentrations in soil in the range between 1 and 50 mg kg^{-1}. In As hyperaccumulator plants, the complex formation of AsIII with GSH and phytochelatins followed by their transportation in root and shoot and finally vacuoles storage constitute important mechanisms for coping with As stress and hyperaccumulation

Table 11.1 Arsenic accumulation potential of different aquatic plants

Aquatic plants	Arsenic accumulation	References
Eichhornia crassipes	597 mg kg^{-1}	Singh et al. (2016)
	600 mg ha^{-1} day^{-1}	Alvarado et al. (2008)
Lemna minor	735 mg kg^{-1}	Singh et al. (2016)
	140 mg ha^{-1} day^{-1}	Alvarado et al. (2008)
Spirodela polyrhiza	372 mg kg^{-1}	Singh et al. (2016)
Wolffia globosa	1000 mg kg^{-1}	Zhang et al. (2009)
Pistia stratiotes	21–24 mg kg^{-1}	Singh et al. (2016)

capability (Souri et al. 2017). The mechanism of uptake of As depends on a number of factor including soil type (pH, organic content, As content, etc,) and As speciation. Different forms of As are taken up by different transporters: aresnate (AsV) is taken by phosphate transporter while As(III) by nodulin26-like intrinsic proteins (NIPs) transporter which belongs to aquaporin family (Limmer et al. 2018; Wu et al. 2017). The uncharged methylated form of As such as MMA and DMA enters inside the plant by the same transporter of As(III). The methylated form of As is supposed to be the biotransformed product of microbes and not reported in plants with potency to accumulate As (Lomex et al. 2012).

11.4.2 Arsenic Biomonitoring and Algal Indicators

Various algal species have been reported for their heavy metal removal efficiency from the wastewater (Rai et al. 2005). Algae being an important component of natural water system play an important role in cycling of matter in the environment (Ye et al. 2012). In the last few years, algae have received more attention because of their efficiency to absorb and sequestrate and their ability to synthesize phytochelatins and metallothioneins which bind with heavy metals in complexation and translocate them into the vacuoles (Suresh and Ravishankar 2004). Metal uptake by algae basically depends on the process of adsorption and metabolism-dependent active uptake and accumulation (Lomax et al. 2012).

11.4.3 As Accumulation in Algae

The ability of algae to absorb metals has been recognized for many years (Bahar et al. 2013a, b; Upadhyay et al. 2016). About 300 years ago, plants were proposed for use in the treatment of wastewater (Hartman 1975). At the end of the nineteenth century, *Thlaspi caerulescens* and *Viola calaminaria* were the first plant species documented to accumulate high levels of metals in leaves. In 1935, Byers reported that plants of the genus *Astragalus* were capable of accumulating up to 0.6% selenium in dry shoot biomass. In natural environments, algae play a major role in controlling metal concentration in lakes and oceans (Sigg et al. 1987). An alga possesses the ability to take up toxic heavy metals from the environment, resulting in higher concentrations than those in the surrounding water (Megharaj et al. 2003; Shamsuddoha et al. 2006). Recently, various dominant algae including *H. reticulatum*, Diatoms, *Pithophora* sp. *Phormidium* sp., and *Oscillatoria* sp. were reported from As-contaminated sites of West Bengal with high As accumulation (Singh et al. 2016). Arsenic accumulation potential of major algae growing in As-contaminated sites is depicted in Table 11.2.

Table 11.2 Arsenic accumulation potential of different algae

Algae	Arsenic accumulation	References
Hydrodictiyon reticulatum	403 mg kg^{-1}	Singh et al. (2016); Rai et al. (2013)
Pithophora sp.	229 mg kg^{-1}	Singh et al. (2016); Rai et al. (2013)
Phormidium sp.	372 mg kg^{-1}	Singh et al. (2016); Rai et al. (2013)
Oscillatoria sp.	394 mg kg^{-1}	Singh et al. (2016); Rai et al. (2013)
Diatom	760 mg kg^{-1}	Singh et al. (2016); Rai et al. (2013)

Bioaccumulation studies reveal the accumulation of the contaminant in the organism via uptake of food or water containing the contaminant. The algal cell wall has many functional groups, such as hydroxyl (-OH), phosphoryl (-PO), amino (-NH), carboxyl (-COOH), sulfydryl (-SH), which confer a negative charge to the cell surface. Since metal ions in water are generally in the cationic form, they are adsorbed onto the cell surface (Crist et al. 1981; Sheng et al. 2004; Schiewer and Wong 2000). Each functional group has a specific pKa (dissociation constant) (Volesky 2007), and it dissociates into corresponding anion and proton at a specific pH. These functional groups are found associated with various cell wall components, e.g., peptidoglycan, teichouronic acid, teichoic acids, polysaccharides, and proteins. Because distribution and abundance of cell wall components vary among different algal groups, the number and kinds of the functional group also vary in different algal groups. Among different cell wall constituents, polysaccharides and proteins have most of the metal binding sites (Kuyucak and Volesky 1989). The cell wall of green algae contains heteropolysaccharides, which offer carboxyl and sulfate group for sequestration of heavy metal ions (Lee et al. 1998; Rai et al. 2013).

11.5 Conclusion

Arsenic contamination in water and soil leads to its accumulation in plants and aquatic animals which may affect human health through food chain contamination. Arsenic-tolerant aquatic and algae may be explored for monitoring of As-affected area; however, arsenic hyperaccumulator aquatic plants and algae may be exploited in removing As from contaminated sites by developing plant-based technologies like phytoremediation, constructed wetland, and algal pond system. Therefore, plant-based system for decontamination of soil and water could provide an alternative method over conventional methods and may serve as eco-friendly and cost-effective technologies.

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Chapter 12
Algae as a Budding Tool for Mitigation of Arsenic from Aquatic Systems

Neha Arora, Khushboo Gulati, Shweta Tripathi, Vikas Pruthi, and Krishna Mohan Poluri

Abstract Arsenic (As) pollution in aquatic environment has become one of the most severe ecological problems affecting the provision of clean drinking water in many countries. To combat this, numerous physicochemical methods have been developed including adsorption, ion exchange, biosorption, solar stills, etc. However, the barrier to the successful deployment of these methods lies in the differential removal and disposal efficiency of As species/wastes generated during the treatment. Plants and algae are currently considered as efficient biotechnological tools for safe As remediation from contaminated soil and water sources. In the current chapter, we will focus on algal (micro and macro)-based As bioremediation mechanism and the influence of environmental factors on its uptake from contaminated aquatic systems. Utilization of algae for As bioremediation has an edge over other conventional technologies as it can efficiently accumulate and metabolize all the As species with adequate efficiency, along with generation of biomass that can be used as biofertilizers and biofuels. Recent studies have shown that algal strains can grow in 500–2000 mg per liter of As waters and can remediate a substantial quantity by rewiring their cellular physiology. In a nutshell, the chapter provides a detailed

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mechanistic overview of algal-based eco-friendly As mitigation processes for generating sustainable environmental solutions.

Keywords Aquatic plants · Bioremediation · Macrophytes · Phytoremediation · Toxic metalloids

12.1 Introduction

The fate of arsenic (As) in the environment and its pernicious behavior made it the most controversial element since its discovery by a German alchemist Albertus Magnus in 1250 A.D. (Singh et al. 2015). Over past decade, As has been widely used in medicines, agriculture, livestock, electronics, and metallurgy which have led to worldwide contamination in aquatic ecosystems (Mitchell 2014; Nriagu et al. 2007). Arsenic is a toxic metalloid having the property of both metal and nonmetal. On the basis of its occurrence, it has been ranked 20th, 14th and 12th among trace elements in the Earth's crust, seawater, and human body, respectively (Mandal and Suzuki 2002). In nature, As is widely distributed in inorganic forms which are more toxic as compared to their organic counterparts. The major inorganic forms of As include arsenate (AsV), As acids (H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$), arsenite (AsIII), and arsenious acids (H_3AsO_3, $H_2AsO_3^-$, $HAsO_3^{2-}$), respectively. On the other hand, the organic forms are resultant of As combining with other carbon- or sulfur-containing molecules, such as arsenobetaine (AB), arsenocholine (AC), arsenosugars, arsenolipids, dimethylarsinate (DMA), and monomethylarsonate (MMA), respectively (Fig. 12.1). Depending on the physiological/biological conditions, As can convert into different forms, i.e., inorganic or organic, and this phenomenon is termed as As speciation. The solubility of As in aqueous medium mainly depends on the pH and presence of other ionic species in the environment. Among the above-mentioned forms, As (V) is the most thermostable and majorly present in oxic environments, whereas As (III) is prevalent in anoxic ecosystems (Gupta et al. 2011; Zhao et al. 2013). Recent studies have suggested the toxicity of As in following the order: MMA (III) > As (III) > As (V) > DMA (V) > MMA (V), respectively (Alexander et al. 2009; Kile et al. 2011; Wen et al. 2011).

In the current scenario, As contamination in the groundwaters is affecting more than 150 million people all around the world. Particularly countries in South-East Asia, namely, Bangladesh, Pakistan, Nepal, India, China, Cambodia, Taiwan, and Myanmar, are at higher risk (Singh et al. 2015). Long-term exposure to As contamination in drinking water and food can cause several types of cancers and skin lesions (Marshall et al. 2007). Contaminated groundwater, food, (fish, shellfish, poultry, and dairy products), and especially plants (rice, wheat and cereals) accumulating As from soil are the major sources by which As gets accumulated in the human body, consequently leading to development of serious health issues (Abdul et al. 2015). According to 2016 world health organization report, arsenic has been found to be associated with developmental effects in newborn baby, cardiovascular diseases, neurotoxicity, and diabetes. Significant amounts of As exposure to human body results in the development of arsenicosis, which is the common term used for the

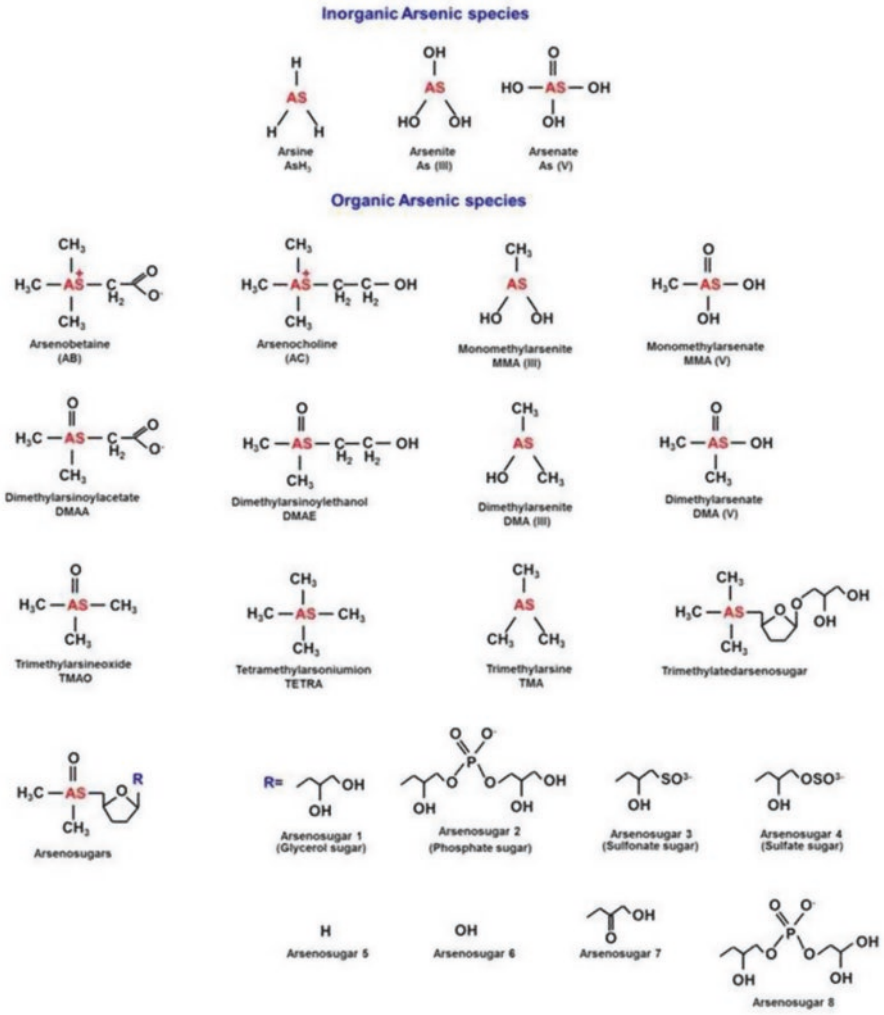

Fig. 12.1 Chemical structures of various inorganic and organic forms of arsenic species

health effects related to As toxicity such as skin pigmentation, skin cancers, internal cancers (bladder, kidney, lungs), diseases related to blood vessels, leg and feet pigmentation, diabetes, high blood pressure, reproductive disorders, and impairment of respiratory system. Recently, it has been reported that some of the arsenolipids are exceptionally toxic (Meyer et al. 2014). Exposure to inorganic As species is associated with numerous disorders including dermatitis, keratosis, melanosis, irritations of the skin mucous membranes, and vascular diseases such as blackfoot disease (BFD), hypertension, etc. (Mandal and Suzuki 2002). Therefore, remediation of As in contaminated water and soil is quintessential to reduce the degree of health risk to human kind.

In recent studies, it has been reported that arsenite is able to bind to the sulfhydryl groups of enzymes, thereby inhibiting more than 200 enzymes in human resulting in functional impairments (Ratnaike 2003). On the other hand, arsenate being the structural analog of phosphate competes with the uptake of the phosphate ions by the cells. This, in turn, causes interference with the normal cellular processes like oxidative phosphorylation by replacing the phosphate group in the nucleic acid, which leads to mutations and cancer (Frankenberger Jr 2001). Further, the formation of free radicals in the cytoplasm due to As uptake may also result in chromosomal and cellular damage (Lièvremont et al. 2009). Additionally, it has been reported that As (III) residing in anaerobic conditions is 10 times more toxic than As (V) present in aerobic conditions while 70 times more toxic than organo-As species (Fowler 2013). The As toxicity and bioavailability largely depends on As speciation making it essential to analyze different forms of As both qualitatively and quantitatively using As speciation analysis.

12.2 Arsenic Speciation in Water

Arsenic speciation in water is mainly dictated by two driving factors, namely, the redox potential and the pH (Fig. 12.2). As (III) is predominantly present in the groundwaters, while As (V) is prevalent in the surface waters (Kumaresan and

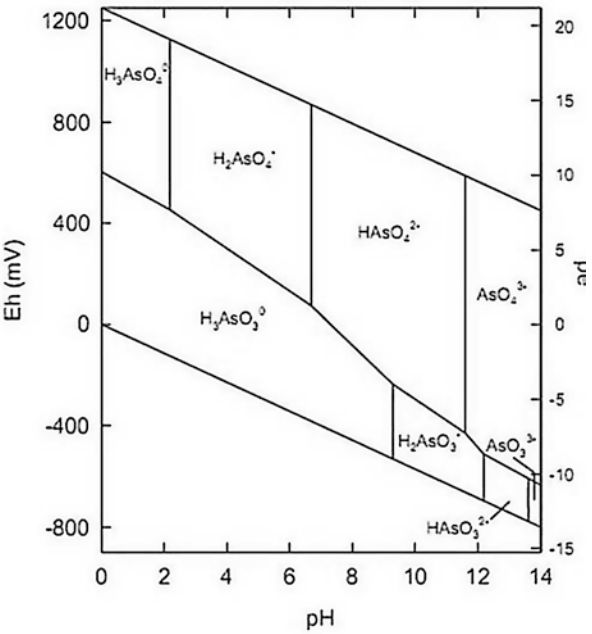

Fig. 12.2 Distribution of different arsenic species in water as a function of pH and redox potential at 25 °C and one bar total pressure (Brookin 1988)

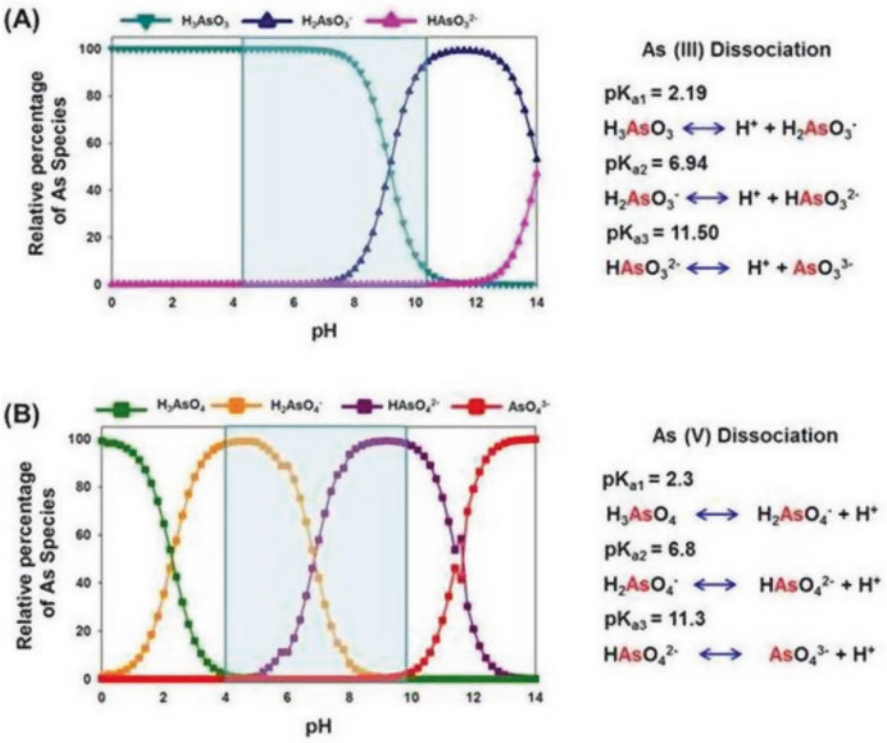

Fig. 12.3 MINTEQ program-based analysis of arsenic speciation as a function of pH, (A) As (III), (B) As (V) (Felmy et al. 1984; Arora et al. 2017)

Riyazuddin 2001). Under oxidizing conditions, at a lower pH (below pH 6.9), As (V) is primarily present as $H_2AsO_4^-$, whereas the $HAsO_4^{2-}$ form dominates at higher pH (Fig. 12.3). On the other hand for As (III), at pH < 9.2, the uncharged arsenite species (H_3AsO_3) dominate in the reducing conditions (Brookin 1988; Yan et al. 2000; Smedley and Kinniburgh 2002). The distribution of As species as a function of pH redox conditions (Eh) is summarized in Fig. 12.2 (Brookin 1988; Yan et al. 2000). In addition, the presence of differential species of As (III) and As (V) concerning changes in pH is depicted in Fig. 12.3. Further, $HAsS_2$ can also be formed in the presence of sulfur at lower pH conditions (Scow et al. 1981). It has been reported that anionic As species in water acts as ligands as they can form bonds with organic sulfur, carbon, or nitrogen (Brookin 1988; Yan et al. 2000; Smedley and Kinniburgh 2002). As (V) is reported to react with reduced nitrogen groups, while As (III) binds with sulfhydryl groups such as organic di thiols, cysteine, protein, and enzyme (Kumaresan and Riyazuddin 2001). However, both As (III) and As (V) are capable of reacting with carbon resulting in the formation of organoarsenicals. The formation of complexes of As with organic material in the environment not only prevents its sorption or coprecipitation but also promotes its mobility in soil and aquatic systems (Kumaresan and Riyazuddin 2001).

12.3 Biogeochemical Cycle of Arsenic

Arsenic is ubiquitous in nature and widely distributed among all the compartments of the environment. Both the biotic (microflora) and abiotic components (pH) of environment play a deciding role in the As fate in nature. Biogeochemical cycle of As is comprised of biotransformation reactions that oscillate between two oxidation states namely, As (III) and As (V) as discussed above (www.greenfacts.org/en/arsenic). The combined array of microbial processes, together with inorganic and physical processes, constitutes to the global As cycle (Fig. 12.4).

The mobility and bioavailability of As is extremely dependent on As (V) reduction and As (III) oxidation (Yamamura and Amachi 2014). The three major modes of As biotransformation are (1) redox transformation between arsenite and arsenate, (2) reduction and methylation of As, and (3) biosynthesis of organoarsenic compounds which include different types of arsenosugars and arsenolipids (Fig. 12.1). Various thermal processes such as coal-fired power generation, smelting, burning vegetation, and volcanism and bioprocesses such as biomethylation and microbial reduction release As and volatile methylated derivatives of As in atmosphere either in aerobic or anaerobic condition (Bundschuh et al. 2011). The microbial species that are involved in As biogeochemical cycle such as bacteria, fungi, protozoans, and microalgae have some specialized machinery (enzymes of oxidation and reduction) to resist elevated concentration of As in their surrounding and metabolize them. The suggested biotransformation reactions taking place in microorganisms include (a) interchangeable conversion of oxidation states of As by oxidation and reduction of As (III) and As (V) (Zouboulis and Katsoyiannis 2005), (b) array of methylation and demethylation by microorganisms (Stolz et al. 2006), and (c)

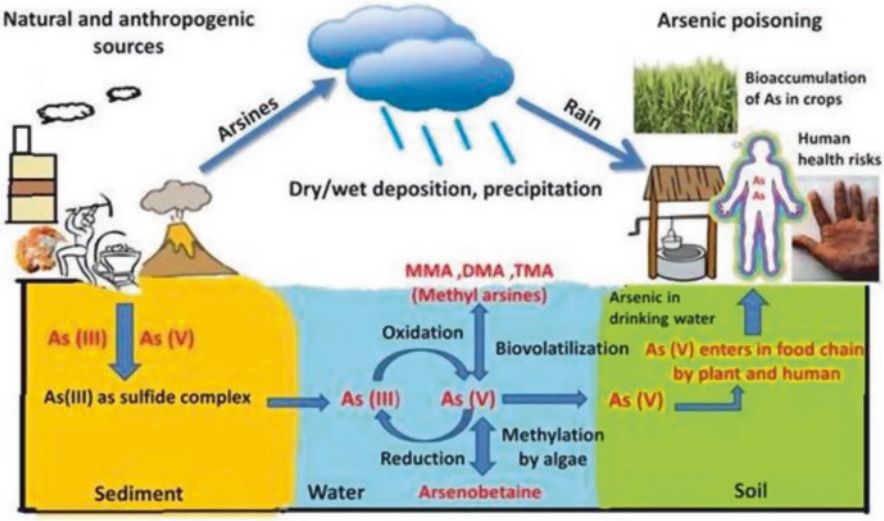

Fig. 12.4 Biogeochemical cycle of arsenic

presence of intracellular metal-chelating cysteine-rich polypeptide such as glutathione. Phytochelatins are having thiol group present in some photosynthetic eukaryotes and microalgae that binds preferably to arsenite to form organo-metallic compounds (Meyer et al. 2007). Marine environment plays a very important role in the biogeochemical cycle of As as it is capable of accumulating As more than 3–4 times than freshwater phytoplanktons (Edmonds and Francesconi 1981). Conversion of toxic inorganic As to less toxic organic form and production of volatile As compound can be the most important step in completing the biogeochemical cycle of As (Michalke and Hensel 2004).

12.4 Algae as Phytoremediators of Arsenic

Several conventional physicochemical and polymer-based techniques such as oxidation, coagulation-flocculation, adsorption, ion exchange, and membrane-driven technologies are reported for efficient As removal (Mondal et al. 2013; Lim et al. 2014; Jadhav et al. 2015). However, the above-listed conventional methods suffer from a number of drawbacks considering the economic factors and environment concerns, which has led to a growing interest in the development of cheaper, efficient, environment-friendly, and pH-independent technologies without any production of secondary toxic and As-laden discards. In this regard, bioremediation techniques utilizing biological materials (live/dead) can potentially contribute to mitigating As in a sustainable and eco-friendly manner (Kumar et al. 2015). Bioremediation mainly involves two modes: biosorption and bioaccumulation. Biosorption is a metabolically passive process to remove heavy metals via nonliving biomass such as biochar, fungal biomass, methylated yeast biomass, chicken feathers, algal biomass, alginate, and orange waste gel from an aqueous solution (Tuzen et al. 2009; Davis et al. 2003). The advantages of using biosorption for removal of As include reusability of bioadsorbent, low operating cost, specificity for heavy metals, short operational time, and no production of arsenic-laden discards or secondary toxic compounds (Sari et al. 2011). On the other hand, bioaccumulation is an active mechanism requiring energy from a living organism (bacteria, yeast, fungi, algae, and plant) to absorb heavy metal onto its cell surface and its subsequent transport into its cytoplasm, which is then metabolized (Kumar et al. 2015). This technique has an additional advantage of detoxification of As compounds (arsenite, arsenate, MMA, DMA, etc.), thereby reducing the metalloid pollution in the environment.

Considering the green and renewable sources for mitigation of toxic materials, bioremediation of As using plants and algae has gained significant importance. The current chapter discusses the role of algae as a budding tool in mitigation of As. Algae are recognized as the oldest life forms which are the basis of the marine food chain as they produce ~ 50% of the oxygen we inhale (Brennan and Owende 2010; Frassanito et al. 2010). They are classically defined as oxygen-evolving thallophytes (plants lacking root, stems, and leaves) containing chlorophyll or chlorophyll-like

pigments as their primary photosynthetic apparatus, and lack a sterile covering around the reproductive cells (Andersen 1992; Acreman 1994). Algae are practically found in every type of environment ranging from freshwater to salt water and hot springs to snowfields and can tolerate a wide range of pH, temperature, turbidity, light, etc. (Acreman 1994; Frassanito et al. 2010). They are also extremely diverse in morphology and size ranging from picoplankton (0.2–2 μm) in diameter to giant kelps (60 m) (Frassanito et al. 2010). Depending on the coloration of their pigments, ecological habitant, and structural and reserve polysaccharides including both prokaryotic and eukaryotic, they can be classified into ten distinct groups: Chlorophyceae (green algae), Xanthophyceae (yellow-green algae), Diatomaceae (yellow/golden-brown algae), Phaeophyceae (brown algae), Rhodophyceae (red algae), Chrysophyceae (golden-brown algae), Chrysophyceae (diverse pigmentation), Dinophyceae (yellowish green to deep golden algae), Euglenineae (pure green algae), and Chloromonadineae (distinct green color), respectively (Frassanito et al. 2010). Further, another algal group, Cyanophyceae (blue-green algae) have been placed in a distinct bacterial domain, hence their name cyanobacteria (Williams and Laurens 2010). Till date, approximately 32,260 species of living algae have been reported in Algal Base. Algae can either be autotrophic (utilize inorganic carbon sources such as CO_2 and light), heterotrophic (require organic carbon sources and nutrients) or mixotrophic (can utilize both inorganic and organic carbon sources) to divide and grow (Brennan and Owende 2010; Arora et al. 2016).

Algae are one of the most promising alternatives due to their high biomass production (compared to plants), cheap availability in both freshwater and salt water, large surface-to-volume ratio (high As binding), no seasonal limitation, rapid metal uptake capacity, phototaxy, potential for genetic engineering, eco- and user-friendly, and applicability in both low- and high-contaminated sites (Tuzen et al. 2009; Chekroun and Baghour 2013; Kumar et al. 2015). The biosorption of As by algae has been attributed to the presence of various functional groups present on its cell wall (Tuzen et al. 2009). The carboxylic groups are the most abundant acidic functional group followed by sulfonic, hydroxyl, and amino that aid in the binding of the As to the cell surface of algae via electrostatic attraction, ion exchange, and complexation, respectively (Davis et al. 2003; Arora et al. 2017). After the adsorption, algae uptakes the As inside its cells and metabolizes (discussed in later sections). In this context, different domains of algae (macroalgae, cyanobacteria, microalgae, and diatoms) have been extensively studied by various researchers throughout the world to remove As (Table 12.1). It is noteworthy that various algae are capable of tolerating high concentrations of As (up to 2000 mg L^{-1}) showing elevated intracellular As accumulation followed by its subsequent metabolism and conversion to less toxic compounds (Table 12.1). Among the macroalgae tested, the maximum removal (95 mg g^{-1} and 60 mg g^{-1}) in only 300 min was shown by *Colpomenia sinuosa* when grown in 100 mg L^{-1} of As (III) and As (V), respectively (Table 12.1).

Further, various studies have been conducted on different macroalgae to evaluate effects of pH, biomass, contact time, temperature, and metal concentration on As removal. As noted in the previous section, the acidity (pH) of the solution alters the

Table 12.1 Summary of arsenic (III, V) removal (mg g^{-1} dry cell weight), IC_{50} value, and time of incubation by various macroalgae, cyanobacteria, microalgae, and diatoms reported in the literature

Algae	As (III) mg/L	As (V); mg L^{-1}	IC_{50} (mg L^{-1})	Time of incubation	Arsenic removal (mg g^{-1} dry weight)	References
Macroalgae						
Cladophora	–	6	–	16 d	2.67	Jasrotia et al. (2014)
Ulothrix cylindricum	10	–	–	90 min	67.2	Tuzen et al. (2009)
Lessonia nigrescens	–	200	–	120 min	45.2	Hansen et al. (2006)
Mougeotia genuflexa	10	–	–	60 min	57.48	Sari et al. (2011)
Colpomenia sinuosa	100	100	–	300 min	As (III) – 95.6 As (V) – 59.9	Abtahi et al. (2013)
Ulva fasciata	–	10	–	90 min	2.82	Christobel and Lipton (2015)
Sargassum wightii	–	10	–		4.35	
Gracilaria corticata	–	10	–		4.27	
Polyphysa peniculus	–	10	–	7 d	As (III) – 0.03 As (V) – 0.053	Granchinho et al. (2004)
Cyanobacteria						
Microcystis aeruginosa	–	50	1.2	30 d	–	Yan et al.(2014)
	13	18	As (III) – 465 As (V) – 23.94	15 d	As (III) – 0.09 As (V) – 0.26	Wang et al. (2013)
	1.29	1.29	–	1 d	As (III) – 0.009 As (V) – 0.02	Huang et al. (2014)
Oscillatoria tenuis	–	50	3.8	30 d	–	
Anabaena affinis	–	50	2.6	30 d	–	
Phormidium tenue	–	5	–	1 d	80.51	Bhattacharya and Pal (2012)
Phormidiurn sp.	–	7	–	15 d	2.8	Ohki et al. (1999)
Nostoc minutum	–	1000	989.30	7 d	0.037	Ferrari et al. (2013)

(continued)

Table 12.1 (continued)

Algae	As (III) mg/L	As (V); mg L^{-1}	IC_{50} (mg L^{-1})	Time of incubation	Arsenic removal (mg g^{-1} dry weight)	References
Synechocystis sp. PCC6803	65	90	–	15 d	As (III) – 0.9 As (V) – 1	Yin et al. (2012)
Microalgae/diatom						
Dunaliella sp.	–	10	–	4 h	As (V) – 0.57	Takimura et al. (1990)
	10	10	–	15 h	As (III) – 0.27 As (V) – 0.56	Takimura et al. (1996)
D. tertiolecta	–	0.002	–	7 d	As (V) – 0.013	Foster et al. (2008)
D. salina	10	1000	–	14 d	As (III) – 0.37 As (V) – 2.74	Yamaoka et al. (1999)
	–	1.12	As (V) – 41.5	9 d	As (V) – 0.27	Wang et al. (2016)
Chlorella sp.	0.75	0.75	As (III) – 93.8 As (V) – 0.57	7d	As (III) – 1.04 As (V) – 1.26	Bahar et al. (2016)
C. vulgaris	–	1000	–	–	As (V) – 2.7	Murray et al. (2003)
	–	200	–	7 d	As (V) – 45.4	Jiang et al. (2011)
	50	–	–		As (III) – 0.53	Ohki and Maeda (2001)
	–	1000	–	10 d	3.6	Maeda et al. (1985)
	100	–	–	36 h	0.37	Ohki et al. (1999)
	–	–	As (III) – 0.1 As (V) – 0.15	72 h	–	Munoz et al. (2016)
	–	1	–	7 d	0.008	Baker and Wallschläger (2016)
C. pyrenoidosa	2000	2000	–	15 d	As (III) –703.6 As (V) – 645.6	Podder and Majumder (2016)

(continued)

Table 12.1 (continued)

Algae	As (III) mg/L	As (V); mg L^{-1}	IC_{50} (mg L^{-1})	Time of incubation	Arsenic removal (mg g^{-1} dry weight)	References
C. minutissima	500	500	–	10 d	As (III) – 145 As (V) – 156	Arora et al. (2017)
S. obliquus	–	0.75	As (V) – 33.5	6 d	As (V) – 6.33	Wang et al. (2013)
Chlamydomonas reinhardtii	–	0.75	As (V) – 0.57	6 d	As (V) – 10.2	
	–	180	–		As (V) – 1.76	Fujiwara et al. (2000)
	–	18	As (V) – 54	–	–	Miyashita et al. (2011)
	17.29	–	As (III) – 224.9	4 d	As (III) – 0.007	Yan et al. (2014)
Scenedesmus sp.	0.75	0.75	As (III) – 196.5 As (V) – 20.6	8 d	As (III) – 0.61 As (V) – 0.76	Bahar et al. (2013)
Scenedesmus sp. IITRIND2	500	500	–	10 d	As (III) – 161 As (V) – 161	Arora et al. (2017)
S. obliquus	0.1	0.3	As (III) – 0.07 As (V) – 0.16	96 h	–	Fuhua et al. (1994)
S. quadricauda	0.03	–	–	96 h	As (III) – 0.03	Zhang et al. (2013)
	100	–	–	96 h	As (III) – 42.3	
Nannochloropsis sp.	12.9	–	–	12 d	As (III) – 2.01	Upadhyay et al. (2016)

speciation of As which in turns can modulate its biosorption. It has been reported that in *Ulva fasciata*, *Sargassum wightii*, *Gracilaria corticata*, *Ulothrix cylindricum*, and *Maugeotia genuflexa*, the As biosorption improved when the pH was increased from 2 to 6, showing maximum uptake at pH 6 (Tuzen et al. 2009; Sari et al. 2011; Christobel and Lipton 2015). Such an enhancement in the biosorption

was attributed to the decrease in positive charge on the algal biomass which restricted the binding of As onto the cell surface (Christobel and Lipton 2015). Once the pH is increased, the amount of protons decreases in the solution resulting in more negatively charged biomass, i.e., binding of As to carboxyl and sulfonate groups present on the cell surface. For example, at pH 6, H_3AsO_3 is the major As (III) species in the solution, which interacts with the unprotonated amino groups (Tuzen et al. 2009). Moreover, a reduction in the biosorption above pH 6 could be due to the dominance of OH^- groups which directly competes with $H_2AsO_3^-$ (major species at alkaline pH) and negative charge of carboxyl, hydroxyl, and amide groups. This results in the repulsion of $H_2AsO_3^-$ ions, leading to reduced biosorption (Tuzen et al. 2009; Sari et al. 2011). On the contrary, *Lessonia nigrescens* showed maximum biosorption at pH 2.5 (3.41 mg g^{-1}) as compared to 4.5 (3.09 mg g^{-1}) and 6.5 (2.96 mg g^{-1}) (Hansen et al. 2006). These findings suggested that this macroalga can be used for mitigation of acid wastewaters such as copper smelting having high As concentrations (400–1000 mg L^{-1}), respectively. Another crucial factor affecting the As removal is the amount of biosorbent (biomass), as it determines the number of active sites present for adsorption of the metalloid. It has been reported that As biosorption showed a positive correlation with the amount of biomass up to a certain extent after which it gets equilibrated. For example, *M. genuflexa* (4 g L^{-1}) adsorbed 96% of 10 mg L^{-1} As (III) present in the media and increasing the biomass by fourfold (16 g L^{-1}) marginally enhanced (98%) As removal (Sari et al. 2011). Similar to biomass, contact time and As concentration have also been shown to have a direct relation with biosorption capacity, showing a rapid uptake of As during initial time and then equilibrating at later time points (Christobel and Lipton 2015). Furthermore, increasing the temperature from 20 to 50 °C resulted in a reduction in biosorption of As (III) from 96% to 60% in *M. genuflexa*, indicating destruction of active sites on the algal cell surface (Sari et al. 2011).

Studies on removal of As (III, V) by cyanobacteria showed that *Nostoc minutum* can tolerate maximum As (V) loads as it has highest minimum inhibitory concentration (IC_{50}) of 986.30 mg L^{-1}, which is defined as the concentration of the compound that results in 50% of cell death (Table 12.1). Though this cyanobacterium could tolerate high levels of As (V), exposure to As (III) in the range of 5–20 mg L^{-1} resulted in cell death (Ferrari et al. 2013). However, *Microcystis aeruginosa* was able to tolerate high levels of As (III) with an IC_{50} value of 465 mg L^{-1} and accumulation of 0.09 mg g^{-1} when exposed to 13 mg L^{-1} of arsenite (Table 12.1). On the other hand, microalgae of genre *Chlorella*, *Scenedesmus*, and *Chlamydomonas* have known to show maximum As (III, V) tolerance and accumulation (Table 12.1). Unfortunately, only a few studies have been conducted to examine the effect of the abovementioned parameters affecting As accumulation in cyanobacteria and microalgae. Zhang et al. showed that *Scenedesmus quadricauda* accumulated more As at pH 8.2 (25.23 μg g^{-1}) as compared to pH 9.3 (8.39 μg g^{-1}) (Zhang et al. 2013). Further, reduction in As uptake was recorded in *Chlorella pyrenoidosa* when the As concentration (III, V) was periodically increased from 50 to 2000 mg L^{-1} (Podder and Majumder 2016). Moreover, a difference between As (V) removal in batch and continuous cultures of *Dunaliella tertiolecta* was reported (Duncan et al. 2013b).

They stated that in case of batch cultures of *D. tertiolecta*, the As accumulation peaked on the 7th day (11 μg g^{-1}) and subsequently decreased on the 42nd day (7 μg g^{-1}). This was due to the death of the algal cells as the nutrients got exhausted in the growth media with time. The above hypothesis was proven to be true as the heat-treated algal cells also showed similar As accumulation (6 μg g^{-1}). On the other hand, higher accumulation (13 μg g^{-1}) was observed in the continuous culture of the microalga as it had more live cells (Duncan et al. 2013a). Another crucial factor that affects As (V) bioaccumulation inside live algal cells is the phosphate concentration in the growth media (discussed in detail in the next section). Lastly, it is crucial to note that As toxicity and mitigation and its response to above-discussed factors can vary with the speciation of alga and its source of isolation.

12.5 Arsenic Speciation in Algae

Arsenic species are distributed among different cellular fractions of algae including the lipid-soluble (lipid), water-soluble (cytosolic), and residual (cell membranes and debris) fractions (Foster et al. 2008; Duncan et al. 2010, 2013a, b). For example, the organoarsenic species like arsenolipids and arsenosugars can be found in lipid-soluble or water-soluble fractions of algal extracts (Wrench and Addison 1981; Edmonds et al. 1997; Foster et al. 2008; Duncan et al. 2013a). In contrast, the inorganic As forms are only present in water-soluble and other residual cellular fractions (Yamaoka et al. 1999; Foster et al. 2008; Karadjova et al. 2008; Duncan et al. 2010, 2013a, b; Zhang et al. 2013). Further, the methylated As species including MMA (V), DMA (V), and TMA have been reported to be present in concentrations that are threefold lower as compared to the inorganic species (Lai et al. 1997; Levy et al. 2005; Karadjova et al. 2008; Miyashita et al. 2011). It has also been demonstrated that the treatment of algae with higher As concentration or longer time exposures induces the transformation of inorganic As species to methylated arsenicals (Yin et al. 2011, 2012; Zhang et al. 2011). The other methylated As species in algae includes less toxic trimethylarsine (TMA) and trimethylarsine oxide (TMAO) (Maeda et al. 1992; Ohki et al. 1999; Yamaoka et al. 1999; Yin et al. 2011). The higher volatility of TMA diminishes the cellular burden of As, which is a potential route for detoxification of As by the algae (Bentley 2005; Ye et al. 2012; Zhao et al. 2013). Quin et al. suggested that the As (V) reduction to As (III), followed by the methylation of As (III) to the volatile TMA, can significantly contribute to the global cycling of As (Qin et al. 2006).

Arsenosugars are more complex, but minor, As compounds formed as a result of transformation of As (V) by several freshwater algae including *Chlorella*, *Monoraphidium*, *Synechocystis*, *Nostoc*, and *Chlamydomonas* (Murray et al. 2003; Levy et al. 2005; Miyashita et al. 2011, 2012). Marine algae belonging to the genera *Dunaliella*, *Phaeodactylum*, and *Thalassiosira* have been reported to form arsenoribosides, with concentrations more than 12% in water-soluble fractions and between 6% and 10% in lipid-soluble fraction, respectively (Duncan et al. 2013a; b,

2014a). The biotransformation of arsenate to oxo-arsenosugars by freshwater unicellular green alga *Chlamydomonas reinhardtii* and to oxoarsenosugar-phosphate by cyanobacteria *Synechocystis* has also been reported (Miyashita et al. 2011, 2012). On the other hand, the presence of phosphate sugars has so far been reported in only *Chlorella* sp., *M. arcuatum*, and *Synechocystis* with concentrations of up to 12% of total cellular As (Levy et al. 2005; Miyashita et al. 2012).

Little is known about the toxicity and structures of arsenolipids or lipid-soluble As species as compared to the water-soluble As species as they are present in lower quantities in the marine environment (Dembitsky and Levitsky 2004; Duncan et al. 2013a, b, 2014a). Arsenolipids synthesis has been reported in diatoms such as *Phaeodactylum tricornutum*, *S. costatum*, and *T. pseudonana*; in blue-green algae, namely, *Oscillatoria rubescens* and *Synechocystis*; and in green algae including *C. vulgaris*, *C. ovalis*, *C. pyrenoidosa*, and *D. tertiolecta*, respectively (Lunde 1973; Wrench and Addison 1981; Foster et al. 2008; Murray et al. 2003; Duncan et al. 2013a, b, 2014b; Xue et al. 2014). The presence of three arsenolipids, one phosphatide, and other two glycolipids has also been reported in the unicellular marine phytoplankton, *D. tertiolecta* (Wrench and Addison 1981). Foster et al. also demonstrated the presence of 20–29% and 29–38% arsenolipids in *P. tricornutum* and *D. tertiolecta*, respectively (Foster et al. 2008). Further, the production of arsenosugar phospholipid in response to As(V) exposure has been reported in a freshwater microalga *Synechocystis* sp. PCC 6803 (Xue et al. 2014). Additionally, the degradation of dead cells break down the arsenoribosides/arsenolipids into methylated As species (Hasegawa et al. 2001; Duncan et al. 2013b).

Arsenobetaine (AB) is a stable, nontoxic, quaternary arsenium compound, which is a major chemical form of As found in marine animals. Arsenobetaine is the common As form observed among marine fishes that is excreted by them without any further biotransformation. It has been reported that the arsenobetaine concentrations are low in the marine algae (Nischwitz and Pergantis 2005). The concentration of arsenobetaine is very low in freshwater organisms as compared to the marine organisms (Nischwitz and Pergantis 2005). This can be related to higher salt content in marine water than in freshwater. Arsenobetaine is structurally similar to nitrogen-containing betaine compound known as glycinebetaine, which serves as an osmolyte in organisms living under saline conditions (Francesconi 2010). Studies have suggested that arsenobetaine formation is the resultant of degradation of arsenosugars (Caumette et al. 2014; Miyashita et al. 2016). Arsenobetaine was detected and identified in brown algae *Ascophyllum nodosum*, *Laminaria digitata*, *Padina pavonica*, and *Fucus vesiculosus*, in freshwater marine algae *U. lactuca* (green algae), and in species of red algae (Nischwitz and Pergantis 2005). The presence of significant amounts of arsenobetaine has also been reported in red Antarctic alga *Phyllophora antarctica* (Grotti et al. 2008), *U. rigida*, and *Enteromorpha compressa*, respectively (Llorente-Mirandes et al. 2010).

12.6 Detoxification Mechanism of Algae-Based Arsenic Remediation

High concentrations of organic forms of As in algae were first reported in 1922 by Jones (Geiszinger et al. 2001). However, it took another 59 years to confirm the presence of two organic species arsenoribosides and arsenosugars in the brown algae *Ecklonia radiata*. It has been reported that algae can accumulate As more efficiently (3000 times higher) than any other upper members of the food web, which makes it imperative to study the detoxification and metabolism mechanism (Bottino et al. 1978). Marine water and freshwater algae can potentially uptake various As species including As (III), As (V), DMA, MMA, etc. and reduce them with subsequent methylation into organic forms before excretion and reintroduction into the surface waters (Markley and Herbert 2010). Interestingly, the toxicity of As species especially inorganic forms (III, V) differs between fresh and marine algae. Arsenite is believed to be more toxic to the marine algae as they can tolerate high concentrations of arsenate, while the opposite is true for the freshwater algae (Levy et al. 2005; Wang et al. 2015). The reason for such kind of differential toxic response is the more bioavailability of As (III) and high concentrations of phosphorus in marine systems (Rahman et al. 2014). Further, different classes of algae occurring in various habitants can have a distinct level and speciation of As species. For example, Phaeophyceae (brown algae) has decreased proportions (22%) of inorganic As as compared to Rhodophyceae (red algae, 43%) and Chlorophyceae (green algae, 47%) (Sanders 1979). Moreover, little variation was observed in the actual concentration of inorganic forms. Presence of organic forms is advantageous as these compounds are less toxic to algae and other trophic level organisms thereby do not affect the marine invertebrates (Sanders 1979).

12.6.1 Arsenic Adsorption and Absorption by Algae

The first step toward the uptake of As by algae is its adsorption by the functional groups present on algae's cell wall. Both As (III, V) have shown to efficiently bind to the hydroxyl, carboxyl, and amide groups present on the cell wall of the algae (Jasrotia et al. 2014; Sari et al. 2011; Tuzen et al. 2009). This step is followed by absorption in which the As species enter the algal cells. Arsenite absorption is an active process thus requires little or no energy to enter the cells (Bottino et al. 1978; Munoz et al. 2016). On the other hand, arsenate absorption is an endergonic process that directly competes with the photosynthetic energy available to the algal cells (Bottino et al. 1978). Further, As (III) is reported to be transported across the plasma membrane via aquaglyceroporine (AQP) and hexose permeases, while As (V) being an analog of phosphate gets internalized through phosphate channels (discussed later) (Wang et al. 2015). Algal cells can show three types of resistance mechanisms against As: (1) avoidance, i.e., efflux As out of the cell, (2) reduce the toxicity of As

by detoxification to methylated forms, and (3) some of the enzymes that are sensitive to As get mutated and become less sensitive (Kobayashi et al. 2003). Among these mechanisms, most of the algae detoxify the As species by converting them to pentavalent or trivalent methylated derivatives (MMA, DMA) followed by their subsequent transformation to arsenosugars and lipids, respectively (Wang et al. 2015).

12.6.2 Arsenite Detoxification Mechanism

On entering the algal cell, As (III) reacts avidly with sulfhydryl groups (S-H) of proteins and can potentially inhibit the enzymes such as glutathione reductase, glutathione peroxidases, thioredoxin reductase, and thioredoxin peroxidase (Benson et al. 1981; Katsoyiannis and Zouboulis 2004). In order to alleviate its toxicity, free thiols (GSH) bind to As (III) forming As- GS that trigger the formation of phytochelatins (PCs) (Munoz et al. 2016). Phytochelatins subsequently chelate As through a stronger interaction by using their multiple cysteine residues. Thus, the resultant reduced and oxidized PCs maintain the redox potential inside the microalgal cells. They, in turn, utilize NADP/NADPH and glutaredoxin as cofactors and reducing enzymes, thereby preventing oxidative damage to the algal cells (Munoz et al. 2016). The complex (GS-As (III)-PC) is then compartmentalized inside vacuoles via ATP-binding cassette subfamily C member 1, 2 (ABCC1/ABCC2) pumps, which further lowers the concentration of As. As (III) is then methylated to MMA and then to DMA or TMA (trimethylarsine oxide) followed by their excretion in the medium (Baker et al. 1983; Munoz et al. 2016). In a recent study by Wang et al., a halotolerant microalga, *D. salina*, showed a positive correlation of production of PCs with As (III) concentrations (11.2–11,200 μg L^{-1}) in the growth media, while the GSH levels increased only at low As exposures (11.2–112 μg L^{-1}) (Wang et al. 2017). Further, they reported that GSH acts as a substrate for PCs' synthesis in *D. salina*.

Contrary to the above detoxification pathway, exposure of 10 μM of As (III) for 12 h to a cyanobacterium, *M. aeruginosa* did not result in the formation of methylated forms (DMA, MMA) (Yan et al. 2014). However, they reported elevated oxidation of As (III) to As (V) under phosphate-deprived condition as compared to phosphate sufficient in the cyanobacterium indicating that phosphorus plays a vital role in the interconversion of inorganic As species. Interestingly, when this cyanobacterium was exposed to 100 μM of As (III) for 15 days, though As (V) was the prominent species, DMA and MMA were also detected (Wang et al. 2013). Furthermore, when the two forms of As (III), $H_2AsO_3^-$ and H_3AsO_3, were compared for their effect on *S. quadricauda*, the latter one was found to be more toxic (Zhang et al. 2013). The above results indicate that various factors such as time of incubation, phosphate concentration, and As concentration can modulate the interconver-

sion of arsenite and its detoxification mechanism. Detailed mechanistic studies depicting the pathway analysis are essential to underpin the arsenite detoxification processes.

12.6.3 Arsenate Biotransformation and Mitigation by Algae

Arsenate detoxification mechanism has been extensively studied in various algal and cyanobacterium species (Table 12.1, Fig.12.5). As arsenate and phosphorus are biochemically analogous, it has been postulated that it enters the algal cells via phosphate specific transport (PST) and phosphate inorganic transport (PIT) systems (Guo et al. 2011; Murota et al. 2012). Studies on As-resistant random mutants of *C. reinhardtii* (AR3) showed tenfold resistance toward As (V) as compared to the wild type (Kobayashi et al. 2003; Murota et al. 2012). The mutants showed suppressed influx and stimulated efflux of As (V) indicating that mutants have disrupted P_i (phosphate) transport gene homolog (PTB1), thus providing proof of concept for the phosphate channels involved in arsenate uptake. This also reflects that the As absorption and transport into the algal cells depend on the relative phosphate concentrations in the environment (Benson et al. 1981). Generally, under low phosphate concentrations, arsenate uptake increases as both compete with each other for the same phosphate transporters (Knauer and Hemond 2000; Markley and Herbert

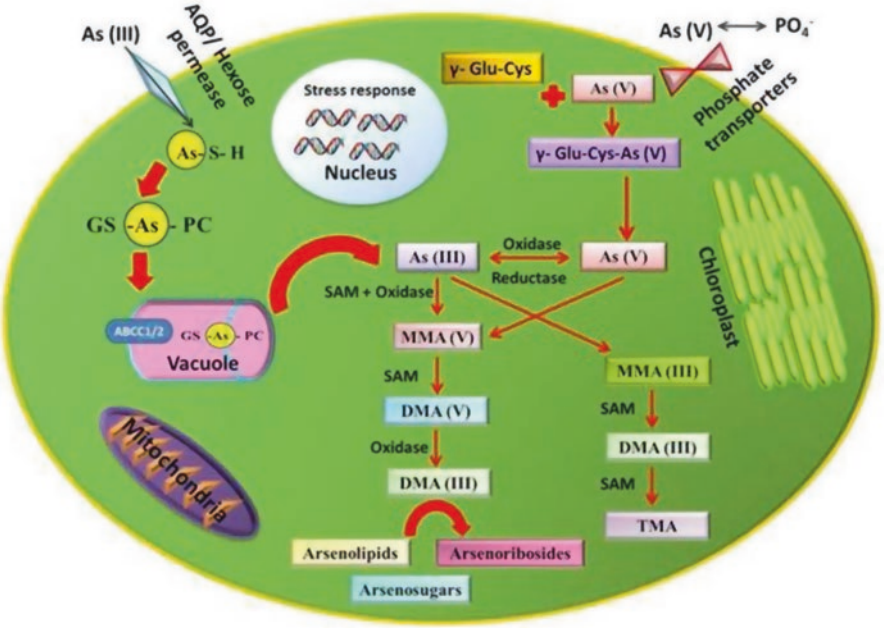

Fig. 12.5 Arsenic bioremediation mechanism by algae

2010). Further, arsenate under phosphate-deprived conditions enhanced the growth rate of *Chlorella* sp. as it acts an alternative substrate for inorganic phosphate (Knauer and Hemond 2000). However, under phosphate sufficient or rich/excess conditions, many algae and cyanobacteria were able to tolerate high levels of As (V) in the medium, as it selectively uptakes phosphates instead of As. For example, when *M. aeruginosa* was grown in phosphate deprived or limited along with 10 μM of As (V), the levels of arsenate decreased in the medium just after addition and then subsequently stabilized. On the other hand, in case of phosphate excess cultures, no change in As (V) was recorded in log phase, but as the cells reached stationary phase, little decrease in arsenate was observed (Guo et al. 2011). Similar results have been reported for various algal/cyanobacterial strains including *Skeletonema costatum*, *Chlorella* sp. CE-35, *Dunaliella* sp., *C. vulgaris*, *Chlorella* sp., and *Monoraphidium arcuatum* (Sanders and Windom 1980; Levy et al. 2005; Duncan et al. 2010; Yan et al. 2014; Bahar et al. 2016; Wang et al. 2016; Wang et al. 2017). Moreover, it was reported that under phosphate-deprived conditions, the activated PIT system does not discriminate between phosphate and As (V) (Guo et al. 2011). Interestingly, for phosphate excess conditions, PST gets activated which is 100 times more specific toward the P_i, thus allowing the alga to selectively uptake phosphate from the growth medium. The earlier work by Thiel (1988) provided kinetic evidence that in *Anabaena variabilis*, phosphate and arsenate do not compete with each other for entry into the cell, yet arsenate inhibited the phosphate uptake (Thiel 1988). They postulated that such an inhibition of phosphate by arsenate took place from inside the cell as preincubation of phosphate-starved cells with As (V) significantly reduced the phosphate transport. However, un-starved cells were unaffected by preincubation with As (V) suggesting the involvement of different transport proteins between starved and un-starved cells.

Once As (V) is inside the algal cell, it induces its toxicity by substituting adenine in molecules such as ATP, ADP, and AMP and effectively decoupling the energy metabolism (Wurl et al. 2013). These As-substituted molecules have low energy and can be easily hydrolyzed as compared to their phosphate analogs (Munoz et al. 2016). In order to ameliorate the toxic effects of As (V), various phytoplanktons emerged with different strategies to mitigate As inside them. Overall the proposed pathway for As (V) mitigation starts by its reduction to As (III) and then its subsequent methylation to MMA (V) by utilizing S-adenosylmethionine (SAM) and oxidase (Baker and Wallschläger 2016). MMA gets converted to DMA (V), which is further reduced to DMA (III), followed by its conversion to a range of organoarsenicals such as arsenosugars, arsenolipids, arsenoribosides, and arsenobetaine (Markley and Herbert 2010; Miyashita et al. 2012; Murray et al. 2003). However, the above interconversion of As (V) into various other As forms depends on the algal species, relative phosphate concentration, growth phase, and incubation time. For example, in *M. aeruginosa*, *Valonia macrophysa*, and *Ostreococcus tauri* (marine microalgae), As (V) was reduced to As (III) followed by its subsequent conversion to DMA in stationary phase, while no MMA was detected (Sanders and Windom 1980; Guo et al. 2011; Wang et al. 2013; Zhang et al. 2013). On the other hand, for *Closterium aciculare* (green algae), *Nostoc* sp., and *C. vulgaris* apart from As (III) and DMA,

MMA was also detected in the stationary phase (Hasegawa et al. 2001; Jiang et al. 2011; Miyashita et al. 2012; Baker and Wallschläger 2016; Munoz et al. 2016). Recently, effect of phosphate levels on As speciation and its subsequent excretion into the medium has been demonstrated in *C. vulgaris*, which showed that under high phosphate condition, As (V) was reduced to As (III) and then methylated to DMA which was subsequently excreted out from the algal cells (Baker and Wallschläger 2016). On the other hand, under medium phosphate condition, both MMA and DMA were excreted out of the cells, while for low phosphate As (III) was predominant in the media. Further, effect of various elements on As (V) accumulation was evaluated in *D. salina*, which showed that addition of 10 mg dm^{-3} each of tin, gallium, bismuth, strontium, and vanadium; 1 mg dm^{-3} each of iron, antimony, zinc, copper, cobalt, and nickel; and 100 mg dm^{-3} of lithium increased As accumulation while potassium, nickel, and cadmium less than 100 mg dm^{-3} decreased its uptake by the algal cells (Yamaoka et al. 1994).

Additionally, the organoarsenicals also differ with the class of algal species as in brown algae sulfonated (SO_3-ribose) and sulfate (OSO_3-ribose) are dominant, while for red and green algae, glycerol and phosphate (PO_4-ribose) arsenoribosides are present (Foster et al. 2008). Moreover, in marine algae a major As-containing product O-phosphatidyl trimethyl arsonium lactate has been reported (Nissen and Benson 1982). It is a carboxy-lecithin in which the nitrogen of the trimethyl ammonium group is replaced by an As atom. Further, the most common arsenosugars, oxo-arsenosugar-glycerol (Oxo-Gly) and oxo-arsenosugar-phosphate (Oxo-PO_4), occur mostly in marine microalgae sp., Chlorophyta, and Rhodophyta (Miyashita et al. 2012).

12.7 By-Products of Algae and Their Potent Uses

Algae not only have the capacity to accumulate and mitigate high amounts of different As species, but the algal biomass obtained after removal can be utilized for production of various industrial products such as biofuel (biodiesel and bioethanol) and fertilizers. Production of these products in synergy with As bioremediation can provide a potential means for biorefinery approach, thereby making the process economically viable. Overview of these products has been described in the following subsections.

12.7.1 Algal Biofuels

The increase in global energy demands especially in the transportation sector and the negative impact of petroleum-based fuels on the environment and mankind have led to renewed interest in the renewable energy (Ho et al. 2014; Quinn and Davis 2015). Further, the greenhouse gasses emissions are expected to increase from 31 to

37 Gt in the year 2035, which intimately affects the quality of life, global stability, and economic prosperity (Mata et al. 2010; Ho et al. 2014). Biofuels (including bioethanol and biodiesel) are being recognized as one of the most promising fuels especially for transportation sector that can replace fossil fuels shortly. Currently, biofuels contribute to only 10% of the global energy demand as more than 2/3 of it comes from terrestrial-based food feedstock such as corn, sugarcane molasses, and wheat starch feed for bioethanol, while soybean, rapeseed, and canola oil for biodiesel production, thus posing a direct threat to the food resources (Ho et al. 2014; Doshi et al. 2016). To overcome this fuel versus food feud, there is a shift toward nonfood crops such as lignocellulosic (sugarcane bagasse, firewood, perennial grass for bioethanol and jatropha, palm for biodiesel) and algal biomass known as the second- and third-generation biofuels (Al Abdallah et al. 2016). However, second-generation biofuels require large-scale production of nonedible plant biomass leading to eutrophication, resource depletion, and competition with the food crops (Ward et al. 2014) (Maity et al. 2014). Algal biomass can potentially overcome the above problems associated with second-generation biofuels as they can be cultivated year-round using non-potable water without addition of any herbicide or pesticides such as wastewater and seawater, thereby minimizing the land-, water-, and nutrient-associated costs (Brennan and Owende 2010). Further, they have a rapid growth rate as compared to their counterparts with an additional benefit of biofixation of waste CO_2 from the atmosphere.

In a recent report, it was predicted that algal yield of 40,000 liters per hectare per year grown in 30 million hectares could replace 1200 billion liters of petroleum used in the USA per year (Sirajunnisa and Surendhiran 2016). Both microalgae and seaweeds comprise of different types of carbohydrates that can be used as a substrate for yeast cultivation and its subsequent production to bioethanol (Gupta et al. 2012; Meyer et al. 2014; Simas-Rodrigues et al. 2015; Kostas et al. 2016). Microalgae that are reported to accumulate high carbohydrate (> 40%) content in form of storage starch and cell wall components (cellulose, pectin, and hemicellulose) include *Chlorella*, *Chlamydomonas*, *Dunaliella*, *Scenedesmus*, *Tetraselmis*, *Anabaena*, *Spirulina*, *Spirogyra*, etc. (Ho et al. 2013a; Hernández et al. 2015). On the other hand, macroalgal biomass is reported to contain high levels of polysaccharides and sugar alcohols such as mannitol and laminarin in various red (*Kappaphycus alvarezii*, *Gelidium amansii*, *Gelidium elegans*), green (*U. lactuca*, *U. pertusa*), and brown seaweeds (*Laminaria japonica*, *Laminaria hyperborean*, *Undaria pinnatifida*, *Alaria crassifolia*, *Sargassum fulvellum*), respectively (Ghadiryanfar et al. 2016). However, to utilize this carbohydrate-rich biomass of algae, the polysaccharides should be hydrolyzed to simple fermentable sugars such as glucose (Ho et al. 2013a). The pretreatment step requires acid, alkali, or enzymatic hydrolysis (Bibi et al. 2016; Chng et al. 2016). Acid and alkali hydrolysis are fast, easier, and cheap methods but produce various toxic compounds such as furfurals and phenolics that inhibit yeast growth (Ho et al. 2013a). On the other hand, enzymatic hydrolysis is an efficient method but is much slower and expensive (Ho et al. 2013b). Hence, the current research is a focused improvement of enzyme processing technologies. After the hydrolysis, the monomer sugars are fermented by

the yeasts (*Saccharomyces cerevisiae* and *Zymomonas mobilis*) to produce bioethanol, which should be then concentrated and purified (Bibi et al. 2016).

The lipid portion of the algal biomass, particularly the neutral lipids, can serve as a propitious feedstock for biodiesel production. The triacylglycerols (TAGs) extracted from algal biomass are chemically similar to conventional plant oils and can be transesterified to form biodiesel which can be blended with any proportion of diesel fuel (Ashokkumar et al. 2015). The transesterification is a stepwise reaction of TAG with methanol/ethanol in the presence of a catalyst (acid/alkali/enzyme) to form fatty acid methyl or ethyl esters (FAMEs/FAEEs) and glycerol as a by-product (Ho et al. 2014). The obtained biodiesel has the edge over conventional petro fuels as it is nontoxic, biodegradable, and CO_2 neutral (Arora et al. 2016). The lipid content in various microalgae such as *Botryococcus braunii*, *C. emersonii*, *D. tertiolecta*, *Nannochloropsis*, *Neochloris oleoabundans*, *Porphyridium cruentum*, etc. was found to be as high as 60–70% of dry cell weight (Maity et al. 2014). Further, the microalgae and their mutant stains accumulate large amounts of lipids particularly TAGs under adverse conditions such as nutrient limitation, salinity, temperature, light intensity, and heavy metal stress (Arora et al. 2017; Mehtani et al. 2017). However, till date, the cost of algal biodiesel production is estimated to be \$5–10.31/gal which is higher than the selected benchmark for petroleum (\$ 3.75/gal) and conventional biodiesel (B100) made from plant oils and animal fats (\$ 4.21/gal) (Zhang et al. 2017). The above cost can be reduced to about \$ 2.76–4.92/gal by improving the algal biomass productivity, oil content, and cultivation cost (Zhang et al. 2017). Thus, integrating As removal with algal biodiesel production not only reduces the cultivation cost by eliminating the feedstock requirement but the stress generated due to As leads to increase in the oil content. In a recent study, two microalgal strains, *C. minnutissima* and *Scenedesmus* sp. IITRIND2, were shown to tolerate 500 mg L^{-1} of As (III) and As (V) along with accumulating lipid content of 50% dry cell weight indicating the feasibility of such a hybrid approach (Arora et al. 2017). Furthermore, *Nannochloropsis* exposure to 100 μM to As (III) resulted in an increase in lipid productivity by threefold (20–27 mg L^{-1} d^{-1}) (Upadhyay et al. 2016).

12.7.2 Algal-Based Fertilizers

The rapid increase in population has led to the excessive utilization of chemical fertilizers to improve the agricultural yield (Osman et al. 2010). This abuse of synthetic agrochemicals has led to massive ecological degradation, eutrophication, pollution, soil infertility, and biodiversity loss (Garcia-Gonzalez and Sommerfeld 2016; Renuka et al. 2016). Biofertilizers are the products that contain living microorganisms or products derived from these organisms that aid plant growth, restore soil fertility, and improve water flow, thereby increasing the overall crop yield (Garcia-Gonzalez and Sommerfeld 2016). In this regard, algae-based biofertilizers are being widely utilized to substitute the chemical fertilizers as they release various

biologically active substances (gibberellin, auxin, cytokinins, vitamins, amino acids, polypeptides, antibacterial, and antifungal) into the soil which promote the crop growth (Osman et al. 2010). For example, cyanobacteria belonging to the genera *Nostoc*, *Anabaena*, *Tolypothrix*, and *Aulosira* are capable of fixing atmospheric nitrogen and are used as inoculants for paddy crop growth (Painter 1993). Further, it has been reported that *Anabaena* in association with water fern *Azolla* contributed to 60 kg/ha/season along with enrichment of soil with organic matter. On the other hand, macroalgae are used as soil fertilizers in coastal regions all over the world as they can have the ability to increase the water-binding capacity and mineral composition of the soil (Pulz and Gross 2004). Moreover, eukaryotic unicellular green microalgae have also been utilized for soil conditioning to control soil erosion in temperate climate zones along with improvement in root volumes, chlorophyll formation, and increase in the shoot and plant heights (Odjadjare et al. 2017). In a recent study, when rice plants grown in 50 μM of As (III) were inoculated with *Nannochloropsis* sp., there was a significant enhancement of root, shoot length, and biomass (Upadhyay et al. 2016). Further, they reported that the overall accumulation of As in rice root decreased from 24.09 to 20.6 mg Kg^{-1} dry weight in *C. vulgaris* cultures while from 29.96 to 11.67 mg Kg^{-1} dry weight in *Nannochloropsis* cultures. Hence, algae can provide a copious and eco-friendly mitigation approach which not only act as natural fertilizers but can potentially reduce the uptake of As by crops.

12.8 Concluding Remarks and Future Avenues

The current scenario of As poisoning posing a global health risk has spurred the development of novel mitigation strategies particularly involving biological sources to limit the negative impacts of As on humans and other life forms. Algae (macroalgae, microalgae, and cyanobacteria) have recently emerged as one of the most favorable alternatives which have the potential to replace the conventional technologies due to their low, copious, eco-friendly, and specificity characteristics. Apart from the above advantages of the algae over conventional treatment technologies, it can be utilized for generation of various by-products such as biofuels and biofertilizers. A wealth of information is available on the As toxicity, transformation, and mitigation by various divisions of algae, but still, a number of knowledge gaps need to be addressed. Firstly, several biotic and abiotic factors that influence the As biosorption, transformation, and secretion by algae such as species, strain, tolerance, life stages, metal concentration, and environmental parameters (light, temperature, nutrients, pH, salinity, etc.) have to be thoroughly explored and defined for every prospective algal species capable of removing different As species. Secondly, there is a vast lacuna on the genes and enzymes involved in the adsorption and transformation of As species that warrant further investigation. These genes and enzymes can be identified and functionally characterized by utilizing the omics technologies such as genomics, transcriptomics, proteomics, and metabolomics, which will shed

light on the molecular pathway for As toxicity, bioaccumulation, and removal by an algal species. The above-identified genes then can be utilized to genetically manipulate different algal species and make then hyperaccumulators of As. Additionally, the expression of the As-related genes under various nutrients and physicochemical conditions needs to be explored. Thirdly, the potential of algae for remediating real wastewaters, drinking water, and As-laden discards needs to be established along with its synergistic studies with other heavy metal contaminants such as cadmium, nickel, lead, and selenium that are present with As. Lastly, in order to deploy algae-based mitigation of As on a large scale, detailed life cycle assessment and techno-economic analysis should be precisely determined.

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Chapter 13
A Glimpse on Uptake Kinetics and Molecular Responses of Arsenic Tolerance in Rice Plants

Arnab Majumdar and Sutapa Bose

Abstract With ever-increasing arsenic (As) pollution in agricultural lands due to various and extensive anthropogenic activities, crop plants, rice to be particular, appeal for immediate attention with possible restriction mechanisms. Arsenic toxicity on rice plants also reveals simultaneous tolerance capacity of intracellular molecules that bind to arsenate/arsenite [As(V)/As(III)]. Interchange of As(III) and As(V) depends on the redox status of the rice field environment and that triggers the transportation competition between arsenate [AS(V)] to phosphate (PO_4^{3-}) and arsenite [AS(III)] to water molecules as well as silica. Phyto-tolerance of As by rice plants is a dependent variable of As transport. Furthermore, studies suggest that inorganic forms of As are more mobile and toxic compared to organoarsenic compounds like monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA). In response to As toxicity, various reactive oxygenic species (ROS) develop which in turn are subdued by the stress suppressor enzymes along with some specialized peptide molecules derived from glutathione (GSH), known as phytochelatins (PC). These protein molecules are well known to form PC-As complex that minimizes the toxicity by chelation. In addition, rice plant root cells are also equipped with Si transporter Lsi1 (OsNIP2;1) as well as aquaglyceroporin (AqpS) molecules that involve ATPase complex and effluxes out the As from the cellular matrix, sometimes transforming into volatile form engaging methylation cascade enzymes. Studies also reported, after analyzing rice genome, the presence of As tolerance gene regulating the quantitative trait locus (QTL) of phosphate uptake controller that suppresses As uptake and holds subsequent tolerance capacity in rice plant.

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13.1 Introduction

Arsenic ($_{74.922}As^{33}$), being nature's one of the elemental constituents, can prevail in its adjacent environment depending on its chemical forms known. Among all primary inorganic forms of As, arsenate [As(V)] and arsenite [As(III)] are the most abundant forms that find their way to soil and aqueous media, respectively, influenced due to various environmental factors. In the course of As toxicity to the higher trophic level, food stuff contaminated with As is the major roll player. Arsenic translocation to the subjective crops cultivated in contaminated soil applying As-rich groundwater finds its accumulation to the grains of cultivated crops leading by rice and other cereals compared to other crops (Williams et al. 2007). Translocation of inorganic As from soil to grains takes place through series of transporter proteins of other use (Ma et al. 2008; Norton et al. 2010a; Dixit et al. 2016). Organic forms of As-like monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) also get transported through these transporter proteins. Toxicity of As continues to grow over the world including the United States, part of Europe, and China but affects mostly the Southeast Asia. India, Bangladesh, Pakistan, and Myanmar are facing As toxicity and its subsequent health hazards to its maximum extent (Muehe and Kappler 2014). Report says that the primary source of As in Gangetic delta basin might be through the weathering of the Himalayan rock and coal containing sulfide minerals associated with As ore that subsequently gets deposited to the Ganga bed and extracted in these decades (Acharyya 2005). Repetitive application of such As-contaminated groundwater for crop cultivation, especially rice as a submerged cereal crop and being the staple food around the globe, particularly in South and Southeast Asia, the toxicity of As is directly linked with rice cultivation and its consumption. Rice production in India prefers two different water application processes; in winter application of groundwater and in monsoon, rain-fed cultivation is preferred. But this presence of As in soil-water system hinders the rice plant interstructural growth leading to the excess production of stress-responsive mechanism in rice plant (Apel and Hirt 2004; Chauhan et al. 2017). Stress regulator enzymes or reactive oxygen species (ROS) scavenging enzymes and some other thiol molecules like phytochelatins (PCs) are the primary molecules that react in adverse conditions to maintain the plant cell homeostasis (Pastori and Foyer 2002; Srivastava et al. 2016; Chauhan et al. 2017). But these processes of the enzymatic reaction are always associated with some transportation media that either play a role to take up the As inside the plant cell or flux out. Various transporter molecules are involved in rice plant in this process that are meant for the transportation of some other elements like silicon, phosphate, glucose, water, etc. These transporter proteins also help to tackle partially As stress intracellularly or extracellularly, and other

influential elements like iron, silicon, and phosphate ion play crucially in such environment to hinder the As load to the rice grain.

13.2 Influencing Factors in As Loading to the Rice Root System

Loading of arsenic to the rice plant system depends on the form of As present in abundance in the associated environment and it gets changed accordingly in altered redox potential capacity of that environment. As(V) and As(III) are interconvertible depending on the redox change, and that leads to the generation of ROS accordingly (Shri et al. 2009; Tripathi et al. 2007). As(III) prevails in waterlogged anaerobic conditions that help to get dissolute the As(III) to the solution and react with near-most soil-water components. In anaerobic setup, submerged rice plant roots release some atmospheric O_2 to its rhizospheric zone via root cell aerenchyma tissues that play crucially in respiration and infiltration of oxygen to the root cells. This release of oxygen to the root surface area is referred to as radial oxygen loss (ROL), and in the presence of oxygen, the redox status of As changes with other elements present (Armstrong 1980; Colmer 2002; Colmer et al. 2006). As O_2 prevails in the rhizospheric area, Fe (II) ions get converted to Fe (III) ions via oxidation and generate different iron oxides/hydroxides precipitating down, known as "iron plaque" having a characteristic brick red to orange color. These iron complexes are perfect adsorbent of As and act as a sink for the As species from surrounding rhizosphere making an As-rich layer of iron plaque on the root surface, reducing directly the chance of As transportation inside the root cell system (Meharg 2004; Liu et al. 2006). Formation of iron plaque is enhanced greatly with a higher degree of ROL and root porosity. Reports suggest that rice cultivars that have a greater capacity of oxygenic supplementation to the root zone via ROL mechanism also have a strong correlation of root iron plaque formation and that in turn adsorb more As species (Mei et al. 2009; Wu et al. 2011a, b). With higher ROL, chances of As(III) oxidation to As(V) get enhanced which is more susceptible to the Fe-plaque adsorption (Fig. 13.1). Pot culture of rice seedlings of different cultivars in As-stressed condition, supplemented with Fe salts, has been proven to be efficient in As stress tolerance in rice seedlings. In a study by Nath et al.(2014), Fe supplementation helps to reduce the As load to the rice intracellular growth reflecting from the less production of stress-responsive enzymes like superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation via malondialdehyde (MDA) production. Uptake of As is lesser compared to the As-induced rice seedlings with negative detection of genetic markers that get activated in the presence of As to trigger the intracellular resistance mechanism, pointing toward the efficacy of Fe application in terms of As load reduction. Similar studies have been carried out by Meng et al. (2002) and Dwivedi et al.

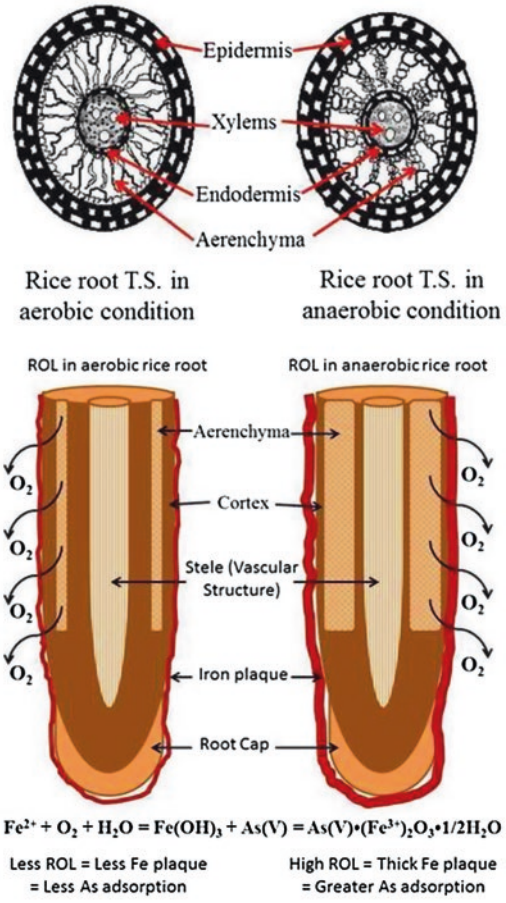

Fig. 13.1 Rice root aerenchyma and Fe-plaque alteration with rhizospheric environments. Upper segment of this image represents the root stele aerenchyma tissues that are being synthesized well in anaerobic condition. Lower segment represents the Fe-plaque formation with changes in ROL and oxygen supply. Anoxic condition helps to maintain higher ROL and thicker Fe-plaque resulting in lesser accumulation of arsenic to the root system

(2010), showing the positive relation between iron plaque and As adsorption and its subsequent effect on the reduction of As translocation from root to the shoot part of the rice plant.

Although ROL is one of the important parameters of reducing As load to the rice plant, it has some inverse relation with silicon that also plays crucially in reducing the As stress to the rice system. Studies by Kotula and Steudle (2008), Fleck et al. (2010), and Wu et al. (2015) showed that addition of silicon to the rice plant growth medium results in developing a suberized exodermis and lignified sclerenchyma cells in rice root system that hinders the process of oxygen loss to the rhizosphere, and thus, the decrease of ROL takes place over a period of time. Decreasing ROL might attribute in more As uptake by the root system, but as silicon has been supplemented, that process gets diminished. Silicon shows a structural similarity and hence competes for the transportation process via a series of transporter proteins (Ma et al. 2008; Bogdan and Schenk 2008), and reports say that As(III) strongly competes for the silicic acid transporters which can be minimized by the needful addition of silicon to the rice growth medium resulting in a manifold reduction of

As transportation. Studies by Tripathi et al. (2013) showed involving different rice cultivars that the supplementation of silicon to the rice seedlings can efficiently restrict As uptake by the rice root system and subsequently translocated to the upper part of the rice plant. This resulted in less production of stress-responsive plant enzymes that become functional in the presence of As, like SOD, CAT, guaiacol peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione *S*-transferase (GST), and some others. In another study by Sanglard et al. (2016), the addition of silicon has been found precisely helpful in reestablishing metabolic impairment caused by As stress and subsequent alleviation of As toxicity in rice plant. Report suggested that the damaged metabolic pathways can be rejuvenated back by supplementing silicon to the rice plant growth environment.

13.3 Role of Stress Regulators in Arsenic Alleviation

Toxicity of As(V) lies on a strategy that interferes with phosphorylation of phosphate and its subsequent metabolism and ATP production, whereas As(III) toxicity is related to the binding of As to the sulfhydryl group of any protein molecule and hinders the chemistry (Hughes 2002). In vivo interactions between cellular factors and ROS components are triggered by these actions which in turn produce glutathione and its derivative PCs, a group of stress-responsive peptide molecules. Glutathione (GSH) is a tripeptidyl molecule formed by a single set or a series of glutamate-cysteine-glycine and a thiol group, which acts as an electron acceptor or donor in diversified biochemical reactions. In the presence of toxic metals or metalloids, plant cells start to synthesize GSH which is a two-step energy-dependent reaction. Figure 13.2 depicts the synthesis pathway of GSH and PCs in plant root and leaf cell matrix with fluxes from the extracellular environment to the intracellular matrix and intermediate passage from root to leaf through the xylem and phloem tissues.

At the very beginning of GSH synthesis, L-glutamate and L-cysteine produce the γ-glutamylcysteine (EC) in the presence of EC synthetase (ECS), after the entry of sulfate molecule inside the root cell. Next, the EC reacts with GSH synthetase to produce GSH by adding a glycine to the C-terminal domain of the EC. GSH often gets oxidized to glutathione disulfide (GSSG) due to the presence of glutathione reductase (GR) which again converted back to GSH for maintaining the balance of the cellular function (Kao 2015). In the presence of As toxicity or any other abiotic stresses, the normal ratio of GSH/GSSG gets disturbed due to the production of ROS, altering GR concentrations also. GST, on the other hand, performs GSH electrophilic interactions balancing the detoxification processes. In the list of ROS scavenging enzymes, GPX is an important player which belongs to the group of multiple isozyme families that play the reduction of lipid hydroperoxides and hydrogen peroxides in the presence of GSH (Kao 2015). PCs, on the other hand, are metal-binding peptide molecules [generic formula $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, where n is the repetitive unit from 2 to 20] that bind with As and cadmium species with a strong affinity resulting in subsequent detoxification (Cobbett 2000; Duan et al. 2011). PC

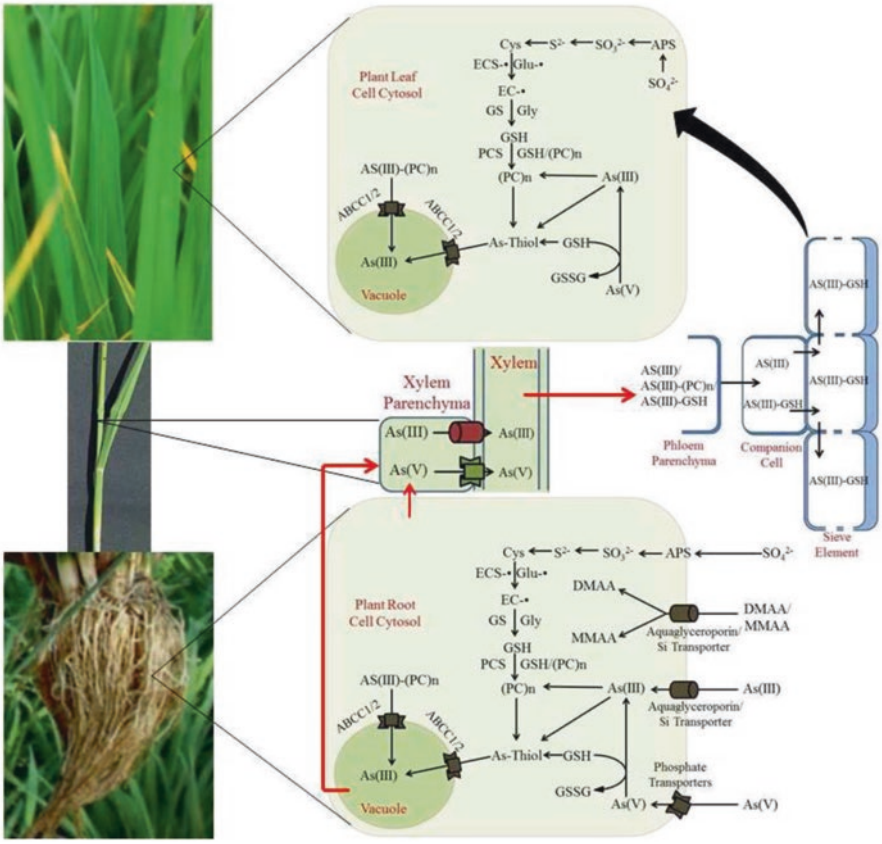

Fig. 13.2 Schematic representation of synthesis pathway of GSH and PC activity with subsequent translocation of arsenic via plant transporter network. The network consists of four parts of arsenic translocation involving different transporter molecules from root to xylem to phloem to plant leaf cell as well as the synthesis pathway of glutathione in root and leaf cell matrices. Abbreviations are mentioned in full in respective text

synthase, the enzyme that synthesizes PC from GSH, performs a transpeptidation reaction by removing a Glu-Cys moiety from the donor to the acceptor molecule. High-performance liquid chromatography associated with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) and molecule-specific electrospray ionization mass spectrometry (ES-MS) have been used for the isolation and screening of PC-As complexes, identifying various conjugates like As(III)-(PC2)2, As(III)-PC3, GSH-As-bound PCs, and MMA-PC2 (Lemos Batista et al. 2014; Raab et al. 2005). Plant cell vacuoles are used for storing such complexes temporarily and transported later via transporter molecules from the ATP-binding cassette (ABC) superfamily, like an ABCC1/2 gateway (Bleeker et al. 2006).

13.4 Role of Sulfur and Thiol Compound in Arsenic Tolerance

Sulfur and sulfur-containing molecules are crucial for plant's nutritional balance as well as for maintaining plant tolerance mechanisms, involving the synthesis of cysteine, GSH, and PCs, toward toxic metalloids like As. In As-stressed conditions, sulfur amelioration strongly helps in plant's tolerance capacity by hindering As uptake, translocation, and accumulation (Zhang et al. 2011; Awasthi et al. 2017). Sulfur gets transported inside a plant cell via a diverse group of sulfate transporter (as classified from 1 to 4) as a form of inorganic sulfate (Davidian and Kopriva 2010) and becomes activated by ATP sulfurylase, converted into adenosine 5′-phosphosulphate (APS). This form of APS then gets reduced to a form of sulfite by APS reductase (APR), which, in turn, is reduced to a sulfide by cysteine synthase to make it incorporated in *O*-Acetylserine to produce the final product, cysteine (Takahashi et al. 2011). From this, the action of sulfur starts that triggers the synthesis of GSH and PCs subsequently. But other than a protein thiol, nonprotein thiols are also proved to be excellent in developing plant tolerance in stressed conditions. Srivastava et al. (2014) reported a nonprotein sulfur compound, thiourea (TU), to be efficient in reducing As stress in rice seedlings. Thiourea is an organic compound structurally similar to that of urea with the exception of replaced oxygen by a sulfur molecule and has a generic formula of CSN_2H_4. The study supports TU amelioration by analyzing sulfate uptake kinetics; GSH redox coupling by high-performance liquid chromatography-inductively coupled plasma mass spectrometry, electrospray ionization mass spectrometry (HPLC-ICP-MS/ESI-MS), and real time polymerase chain reaction (RTPCR) of gene expression profiling can be done. The study suggested the efficacy of TU in the reduction of As uptake with consequent growth days and production of the lesser amount of stress-responsive enzymes, indicating the induced tolerance of rice plant toward As stress in the presence of thiourea. In some other studies, sulfur amendment was observed to be beneficial for the rice plant in As-induced conditions where plants showed a lower uptake and accumulation of arsenite [As(III)] due to the lower gene expression of Lsi2 transporter protein (Dixit et al. 2015a). Application of sulfur to rice seedlings hinders the expression of a Lsi2 protein that primarily acts as a silicon transporter molecule but also allows to pass through the As(III) molecules having a structural similarity with silicon. Thus, downregulation of this transporter molecule also makes the rice plant resistant to As with much less As translocation from the extracellular environment to the inside cell matrix. In another extensive study by Dixit et al. (2015b), sulfur amelioration in rice seedlings was tested for the reduction of As stress in metabolic pathways. Proteomics of rice leaf followed by amino acid profiling using matrix-assisted laser desorption/ionization-time of flight (MALD-TOF) identified diverse proteins that belong to the glycolytic pathway, Krebs cycle, amino acid synthesis pathway, photosynthesis, and many more. All of these identified protein synthases were hindered in the presence of As, whereas sulfur supplementation has been proved to enhance tolerance and express more associated proteins. High-concentration sulfur amendment attributes

to the downregulation of phosphate transporter gene OsPT23 and aquaporin gene OsTIP4;2 as well as upregulation of PC synthase genes (OsPCS1, OsPCS3, and OsPCS13) and some ABC transporter genes (OsABCG5,OsABC6, and OsABCI7_2) with subsequent reduction of As load in rice grain (Zhang et al. 2016).

13.5 As Transporter Proteins and Intracellular Translocation

13.5.1 Transportation of Arsenate

Iron plaque around rice root surface plays crucially in alteration of As species and their adsorption which in turn influence the surrounding rhizospheric As species to get through the transporter molecules inside the rice root (Liu et al. 2006). In soil to root passage and subsequently to the shoot part, translocation of arsenate competes with phosphate (PO_4^{3-}) through the adsorption sites of phosphate in plant root surface (Lee et al. 2016; Bakhat et al. 2017). In a survey from China, similar result of phosphate amendment and decreased As accumulation in rice varieties with a lesser amount of shoot translocation has been documented (Lu et al. 2010). As(V) is an analogue of phosphate allowing its competitive transportation through the phosphate transporters, and studies also suggest that presence of high concentration of arsenate induces phosphate signaling molecules and even directs to misleading sensing of phosphate (Singh and Ma 2006). To induce tolerance capacity against As, rice plants are tested with over-application of phosphorus amendment that resulted in preference of phosphate transporters to take up phosphate molecules over As(V) leading toward an induced As resistance (Kobayashi et al. 2005). Other studies reported the effect of intra- and extracellular phosphorus amelioration could alleviate As toxicity by avoiding the uptake of As(V) from rhizospheric zone (Lihong and Guilan 2009). In a study by Bucher (2007), it was found that the phosphate transporter 1 (Pht1) family and its regulating genes, accounting more than 100 in numbers, are more likely to be present around the root stele (vascular cell system). Earlier reports on Pht1 transporter family documented 12 transmembrane domains having partially duplicated subdomains in some of these major domains (Saier Jr 2000), showing exclusively their expression around root surface where availability of phosphate is low (Bucher 2007). Reports show in *Arabidopsis thaliana*, two phosphate transporters (Pht 1;1 and Pht 1;4) are present with both arsenate-rich and phosphate-rich system that control arsenate transportation considerably through these protein channels in a wild-type model organism. But the double mutated gene (Pht 1;1Δ4Δ) of *A. thaliana* showed arsenate transportation and resistance to a much greater extent compared to the wild organism (Shin et al. 2004). Several reports documented that in both low- and high-phosphate environments, two phosphate transporter genes in *A. thaliana* were expressed significantly as identified by T-DNA insert knock out test (Misson et al. 2004; Shin et al. 2004). A study by Ai

et al. (2009) finely emphasized on the Pht1 family in rice, detecting two members, OsPht1;2 (OsPT2) and OsPht1;6 (OsPT6), in the presence of phosphate surrounding root system. The report showed the expression of the first gene to be efficient in the stele of primary and lateral roots, whereas the second one was better expressed at epidermal and cortical cells of younger lateral roots. In transgenic rice, knocking out either of these two genes by RNA interference resulted in the decrease of phosphate transportation from root to shoot. In another hydroponic study by Wu et al. (2011a, b), a rice mutant was used to check the ability of phosphate transportation using a defective OsPHF1 (*Oryza sativa* phosphate traffic facilitator 1) comparing with expression of another transporter gene OsPht1;8 (OsPT8) deducing the capacity of the later one much higher than other transporters. These reports emphasize on the relation between arsenate and phosphate interaction at the cellular level in plant root system.

13.5.2 Transportation of Arsenite and Other Species

Flooded wetland system maintains a reducing environment, promoting arsenite to predominate over arsenate. In a recent study by Zhang et al. (2017), the ratio of silicon to As was tried to find out for the better understanding of As uptake and transportation capacity in the presence of silicon and phosphorus. The study reported that a ratio of 100:1 of Si:As is effective in reduction of As uptake without the supplementation of phosphorus in the growth media, whereas in the presence of a phosphate group, the ratio of Si:As becomes 10:1 in doing the same. Arsenous acid [$As(OH)_3$] shows a structural resemblance with silica hydroxide [$Si(OH)_4$], and Bhattacharjee and Rosen (2007) have shown the competitive nature between silica and As(III) for the transportation from an exoplasmic environment to the root cell matrix through specific silicon transporters. Although As(III) and As(V) get transported via silicic acid and phosphate transporters, respectively, the rate of silicon to As transportation via these mediator proteins is higher as silicon can pass through the xylem sap much more efficiently compared to As(III) and again arsenite can even be transported better than As(V) through thexylem system (Mitani and Ma 2005; Su et al. 2010; Meharg and Zhao 2012). Transporters like Lsi1and Lsi2 (low silicon transporters 1 and 2) act as a gateway of arsenite from surrounding environments to the root cells and from root cells to xylem parenchyma, respectively (Ma et al. 2006; Ma et al. 2007). Recently, Wu et al. (2017) have shown that both Lsi1 and Lsi2 transporter molecules get downregulated in aerobic field conditions compared to their better expression in an anaerobic environment, without showing any such differences for variable As concentrations or rice cultivars. This study pointed out the applicability of aerobic rice cultivation that will result in a decrease in As uptake in rice grain. In a report, the ability of a mutated rice plant was checked deactivating the Lsi1 and Lsi2, respectively, and found to be more effective in reducing arsenite load in Lsi2 mutation compared to Lsi1 mutated plant (Ma et al. 2008). Besides silicon transporters, aquaporin is a group of a diverse transmembrane

transporter protein family denoted as nodulin 26-like intrinsic proteins (NIP) that allow water, glycerol, and glucose with some other smaller molecules including arsenite. OsNIP1;1,OsNIP2;1, OsNIP3;1, OsNIP3;2OsNIP3;3,AtNIP1;1, AtNIP1;2, AtNIP5;1, AtNIP7;1 and LjNIP5;1, and LjNIP6;1 are some of the NIP protein transporters identified from *O. sativa*, *A. thaliana*, and *Lotus japonicas*, respectively, as divided in different plant origin NIP families (Zhao et al. 2010; Katsuhara et al. 2014; Chen et al. 2017). Ma et al. (2008) found that mutation in OsNIP2;1 resulted in a steep decrease in As(III) uptake and further transportation through the shoot to the grain. Chen et al. (2017) reported the role of OsNIP3;2 in As(III) uptake through the lateral roots of rice where this particular gene is predominantly localized within the stele of lateral and primary roots. Mutation of this gene led to a markedly decrease in As uptake in root but not the shoot part. Interestingly, apart from the NIP transporter proteins, in a study, Mosa et al. (2012) have reported the activities of another transporter group, plasma membrane intrinsic proteins (PIPs) having members OsPIP2;4, OsPIP2;6, and OsPIP2;7, participating similarly in arsenite transportation. Extensive studies on organoarsenic species like DMAA, MMAA, and their transport mechanisms are yet to be done. Some trials reported that the transportation might takes place via the same phosphate and silicon transporter with a low intensity passage of organoarsenicals, although the rate of inorganic As transportation is much higher than any organoarsenic species (Li et al. 2009; Jia et al. 2012). In studies on these methylated forms of arsenicals, it was reported that during translocation process from root to shoot, the number of methyl group plays a vital role, as more number of methyl group results in higher translocation rate of As compared to inorganic forms (Raab et al. 2007; Arao et al. 2011; Jia et al. 2012). Researches were done on rice xylem and phloem translocation and found to be more compatible for DMAA which is ~tenfold more efficient to be transported through this stele compared to arsenite (Ye et al. 2010; Carey et al. 2010; Carey et al. 2011). DMAA is not preferentially adsorbed to the root system of rice but can get passed through the shoot to the grain at a higher magnitude compared to inorganic forms of As leading to the deposition of organoarsenicals to the grain in high concentrations (Zhao et al. 2013).

13.6 Gene Response Toward Arsenic Stress

Arsenic uptake and subsequent accumulation depend on the environmental factors, As contamination intensity, and even rice genotypes (Tripathi et al. 2012; Rai et al. 2015). This detoxification and tolerance depend on the efflux rate of toxicants from cellular matrix or sequestration within cell vacuoles and other organelles and sometimes chelation by metal(loid)-binding peptide molecules (Clemens 2006; Briat 2010). Ionomics is the study of the ionome that involves the simultaneous and quantitative assessment of the elemental composition of a plant identifying the extracellular stimuli that might affect the gene response of the subjective plant creating an intracellular stress. Recently, in a study by Feng et al. (2017), a combination of

ionomics and transcriptomics has been applied to identify the distributional transport of As in brown rice. The report has shown the significant difference in rice genotypes with their As translocation and subsequent accumulation through panicle nodes and intermediate nodes. In another study, As-tolerant type 1 (ATT1) rice mutant has been used compared to a wild rice genotype, showing the response of rice genome toward As toxicity and their subsequent identification (Hwang et al. 2017). Genes that are responsible for the regulation of photosynthesis, lipid biosynthesis, or mitochondrial electron transport get hindered, whereas some cysteine-containing genes were upregulated in this process indicating the role of this amino acid toward the tolerance against As. In some previous studies, upregulation of two phosphate transporter genes (Os04g10690 and Os09g38410) was identified by Huang et al. (2012) using microarray technique and shown to be expressed fine in the application of high arsenate dose. Norton et al. (2008) reported similarly the effect of As application on sulfate transporter gene induction and their upregulations (Os03g09940, Os03g09970, Os09g06499, Os08g31410, and Os09g06510). The positive correlation of sulfur-containing amino acid synthesis has been demonstrated by Kumar et al. (2014), mentioning the upregulation of cysteine synthase and serine acetyletransferase, while other essential amino acid syntheses were obstructed due to the downregulation of their respective genes. Dasgupta et al. (2004), for the first time, reported the arsenate tolerate gene mapping determining the quantitative trait loci (QTL, a segment of the gene that correspondence to a particular trait or phenotypic characteristic) of arsenate tolerance gene at the upper part of chromosome 6. This report also supports the findings by Wissuwa and Ae (2001) that the QTL of phosphate uptake and arsenate tolerance in rice might be the same, as identified in rice genome project map. In another study by Norton et al. (2010a, b), more QTLs were identified at chromosomes 1 and 5, responsible for the leaf phosphate accumulation resulting in As(V) tolerance. Also, two silicon uptake regulator QTLs were also identified at chromosome 5, near the R569 gene marker, and the second one was located at chromosome 10, near the G1082 marker. These QTLs are responsible for the preferential uptake of Si suppressing the As stress. Details of these QTLs are provided in Table 13.1. Although this report shows only rice leaf

Table 13.1 Different quantitative trait loci in rice with their chromosomal location that regulates arsenic transportation (Norton et al. 2010a, b)

Trait determinants	QTLs	Chromosome number	Nearest gene marker
As	qAs1	1	R117
	qAs3	3	R1618
	qAs5	5	C624
	qAs6.1	6	A12361
	qAs6.2	6	AB0603
P	qP1	1	RZ14
	qP5	5	C624
Si	qSi5	5	R569
	qSi10	10	G1082

QTLs for As regulations at chromosomes 1, 3, 5, and 6, Zhang et al. (2008) have shown two rice grain trait loci also at chromosome 6. Suppression of a gene activity and its subsequent production by another gene is termed as "epistasis," which plays also in As tolerance in rice plant. Norton et al. (2010b) have shown the effect of epistatic gene activity, apart from the QTLs interference, can be manipulated between chromosome 1 to chromosome 12 resulting in a drastic epistasis of suppressed As regulator loci leading to a decrease in As uptake. Transporter proteins and their producer genes that are responsible for the passage through ATP-binding cassette pathway (ABC) are a leading family of genes that can get activated in the presence of high concentration of As and not so active in a low dose of As (Song et al. 2014). This C-type ABC transporter family in rice (OsABCC1) is involved in the detoxification and reduction of As load in rice grain while being expressed in most of the rice plant parts: root, leaf, nodes, peduncles, and rachis. The study says that knockout experiment of this OsABCC1 gene resulted in higher As accumulation in rice grain comparing to wild-type rice indicating its role in As sequestration by restraining As in cellular vacuoles at nodal stele. Shi et al. (2016) have shown the presence of two orthologous (these are similar functioning differentiated genes present in different species, diverged during the course of evolution) HAC 1 (high As content 1, as in *A. thaliana*) in rice, OsHAC1;1 and OsHAC1;2, which are responsible for the reduction of As as arsenate reductases, regulating the toxicity of As in rice. Xu et al. (2017) reported a similar OsHAC4 gene in rice, and its mutation led to the higher As(III) accumulation in root and shoot with a decrease in the reduction of As(V). Also, due to overexpression of this gene, identified using RT-PCR, As(V) tolerance capacity has been increased. Study shows the localization of this gene predominates at the epidermis and exodermis root cells that most likely participate in As(V) uptake and efflux processing of As(III). In a previous study, a gene marker OsCLT1 (*O. sativa* CRT-like transporter 1; where CRT stands for chloroquine resistance transporter) was tested for rice glutathione and γ-glutamylcysteine homeostasis (Yang et al. 2016). This study was pointed out that this gene can be triggered up in As-stressed condition, and the transporter helps in export of glutathione and γ-glutamylcysteine from plastid to cytosol matrix and thus maintains the homeostasis with subsequent reduction in As load. Tiwari et al. (2014) have reported another protein molecule, NRAMP (natural resistance-associated macrophage protein in rice, OsNRAMP1), that plays an important role in xylem-mediated translocation of As and its subsequent reduction.

13.7 Conclusion

In As-stressed conditions, rice plant develops their resistance and tolerance mechanism that helps to reduce the As load in cellular matrix. Studies on stress-responsive enzymes and their synthesis pathways have elucidated the role of those protein molecules in As tolerance. Furthermore, rhizospheric environments with some elemental interference can also cut down the chance of As transportation through the

transporters that meant to be for some other molecular passages. Scientists have shown that genes are either upregulated or downregulated for balancing As stress mediated by their respective gene products. The vascular system also contributes to competitive translocation of As and silicon/phosphate via different gateway proteins. Hence, natural resistance within rice intracellular matrix works in the presence of As overload but can also get alleviated applying agricultural practices or genetic modifications.

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Chapter 14
Transcriptomics of Arsenic Tolerance in Plants

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Abstract Transcriptome analysis is a potent method for characterizing the global response to stress conditions of any organism. Main high-throughput techniques of genome-wide transcriptomic investigation are RNA microarray and RNA-seq. Global differential expression of genes upon plant exposure to arsenite As(III) and/or arsenate As(V) studied using different methods is presented in this chapter. Microarray studies of rice (*Oryza sativa*) response to As revealed that there is a set of genes expressed differently upon As(III) and As(V) challenge. As(V) was found to affect cell wall proteins and primary and secondary metabolism, while As(III) treatment affected hormonal and signaling processes. In *Arabidopsis thaliana*, As(V) treatment resulted in a repression of transcription of genes involved in the phosphate starvation response and some transcription factors. Of the genes involved in oxidative stress response, some were found to be upregulated, whereas others were downregulated. RNA-seq analysis of rice transcriptome revealed that genes involved in heavy metal transport, transcription, hormone biosynthesis, and lipid metabolism respond to As(III) exposure in rice. Differential regulation of miRNAs was also discovered. Differential gene expression upon As(III) and As(V) challenge with implication on metabolic pathways involved in plant response to As is discussed in this chapter.

Keywords Metalloids · mRNA · Microarray technology · Plant molecular biology · Secondary metabolism · Soil pollution

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14.1 Introduction

The transcriptome is a total pool of RNA transcripts present in a cell at a particular moment. Transcriptomics assumes that cells react to environmental changes by adjusting a number of specific transcripts, such as mRNAs, transcripts of noncoding genomic regions, and regulatory RNA molecules. The ideal case of transcriptomic analysis is the genome-wide detection of transcript levels in control and study samples, followed by functional annotation of transcripts that are deemed as differentially regulated. There are several methodologies enabling researchers to study changes in the transcriptome. Most popular currently are DNA microarrays, serial analysis of gene expression (SAGE) (Velculescu et al. 1995), suppression subtractive hybridization (SSH), and RNA sequencing. Data obtained with SSH, DNA microarray technology, and RNA-seq are currently available and will be discussed here. SSH is a method developed in the 1980s and 1990s (Lisitsyn et al. 1993; Diatchenko et al. 1996, 1999). It provides a tool for comparative analyses of two populations of mRNA. In general, both populations are first converted into cDNA libraries, which are then hybridized. Hybrids are removed, whereas unhybridized cDNA fragments are regarded as differentially regulated transcripts. Those transcripts are cloned, sequenced, and annotated. Similarly, DNA microarray and RNA-seq methods rely on isolation of total RNA – which may be followed by mRNA enrichment step – and reverse transcription to create a library of cDNA molecules. DNA microarray analysis requires preparation of a chip with immobilized DNA fragments to which fluorescently labeled sample cDNA is hybridized and a fluorescent signal is read and analyzed (Grunstein and Hogness 1975; Gergen et al. 1979). It is thus necessary to have prior knowledge regarding expected transcript sequences before the experiment. This approach limits the analysis to sequences immobilized on the chip with no possibility of additional information. A gene may have more than one complementary probe on a gene chip. Thus the number of responsive probe sets might not always be equal to identified responsive genes. RNA sequencing emerged with the development of sequencing-by-synthesis technology (Ronaghi et al. 1998; Margulies et al. 2005). This approach allows analysis of theoretically all the RNA molecules present in a cell at the time of the experiment, with the limitations set by the need of reversed transcription step (lower efficiency of reversed transcription of fragments with high GC content or long homopolymer stretches). RNA-seq offers many advantages to microarray analysis: it does not require prior knowledge of analyzed sequence, and it allows whole transcriptome analysis and provides a direct measure of transcript abundance.

Although today transcriptomics is a well-established experimental approach to study cell or organism stress response to the variable environment, there are only a few reports presenting transcriptomic data on arsenic (As)-treated plants. It is because of the large size and complex organization of plant genome and the lack of sequenced and annotated reference genomes for many plant species. Here, we discuss studies conducted on rice (*Oryza sativa*), *Arabidopsis thaliana*, barrel clover (*Medicago truncatula*), purple willow (*Salix purpurea*), Indian mustard (*Brassica*

juncea), and Abyssinian kale (*Crambe abyssinica*). With the exception of *A. thaliana*, these are economically important species, with rice forecasted paddy production of 754.6 million tonnes (500.8 million tonnes, milled basis) in 2017 (FAO 2017).

14.2 Transcriptomics of Arsenic in Rice

Majority of As-toxicity studies are performed on rice (*O. sativa* L.). Rice is a staple food for a large percentage of the human population and major crop accounting for one-fifth of the calories consumed by people worldwide (Smith 1998). Rice is grown in flooded fields what makes inorganic As readily available for plant intake and accumulation. Arsenic is accumulated in rice grains. Prolonged ingestion of contaminated rice can cause intoxication leading to severe health issues. This is a serious problem especially in Southeast and East Asia.

In a study by Chakrabarty and co-workers, gene expression in rice (*O. sativa* ssp. *indica* cultivar IR64) under As(III) and As(V) challenge was examined by DNA microarray (Chakrabarty et al. 2009). Ten-day-old seedlings, grown with or without 25 μM As(III) or 250 μM As(V), were used (Table 14.1). Study found that 72 genes were differentially regulated by As(V), while 27 genes were differentially regulated by As(III), although the toxic effect on seed germination was stronger in the case of As(III)-treated plants, as previously reported (Fitz and Wenzel 2002; Meharg and Hartley-Whitaker 2002; Abedin and Meharg 2002). As(V)-regulated genes are involved mainly in cell wall metabolism and primary and secondary metabolic processes, while As(III)-regulated genes are involved in hormonal and signaling processes. Both forms of As caused differential regulation of genes involved in photosynthesis, plant defense, and signal transduction. Altogether around 1% of genes on the array chip was differentially regulated (Chakrabarty et al. 2009). As(V) challenge led to increased transcription of a number of genes commonly implicated in xenobiotic stress, heavy metal response, and detoxification. Among them are ten glutathione *S*-transferase genes, one glutaredoxin-encoding gene, ten cytochrome P450-related genes, four metallothionein genes, 11 heat shock protein genes, and a gene encoding a germin-like protein with both superoxide and oxalate oxidase activities. Another group of genes upregulated upon As(V) treatment were genes encoding various types of transporters: one sulfate transporter, two metal transporters, two glutathione-related transporters, two multidrug and toxic compound extrusion (MATE)-efflux family proteins, one zinc-iron transport family protein, and a multidrug resistance protein. All these proteins are potentially capable of active efflux of inorganic As, as well as its organic methyl or glutathione conjugates. Transcription factors comprise another group of genes undergoing increased expression when rice seedlings are challenged with As(V). Considering the number of genes differentially regulated in such conditions, this is hardly a surprise. Some F-box, U-box proteins, and protein kinases possibly involved in signal transduction were also upregulated in As(V)-treated rice seedlings. The glycine-rich cell wall structural protein 2 precursor exhibited

Table 14.1 List of plant species, arsenic source, and treatment conditions for presented transcriptomic studies

Plant species	Form and concentration of arsenic	Time of treatment	Growth conditions	Organs analyzed	Transcriptomic technology	References
Rice (*Oryza sativa*) ssp. *indica* cultivar IR64	As (III) as 25 μM $NaAsO_2$, As(V) as 250 μM Na_2HAsO_4	10 days	Solid MS medium 26 °C, 16 h day (110–300 μmol m^2 s^{-1} PAR)	Whole seedlings	DNA microarray	Chakrabarty et al. (2009)
Rice (*O. sativa*) ssp. *japonica* cultivar Nipponbare	As(III) as 20 μM, 80 μM $NaAsO_2$	6 h, 24 h	½ Hoagland liquid medium, 28 °C (day): 25 °Cr (night) 16 h day	Roots, shoots	RNA-seq	Yu et al. (2012)
Rice (*O. sativa*) cultivars Bala (ssp. *indica*) and Azucena (ssp. *japonica*)	As(V) as 13.3 μM disodium hydrogen arsenate	7 days	Hydroponic culture, 25 °C, 12 h day (300 μmol m^2 s^{-1} PAR)	Roots	DNA microarray	Norton et al. (2008a, b)
Rice (*O. sativa*) cultivar TN67	As(V) as 25 μM Na2HAsO4•7H2O	1 and 3 h	26 °C	Roots	DNA microarray	Huang et al. (2012)
Arabidopsis thaliana ecotype Columbia	As(V) as 100 μM potassium arsenate	3 days, 10 days	Solid MS medium, 25 °C, 16 h day	Whole plant	DNA microarray	Abercrombie et al. (2008)
Medicago truncatula (Gaertn.) cultivar Jemalong	As(III) as 25 μM sodium arsenite	5 days	Solid BNM medium, 22 °C (day):16 °C (night), 16 h day	Roots	DNA microarray	Lafuente et al. (2015)
Purple willow (*Salix purpurea*) cultivar Fish Creek	As(V) as 0.5 mg L^{-1} (67 μM) Na_2HAsO_4•$7H_2O$	14 days	Hydroponic, ¼ Hoagland medium, 18–25 °C, 18 h day (500 μmol m^2)	Roots, stems, leaves	RNA-seq	Yanitch et al. (2017)
Indian mustard (*Brassica juncea* L.) Czern. var. TPM-1	As(V) as 500 μM Na_2HAsO_4	4 h (roots only), 24 h, 96 h	Liquid ½ Hoagland medium, 25 ± 2 °C, 14-h day (150 μmol m^{-2} s^{-1})	Roots, shoots	DNA microarray	Srivastava et al. (2015)
Crambe abyssinica cultivar BelAnn	As(V) as 250 μM Na_2HAsO_4•$7H_2O$	24 h	½ MS liquid medium, 22 °C, 16 h day	Whole seedlings	SSH	Paulose et al. (2010)

highest fold change in transcription; its gene was transcribed 146 times more in As(V) challenge conditions than in control seedlings. At the same time, As(V) challenge led to downregulation of many genes, often belonging to the same families as abovementioned upregulated genes. Among them were two glutathione-*S*-transferase genes, peroxidases, one MATE-efflux protein, one phosphate: H^+-symporter, two amino acid transporters, one ATP-binding cassette transporter, one zinc transporter, F-box proteins, U-box proteins, and protein kinases. Rice seedlings exposed to As(III) upregulated transcription of one glutathione-*S*-transferase gene, three glutaredoxin genes, ten peroxidase family-related genes, two metallothioneins, three heat shock proteins, some transporter-encoding genes (sulfate transporter, two metal transporters, multidrug resistance protein), a number of transcription factors, F-box protein genes, U-box protein genes, and some protein kinases. Similarly to the effect of As(V), downregulation of a number of genes encoding proteins of the same groups took place upon As(III) treatment.

Similar technology was employed in a study comparing an effect of As(V) on gene expression of two rice varieties: cultivars *japonica* (Azucena) and *indica* (Bala) (Norton et al. 2008a). Gene expression in roots of hydroponically grown plants exposed to As(V) challenge was analyzed (Table 14.1). Expression of 44% of genes was confirmed, with 1604 of probes significantly upregulated and 1828 probes downregulated in Azucena and 909 probes upregulated and 935 probes repressed in Bala plants. Of these 576 probes (460 genes) were significantly upregulated, and 622 probes (523 genes) were significantly downregulated in both Azucena and Bala samples. Three genes were found to be regulated in the opposite direction in the two plant variants: permease 1 gene was upregulated in Bala while downregulated in Azucena, and Bowman–Birk-type bran trypsin inhibitor precursor and cytochrome P450 CYP99A1 were both downregulated in Bala while upregulated in Azucena in response to As(V). Gene ontology categories were assigned to probe sets using WEGO tool (Ye et al. 2006). Upregulated genes comprised of genes encoding proteins involved in heat and toxin responses, the toxins biotransformation, sulfur, amines, organic acids, macromolecules and cellular catabolism as well as nitrogen biosynthesis categories. Downregulated genes were involved in transport; regulation of metabolism and cell size; metabolism of phosphorus, phenylpropanoids, and aromatic compounds; cellular morphogenesis; cell growth; and responses to auxin. In the transporter category, the phosphate: H^+ symporter gene *OsPT2* and an inorganic phosphate transporter were dramatically downregulated, as well as the transporters of chloride, ammonium, nitrate, sugars, amino acids, and peptides. Five sulfate transporter genes were upregulated under As(V) stress. Seven MATE transporter genes were upregulated, while two other MATE genes were repressed. A glutathione conjugate transporter gene, a gene annotated as a multidrug resistance-associated protein MRP2, and other possible ATP-binding cassette (ABC) family vacuole pumps were upregulated. The expression of a number of metal transporter genes was differentially regulated by As(V) treatment, including two Nramp1 genes, two potassium transporter genes, and a ZIP zinc/iron transporter gene. Genes encoding TIP and NIP types of aquaporins were found to be downregulated, while five major facilitator superfamily protein genes were upregu-

lated. Glutathione-*S*-transferases-encoding genes, the majority of them belonging to *tau* subfamily, were differentially expressed in As-exposed rice. Some of them were up to 30-fold upregulated compared to control plants. Three methyltransferase-encoding genes were found to increase their expression upon As stress, while some heat shock proteins and chaperones exhibited differential expression in these conditions. Gene-encoding peroxidases were mostly repressed, while cytochrome P450 genes were differentially expressed with 8 being upregulated and 12 genes being downregulated. Expression of genes encoding proteins involved in cell growth and cell cycle was downregulated by As(V), including expansins, tubulin, actin, and microtubule genes.

The effect of As(V) exposure on expression of early response genes in rice roots was studied by DNA microarray (Huang et al. 2012). 1690 genes were found to be upregulated after 3 h of treatment, and 698 were found to be downregulated. Gene ontology annotations showed that the most upregulated biological processes were response to heat, regulation of transcription, toxin catabolic process, secondary metabolic process, cellular lipid metabolism, and jasmonic acid- and ethylene-dependent systemic resistance. Among the most upregulated molecular functions in GO terms were glutathione transferase activity, transcription factor activity, ion binding, calcium ion binding, and oxidoreductase activity. Secondary cell wall metabolism was identified as a downregulated biological process. Sixty-six transporter-related genes were upregulated in As-treated cells, including ABC transporters and tellurite resistance/dicarboxylate transporters. Two phosphate transporters OsPT4 and OsPT19 were upregulated, whereas citrate transporter OsLsi2, aquaporin OsNIP2;1, and two sulfate transporter genes were repressed during early response to As(V) exposure. Transcripts of genes involved with oxidative stress response were identified among As-regulated genes. Among them genes encoding GSTs, glutaredoxins, alternative oxidases, monodehydroascorbate reductases, thioredoxins, peroxiredoxin, and respiratory burst oxidase homolog were upregulated, while class III peroxidase genes were differentially regulated. In the group of phytohormone-related genes, jasmonic acid, abscisic acid, cytokinin, and ethylene biosynthesis and signaling pathways showed significant upregulation. A number of signal transduction-associated genes were identified as As responsive. Ninety-five genes showed increase and 39 genes repression of expression. Receptor-like kinases were the major upregulated group. One MAPK and seven MAPKKK genes were also found to be upregulated under As stress. Among genes involved in calcium regulation calcium-dependent protein kinases, calmodulins and calmodulin-like proteins were upregulated. Two hundred thirty-one transcription factor genes were identified as regulated by As(V). They belong to several TF families, such as AP2/ERF (APET-ALA2/ethylene response factors), HSF (heat shock factors), ZIM (zinc finger proteins expressed in meristem), and MYB and WRKY. Cell wall metabolism was found to be the biological process downregulated at the transcript level in the early response of rice roots treated with As. Twenty-seven genes involved in cell wall biogenesis were repressed, while 13 were upregulated. Downregulated genes included genes encoding cellulose and beta-mannose synthase-like proteins, xyloglucan galactosyltransferases, xyloglucan xylosyltransferases, galactomannan

galactosyltransferases, xyloglucan hydrolases, glycosyl transferases, beta-galactosidases, glycoside hydrolases 9, and polygalacturonases. This work showed that As stress provokes dramatic changes in cell transcriptome in a very short time. Such a profound early response suggests significant toxicity of this metalloid toward rice root cells.

Another study utilized RNA sequencing technology to examine the response in roots and shoots of 14-day-old rice seedlings exposed to 20 or 80 μM As(III) for 6 or 24 h (Yu et al. 2012). *O. sativa* ssp. *japonica* cultivar Nipponbare was used as experimental material (Table 14.1). Authors have found that 7865 genes were differentially regulated upon As challenge in either time-dependent or dosage-dependent manner. It was also shown that roots responded to As dose, while shoots responded to the time of treatment in a more profound manner. It can be explained in a way that roots are the organs where the intake of metalloid takes place, and so As levels in root cells increase relatively quickly even when treated with lower concentrations. Shoot cells, on the other hand, need As to be transported in xylem, so As levels in shoot cells build up gradually with time. Genes involved in transport, phytohormone biosynthesis, and signaling and lipid metabolism along with transcription factors were found to be differentially regulated by As(III) in this study. In root samples, 27 genes were found to be differentially regulated during treatment with lower As(III) concentration, while 72 genes showed altered transcription when treated with high concentration of As(III). Among them a number of ABC transporter family G genes, P-type ATPase genes, phosphate transporter gene, metal transporter gene OsZIP8, nodulin 26-like intrinsic membrane proteins (NIPs: OsNIP3;2 and OsNIP1;1)-encoding genes, and genes encoding P-type heavy metal ATPases (HMAs) – OsHMA5, a xylem loading protein, and OsHMA9 which is a metal efflux protein – were all upregulated. Some of the aquaporin genes were downregulated in As(III) stress, while citrate transporter genes were either not responsive to this condition or also downregulated. A number of genes involved in jasmonate biosynthesis were upregulated in As(III)-stressed roots, indicating that this phytohormone is accumulated and plays a role in plant response to the heavy metal challenge, as was proposed before (Maksymiec et al. 2005; Maksymiec 2007). Possible accumulation of auxins, cytokinins, and ethylene, but not brassinosteroids, was also suggested by the analysis. Forty-two genes encoding proteins involved in lipid metabolism, including both prokaryotic and eukaryotic lipid biosynthesis pathway genes, were found to be differentially regulated upon exposure to As(III) in roots and 32 genes in shoots. Expression analysis of transcription factor (TF) genes revealed that high concentration of As(III) induces expression of more TFs than low As(III) concentration and that more genes are upregulated after 6 h of treatment than after 24 h. Downregulation of TF genes was also found to depend on As(III) dose. NAC and WRKY families of TFs are likely to be responsible for regulation of transcriptional response to As(III) challenge in rice roots. Very interestingly, this study also presented a short analysis of regulator miRNA molecules differentially expressed upon As(III) challenge. They found 13 upregulated and 12 downregulated miRNAs in roots and 20 upregulated and 10 downregulated miRNAs in rice shoots. The analysis of the biological significance of possible miRNA-mRNA

pairs indicated that there are 237 such pairs with possible significance in roots and 128 pairs in shoots, including transporter mRNA-miRNA pairs, lipid metabolism involved mRNA-miRNA pairs and jasmonate metabolism related mRNA-miRNA pairs. These results suggest that described changes in expression of certain genes might be regulated by coordinated actions of both transcription factors and miRNAs.

14.3 Transcriptomics of Arsenic Tolerance in Selected Dicot Plants

14.3.1 Arabidopsis thaliana

A massive amount of genomic and metabolomic data available for *A. thaliana* makes this species a perfect candidate to study the general metabolism of As in plant cells, although *A. thaliana* is neither economically nor industrially important plant. When whole-genome oligonucleotide DNA microarray technology was used to study changes in transcript levels of *A. thaliana* ecotype Columbia exposed to 100 μM potassium arsenate in in vitro cultures (Table 14.1), 46 genes were found to be upregulated in response to As, while 113 genes were deemed downregulated after applying stringent statistical criteria (Abercrombie et al. 2008). Gene ontology (GO) terms were assigned to identify genes using GO annotation bioinformatics tool available at the *Arabidopsis* Information Resource web. Most upregulated categories included unknown function, hydrolase, antioxidant, transferase, kinase, lyase, transporter, and binding activity. Downregulated gene ontology categories included unknown function, hydrolase, binding, transporter, kinase, transferase, and transcriptional regulator activity.

Among upregulated genes encoding proteins involved in antioxidant response were copper superoxide dismutases (CuSOD), peroxidases, and peroxiredoxin Q. Upregulated were also genes encoding *tau* class GST, transporter proteins (plasma membrane intrinsic protein 2B and tonoplast intrinsic protein gamma), metal ion-binding protein (metallothionein-like protein IA and ferredoxin), 5′-adenylylsulfate reductase (APR3), nitrate reductase I (NR I), leucine-rich repeat transmembrane protein kinase and cyclin-dependent protein kinase, cell wall-localized glycine-rich protein, cytochrome b_{561} family protein, and a number of unknown molecular function proteins (e.g., universal stress protein, pentatricopeptide repeat-containing protein, photoassimilate-responsive protein). Other transcript levels were found to be downregulated in As(V) challenge. Among them were genes encoding catalase 3, some peroxidases, FeSOD, lipoxygenase, cytochrome P450, two germin-like proteins, calcium-binding EF-hand family protein, calmodulin-related protein, ferritins 1 and 4, zinc finger proteins, glycosyl hydrolases, xyloglucan endotransglucosylases/hydrolases, lipase class 3 family protein, invertase/pectin methylesterase family protein, and others. Interestingly expression of allene

oxide cyclase involved in jasmonic acid synthesis was also downregulated. A number of transcription factors were repressed during As exposure, among them three WRKY DNA-binding protein family members, two zinc-finger proteins, a NAC (NAM, ATAF, CUC) domain-containing protein, DRE (dehydration-responsive element)-binding protein, and an AP2 (APETALA2) domain-containing transcription factor. This study clearly exhibited that arsenate treatment causes downregulation of expression of genes involved in phosphate starvation, since As may utilize phosphate transporters to get into plant cells.

14.3.2 Medicago truncatula

DNA microarray technology was also used to examine As(III) effect on transcript abundance in the roots of *M. truncatula* (Gaertn.) (cultivar *Jemalong*) in control conditions or inoculated with nitrogen-fixing bacterium *Ensifer medicae* MA11 (*Sinorhizobium medicae*) (Lafuente et al. 2015). Arsenic was provided to pregerminated seedlings in the form of 25 μM sodium arsenite; the exposure continued for 5 days (Table 14.1). Compared to control conditions, non-inoculated roots challenged with As(III) exhibited upregulated expression of 263 genes, while the expression of 528 genes was repressed. Among the upregulated genes were abiotic stress-related genes, such as ATP5a (mitochondrial ATP synthase subunit alpha), glutathione-*S*-transferase, germin-like protein, and 1-pyrroline-5-carboxylate synthase (P5CS) genes. Genes encoding transporter proteins including ABC transporter B family member, sulfate high-affinity transporter, root-specific metal transporters, and phosphate transporter 2 were also found to be overexpressed in As(III)-treated roots. Genes related to sugar metabolism, such as glucosyltransferase-13 and probable mannitol dehydrogenase genes, as well as secondary metabolism genes, like terpenoid synthase gene, isoflavone-7-*O*-methyltransferase gene, glucosyltransferase gene, *O*-diphenol-*O*-methyltransferase gene, and naringenin chalcone synthase gene, were upregulated, suggesting that the general biotic and abiotic stress response mechanism is activated in root cells during exposure to As (Vogt 2010). Downregulated genes included genes involved in As uptake, namely, the gene encoding silicon aquaporin at the plasma membrane NIP2-1, and cell wall biosynthesis- and architecture-related genes, such as pectin-esterase inhibitor genes, genes encoding extensins, and cell wall-specific peroxidases. The gene encoding a small subunit of Rubisco was also found to be repressed in treated root cells. Interestingly, inoculation of *Medicago* roots with nodule-forming bacteria caused a significant decrease in the number of upregulated stress response genes and flavonoid biosynthesis-related genes. It has also reduced the downregulation of cell wall architecture genes, photosynthesis-related genes, and genes involved in carbohydrate metabolism. Altogether the results of this study indicate that inoculation of *M. truncatula* with *E. medicae* had a mitigating effect on the stress imposed by the presence of As in growth medium. It is likely that microorganisms may be able to deal with at least a part of the stressor alleviating its effect on plant cells.

14.3.3 Salix purpurea

Hydroponically cultured purple willow (*S. purpurea*, cultivar *Fish Creek*) was examined in another RNA sequencing study (Yanitch et al. 2017). Stem cuttings of normalized length were cultivated for 2 weeks in Hoagland medium before the addition of 5 mg/L sodium heptahydrate arsenate, after which the treatment continued for another 2 weeks (Table 14.1). The transcriptomic analysis was conducted separately for leaf, stem, and root samples. Differential expression of genes was found not only between control and treated plants but also between different plant organs. Genes were assigned gene ontology terms using a PANTHER tool (Thomas et al. 2006; Mi et al. 2013, 2017). It revealed that in root cells most upregulated gene categories were catalytic activity, transferase activity, biological regulation, carbohydrate metabolism, oxidoreductase activity, cell cycle, and peptidase activity. Most repressed gene categories in roots were protein metabolic processes, RNA-binding protein, translation, structural molecule activity, and ribosomal activity. Root cells can deal with As presence in the environment by decreasing its uptake via the decrease of the number of transporters and aquaporins capable of capturing the As ions and/or by activating intracellular detoxification mechanisms, such as efflux, conjugation, and storage in the vacuole. The transcription of a gene encoding phosphate transporter PHO1 and three genes encoding aquaporin NIP1;1 was upregulated, while the transcription of a gene encoding aquaporin TIP2 was repressed. A number of ABC transporter transcripts were found to be differentially regulated in As(V) challenge with 8 transcripts exhibiting increased abundance and 19 transcripts showing reduced abundance, compared to the control samples. Two vacuolar cation/proton exchanger 2 protein (CAX2) transcripts were upregulated. Glutathione synthase, GST, and phytochelatin synthetase transcripts were found among the ones increased by the treatment. Seven transcripts (from five genes) of S-adenosyl methionine-dependent methyltransferase (SAM-dependent methyltransferase) were upregulated, while six other transcripts (from three genes) were downregulated in this experiment.

Among 15 most abundant gene ontology terms in stem cells, only ubiquitin-protein ligase and steroid metabolism were upregulated, while binding, kinase activity, protein phosphorylation, translation, protein kinase receptor, ribosomal protein, structural constituents of the ribosome, and cellular amino acid catabolic process were all repressed. Upregulated transcripts included a silicon transporter and CAX2 transporter, catalytic subunit 9 of cellulose synthase A, sucrose-phosphate synthase, and salicylic acid carboxyl methyltransferase. Downregulation of expression of 2 other CAX exchangers, 31 ribosomal proteins, 2 cellulose synthase catalytic subunits and a cellulose synthase-like protein, callose synthase, and proteins related to ethylene biosynthesis was reported.

In leaves most upregulated gene ontology categories were hydrolase activity, transporter activity, lipid metabolic process, biological regulation, transcription factor, regulation of nucleobase-containing compound metabolic process, and cellular amino acid biosynthesis. Downregulated groups of genes included RNA-binding

protein, translation, structural molecule activity, and ribosomal protein. A number of transporter genes were found to be differentially regulated in leaves. PHO1 phosphate transporter; Na-dependent phosphate transporter; boron transporter; aquaporins NIP, NIP1;1, TIP1, and SIP1; three CAX2 vacuolar exchangers; and 20 ABC transporters were all upregulated, whereas PHO1-like transporter, one aquaporin of NIP subfamily, two CAX1 proteins, and four ABC transporters were downregulated. Cadmium-induced protein and cadmium resistance protein were also upregulated. Unlike in root cells, phytochelatin synthase transcripts exhibited decreased abundance in As-treated leaves. Very interesting is the differential expression of many genes involved in flavonoid biosynthesis in As-treated willow leaves. Transcripts of genes encoding chorismate mutase, cinnamate-4-hydroxylase, 4-coumarate: CoA ligase, chalcone synthase, chalcone-flavanone isomerase, flavanone-3-hydroxylase, flavonoid-3′,5′-hydroxylase, flavonoid-3′-hydroxylase, dihydroflavonol-4-reductase, anthocyanidin reductase ANR1–1, anthocyanidin synthase, and a leucoanthocyanidin reductase were all upregulated, while abundance of transcripts of chalcone-flavanone isomerase, dihydroflavonal-4-reductase, and flavonol synthase were downregulated. Such a massive upregulation of secondary metabolite biosynthesis in As-stressed leaves may indicate increased oxidative stress in these organs or induction of a general stress response mechanism.

14.3.4 Brassica juncea

A DNA microarray study was also conducted on a plant commonly used in soil phytoremediation to remove heavy metals, such as lead or cadmium, i.e., Indian mustard (*B. juncea* L.). Exposure to As(V) (Table 14.1) highlighted the importance of hormones and kinases in As-dependent signaling of this species (Srivastava et al. 2015). Differential expression of many transporter-encoding genes was additionally reported, namely, genes encoding major intrinsic protein family members (NIP2;1, TIP2, PIP1;2, PIP1;4, PIP2;1 PIP2;2), ABC transporter family proteins (ABCB4, ABCC4, ABCF4, ABCG27, and ABCG32), and mitochondrial transporters (phosphate transporter PHT3;2, dicarboxylate transporter 1, and dicarboxylate carrier 1). Metabolism of several phytohormones was found to be altered during As challenge. Jasmonic acid-related genes were differentially expressed. Allene oxide cyclase 4 and two jasmonate-zim-domain proteins (JAZ1 and JAZ5) were downregulated in roots, 12-oxophytodienoate reductases were upregulated in both roots and shoots, and jasmonate resistance one gene was downregulated in shoots. Genes involved in abscisic acid signaling were induced (ABA-induced PP2C1, ABA-insensitive 1, and ABA-interacting protein 2). Auxin-related differentially regulated genes included those of auxin-responsive proteins from shoots and roots and were upregulated. Two auxin efflux carrier proteins (PIN3 and PIN6) were downregulated in shoots. Farnesoic acid carboxyl-*O*-methyltransferase (FAMT), a gene from salicylic acid biosynthetic pathway, was upregulated in both roots and shoots. A large number of transcription factors representing various families were found to be differentially

regulated by As. A total of 12 redox-related genes were described, of which seven were upregulated and five were downregulated, including monothiol glutaredoxin 17, glutathione peroxidase 6, monodehydroascorbate reductase, and copper/zinc superoxide dismutase 1 in roots and glutathione peroxidase 3 and iron superoxide dismutase 2 in shoots. Genes encoding proteins comprising mitochondrial electron transport chain showed significant upregulation in both roots and shoots, indicating that alteration of energy requirements imposed by cell need to respond to the presence of As puts electron transfer chain under significant stress.

14.3.5 Crambe abyssinica

The last study discussed here was conducted on *C. abyssinica* (cv. *BelAnn*), an oilseed-producing plant that was shown to be a heavy metal accumulator (Paulose et al. 2010). In this experiment 10-day-old crambe seedlings were exposed to 250 μM sodium arsenate. At this As(V) concentration plants showed a significant decrease in biomass, as compared to control plants, but no severe toxicity symptoms. After 24 h of treatment, plants were harvested, frozen, and used for further experimental steps (Table 14.1). One hundred five transcript clones were obtained that represented 38 unique coding transcript sequences. Identified proteins encoded by differentially regulated genes indicated As(V) effect on metabolic pathways related to oxidative stress, defense, ion transport, sulfur assimilation, signal transduction, photosynthesis, and metabolism.

Glutathione-*S*-transferase transcripts comprised the largest group of differentially expressed genes identified in crambe cells. *Tau* and *phi* GST subfamily members were present in studied samples. Additionally, transcripts of other genes encoding proteins that may work together with GST in response to As(V) challenge were found to be differentially regulated, namely, monodehydroascorbate reductase (MDAR), adenosine phosphosulfate kinase (APSK) and adenosine phosphosulfurylase reductase (APR), and sulfite reductase (SiR). Expression of ABC transporter proteins was also found to be differentially regulated including multidrug-resistant proteins (MRPs) and yeast cadmium factor1 (YCF1). Other membrane transporter gene transcripts identified in SSH experiment were MATE family drug transporter and putative cation transporter-associated protein (ChaC). Transcripts of genes encoding proteins involved in oxidative stress response aldo/keto reductase (AKR) and peptide methionine sulfoxide reductase (PMSR) were found to have altered expression in As(V)-treated plants, as well as a gene encoding oxophytodienoate reductase (OPR) involved in jasmonate synthesis and serine palmitoyl transferase (SPT) implicated in sphingolipid biosynthesis. Three proteins connected to ubiquitin-mediated protein degradation pathway were deemed differentially regulated between control and study sample, namely, 20S proteasome beta subunit, ubiquitin 14 (UBQ14), and an ubiquitin-associated (UBA)/TS-N domain-containing

protein. Other identified transcripts encoded glucosidases, heat shock proteins, defense-related protein, pathogenesis-related protein, iron ion-binding oxidoreductase, and a number of proteins of unknown function.

14.4 Plant Response Mechanisms as Seen by Transcriptomic Analyses

Transcriptomic attempts to unravel the mechanism of plant resistance in the presence of As species are presented in this chapter. Plants used as model organisms in these studies are classified in different families (*Poaceae*, *Brassicaceae*, *Salicaceae*, *Fabaceae*). Some of them, like rice and crambe, are effective As accumulators, whereas others (i.e., *A. thaliana*, willow, Indian mustard, and *Medicago*) do not accumulate this metalloid. Despite the differences, there is a rough pattern of gene expression in examined plants that indicate the involvement of a general biotic and abiotic stress response mechanism (Fig. 14.1). Similar mechanisms are engaged when plants are exposed to other nonessential metals, excessive amounts of essential metals, or xenobiotics.

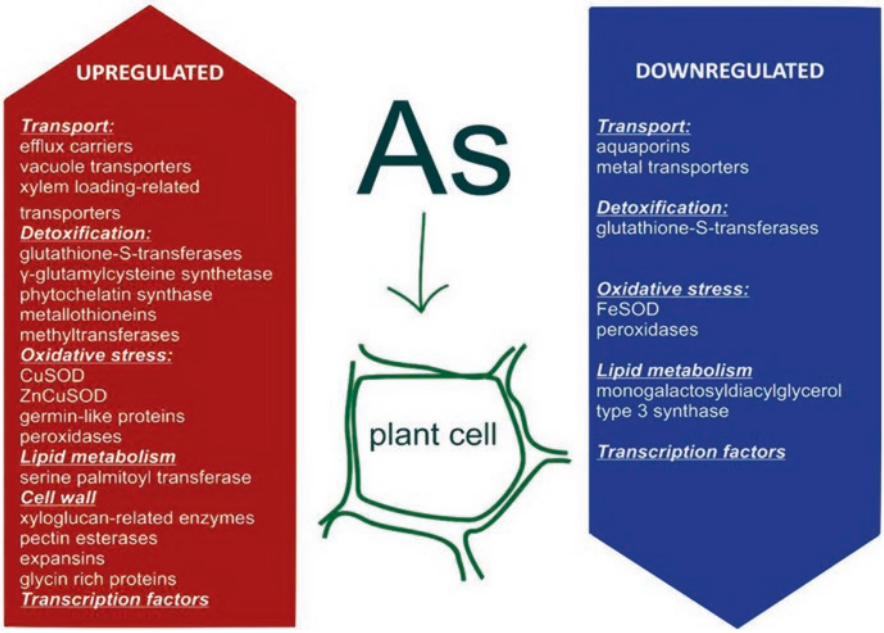

Fig. 14.1 Schematic representation of cell processes differentially regulated by arsenic challenge. Particular proteins encoded by up- or downregulated genes are listed when feasible

14.4.1 Arsenic Transport

Arsenic enters the protoplasm through roots, and these organs are the first to activate their protection arsenal, as shown by Yu et al. (2012). As(III) and As(V) differ in the influx mechanism. As(III) is most probably taken up by silicon transporters, while As(V) utilizes the phosphate route (Ma et al. 2006, 2007, 2008). To prevent As uptake, root cells can downregulate the expression of plasma membrane transporters capable of introducing As into the cytoplasm (Hartley-Whitaker et al. 2001) and upregulate different types of efflux pumps (Lee et al. 2007; Kim et al. 2007; Yu et al. 2012). The expression of transporter-encoding genes was found to be differentially regulated by As in all of the presented transcriptomic studies. Downregulation of NIP2;1, similar to Lsi1 rice transporter, was reported by Lafuente and Huang (Huang et al. 2012; Lafuente et al. 2015), while the same transporter was found to be upregulated during As treatment by Srivastava (Srivastava et al. 2015). Differential regulation of many aquaporin-encoding genes was shown (Yu et al. 2012; Huang et al. 2012; Srivastava et al. 2015; Yanitch et al. 2017) as well as upregulation of MATE extrusion pumps was found (Norton et al. 2008b; Paulose et al. 2010; Srivastava et al. 2015). Another group of transporters transcriptionally regulated by As is the ABC transporters that may be involved in metal export or vacuolar sequestration (Kim et al. 2007). ABC transporter involvement in plant response to As stress was described on transcriptional level (Norton et al. 2008a; Yu et al. 2012; Lafuente et al. 2015; Srivastava et al. 2015; Yanitch et al. 2017). From the root As can be transported to plant aboveground organs (root-to-shoot transport) (Deng et al. 2013). One of the proteins responsible for xylem loading, OsHMA5, was found to be upregulated in rice (Yu et al. 2012).

14.4.2 Detoxification in the Cell

Once inside the cell, the majority of As(V) is reduced to As(III) by As reductase (AR) (Bleeker et al. 2006; Dhankher et al. 2006). Then As(III) may undergo conjugation with glutathione (GSH) and phytochelatins (PCs) (Grill et al. 1987; Sneller et al. 1999; Mendoza-Cózatl et al. 2005). Such conjugates are sequestered in the vacuole, as a final step of detoxification. GSH is synthetized in an ATP-dependent process by γ-glutamylcysteine synthetase and glutathione synthetase that were found to be differentially regulated by As (Huang et al. 2012; Yanitch et al. 2017). GSH is the cell largest reservoir of nonprotein thiol groups (Mendoza-Cózatl et al. 2005); thus its synthesis strongly relies on sulfur assimilation. Genes encoding proteins involved in sulfur metabolism were reported to be upregulated (Norton et al. 2008a; Paulose et al. 2010; Lafuente et al. 2015; Srivastava et al. 2015). Phytochelatins (PCs) are small cysteine-rich peptides synthetized by phytochelatin synthase from GSH, which are capable of metal chelation (Grill et al. 1985; Grill et al. 1989; Cobbett 2000; Schmöger et al. 2000). Synthase-encoding genes were

found to be differentially transcribed in the presence of As (Huang et al. 2012; Yanitch et al. 2017). Both phytochelatin synthase and vacuolar transporters were shown to be upregulated to increase sequestration of metal ions, thereby removing it from the cytoplasm (Grill et al. 1987; Song et al. 2010; Tripathi et al. 2007). Another group of peptides that can bind metal ions are metallothioneins (MTs), relatively small cysteine-rich molecules (Hassinen et al. 2011). Their expression was shown to be correlated with metal accumulation in plant tissues (Hassinen et al. 2009; Zimeri et al. 2005) and with As treatment (Yu et al. 2012; Chakrabarty et al. 2009). Glutathione-*S*-transferases are a diverse family of enzymes that exhibit As-dependent expression in each of described experimental studies (Abercrombie et al. 2008; Norton et al. 2008a; Chakrabarty et al. 2009; Paulose et al. 2010; Yu et al. 2012; Huang et al. 2012; Lafuente et al. 2015; Yanitch et al. 2017); moreover in several studies, GST-encoding genes constituted the biggest group of detected transcripts. This protein family is divided into eight classes of which *tau* and *phi* are involved in detoxification of xenobiotics and are usually stress-responsive (Marrs 1996; Dixon et al. 2002; Dixon and Edwards 2015). One possible mechanism of action of GSTs in the presence of As is performing conjugation of the metalloid with glutathione (Pandey et al. 2015). Another way plant may use GSTs is to mitigate oxidative stress caused by As (Schutzendubel and Polle 2002). It was shown that metals can induce oxidative stress (Gallego et al. 1996; Hartley-Whitaker et al. 2001; Pinto et al. 2003) and that GSTs take part in cell response to oxidative stress (Cummins et al. 1999; Kilili et al. 2004). Transgenic plants overexpressing GSTs are more resilient to oxidative stress conditions (Roxas et al. 2000; Zhao and Zhang 2006; Ji et al. 2010); moreover *tau* class GST from tomato expressed in yeast conferred yeast resistance to oxidative damage (Kampranis et al. 2000). GSTs may also be responsible for the transfer of phytochemicals between cell compartments (Edwards et al. 2000). Arsenic methylates are found in some plant species, but it is controversial whether plants can methylate As themselves or if they take it up from soil microorganisms (Lomax et al. 2012). If plants were performing the methylation reaction, then a good candidate enzyme would be SAM-dependent methyltransferases, which expression was shown to be upregulated in the presence of As (Norton et al. 2008a; Srivastava et al. 2015; Yanitch et al. 2017). It was recently shown, however, that rice was only able to methylate As after transformation with fungal WaarsM methyltransferase gene from *Westerdykella aurantiaca* (Verma et al. 2018).

14.4.3 Oxidative Stress Response

Nonessential metals, such as As, present cells with oxidative stress (Singh et al. 2006). A number of genes encoding proteins typically involved in oxidative stress response were found to be differentially regulated by As in all examined plant species. For example, genes encoding CuSOD and ZnCuSOD were found to be upregulated by As challenge (Abercrombie et al. 2008; Srivastava et al. 2015), while genes encoding FeSOD were found to be upregulated in shoots of *B. juncea* (Srivastava

et al. 2015) and downregulated in *A. thaliana* (Abercrombie et al. 2008) in similar conditions. It should be noted, however, that *B. juncea* experiment was carried on root and shoot material separately, whereas *A. thaliana* samples were prepared from whole plants (Table 14.1). As it was shown, plant organs respond with specific expression patterns and temporal regulation to As treatment (Yanitch et al. 2017). Germin-like proteins were also found to be differentially regulated in the presence of As (Abercrombie et al. 2008; Chakrabarty et al. 2009; Lafuente et al. 2015). These are metal-binding glycoproteins associated with extracellular matrix that usually have oxalate oxidase activity, often supplemented by SOD or phosphodiesterase activity (Bernier and Berna 2001; Nakata et al. 2004; Lu et al. 2010). They are known to accumulate in response to variety of stress conditions, such as bacterial, viral, fungal infections, parasite attacks, xenobiotic and chemical toxicity, and salt and drought stress (Hurkman et al. 1991; Hurkman et al. 1994; Schweizer et al. 1999; Lane 2002; Lou and Baldwin 2006; Zimmermann et al. 2006; Manosalva et al. 2009; Wang et al. 2013). Peroxidases, hydrogen peroxide scavenging enzymes, comprise another group of proteins differentially regulated by As treatment (Asada 1992; Yoshida et al. 2003). Expression regulation pattern seems to be very complex depending on plant species, organ examined, As dosage, and time of treatment (Abercrombie et al. 2008; Norton et al. 2008a; Chakrabarty et al. 2009; Huang et al. 2012; Lafuente et al. 2015; Srivastava et al. 2015). It might be connected with the fact that these enzymes are involved in a variety of cell processes including reactive oxygen species scavenging but also defense, auxin signaling, and cell wall metabolism (Kawano 2003; Passardi et al. 2004; Correa-Aragunde et al. 2015). Despite the complicity of plant response, it is clear that excess levels of As promote differential regulation of oxidative stress-responsive genes.

14.4.4 Hormonal Regulation

Hormonal regulation is crucial for plant ability to acclimate to variable environmental conditions (Peleg and Blumwald 2011) such as nonessential metal exposure, as indicated by transcriptomic analyses. Differential regulation of expression of genes involved in jasmonate (JA) metabolism was reported in several studies (Abercrombie et al. 2008; Chakrabarty et al. 2009; Paulose et al. 2010; Yu et al. 2012; Huang et al. 2012; Srivastava et al. 2015). JA and methyl-JA are active metabolites with roles in cell wall metabolism, defense against herbivore attack and other biotic and abiotic stress factors, and induction of phytoalexin production (Rakwal et al. 1996; Tamogami et al. 1997; McConn et al. 1997; Thomma et al. 1998). It was suggested that JA might act as a regulator of sulfate assimilation pathways in order to enhance As complexation by phytochelatins (Srivastava et al. 2009). It was also shown that JA activates GSH biosynthesis genes in *A. thaliana* (Xiang and Oliver 1998). JA is known to act together with ethylene in the induction of plant defense mechanisms (Penninckx et al. 1998; Lorenzo et al. 2003). Genes encoding proteins involved in ethylene signaling were also found to be among the ones regulated by As.

Downregulation of such genes was described in willow (Yanitch et al. 2017), whereas in rice they were found to be upregulated (Yu et al. 2012) or differentially regulated (Huang et al. 2012). The increase in expression of an ethylene-responsive transcription factor in rice upon exposure to As(V) was also reported (Chakrabarty et al. 2009; Huang et al. 2012). Salicylic acid (SA) is another phytohormone which seems to play an important role in plant response to As(V) challenge (Chakrabarty et al. 2009; Srivastava et al. 2015). It is not surprising given that it is known as a regulator of ion transport (Raskin 1992; Hayat et al. 2007). Transcriptome analyses showed that genes related to metabolism and physiological actions of abscisic acid, auxins, brassinosteroids, and cytokinins were also regulated by As. It is clear that the response to high As levels is coordinated at an organism level, not just a cell level.

14.4.5 Lipid Metabolism

There are several reports indicating As-dependent regulation of the expression of genes involved in lipid metabolism (Abercrombie et al. 2008; Paulose et al. 2010; Huang et al. 2012; Yu et al. 2012; Srivastava et al. 2015; Lafuente et al. 2015). However, not all transcriptomic studies were able to detect these alterations. Gene encoding serine palmitoyl transferase, an enzyme from sphingolipid biosynthetic pathway, was shown to be upregulated by As(V) in *C. abyssinica*. In *A. thaliana* repression of monogalactosyldiacylglycerol type 3 synthase was reported under As(V) stress. As(III) seems to have a stronger impact on lipid metabolism in plants. In *M. truncatula* two lipid-related genes were found to be regulated under As(III), namely, a non-specific lipid transfer protein was upregulated, while a gene involved in lipid metabolism during nodulation, MtEnod8.1, was repressed. Using RNA-seq technology, 59 lipid biosynthesis and metabolism genes differentially regulated by As(III) were identified in rice (Yu et al. 2012). The pattern of up- and downregulation remains quite complex, but the importance of adjustments of biological membrane composition upon As(III) challenge seems clear.

14.4.6 Transcription Factors

Transcription factors (TFs) comprise a significant number of differentially regulated genes identified by transcriptomic methods. It does not come as a surprise considering the number of genes involved in the systemic response to As exposure. General need to rearrange expression patterns during stress creates demand for the action of TFs. TF families bHLH (basic helix-loop-helix), BZIP (Basic Leucine Zipper Domain), MYB (myeloblast DNA-binding domain), WRKY (Wrky DNA-binding proteins), RAV (related to ABI3/VP1), ERN (ethylene-responsive), NAC (NAM, ATAF, and CUC), and WOX (WUS homeobox containing) were among the affected by As treatment.

14.4.7 Cell Wall Reorganization

The cell wall is able to bind certain metal cations present in the environment. For this purpose, it undergoes remodeling during challenge with metals and metalloids, including polysaccharide modifications (Krzesłowska 2011). Differential regulation of expression of genes related to cell wall and polysaccharide metabolisms, such as xyloglucan-related enzymes, pectin esterases, expansins, and glycine-rich proteins, was discovered by transcriptome analyses (Abercrombie et al. 2008; Chakrabarty et al. 2009; Huang et al. 2012; Lafuente et al. 2015). Some peroxidases, already mentioned as oxidative stress-responsive, are also involved in cell wall structural reorganization via decreasing the cross-linking of cell wall compounds (Passardi et al. 2005).

14.4.8 Interaction with Microorganisms

The most common resistance mechanisms that developed in bacteria in response to As challenge are As(V) to As(III) reduction and As efflux. These processes depend on the presence of *ars* genes on plasmid or chromosome (Kruger et al. 2013). Transcriptomic data from *Rhizobium* sp. NT-26 (Andres et al. 2013), *Herminiimonas arsenicoxydans* (Weiss et al. 2009; Cleiss-Arnold et al. 2010), and *Geobacter* strains (Dang et al. 2017) in the presence of As(III) and *Enterobacteriaceae* LSJC7 (Zhang et al. 2016) in the presence of As(V) demonstrated that this metalloid regulates expression of a number of genes involved in many metabolic processes. As it was previously reported, the interaction of plants with microorganisms in rhizosphere modulates metal toxicity (Fitz and Wenzel 2002; Wenzel et al. 2003). Transcriptomic data confirm this observation on the level of transcript accumulation (Lafuente et al. 2015). Inoculation of *M. truncatula* with *Ensifer medicae* led to decreased response to As in terms of gene expression modulation. When compared to non-inoculated plants, inoculated samples exhibited a reduced number of differentially regulated genes and suppressed susceptibility to As. It is supposed that microorganisms present in rhizosphere uptake and detoxify a pool of available metalloid, thus reducing the extent of adverse effect posed by the stressor.

14.5 Concluding Remarks

The picture of plant response to As on a transcriptome level seems to be the one of a systemic nature, involving a variety of mechanisms to fight metalloid itself, as well as the secondary effects of its presence in the cell (Fig. 14.1). This consists of changes in hormonal signaling, remodeling of transcription factor assemblage, activation of the oxidative stress response, and others. Transcriptomic analyses produce a huge amount of data of which only a fraction can be currently understood and

appreciated. With the accumulation of such datasets, the need will grow for synthetic meta-analyses of co-regulated gene clusters but also for experimental verification of the significance of particular transcripts.

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Chapter 15
Agronomics Management for Arsenic Stress Mitigation

Anil Barla, Sreehari Sathyavelu, Fathima Afsal, Megha Ojha, and Sutapa Bose

Abstract Arsenic (As) accumulation in rice is considered as a new recognized disaster in Southeast Asia regions like Vietnam, Bangladesh, China, etc., where rice is considered as staple food. Rice cultivation required a huge volume of water for irrigation purposes. In Bangladesh and West Bengal, India, rice cultivation is mostly dependent on shallow contaminated groundwater tube well. Rice accumulates more As compared to other plants because they are grown mostly in flooded/anaerobic conditions. Rice accumulates As from the contaminated soil and also from the groundwater used for irrigation purpose. Exposure of high concentration of As through ingestion (mainly drinking water and eating contaminated rice) for long time span may affect the human health including cancers, melanosis, hyperkeratosis, lung disease, peripheral vascular disease, hypertension, and heart diseases. About one-third of the population of these regions is affected and suffers from various diseases. Widespread use of groundwater for irrigation suggests that ingestion of crops so produced could be a major source of As poisoning. Arsenic uptake by the rice plants via root tissues enters into plant body and edible parts (grains). Previous findings had reported rice grain samples with As accumulation much higher than the permissible limit (1 ppm) recommended by WHO. Arsenic uptake by rice plants depends on the plant species, physiochemical properties of the soil, redox conditions, and fertilization methods. Increase in concentration of phosphate in the soil through fertilization or by any other method could result in lower arsenate uptake by the plants because phosphate and As compete for the same transport protein (OS PHF1), and this results in competitive inhibition. Similarly, the use of silicon fertilizers results in lower arsenite uptake by the plants. In agricultural fields, providing aerobic conditions at regular intervals, selection of As-resistant

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rice varieties, and adoption of proper mitigation measures can reduce the bioaccumulation of As in rice plants.

Keywords Arsenic · Arsenite · Cytotoxicity · Silica fertilization · Thiourea

15.1 Introduction

Rice is a solid staple, palatable food and is a unique crop cultivated by 3.5 billion people almost cultivated in every continent except Antarctica (IRRI 2013). Rice is the substantial part of the nutrition intake of the people living in Asia Pacific region, Latin America, and the Caribbean and also has increased profoundly in sub-Saharan Africa (FAO 2004). On an average, more than 715 million tons of paddy rice are grown across 100 countries (FAO 2013) with India and China accounting for 50% of the total production (FAO 2006). With the onset of the green revolution, the research and development of technology happened between 1930s, and in the late 1960s, the agriculture increased productivity particularly among the developing countries and the yield of rice tripled prior to the green revolution period. With the recent changes in the global climatic patterns, water management and farming practices have become the key entry point in determining the socioeconomic standard of the population across countries like India and Bangladesh in Southeast Asia. India arose from the brink of the massive famine in 1961 and is now the forefront of the green revolution (IRRI 2016).

The green revolution enhanced the process in the usage of groundwater to a greater extent for the purpose of the paddy irrigation across India and Bangladesh during the dry season (boro). The most densely populated Ganga–Brahmaputra basin poses aquifers that are contaminated with arsenic (As). The source of As in the groundwater is believed to be due to geological changes leading to leaching of As due to oxidation of arsenopyrite or reduction of iron and manganese oxy hydroxide (Smedley and Kinniburg 2002). The As-contaminated groundwater is also the source of drinking water for about 90% of the total population of Bangladesh (WHO 2007) and used for cooking rice (WHO 2001). The use of As-contaminated groundwater for both cooking and irrigation of paddy field could be the major source of exposure of the human body to As. This chapter elaborately deals with the present strategies of As mitigation in a soil-aqueous phase that certainly reduces the chance of As translocation from soil to rice plant and the dependent consumers. Health impacts of As on humans and cattle have been summarized here showing the evidence of health adversity.

15.2 Impact and Causes of Arsenic Accumulation in Rice

The accumulation of As in rice is determined by the plant and soil (Abedin et al. 2002b). The rate of accumulation of As is varied between the different species of rice grown in different regions (Williams et al. 2005). The rice grown in the paddy fields

irrigated with As-contaminated water in a naturally or anthropogenic way like mining and industrial effluents create a threat to the population of West Bengal in India, Bangladesh, and Southeast Asia (Duxbury et al. 2003; Meharg and Rahman 2003). Rice is unique food used to cook porridge and has ten times elevated higher concentration than all other grain crops (Meharg et al. 2008; Williams et al. 2007a).

The intake of As in rice is in various forms as As exists as different chemical species under a wide range of pH and soil condition (Barla et al. 2017). The various forms of As species include inorganic (arsenite and arsenate) and organic species like monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and tetramethylarsonium acid (TMA); arsenobetaine (AB); and arsenosugar (Aposhian et al. 2004). Apart from these there are trivalent MMA and DMA (III) as intermediates of As methylation pathways. The combine effects of contamination of groundwater and rice pose a serious threat to both the water and human resources.

The chronic exposure to arsenate and arsenite has serious implications such as hypertension, diabetes, and premature birth. Around 100 million people suffer in the world through elevated As drinking water supplies (NRC 2001; WHO 2004; Ravenscroft et al. 2009). The consumption of rice contaminated with As is the largest dietary source to the world's population. Around 22 in 10,000 Bangladeshi people suffer from bladder and lung cancer from lifetime exposures (EFSA 2009; Meharg et al. 2008). Apart from the direct dietary exposure, there are also transboundary concerns at global level due to trade (Meharg et al. 2009; Meharg and Raab 2010). The average rice consumption among the population of countries such as Bangladesh, Laos, and Myanmar is 400–500 grams per day (Meharg et al. 2008). UK Bangladeshi consumes 250 gm day^{-1} comprising 5% of total UK population (Meharg 2007). The biogeochemical study of paddy soil reveals that the rice crop grown under anaerobic condition uptakes As from the soil and water into grain through various pathways (Williams et al. 2007b; Xu et al. 2008).

15.3 Physiology of Arsenic Uptake in Rice

The accumulation of various As species in the rice leads to phytotoxicity and loss of the net productivity over a long time. The mechanisms and rate of uptake of the various forms of As species like As(III), As(V), and methylated As such as MMA and DMA depend upon the redox condition in the paddy fields during the growing season (Takahashi et al. 2004). The redox gradients are created between the rice root and the soil through the aeration of the rhizospheric zone by root hair to survive in the reduced environment. This leads to the formation of iron plaque between root surface and in the rhizospheric zone (Fig. 15.1) (Chen et al. 2005; Liu et al. 2006). The pH plays a key role in the protonation or dissociation of the As species and also their transport into the plant cell.

The arsenite and arsenate are redox sensitive and predominant under reducing and oxidizing condition (Zhao et al. 2010a) and interchange themselves by changing pH (Heimann et al. 2007). Arsenate reductase reduces the arsenate into arsenite

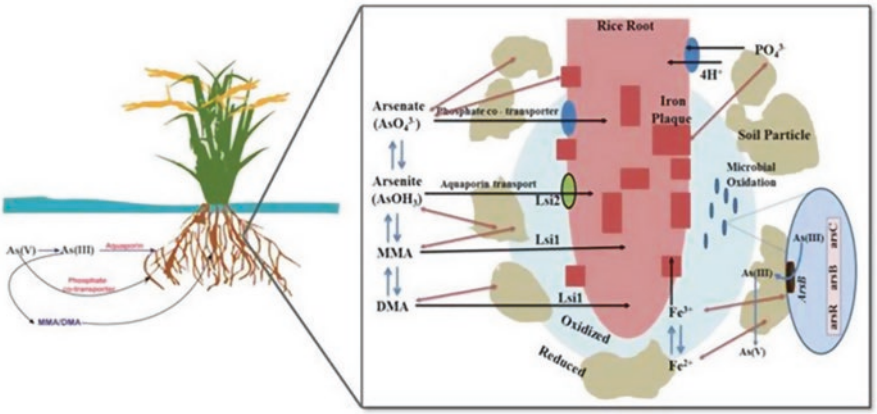

Fig. 15.1 Schematic diagram of arsenic (As) species in root zone of rice. Red arrow showed the adsorption of As species to soil and iron plaque. Black arrows denote the active plant transport of As into the plant. Blue color represents the oxygenation zone around the root due to ROL process. Arsenate enters into plant system through root via phosphate co-transporter, while arsenite enters via aquaporin transporter and Lsi1 transporter. Methylated As species also enters through Lsi 2 transporter

and later gets oxidized by certain microbes to produce energy for their metabolism (Rhine et al. 2006). Arsenite can also be methylated aerobically as well as anaerobically (Cullen and Reimer 1989). The uptake of As in plant bodies takes place through the silicic acid and phosphate pathways (Fig. 15.1) (Ma et al. 2008; Li et al. 2009a; Abedin et al. 2002a; Wu et al. 2011). The uptake of As species like arsenate, MMA, and DMA behaves like silicic acid and phosphate analogues (Li et al. 2009a; Karimi et al. 2009).

15.4 Accumulation of Arsenate into Plants

Arsenate uptake in plants shows analogue with the phosphate because they both belong to the same group (Group VB) in the periodic table. Plants are not capable of discriminating between arsenate and phosphate under starved condition; hence, the ability of uptake of both arsenate and phosphate increases (Lee 1982; Wang et al. 2002). The arsenate predominantly exists as $H_2AsO_4^-$ and $HAsO_4^{2-}$ with high affinity for iron hydroxides and manganese making it less mobile in soil. The studies reveal that under the starved condition, the expression of a phosphate transporter gene is enhanced (Raghothama 1999). The mutant studies performed in phosphate transporter gene show a correlation between arsenate and phosphate sharing a common pathway as the mutated genes involved in phosphate transporter showed tolerance to arsenate (Shin et al. 2004; González et al. 2005; Wu et al. 2011). The rate of

Table 15.1 List of genes encode proteins for phosphate transportation

Genes	Location	References
Ospht 1:6	Epidermal and cortical cell of rice plant	Jia et al. (2011)
Ospht 1:2	Stele of rice root	Jia et al.(2011)
Ospht 1:8	Roots, epidermal cells, root, and shoot	Jia et al. (2011), Wu et al.(2011)
Ospht 1:11	Arbuscular mycorrhizal symbiosis of rice roots	Paszkowski et al. (2002)

uptake of the phosphate and arsenate anions into the cells of the root system and their ability to transporter protein are determined using Michaelis-Menten kinetic parameter K_m and V_{max}. Here V_{max} is the measure of the activity of the transporter protein in the plasma membrane.

The V_{max} for the phosphate is two to three times higher than the arsenate (Wu et al. 2011) resulting in faster uptake of phosphate. The phosphate is usually in lower concentration near the rhizosphere (<10 μm); hence, the high-affinity transporter with low K_m plays a significant role in uptake.

Arsenate and phosphate have similar K_m values. The genome of rice poses 13 genes encoding high-affinity transporter protein named as Ospht 1:1, Ospht 1:13 expressed in different tissues and performs different roles of phosphate uptake (Ai et al. 2009) as listed below (Table 15.1).

15.5 Accumulation of Arsenite in Plants

The arsenite is not an essential nutrient to the plant but in anaerobic condition under the submergence in water provides the reducing environment to reduce arsenate to arsenite and can be precipitated as sulfide minerals such as arsenopyrite (Takahashi et al. 2004; Panaullah et al. 2009; Stroud et al. 2011). The studies on the mechanism of uptake of arsenite by roots reveal that the nodulin 26-like intrinsic proteins (NIPs) are involved (Meharg and Jardine 2003; Bienert et al. 2008; Isayenkov and Frans 2008; Ma et al. 2008; Kamiya et al. 2009).

The plant aquaglyceroporins (Nips) transports undissociated arsenite, and their selectivity for arsenite is relatively low compared to silicic or boric acid (Wallace et al. 2006; Mitani et al. 2011). The Si transporter named Lsi 2 is involved in the arsenite transport into the exodermal and endodermal cells of the roots (Mitani et al. 2011). The mutant studies reveal that Lsi 2 results in the decrease of the total As concentration in the shoots more than 90% (Ma et al. 2007; Ma et al. 2008). There exists a direct competition between Si and arsenite. There is an inhibition of the As accumulation in rice upon addition of silicic acid to the nutrient solution (Guo et al. 2007; Li et al. 2009b) as well as similarity in PK_a values of silicic acid and arsenous acid. The As accumulation is decreased in straw and grain after addition of silica gel as shown in a pot experiment (Li et al. 2009b).

15.6 Accumulation of Methylated Arsenic Species in Plants

The plants are incapable of methylating As, and hence the methylated As species must have been taken up via the root into the plant. The rate of uptake of MMA and DMA is lower than the inorganic form of As. It is transported more efficiently into xylem and phloem (Carbonell et al. 1998; Abedin et al. 2002c). The MMA and DMA are up taken as undissociated molecules through the aquaporin Lsi1 (Li et al. 2009a). The mutant studies revealed that Lsi1 is not only permeable to silicic acid but also to neutral molecules like MMA and DMA.

15.7 Unloading of Arsenic Species into Rice Grain

Arsenite is the predominant species of As in plants as they are able to reduce arsenate into arsenite rapidly (Pickering et al. 2000; Dhankher et al. 2002; Xu et al. 2007). The reduction of the arsenate into arsenite allows the plants to detoxify the arsenite as it shows high affinity to the thiol groups (-SH) of the cysteinyl residues. The proteins with the thiol groups are sensitive to the arsenate leading to enhancement or decrease in the catalytic function of enzymes. The mobility of As species is further enhanced by synthesis of phytochelatins (Zhao et al. 2009a). Although silicic acid and arsenite share the common pathway, the silicic acid has higher mobility compared to arsenite (Mitani and Ma 2005) in terms of root to shoot translocation; from the study on hydroponic rice, the arsenite was found to be in higher concentration in xylem sap even when supplied with arsenate (Wu et al. 2011). The uptake of the methylated arsenite results in the physiological disorder known as "straight head disease" under natural conditions (Iwamoto 1969; Belefant and Beaty 2007).

15.8 Impact of Arsenic on Human Health and Cattle

Arsenic is one of the biggest problems in Southeast Asian countries especially Bangladesh. Many districts of West Bengal also face the problem of As contamination. Exposure of human body to As could be due to consumption of As-contaminated water or rice. The As exposure leads to the chronic symptoms in the human body. Arsenic has an adverse impact both on physical and mental health of humans. High level of As accumulation can lead to cancer and other lethal diseases. Urinary As can act as a biomarker of As exposure (Chen et al. 2009). Chronic exposure of As could be seen as cutaneous abnormalities, which start with skin lesions and later end up taking the shape of a malignant tumor. Arsenic contamination causes various skin abnormalities along with skin cancer. The International Agency for Research on Cancer (IARC) has listed As as a human carcinogen since 1980 (Chen et al. 2009).

Arsenic contamination has an equally severe impact on human social life as on his physical body. Disease associated with As can cause problems like social hazards, poverty, social instability, superstition, and ostracism. It is also associated with poor mental health and physical disabilities. The social pressures and discriminations can lead an individual to slip into depression.

The susceptibility to As toxicity is related to its biological transformation after entering into the human body. Arsenic is first reduced in blood to arsenous acid and later is methylated in the liver where SAM acts as a methyl group donor. The concentration of inorganic As, MMA, and DMA in the urine can reveal the measure of exposure of As through various means, and also their relative amount can give the idea of the relative rate of metabolism in the body. MMA is extremely toxic and is associated with several As-related diseases and symptoms. Very few studies have been done on the effect of As toxicity on maternal health. Increased As concentration is associated with increased systolic pressure in women 6 weeks post-partum. Arsenic exposure during pregnancy causes anemia which increases in intensity during the later phase of pregnancy. Studies of pregnant women exposed to As via drinking water in Argentina showed As concentrations in cord blood similar to those in maternal blood, a finding recently confirmed in Bangladeshi women (Vahter 2009). Both the inorganic and organic forms of As have been isolated from the placental cord. Pregnancy results in the increased methylation of inorganic As to the organic form; this can be supported by the fact that all the As isolated from the infant's urine and blood are as DMA. This means that the lesser toxic forms enter the infant's body from the mother's body even at a higher concentration of As exposure, but a recent study in Bangladesh has reported high MMA concentration in cord blood. Increased concentration of As inhibits the methylation, and so the fetus is exposed more to MMA and inorganic As. High As concentration can lead to impaired fetal growth. Breastfed children show a considerable degree of protection from the As poisoning; this is because breast milk contains various antioxidants which protect the infant from the As-induced oxidative damage. Taurine, found in abundance in breast milk, is a free amino acid, has antioxidant properties, and has potential to protect against As-induced cytotoxicity including lipid peroxidation in murine hepatocytes. Thio redox in levels also is high in breast milk. It is possible that these mechanisms contribute in an integrated way to the protection against certain As-induced developmental defects (Vahter 2009).

Recent year investigations are pointing toward the epigenetic effects of As at a very low exposure level; this is caused either by DNA methylation or histone acetylation. Epigenetic effects lead to an effect on the long-term fetal programming (Cooney 2001). Arsenic poisoning can result not only in fetal deformities but also in some cases stillbirths and spontaneous abortions. There have been various reports of As-induced childhood cancer from across the globe. Moore et al. (2002) studied childhood cancer incidence rates in Nevada at higher water concentrations, up to concentrations in the range of 35–90 $\mu g\ L^{-1}$ (Moore et al. 2002; Smith and Steinmaus 2009). Andrew et al. did a study in 2006, involving both in vitro and in vivo culture, showing that As exposure leads to decrease in the DNA repair mechanism. Their study supported the hypothesis that As acts through a co-carcinogenic mechanism

of action. Arsenic accumulates the most in the keratin-containing tissues like the hair, skin, and nails. The toxicity, therefore, can be easily seen in these tissues in the form of hyperpigmentation which can later transform into cancer or another lethal form of diseases.

The As toxicity is not just caused by direct exposure to As-contaminated water. The staple food of people of Southeast Asia contains a significant amount of As, and there is an increased exposure to As through rice consumption as well. Using As-contaminated water for irrigation and later cooking the rice grains increase the chance of As toxicity of people consuming the rice. With the increase in the concentration of As in rice grain and with an increase in the amount of rice consumption, daily input of consumption increases and thereby the toxicity. The varieties of rice influence the speciation of As in them and thereby differ by the amount of toxicity caused by their consumption.

A study conducted in Patna showed the neurological manifestations in people exposed to As: the level of As in their biological samples was high and patients showed distal hypoesthesia, distal paresthesia, and limb pains, although the prevalence of neuropathy was less in children up to 15 years (33.33%) compared to adults of 16–75 years (41.94%) (Chakraborti et al. 2016).

Arsenic acts as an endocrine disruptor and can alter gene transcription at doses as low as 0.4 μg L^{-1} arsenite. Different doses of As can affect hormone regulation in cells at different levels. Internal conditions of the human body affect gene expression, and also different organs in the body respond differently to As exposure. Inorganic As is diabetogenic in humans, but little is known about pathophysiological mechanisms (Chakraborti et al. 2016). Other than cancer, cardiovascular, kidney, liver, and neuropathy problems, there are various other major and minor discomforts caused by As poisoning (Kapaj et al. 2006). It includes irritability, lack of concentration, depression, sleep disorders, headaches, fatigue, skin itching, burning of eyes, weight loss, anemia, chronic abdominal pain, diarrhea, edema of feet, liver enlargement, spleen enlargement, cough, joint pain, decreased hearing, decreased vision, loss of appetite, and weakness (Chakraborti et al. 2016).

Arsenic poisoning does not only affect the human beings directly. Cattle are exposed to As by consuming the As-contaminated water and feeding on the husk and straw of rice which has a higher level of As when compared to that of grain. This results in the toxicity in cattle. The mechanism of As toxicity in cattle operates on the same basic principle of oxidative damage. Arsenic generates ROS which results in lipid peroxidation. Oxidative damage to hemoglobin has been shown to cause changes in its structure and function, resulting in denaturation, precipitation, and methemoglobin formation inside erythrocytes (Rana et al. 2010). In a study conducted by Rana et al. in 2010, As contamination in cattle was studied in detail. Arsenic in water in the study area was around 0.122 ppm which was significantly higher than the control, uncontaminated area. The As concentration in blood of cattle the contaminated zone was significantly higher than that for the control group. In the cattle population which were exposed to As, there was a decline in SOD and catalase activity as compared to that of the control population. Nitrite level was also found to be reduced. Oral exposure to As in cattle, through contaminated drinking

water, did not produce any specific clinical manifestation of As toxicosis as in human beings. But most cattle in the As-affected area showed general clinical disorders, such as pale mucous membranes of the eye, cachexia, reduced ruminal motility, and rough hair coat (Rana et al. 2010). All sorts of oxidative damage caused by ROS generated due to As toxicity, the effect on the blood of the cattle is the most severe. It includes erythrocytic oxidative damage, the structural integrity of the cell membrane, decreased osmotic resistance, the fragility of erythrocytes, and intravascular hemolysis (Saleh 2009). Loss of membrane integrity leads to the lysis of RBC, causing the release of hemoglobin, severe hematological disorder (Sowemimo 2002). These hematological damages can lead to the development of anemia in cattle. Other than the hematological symptoms, clinical signs of As toxicity in cattle can manifest from gastrointestinal to nervous signs. There have been only a few studies on As toxicity on cattle. Some of the studies have shown that As toxicity along with the metaldehyde has an additive effect and manifests into neurological disorders in Angus cows (Valentine et al. 2007).

15.9 Mitigation Methods of Arsenic in Rice Plant

Arsenic is mainly found in Asian country due to geogenic activity and could not be removed in the soil surface easily. Most of the scientists are trying to mitigate or decrease the As accumulation in the plants that are grown in As-contaminated land especially in rice grain. Reduction of As in rice grain can be achieved by using several strategies. Scientists are working to mitigate As involving geochemical, agricultural, microbial, irrigational, as well as phytoremediation technologies. A recent study by Roy Sarkar et al. (2017) and Singh et al. (2015) showed that intermediate application of some potential phytoremediation wetland plants like *Typha latifolia* and *Pteris vittata* could be used in between two rice cultivation phases. This process enhanced the uptake capacity of subjected wetland plants and resulted in a subsequent reduction in As load to rice grain, cultivated thereafter. There are several methods to mitigate the As in the rice grain.

15.9.1 Different Agronomic Practices

In Bangladesh and West Bengal, most of the tube wells are shallow depth and contaminated with As. During irrigation, dissolved As comes out and gets accumulated in the agricultural soil. From the soil, the plants uptake the As and accumulate in their bodies especially in their edible parts. Many scientists try to reduce As toxicity in plant bodies by changing the irrigation practices. Spanu et al. (2012) showed that the sprinkler irrigation practices minimize the As concentration in the rice plants. A similar study has been done by Barla et al. (2017) and suggested that the periodically water-saturated rice field had low As concentration than the continuous

water-saturated rice field due to alternative dry and wet condition. Aerobically grown rice also showed less As concentration than the conventional rice growing practices (Xu et al. 2008). Many methods of water management or irrigational practices alternative to continuous irrigation practices have been described by the various author for their effect on rice grain As accumulation. The methods used were intermittent or periodically flooding (Somenahally et al. 2011; Sarkar et al. 2012), raised beds (Duxbury and Panaullah 2007), and aerobic grown (Sarkar et al. 2012). Here, all methods were applied on single or selected rice cultivars and cultivated on agricultural fields (Duxbury and Panaullah 2007; Somenahally et al. 2011; Sarkar et al. 2012) or pot experiment (Duxbury and Panaullah 2007; Xu et al. 2008). All authors showed that the application of less water consumption and reducing environment causes reduction of the total As in rice grain. The effect of changing irrigation practices, i.e., from continuous flooding to saturation irrigation, was observed to be insignificant, but their outcome becomes more significant when periodic water irrigation practices such as intermittent irrigation (Sarkar et al. 2012) or flooding, raising beds (Duxbury and Panaullah 2007) are practised instead of conventional irrigation practices. In the pot experiment, Xu, McGrath, Meharg, and Zhao (Xu et al. 2008) showed considerable As the reduction in rice grains of close up to 90% by shifting from traditional irrigation practices to an aerobic irrigation practices. The main reason for the enhanced As accumulation in paddy rice is the soil chemical transformation under the flooded condition (Spanu et al. 2012). In flooded irrigation, reduced condition prevailed, that triggered the reduction of oxide or hydroxide of Fe and Mn and got dissolved in solution. The oxyhydroxides of Fe and Mn released the As from soil to water phase (Takamatsu et al. 1982; Barla et al. 2017). This is easily taken up by the paddy plants. In aerobic or intermittent or deficit irrigation practices, most of As were locked in the oxyhydroxides of Fe and Mn and form precipitate due to alternate dry and wetting condition (Liao et al. 2013).

15.9.2 Silicon Fertilization

Silicon is mostly found in Earth's crust as oxide or silicate forms, and plant takes up silicon in the form of silicic acid which is then precipitated in plants as amorphous silica (Epstein 1999). In Earth's crust, Si is found in plentiful amount, but most of them are insoluble and not available for the plants. Only dissolved Si, adsorbed Si, and amorphous Si are available for the plant uptake. Rice is more efficient in As accumulation than other cereal crops (Williams et al. 2007a, b), and there are two main reasons. First, anaerobic environment prevails in the agricultural field, and it leads to mobilization of As(III) that frequently enhances the bioavailability of rice (Takahashi et al. 2004; Xu et al. 2008). Second, arsenite under anaerobic condition can follow the same pathways with Si for rice (Ma et al. 2008). Mainly two Si transporters, protein Lsi1 and Lsi2, are responsible for transporting silicic acid into root cells and from cells to apoplast in the direction of stele for the translocation toward shoots (Ma et al. 2006; Ma et al. 2007). Both transporters are highly present in rice

roots and also transport arsenite (Ma et al. 2008). Lsi2 plays an important role for transporting arsenite from root to shoot and in rice grain (Li et al. 2009a). Fleck et al. (2013) and Liu et al. (2014) reported that the Si fertilization in soil might limit the As entering into a rice plant. A similar study has been done by Zhao et al. (2011b) and suggested that agronomic practice like Si fertilization can be an effective alternative to control As in rice plants. Silicon fertilization may affect the metal concentration in plants. It was found by Schaller et al. (2012) that Si application may alter the ratio of nitrogen (N) and phosphorus (P) and carbon concentration was declined in *Phragmites australis.* Song et al. (2014) also showed that the Si application in soil showed the negative correlation with nitrogen and phosphorus. Meanwhile, it also affects the heavy metal, especially in As. Li et al. (2009b) found that Si application in the agricultural field significantly decreased the inorganic As in the husk and rice grain. Fleck et al. (2013) also found that Si addition can decrease the As in rice grain significantly.

Silicon fertilization enhanced the As concentration in soil solution (Seyfferth and Fendorf 2012; Lee et al. 2014). The increment of As concentration in soil solution by Si addition was due to competitive adsorption site between silicate and arsenite with silicic acid on the soil. Rice has the capacity to accumulate Si and about 100 g Kg^{-1} Si amount in the shoots (Ma and Yamaji 2006). Arsenite is chemically similar to Si (H_3AsO_3, pK_a = 9.2; H_4SiO_4, pK_a = 9.8) (Teasley et al. 2017). So, arsenite shares the similar transporters of Lsi1 and Lsi2 to enter the plants. So, elevated Si in the soil competed with arsenite for the same adsorption site or transporters and decreased As accumulation in rice plant. The decrease in inorganic As in rice plant can be interpreted by the competitive suppressive effect of Si on arsenite uptake and transport toward the aboveground parts of the plant.

Silicon fertilization also stabilized the ferrihydrite, so it provides more surface area for As adsorption onto Fe phases (Schwertmann and Thalmann 1976; Seyfferth 2015; Amaral et al. 2016), and excess Si may restrain re-release of As by following Si polymerization over As-bearing ferrihydrite minerals (Swedlund and Webster 1999).

15.9.3 Redox Molecules Amendment

In rice plant, As toxicity is mainly induced with the stimulation of sulfur deficiency, oxidative stress, and imbalance of redox condition (Srivastava et al. 2011b; Rai et al. 2011; Finnegan and Chen 2012). Sulfur is a vital part of plant growth. Sulfur-containing compounds in plant affect the As uptake, its translocation and its accumulation in rice plants. Rice plants take sulfur in the form of inorganic sulfate (Davidian and Kopriva 2010) and then gets activated to adenosine -5′- phosphosulfate with the help of adenosine triphosphate sulfurylase. Further then, it is reduced to sulfite by APS reductase (APR). Again sulfite gets reduced to sulfide and incorporated by cysteine synthase and finally forms cystine (Takahashi et al. 2011). In the plant, shoot chloroplast is the place where sulfur reduction occurs, and glutathione

and phytochelatins are the important sulfur-containing compounds which take part in As complexation and vacuolar sequestration and maintain the redox homeostasis (Song et al. 2010; Noctor et al. 2012; Bianucci et al. 2012). Thiourea is used earlier under salt, salinity, and UV stress on *Brassica juncea* (Indian mustard) (Srivastava et al. 2011a; Pandey et al. 2012). It shows the positive effect of Indian mustard under salt and salinity stress and also improves the mustard yield and oil content.

In rice plant, TU enhances the sulfur-containing compound capability in plant body that causes vacuolar sequestration of As to occur in shoot components and also manage the oxidoreduction homeostasis (Srivastava et al. 2014). Their usages generate the reduced redox state and shift in oxidoreduction state toward reducing direction and are responsible for partial stress amelioration. It conjointly affects the loading of As into vascular tissue for root to shoot transportation. The usage of TU in rice plant with success minimize the As concentration in rice grain. It additionally increases the rice grain yield compared to normally grown rice.

15.9.4 Microbial Remediation

Remediation of As with the help of microorganism is cost-effective and environmentally friendly technology (Valls and De Lorenzo 2002). Volatilization of As is the natural process in the environment by which As is decreased from soil and water (Jakob et al. 2010). Volatilization of As is an enzymatic process in which arsenate gets reduced to arsenite and series of methylation reaction takes place (Michalke et al. 2000; Mukhopadhyay et al. 2002). This process can be a useful tool for As remediation (Cox and Alexander 1973). Aerobic and anaerobic microorganisms such as bacteria and fungi are mainly responsible for volatilization of As (Meyer et al. 2008). Singh et al. (2015) found three As-resistant strains which are useful for removing or decreasing the As concentration in soil through the transformation of inorganic As into methylated As. They are *Bacillus altitudinis*, *B. megaterium*, *and Lysinibacillus* sp. Banerjee et al. (2013) also found *Brevibacillus brevis* a bacterial strain which is useful for As mitigation.

In root system, oxidoreduction of iron and iron plaque formation play an important role in affecting As uptake by rice plants (Chen et al. 2005; Liu et al. 2006). They are mostly governed by oxygen release from rice roots. Rice root surface is usually coated with iron and manganese oxide and As present in the precipitated form on these oxides (Liu et al. 2006; Frommer et al. 2011). Radial oxygen loss from rice root promotes microbial abundance (Bais et al. 2006). Jia et al. (2014) suggested that the rice plant which has high ROL promotes As(III)-oxidizing bacteria resulting in As sequestration which takes place around the rice roots and rhizospheric soil, which limited the As uptake into rice plants.

Nitrate fertilization also reduces the As uptake. Nitrate reduces reduction of Fe(III) and instead causes oxidation of Fe(II), leading to As being absorbed in the Fe(III) mineral surface and thereby precipitating (Chen et al. 2008). A lot of controversy is associated with the application of phosphorus as a fertilizer and its effect

on the uptake of As by the plants; some pot experiments have shown decrease of As concentration in the plants on addition of phosphorus, whereas there are other cases in which phosphate was found to be effective in exchanging absorbed inorganic form of As from the solid phase, thereby increasing As availability to rice roots (Islam et al. 2016). Organic matter in the paddy fields influences the mobilization of As. Soil microbes utilize the organic matter and during this process consume the oxygen that leads to decrease in redox potential; this causes dissolution of As from FeOOH (Smedley and Kinniburgh 2002; Rowland et al. 2009). It also influences As availability by desorbing As from soil surface exchanging sites (Weng et al. 2009) and dissolved organic matter complexing As species (Liu et al. 2011). There have been recent reports which indicated that total amount of As in grain was higher in As-contaminated soil with higher organic matter (Islam et al. 2016).Various forms of Ferric ions have been used to immobilize As ion (Warren et al. 2003).

15.9.5 Cooking Techniques

Processing and cooking of rice after harvest influence the concentration of As in the grains. Rice is not just cultivated in an excess amount of water but is also cooked using a substantial amount of water. Washing the brown or polished rice several times before cooking decreases the As concentration. Washing grains of polished rice three times before cooking reduces the total As from 71–83% (Naito et al. 2015). Sengupta et al. (2006) reported that washing of long-grain rice 5–6 times may remove 28% of total As, whereas washing long-grain white rice 3 times removed 8–17% of total As (Mihucz et al. 2007). Rice variety, the percentage of water absorbed, the manner of preparation, and duration of cooking influence the amount of As in the cooked rice and its subsequent consumption. Parboiled and non-parboiled rice has shown the concentration of rice husk > bran brown rice > raw rice > polish rice (Rahman et al. 2007). In a survey performed by Duxbury et al. in 2003, they found that there was a reduction in As concentration by 19% on parboiling and milling the rice before consumption. Cooking rice in excess of water reduces the amount of inorganic As by 40% from long grained polished, 60% from parboiled, and 50% from brown rice (Islam et al. 2016). When rice is cooked with excess water and gruel is discarded, the concentration of As in rice decreases; this can be due to release of water-soluble As at high temperature and decantation of cooking water after cooking, instead of rice being dipped in it for a longer period of time and absorbing the same (Rahman et al. 2006).

Soil removal can act as a last resort to mitigate the problem of As accumulation. Since the top soil is the one which is contaminated with the As the most, only the upper 10–15 cm of soil needs to be removed. The topsoil removal need not be only in the field which it is grown but also of the tube well or irrigation site. In heavily contaminated field, topsoil removal is the most efficient method to mitigate the As problem (Brammer 2009).

Although the problem of As is highly prevalent in Southeast Asian countries, there are various simple, cost-effective methods available to mitigate the problem. From cultivation to the post-harvest stage, the problem can be addressed at each stage and that too in a cost-effective manner.

15.10 Conclusion

The recent advancements in mitigation techniques and awareness about the impacts of As on wide range of biota need to be spread at a global level. The steps taken by the policy makers must be in coherence with the established research results so that the policies fulfill the three pillars of sustainability like environment-friendly, economically viable, and socially acceptable.

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Chapter 16
Environmental Chemistry, Fate and Speciation of Arsenic in Groundwater-Soil-Crop Systems

Indranil Das, S. K. Sanyal, and K. Ghosh

Abstract Arsenic (As) is a toxic metalloid having a natural origin in the earth's crust. Among the several sources of As pollution, geogenic As pollution through contamination of the groundwater in the deltaic basin of Bengal (region of Ganga and Padma river) covering India and Bangladesh is of great concern to the world as it paved its way for As to adversely affect the soil-plant-animal continuum. Arsenic in soil and water is transformed chemically and biochemically through different processes, namely, oxidation, reduction, methylation, and demethylation. Regarding the fate, As mobility depends upon the clay percent and mineralogical makeup of the soil, whereas As retention is facilitated by different soil physicochemical properties, adsorption and ion exchange process, organic fraction-As complexation equilibria, surface charge characteristics, and other nutrient element interactions in soils. Precipitation-coprecipitation and microbial transformation also govern the fate of As in soil and water. After interaction with soil and water, As is further translocated or metabolized to plant body in several inorganic and organic forms. In plant body, As accumulation pattern, in general, was observed to follow the order root > stem >leaf > economic produce. Several workers attempted to derive the toxicity symptoms and values in the plant as well as man's edibility. Finally, speciation of total loading of As for the affected soils and the crops into arsenite and arsenate oxyanion species is important for characterizing the *net* toxicity of As in the given soil-crop systems.

Keywords Arsenic · Transformations · Mobility · Retention · Fates · Speciation

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16.1 Introduction

The word "arsenic" is derived from the Persian word زرنیخ *Zarnik* which meant "yellow orpiment." *Zarnik* was further changed by the Greeks as *arsenikon*. Since ancient times arsenic (As) has been known and used in Persia and elsewhere and also known as the *poison of kings and the king of poisons*. Arsenic was often included during the Bronze Age in bronze mostly as an impurity to render the alloy harder. It was believed that in 1250 AD, Albertus Magnus first isolated the element (Emsley 2001). Arsenic has also been used as a pest killer, thereby contaminating human foods and environment with As, resulting in adverse effects on the health of humans over generations. By exploiting As-contaminated underground water for drinking water resources, As intimidates the health hazards of million population in many parts of the world (Nriagu 2002).

Arsenic, which is placed in group V and period 4 of the periodic table (very similar chemically to its predecessor in group V, namely, phosphorus), is a natural metalloid having *atomic mass* of 74.921 and *atomic mass units* (amu) of 60 with 33 *atomic number* (*Z*) (Henke and Hutchison 2009). Arsenic is most commonly found in −3, 0, +3, and +5 valence states (Shih 2005). Arsenic is physically a silver-gray brittle having hexagonal or rhombic crystalline structure, resembles the metallic look, and exists in yellow, gray, and black allotropic forms of which the first two forms are solid modifications with specific gravities of 1.97 and 5.73, respectively (Cotton et al. 1999). It is odorless unless heated to evolve pungent odor like garlic, due to the formation of As trioxide upon oxidation. Regarding taste, As is tasteless. Arsenic is soluble in only oxidizing acids (nitric acid, cold hydrochloric, and sulphuric acids) but non-soluble in water. Except for the gaseous forms of As like AsH_3 and As_2O_3, As compounds mostly are nonvolatile (HSDB 2001). As regards the physical properties, As with an atomic weight of 74.92 sublimes at 612 °C and melts at 817 °C at 28 atmospheric pressure. Furthermore, its vapor pressure is 1.0 mm Hg at 372 °C and has a specific gravity of 5.727 at 14 °C.

16.2 Origin of Arsenic

Arsenic has been detected in the atmospheres of Jupiter, Saturn, meteorites, and Moon rocks (in trace amounts) of our solar system, whereas in earth, it is largely concentrated in the core and crust (clay- and sulfide-rich portions). In crustal rocks As is concentrated through the transportation from hydrothermal fluids (Henke 2009). The wider distribution of As accounts for 0.0005% of the earth's crust. Arsenic is found in rocks (igneous and sedimentary) as a compound form of inorganic As and often found as arsenides of nickel, cobalt, copper, and iron which are obviously the intrinsic part of sulfide ores. There are about 320 As-bearing minerals in nature (Fleischer 1983). The common primary minerals of As are arsenopyrite (FeAsS), orpiment (As_2S_3), realgar (AsS), and minor primary minerals like niccolite

(NiAsS), cobaltite (CoAsS), and energite (Cu_3AsS_4). The secondary minerals, scorodite ($FeAsO_4$, $2H_2O$) and oxides, namely, claudetite (As_2O_3), arsenolite (As_4O_6), and the pentoxide (As_2O_5), are also present (Sanyal and Nasar 2002). At the time of volcanic eruptions, As may be emitted in nature through atmospheric fluxes. The global emission of As into air, water, and soil is compiled by Nriagu (1988) as 18.8, 41.0, and 82 thousand metric tonnes per year, respectively. Additionally, As gets entry to the lithosphere, hydrosphere, and atmosphere through several anthropogenic activities such as mining (Grossl et al. 1997) and industrial (from manufacturing process of glass pigments, enamels, antifouling paints, textile, wood preservation fireworks, industrial wastes) and agriculture operations (from the use of agrochemicals) (Chakravarty and Das 1997; Sanyal 1999, 2000). Presently As acid (H_3AsO_4), sodium arsenite and arsenate ($NaAsO_2$ and Na_3AsO_4), and dimethyl As acid (DMA) are being used as defoliants, while disodium methanearsonate (DSMA), monosodium methanearsonate (MSMA), and methyl arsonic acid (MAA) are used (since the mid-1970s) as herbicides (Onken and Hossner 1996). Extensive use of these materials may facilitate As contamination in soil and water. In addition to the factors mentioned above, the increasing accumulation of As in groundwater resource may be of geologic origin, especially due to the occurrence of arsenopyrite minerals (Wells and Elliot 1971).

16.2.1 Arsenic in Water Bodies

The cycle of As in natural water has been reviewed by Fergusen and Gavis (1972). Reports of As contamination in natural water bodies, namely, ocean, rivers, lakes, and well water, are known worldwide. Braman (1975) reported that a considerable amount of inorganic and organic As is present in tidal and saline bay water. Further seawater contains a considerable amount of organo-As compound, a by-product of biological transformation by microbes in the sea (Johnson 1972). A significant concentration of As was reported by Grimmett and McIntosh (1939) and Lanchester et al. (1971) in hot springs due to the liberation of fumaric acid evolved from such springs. Besides, a high concentration of As may result from thermal activities and through rocks, enriched with As (White et al. 1963). High values of As contamination in rivers and lakes of many developed countries like the United States, New Zealand, etc. may probably be attributed to industrial contamination (Brown et al. 1973; White et al. 1963). Some good waters of the United States also found to be contaminated by profuse As loading might have the accessibility to sediment contamination (Feinglass 1973). It has been assumed that surface water bodies like ocean have a self-purifying effect of the toxin by deposition in the sediments, thereby posing little hazards to the environment (Sollin 1970) and so also the case of other water bodies containing more As than most natural water bodies (Table 16.1).

Table 16.1 Arsenic concentrations in water except groundwater

Source	Arsenic (μg As L^{-1})
Rainwater and snow	< 0.002–0.59
Rivers	0.20–264
Lakes	0.38–1.00
Seawater	0.15–6.00
Ponds (West Bengal, India)	4–70
Canals (West Bengal, India)	40–150

Source: Sanyal et al. (2012); Sanyal (2017)

16.2.2 Origin of Arsenic in Groundwater

Geothermal environments specifically deposits of a volcanic eruption, earth's internal heat systems, and basin-fill alluvial deposits of lake sediments are usually the original sources of high As concentration in groundwater (Welch et al. 1988). Widely distributed groundwater As contamination in parts of West Bengal [covering nine districts, namely, Malda, Murshidabad, Burdwan, Nadia, Howrah, Hoogly, 24 Parganas (North and South), and Kolkata], India, and Bangladesh encompassing the deltaic region of the Bhagirathi and Ganga-Padma rivers has been reported by several workers (Sanyal et al. 2015; Sanyal 2017). Two major hypotheses regarding the origin of As, each of geogenic origin, are projected (Sanyal 2005). According to the first hypothesis (Mandal et al. 1996), iron pyrites may be responsible for As contamination of groundwater. In situ iron pyrite is formed when sulfur combines with iron-bearing minerals associated with As, as a part of the alluvial sediment brought along by the rivers or formed in situ. With the use of water as agricultural irrigation, particularly for cultivation of summer (*boro*) paddy, lowering of water table causes oxidation of arsenopyrite in aquifer sediments through the invasion by atmospheric oxygen. Further, on the decomposition of pyrite, As mobilization is facilitated with the formation of iron sulfates and sulfuric acid (Sanyal 1999, 2005, 2017).

This hypothesis is inconsistent within the marginally alkaline groundwater of the affected deltaic region *nor* with its meager concentration of sulfate, or high concentrations of bicarbonate, iron (II), arsenite, calcium, and magnesium facilitate an anoxic (reduced) aquifer conditions (Sanyal 2005). Some workers (Bhattacharya et al. 1997, 2003; Nickson et al. 1998) introduce the second hypothesis that the organic matter-enriched burial sediments deplete dissolved oxygen in groundwater, thereby causing extremely reduced conditions in groundwater aquifer manifested by elevated groundwater table, surface layers having fine grain and widely cultivated wetland paddy, as well as microbial oxidation of sedimentary organic matter. Under such condition, the concentrations of sedimentary organic matter drive As to get released from As-enriched iron oxyhydroxide, which is efficient As scavengers by reducing them in groundwater devoid of oxygen. In spite of these hypotheses, the exact geochemical reaction sequence for As release in groundwater from the aquifer sediments is still a debated matter (Sinha Ray 1997; Sanyal 1999, 2017; Sanyal and Nasar 2002; Ghosh et al. 2004; Sanyal et al. 2015).

16.3 Chemical and Biochemical Transformation of Arsenic in Soil and Water

16.3.1 Arsenic Oxidation

Air and pure oxygen (O_2) were the agents for oxidation of arsenite in water (Bissen and Frimmel 2003; Burkitbaev 2003; Hering and Kneebone 2002; Bisceglia et al. 2005). Sometimes even in anoxic condition, microorganisms, ferric species, nitrate (NO_3^-), natural organic matter (NOM), or Mn(III,VI)(oxy)(hydr) oxide compounds can considerably oxidize As in natural waters. (Craig et al. 2003; Price and Pichler 2005; Langner et al. 2001; Evangelou et al. 1998; Schreiber et al. 2003; Redman et al. 2002; Stollenwerk 2003). The arsenite to arsenate oxidation maybe further facilitated by higher specific surface areas of poor crystalline solid oxidants (at least some Fe(III) oxyhydroxide oxidants) by the photo-catalyzed reactions (Stollenwerk 2003).

The following reaction is one of the examples of orpiment oxidation to inorganic arsenite in the aqueous solutions (Lengke and Tempel 2002):

$$As_2S_3 + 6O_2 + 6H_2O \rightarrow 2H_3AsO_3^{\ 0} + 3SO_4^{\ 2-} + 6H^+ \tag{16.1}$$

Under oxidizing and near neutral pH conditions, this inorganic arsenite may undergo slow oxidation to form inorganic arsenate by the following reaction:

$$2H_3AsO_3^{\ 0} + O_2 \rightarrow H_2AsO_4^{\ -} + HAsO_4^{\ 2-} + 3H^+ \tag{16.2}$$

16.3.2 Arsenic Reduction

Among the reversible reaction within As(III) and As(V), the transformation of As(V) to As(III) in general is faster in reducing conditions compared to the conversion of As(III) to As(V) under oxidizing environments (Stollenwerk 2003). In natural subsurface environments, the common reductants are hydrogen sulfide (H_2S) and organic carbon associated with or without the activities of microorganisms (Stollenwerk 2003). Under acidic conditions, As(V) reduction by H_2S is rapid, whereas in natural environments, microorganisms are responsible for the reduction of arsenate to arsenite and arsenite to arsine or dimethyl arsine [$(CH_3)_2AsH$](Craig et al. 2003; Stollenwerk 2003). *Pseudomonas fluorescens* and *Anabaena oscillaroides* are known to act as arsenate-reducing bacteria (Cullen and Reimer 1989).

16.3.3 Arsenic Methylation and Demethylation

Arsenic in inorganic form may be converted through methylation into MMA(III), DMA(III), trimethylarsine, trimethylarsine oxide, and various other methylated forms. In the methylarsenic pesticides-contaminated areas, methyl thioarsenates may also form (Wallschläger and London 2008). At an unnamed contaminated site, Wallschläger and London (2008) specifically confirmed the existence of $(CH_3)AsO_2S^{2-}$, $(CH_3)AsOS_2^{2-}$, $(CH_3)_2AsOS^-$, and $(CH_3)_2AsS_2^-$ species in groundwater.

Biotic processes are entirely or almost entirely responsible for the methylation of As (Frankenberger and Arshad 2002). Some specific fungi (yeasts also) and bacteria possess the ability of methylating As microbially (Bentley and Chasteen 2002; Cullen and Reimer 1989) which can be described by *Challenger mechanism* (Bentley and Chasteen 2002; Dombrowski et al. 2005). This mechanism inherits a series of reduction and oxidative methyl transfer reactions starting from the reduction of inorganic forms of As(V) to inorganic As(III) and ending with the final product of trimethylarsine. The naturally occurring reductants of the Challenger mechanism are probably sulfur-containing analogue of alcohol (thiols) and, in particular, glutathione (a tripeptide derived from glutamic acid, cysteine, and glycine involved as a coenzyme in oxidation-reduction reactions in cells) and lipoic acid (6,8-dithiooctanoic acid) (Bentley and Chasteen 2002). The outcome of the Challenger mechanism regarding the production of methylated species could be excreted or retained by the microorganisms or be transformed into the succeeding As species in the sequential order.

The process of Biomethylation might generate more complex alkyl arsenic groups, namely, $As(C_2H_5)(CH_3)_2$, which has been found in dumping place (landfill), and sewage gas which evolved from digestion and most likely exists in natural gas (Bentley and Chasteen 2002).

The process of removal of methyls from organoarsenicals known as demethylation refers to the ultimate transformation of the organoarsenicals into inorganic As. Demethylation of As may take place through the exposure to ultraviolet radiation (Cullen and Reimer 1989) and also by the important role of microorganisms. Though MMA(V) and DMA(V) are extremely steady in water under sterile conditions (Cullen and Reimer, 1989), bacteria have the capability to demethylate them and also another methyl form of As species into inorganic forms of As (Frankenberger and Arshad 2002; Cullen and Reimer 1989; Santosa et al. 1996). In the soil samples of organic wetland and forest floor of Fichtelgebirge mountains of Germany, Huang et al. (2007) found DMA(V) and arsenobetaine $[(CH_3)_3As^+CH_2COO^-]$ as the dominant organoarsenicals, where the latter form rapidly demethylates to an unidentified As species perhaps dimethylarsenoylacetate $((CH_3)_2As(O)CH_2COO^-)$, which yielded to DMA(V) upon demethylation under incubations at 5 °C of aqueous extracts of the said soils. However, the conversion of DMA(V) to MMA(V) in the extracts of these soils is much sluggish and is succeeded by the fast transformation of MMA(V) to inorganic As through demethylation (Huang et al. 2007).

Lehr et al. (2003) observed that *Mycobacterium neoaurum* is competent to demethylate MMA(III and V) to inorganic As but incompetent of transform DMA(V) or trimethylarsine oxide which indicates that at least some MMA(V) transfer methyl group reductively to form inorganic As(III), which is none other than a reverse reaction of the challenger mechanism.

16.3.4 Chemical Aspects of Arsenic in the Groundwater-Soil Environment

Waters in nature might have As in one or more dissolved form, and their chemistry would depend on the chemical nature of water. At the expense of time, these dissolved As forms in water may (1) possibly interact through methylation or demethylation by biological organisms; (2) take part in reactions from abiotic or biotic oxidation, reduction, or other types; (3) sorb onto solid phases via ion exchange reactions; (4) precipitate; or (5) coprecipitate (Henke and Hutchison 2009).

Apart from the organic forms, As in groundwater is generally present as dissolved, proton accepting/donating oxyanions, namely, arsenites ($As^{III}O_3^{3-}$; $H_nAs^{III}O_3^{(3-n)-}$, with n = 1, 2) or arsenate ($As^{V}O_4^{3-}$, $H_nAs^{V}O_4^{(3-n)-}$, with n = 1, 2), or both. The toxicity of As species in groundwater as well as in soil depends upon the oxidation and redox status and pH. The toxicity follows the order: arsine [AsH_3; valence state of As: −3] > organo-arsine compounds >arsenites (As^{3+}form) and oxides (As^{3+} form) > arsenates (As^{5+} form) > arsonium metals (+1) > native As metal (0) (Sanyal et al. 2015; Sanyal 2017). The arsenites have much more solubility, mobility, and toxicity compared to arsenates in water-soil systems (Cullen and Reimer 1989; Korte and Fernando 1991; Sanyal 1999, 2005, 2017). In addition to its activity, As(III) is also known to dominate in the soils of the As-contaminated belt of West Bengal, India, and Bangladesh as these soils and groundwater eventually had normal pH range and hence is a matter of worry. The fact behind the predominance of As(III) can be explained through Henderson's equation which explain that under normal pH of soils and groundwater, this As form remained as a neutral molecule, namely, arsenous acid, $H_3AsO_3^0$ having $pK_a = 9.2$ (Sanyal et al. 2015; Sanyal 2017), and because of electroneutrality this form is less accessible for detention by the charged mineral surfaces in soils and sediments.

Similar to the water-soil system, the toxic effect of As in soil-crop system also depends on its chemical forms, specifically the oxidation state (Woolson 1977; Sun and Doner 1998). Under reducing conditions (E_h = 0–0.1 V), arsenite oxyanion species ($H_3As^{III}O_3$, $H_2AsO_3^-$, $HAsO_3^{2-}$) along with arsenous acid ($H_3AsO_3^0$) dominate in soil (Deuel and Swoboda 1972; Haswell et al. 1985; Sadiq 1997; Ghosh et al. 2004; Sanyal 2005; Sanyal 2017), but elemental As and arsine can also prevail (Walsh and Keeney 1975). In well-aerated or oxidized soils (redox potential, E_h = 0.2–0.5 V), As present would remain as $H_2AsO_4^-$ and $HAs^{V}O_4^{2-}$ ions (pentavalent As forms) in acidic soil or as $HAsO_4^{2-}$ in the alkaline soil and As acid species ($H_3AsO_4^0$ and

$H_2AsO_4^-$) which are steady in these conditions (Welch et al. 1988). The pore water of aerobic soils has access to oxygen; hence the predominant species is arsenate, with a meager amount of arsenite and MMA in mineralized areas.

Besides oxidation and reduction, the other reactions commonly occurring with As in soils are adsorption, dissolution, precipitation, biotransformation, and leaching (Bhumbla and Keefer 1994). In acidic soils only, sorption of As is facilitated by the exposed surfaces of aluminum oxides/hydroxides and clay, whereas, in calcareous soils, carbonate minerals are responsible for adsorption (Sadiq 1997; Goldberg and Glaubig (1988). Precipitation is another mechanism which limits As concentrations in all types of (alkaline, acidic, oxic, and suboxic) soil solutions leading to the formation of both iron arsenate and calcium arsenate (Sadiq et al. 1983; Sadiq 1997). Three major modes of biotransformation, namely, redox shifting within arsenite and arsenate, As reduction and methylation, and the biosynthesis of organoarsenic compounds of As species, present in the soil (Andreae 1983). Leaching from soil does not seem to be an important avenue for As loss. According to Devenport and Peryea (1991), administering phosphatic fertilizers can significantly enhance the As leaching from lead arsenate pesticide-contaminated soil.

It is needless to say that the groundwater or soil solution being closer to an open system (considering the thermodynamic aspect) cannot be expected to attain thermodynamic equilibrium as both the solutions are exposed to several forces and fluxes and also perturbed by anthropogenic activities due to drafting. Thus, the ratio of concentrations of As^{III} over As^{V} usually in agricultural soils mismatched with the calculated value from the observed redox potential (E_h) by employing Nernst's equation (at 25°C) for the equilibrium redox reaction (which is strictly speaking applicable only to a thermodynamically closed system), namely:

$$As^{V}O_4^{3-} + 2H^+ + 2e = As^{III}O_3^{3-} + H_2O \tag{16.3}$$

$$E_h - E^0{}_h - 0.0295\log\left[\left(As^{III}O_3^{3-}\right)\right]/\left(As^{V}O_4^{3-}\right)] - 0.059pH \tag{16.4}$$

where the terms refer to the equilibrium concentrations of the respective ionic species in the dilute soil solution and $E^0{}_h$ is the standard redox (reduction) potential of the ($As^{V}O_4^{3-}/As^{III}O_3^{3-}$) redox couple at 25°C. Equation 16.4 thus revealed that the magnitude of As^{III} (soluble As form) in the soil should increase significantly with declining E_h and escalating pH. Further, at a greater pH, the abundance of OH^-ion would facilitate the dislocation of As ^{III} and As^{V} species from their exchange sites via competitive ligand exchange mechanisms (Sanyal et al. 2015; Sanyal 2017). Thermodynamic calculation suggests that the ratio of As(V) to As(III) in oxic seawater (pH: 8.3, pE: 12.5) should be about 10^{15}. In reality, this ratio is only about 15–250 (Andreae 1979), thereby suggesting that the As(III)-As(V) couple is not in equilibrium and that the proportion of As(V) to As(III) is kinetically controlled. Indeed, a coupled adsorption-redox transformation reaction at solid/liquid interfaces (such as soil/soil solution interface) may be critical to the amount and rate of As mobilization in soil and sediment environments (Sun and Doner 1998).

The stability diagram for dissolved As species as an effect of E_h and pH at 25 °C is illustrated in Fig. 16.1.

The balance in aqueous solutions (which include groundwater, surface water, laboratory solutions, and pore waters from soils, sediments, or rocks) that exist between the major dissolved species and precipitates is sometimes predicted or explained through the E_h-pH diagrams. Interestingly, many natural aqueous systems usually do not attain equilibrium due to the formation of *metastable* species which are not anticipated by E_h-pH functions. *Metastable species* are compounds, other substances, or ions that exist under redox, pH, pressure, temperature, or other conditions where chemical equilibrium endorse their instability and absence. The biological activity generates these species (such as As(III) in oxic seawater) (Henke and Hutchison 2009).

The major limitation of E_h-pH diagrams is their specificity toward temperature and pressure (typically 25 °C and 1 bar pressure with O_2 and H_2 gases) and often within the periphery of simplistic chemical conditions. As a result, fluctuations in temperature, pressure, and gas composition or the addition of new components (such as iron or calcium) would deviate the stability fields and generate new chemical species with the exclusion of others. For example, the application of 0.001 M total sulfide to 10^{-6} M total As solution resulted in the reduction of size of the $H_3AsO_3^0$stability field, exclusion of As^0, and yield orpiment (As_2S_3) and realgar (AsS) under intensely reduced ($-E_h$) state, whereas the extremely elevated concen-

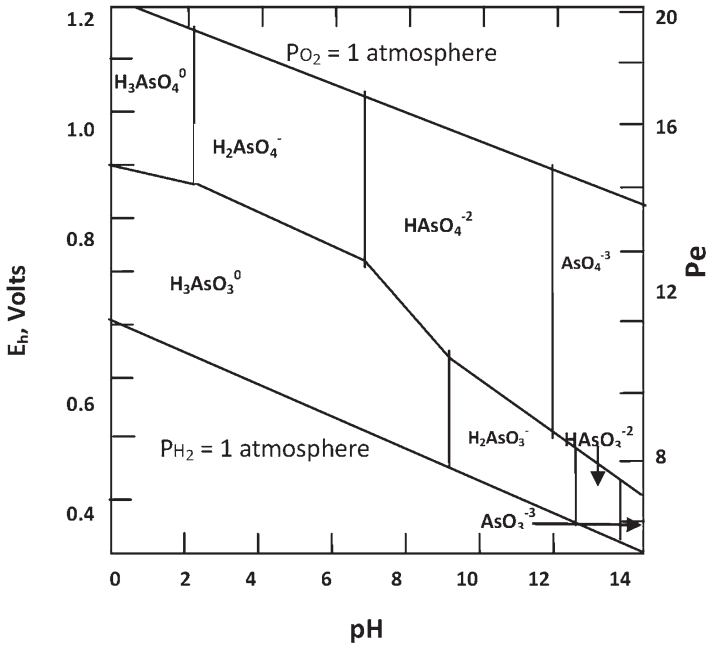

Fig. 16.1 Fields of stability for dissolved forms of arsenic as a function of E_h and pH at 25 °C (Source: Ghosh et al. (2004); Sanyal (2005))

tration of As (above about 0.16 M) in water sample could lead to precipitation of arsenolite and claudetite in the identical $H_3AsO_3^0$field (Henke and Hutchison 2009).

Arsenic distribution in different size-fractions of soil has been related to the stability of the primary minerals in which it is found and the extent of weathering (Huang Yan-Chu 1994). Using an equilibrium thermodynamic approach, Sadiq et al. (1983) observed that calcium arsenate was the most persistent As mineral following manganese arsenate from well-oxidized soils and alkaline soils, whereas in reduced soils (pH < 7.0), As(III) oxides and As sulfides were stable, while the arsenite minerals are too soluble to persist in soils. Sadiq et al. (1983) further established the order of stability of arsenates in well-aerated soils as $Cd_3(AsO_4)_2 > Pb_3(AsO_4)_2 > Ca_3(AsO_4)_2 > AlAsO_4 > FeAsO_4 > Zn_3(AsO_4)_2 > Ni_3(AsO_4)_2$.

Inorganic As fractions in soil from the contaminated sites were characterized sequentially into different soil As fractions which include water-soluble As (Ws-As), aluminum-trapped As (Al-As), iron-trapped As (Fe-As), and calcium-trapped As (Ca-As). The observation perceived from this scheme followed the order as Ws-As < Al-As < Ca-As < Fe-As. Among the fractions, Fe-As constituted 45–74.7% of the total soil As (Sanyal et al. 2012; Banik and Sanyal 2016; Sanyal 2017). Das et al.(2011) studied the sequential fractionation of As in some selected As-affected and As-unaffected soils of West Bengal which revealed that the proportion of different As sequential forms increased in the order internally bound As in soil aggregates (20.7%) > freely exchangeable As (20.3%) > calcium bound As (18.7%) > chemisorbed As (17%) > residual As (15.7%) > labile As (3.29%). The fluctuation in these As fractions may be credited to the mineralogical configuration of soils (also revealed from the statistical study) along with surface area, pH, total and Olsen-extractable As, amorphous iron, and, to a smaller extent, calcium and magnesium content of these soils. The poor interaction of exchangeable forms of soil As toward crop amenability implies the transformation of As has through minerals upon redundant exposure to As-contaminated irrigation water.

16.4 The Fate of Arsenic in Water and Soil Systems

Following the As transformations in the soil-water system, there are different fates of As attributed to different chemical and physical properties of soil. These fates are discussed in details below.

16.4.1 Arsenic Mobility in Soil

Arsenic mobility in soil is examined through transport processes through soil by several workers. Carey et al. (1996) used concentrated ionic solutions of copper, chromium, and As (chromated copper arsenate, used for timber protection) to drain through unperturbed soil monolith lysimeters containing the surface and subsurface

horizons of two free-leaching New Zealand soils. The breaks through curves so obtained were successfully designed using the similar Gompertz equation used for biomass growth. The variation within the soil horizons reflected in the Gompertz parameter values was related to the variation in soil physicochemical properties influencing leaching. The comparative ease of solute ion breakthrough followed the order: copper < arsenate < dichromate. The essence of this study further calls for a future investigation in fields about prevention of heavy metal spills in the soil.

Laboratory experiment on As transport horizontally and vertically through a soil profile of the contaminated zone revealed important information in respect of assessing the retention and released pattern of As during infiltration and percolation of As-affected groundwater, thereby furnishing the soil to become an effective sink (Mukhopadhyay and Sanyal 2002; Das et al. 2014). These findings are in agreement with the breakthrough curves derived from the affected soils (Carey et al. 1996; Mukhopadhyay and Sanyal 2002; Das et al. 2014), and these were justified by field observations of steep decline in As content of different horizons of the soil profile of the contaminated zone (Ghosh et al. 2002; Das et al. 2014). The transport data were interpolated to the formulations by irreversible thermodynamics to derive proper reflection coefficient (σ), mechanical filtration capacity of soil (L_p), and solute permeability (ω) and multifarious hydrodynamic frictional coefficients. Such experimental findings revealed a high degree of As retention (as reflected by the shifting of breakthrough curves to the left of inflection point, at $P_v = 1.0$) by the As-affected soil, thereby elucidating the nature of surface soils in partitioning As from flowing water. Presumably, the soil clay fraction in the soil might have been responsible for such retention. Actually the water of canal, ditches, and ponds of the entire contaminated belt was virtually found to be toxicant-free which further supports the hypothesis that the soil acts as a sink of the toxin. In this context, it is also necessary to adjudge the soil, which acts as a sink may also serve as the source whenever the carrying capacity of the toxins exceeds (Mukhopadhyay and Sanyal 2004; Das et al. 2014). Saha and Sanyal (2005) and Das et al. (2014) observed As retention to correlate with the amount of clay of the As-affected soils in vertical transport studies.

Das et al. (2014) studied the mobility study of As in solutions from both contaminated and uncontaminated soils of West Bengal, India, under laboratory incubated condition. The vertical column study of infiltrating As revealed that the leachate concentration progressively increased with the expense of flow time which essentially indicates a reduced extent of As retention over longer time intervals. The observed sigmoid shape of the breakthrough curves (BTCs) of As in the contaminated soils demonstrate hydrodynamic dispersion of As in soil throughout the flow. Nevertheless, the BTCs achieving the inflection point at a lower relative concentration (than 0.5) indicate a greater extent of As confinement by As-contaminated soils (S_1, S_3, and S_2 soils) (Fig. 16.2). The above effects might be attributed to the differences in clay composition, amorphous Fe and Al content, mineralogical characteristics, and the specific surface area of the soils. The fast and sharp rise in the As concentration of the leachate from the As-unaffected (K) soil suggests lesser retention of As in comparison to the contaminated soils. Carey et al. (1996) also recipro-

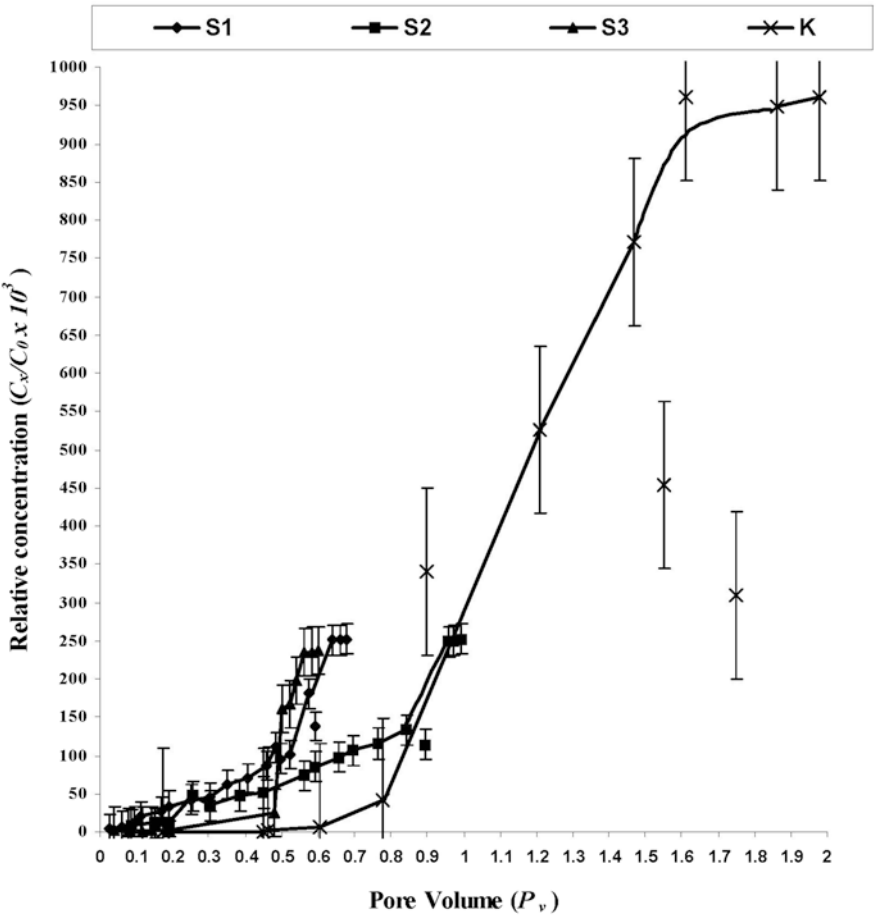

Fig. 16.2 BTCs for conveyance of aqueous arsenic solution into the experimented soils from arsenic-affected area (S_1, S_2 and S_3) and from unaffected area (K). (Source: Das et al. (2014))

cated such sharp increase in BTCs for chromium concentration in soils as the minimal detention of dichromate. However, the delay in the solute flux at the advanced stages in the K soil (beyond $1.6P_v$; Fig. 16.2) might partially be attributed to the swelling nature of the montmorillonitic clay in this soil and partially to the dispersion of the aggregates.

The lateral passing of the aqueous As through the abovementioned soils (Das et al. 2014) explained the As building-up trend, as implied from the lesser solute permeability (ω) and mechanical filtration capacity (L_p) (Fig. 16.3) as well as the higher values of the reflection coefficient (σ) in the As-contaminated soils than the uncontaminated one. Such a pattern was related to the enhanced clay and amorphous Fe and Al contents and specific surface area in the As-contaminated soils. One of the hydrodynamic frictional coefficients (f_{sm}) indicates a greater extent of

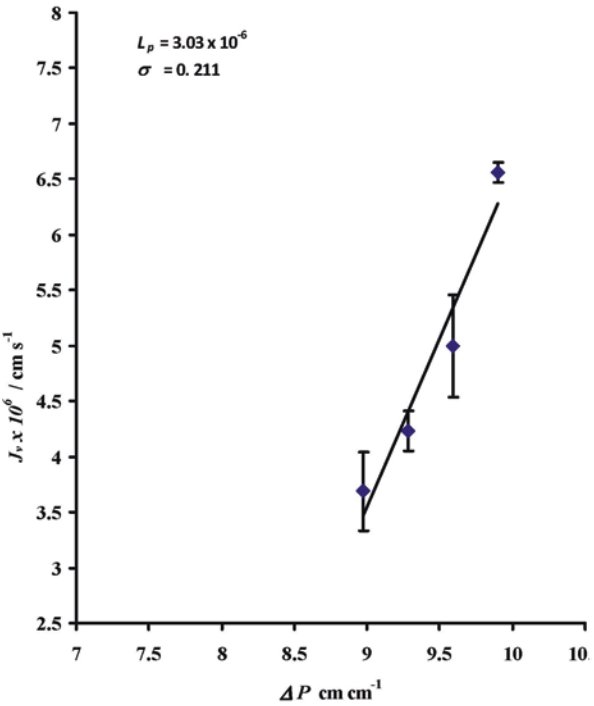

Fig. 16.3 Diagrammatic representation of volume flux (J_v) against pressure gradient (∇P) for aqueous arsenic in the arsenic-affected soil. (Source: Das et al. (2014))

frictional interplay between As and soil during travel of As through the As-contaminated soils. This coefficient provides As more attainable to retain by the soil colloidal phase, apparently due to the enhanced clay content, CEC, amorphous Fe and Al content, illite-kaolinite mineralogy, and the specific surface area of the As-contaminated soils. Thus, the soils having these properties along with greater organic matter content can have the capacity for As accumulation than the sand-predominated, vermiculite-montmorillonite-enriched alluvial soils. Besides, native soil organic matter and the advocated organic matter facilitate As sorption, thereby combating its toxic effect in the soil-crop system*s* vis-a-vis entry to the human food web.

Williams et al. (2003) conducted experiments on sorption and mobility of As(V) in the experimental subsurface systems exploiting column study and utilizing unidimensional advection-dispersion equation of both linear and nonlinear adsorption equilibrium model (LLE and NLLE) where the latter predicted better output than the former. These predicted models differ in As(V) breakthrough as it occurred more rapidly due to adsorption nonequilibrium and the lower recovery from the total amount of As(V) due to the presence of an irreversible or slowly desorbing fraction.

16.4.2 Retention of Arsenic in Soil and Factors Affecting It

16.4.2.1 Arsenic Retention Through Soil Properties

Numerous soil properties influence As retention by soils, soil minerals, and sediments. Physicochemical properties like nature (clay mineralogy) and amount of solid components (i.e. clay), sorption potential and nature of clay colloids (clay, oxides or hydroxides of aluminum, iron and manganese, calcium carbonates, or organic matter), soil pH, organic carbon, and amorphous Fe content of the soil, among others, and also the amount of As present in soils govern As retention in soils (Jacobs et al. 1970; Livesey and Huang 1981; Zhou 1986; Carey et al. 1996; Manning and Goldberg 1997; Mukhopadhyay 2002; Sanyal and Nasar 2002; Ghosh et al. 2004; Mukhopadhyay and Sanyal 2004; Saha et al. 2005; Sanyal 2017). The increase in As adsorption from solution is associated with the increase of oxides of free iron, magnesium and aluminum, or clay content of the soil, while on the contrary, removal of amorphous iron or aluminum components by oxalate treatment removes or appreciably decreases the As adsorption capacity of the soil (Dickens and Hiltbold 1967; Jacobs et al. 1970; Galba 1972; Wauchope 1975; Livesey and Huang 1981). Bhumbla and Keefer (1994) observed that the amorphous oxides having loose and highly hydrated form can strongly sorb As, also permitting other hydrated ions to diffuse freely throughout the structure, without imposing restrictions as in outer surfaces of more crystalline solids.

Barry et al. (1995) reciprocated the above findings for the forest soil profile where the greatest As sorption achieved with the persistence of clay and oxyhydroxides of iron and aluminum. Manning and Goldberg (1997) observed the greater affinity for arsenite and arsenate with the arid-zone soils having highest citrate-dithionite extractable iron and clay content and exhibited similar adsorption behavior that of pure ferric oxide. Adsorption isotherms revealed that the arsenate forms are adsorbed firmly than arsenite (Sanyal et al. 2015; Sanyal 2017). Again, the rate of arsenate desorption from soil was reported to increase with the increase in the (CaO + MgO): (Al_2O_3 + Fe_2O_3) ratio in soil (Galba 1972; Galba and Polacek 1973).

Ghosh (2006) observed the equilibrium As sorption/desorption parameters at 35 °C and 45 °C for three As-affected soils and one unaffected soil. The values of the Freundlich K (μg g^{-1}) ranged from 13.5 to 70.7 at 35 °C and 22.9 to 176 at 45 °C. A higher sorption capacity was found in the As-contaminated soils rather than uncontaminated soil at 35 °C. In general, Freundlich K was positively correlated with organic carbon and clay content of the given soils. The adsorption capability of the soils was greater at the elevated temperature. It has been observed that the Freundlich equation, by and large, gave a superior equilibrium As sorption data than did the Langmuir equation. This can be explained as Freundlich equation, though empirical, suggests that the proclivity for As adsorption diminishes exponentially with increasing surface saturation, which is nearer to reality than the presumption of a constant binding energy theory of Langmuir equation (Sposito 1984; Sanyal and De Datta 1991; Sanyal et al. 1993; Sanyal 2017). At the time of desorption, the extent of As adsorption at a specific equilibrium solution concentration was

greater compared to sorption, which reflects the extent of hysteresis effect observed for the given As sorption/desorption processes. Generally, higher Freundlich K values are obtained during desorption, compared to those for the sorption run. The difference between the amounts of As sorbed and desorbed was noted to be lower in the As-unaffected soil as compared to those in the As-affected soils at 35 °C. This may be due to the lower clay content of the As-unaffected soil compared to the remaining soils. These observations imply that the sorbed As had undergone transformations that imparted to it a higher degree of preference for the soil surface. The results also revealed negative free energy change, accompanying the given As adsorption process (except for the As-unaffected soil at 35 °C) which essentially suggest the process to be thermodynamically favorable. Thus, the stability of the As reaction products with the soil components in the As-affected soils, relative to As in soil solution, tends to facilitate As retention by these soils, which therefore act as an effective sink for As.

Interferences for Arsenic Adsorption and Ion Exchange

In both natural condition and water ameliorating systems, As adsorption and ion exchange processes are interfered by dissolved organics and anions either by direct competition with As for sorption sites or may dislodge As from the adsorption sites. Vanadium could restrict As sorption onto mineral surfaces by imparting hindrance to the adsorption of As(V) onto iron (III) (oxy)(hydr)oxides. Phosphorus and As belong to the same group in the periodic table, and their oxyanion, phosphate (PO_4^{3-}), and inorganic As(V) possess similar charge and tetrahedral structure; hence they compete for the sorption sites on clay minerals and different iron and aluminum compounds over an extensive variety of pH conditions (Gao and Mucci 2001; Stollenwerk 2003; Su and Puls 2003). Phosphate can also desorb As from humic acids and some mineral surfaces (Mok and Wai 1994; Stollenwerk 2003; Lafferty and Loeppert 2005).

Other than phosphate, silica is in competition with As(V) for sorption/ion exchange sites on the diverse iron(III) and aluminum compounds (Clifford and Ghurye 2002; Holm 2002; McNeill et al. 2002; Su and Puls 2003; Zhang et al. 2004; Smith and Edwards 2005) or polymerize on adsorbent surfaces and wipe out positive surface charges responsible for As sorption (Stollenwerk 2003).

Dissolved organic compounds are also found to contend with As for sorption and ion exchange sites on an assortment of sorbents (Stollenwerk 2003); particularly, fulvic acid is found to meddle with As(V).

Arsenic Retention by Organics

Studies on contaminated bottom sediments in New Jersey (Faust et al. 1987) revealed a low correlation between As total organic carbon ($r = 0.42$). A similar type of trends was reported by Ghosh et al. (2002) while examining the selected

As-contaminated soils of West Bengal, India. The residence of dissolved organic carbon (DOC) may contend with As for sorption sites on mineral surfaces, therefore enhancing its potential bioavailability. In accordance with earlier statement, Grafe et al. (2001) studied the sorption of arsenate [As(V)] and arsenite[As(III)] on goethite (α-FeOOH), in association with any one of peat humic acid (HAp), a Suwannee River fulvic acid (FA) (International Humic Substances Society, St. Paul, MN) or citric acid (CA). Both HAp and FA decreased As(V) adsorption at different pH as HAp decreased As(V) sorption within pH 6 and 9, while FA hindered As(V) sorption to the extent of 17% within pH 3 and 8, but CA did not affect. In between pH 3 and 8, arsenite sorption was reduced in all these acids and falls under the sequence: CA > FA ≅HAp. The differential pH divisions for decreasing As(V) sorption by HAp and FA indicate that multifunctional group instead of a single group of these complex organic polymers are the binding agents toward the α-FeOOH surface. Thus this experiment revealed that in a crystalline iron oxide-dominated solid phase, DOC substances are competent to increase the As bioavailability in soil-water systems.

Macalady and Ahmann (2002) in this context propounded that natural organic matter (NOM) has a universal presence in natural or artificial water bodies and sediments which can bind As along with metals also. These authors developed a scheme of such NOM which is characterized by several functional groups (like an alcoholic, carboxylic groups, ethers, aromatic moieties, amino groups, and sulfhydryl groups) and further proposed a conceptual model dealing with important considerations. The NOM forms inner-sphere complexes with a metallic surface, thereby exposing competitive behavior toward the metal; henceforth, metals are less accessible to As. These NOM can form aqueous complexes (ternary complexes formed from Al-NOM with As) with cationic metals.

16.4.2.2 Soil Humic-Arsenic Complexation Equilibria

Both natural and synthetic humic or fulvic acids assume a vital part in holding As [As(III) or As(V)] and hence in a reduction of the aqueous levels of As in acidic environments as compared to what the clays and hydrous oxides do. However, under alkaline conditions, the proportion of As supports the fluid phase (Thanabalasingam and Pickering 1986; Sanyal 2005). The soil organic fraction including humic acid and fulvic acid is known to combine with metal ions, clays, pesticides, and several organics because of its high specific surface area and chelating abilities (Datta et al. 2001; Sanyal 2002, 2017; Mukhopadhyay and Sanyal 2004; Ghosh et al. 2012).

Adsorption by humic acids for As(III) or As(V) varies according to adsorbate concentration, pH, and the valence state (Thanabalasingam and Pickering 1986). At pH 5.5, the amount of As adsorbed followed the Langmuir relationship, with calculated capacities for humic acid, having low ash and low calcium, of the order of 70 and 90 mmol/kg for As(III) and As(V), respectively, and for humic acid with high ash and high calcium, of 90 and 110 mmol/kg for As(III) and As(V), respectively. The organic moiety produces associated counterions as humate salts of complexes

that could disintegrate at high pH to produce hydrate oxide form of aluminum and iron, which are capable to sorb As. Therefore, at high pH, adsorption of As still occurred but by a different chemical species. The humic acids acted as an anion exchanger in which the basic amino functional groups seem to be involved in retaining the acidic groups with the affinity sequence being (Thanabalasingam and Pickering 1986) as follows:

$$H_2PO_4^- > H_2AsO_4^- > SO_4^{2-} > CO_3^{2-} \text{for As(V), and}$$
$$H_2PO_4^- > H_3AsO_3 > F^- > SO_4^{2-} > CO_3^{2-} \text{for As(III)}$$

Indeed the nitrogen contents of the HAs studied were found to be approximately in the same ratio as the maximum apparent As sorption capacities of the HA samples. The HA samples were shown to be capable of contributing more to As retention process thereby reducing its aqueous levels under specific soil conditions of soil acidity rather than the effect of clays and hydrous oxides..

The activity of fulvic acid at a concentration of <10 mg L^{-1} generally decreased As adsorption in the pH range of 5–7 (Xu et al. 1999). Both laboratory and field observations suggest that under acidic environment, As leaching through soils or sediments to surface and groundwater would be favored under reducing conditions, whereas under oxidizing conditions its movement gets restricted due to increased sorption (Xu et al. 1999).

Ghosh et al. (2012) studied the complexation of fulvic acid (FA) and humic acid (HA), derived from compost, oilcake, and top soils collected from As-affected and unaffected sites of West Bengal, India. These HA/FA samples were assessed through pH potentiometry, viscometry, visible spectrophotometry, and surface tension measurement. Potentiometric characterization of the HA/FAs exhibited that carboxylic (–COOH) acidity was higher compared to phenolic (OH) group acidity, a finding which agrees with the reporting of Ghosh and Schnitzer (1980) and Mukhopadhyay and Sanyal (2004). Total acidity (phenolic and carboxylic) recorded the highest value in the fulvic acid fraction of compost and lowest in the humic acid fraction of the As-unaffected soil. Significantly higher total acidity in the FA compared to HA fractions can be attributed to larger quantities of complex polymers derived through polycondensation process in HA. Further, the total acidity vis-a-vis carboxylic and phenolic OH group in the HA/FA extracted from manures was significantly higher (Fig. 16.4) compared to those extracted from soil, which essentially indicates the greater magnitude of degradation of organic matter in soil reflecting lower acidity in the HA/FA moieties. It is of importance to observe that the total acidity of both FA and HA of the As-contaminated soil enhanced after incorporation of compost in a crop field. The potentiometric titration by using fresh additional alkali to reach stable pH of higher range of these HA/FAs also exposed the coiling nature of these moieties (by steric hindrance) (Yee et al. 2009), which remained not folded or more flexible under thermodynamic obligation, further on to aliphatic/aromatic balance of HA/FAs (Sanyal 1984, 2017). The FA of the As-unaffected soil has the highest flexibility compared to the lowest flexibility of the HA of compost. The B_{expt} values

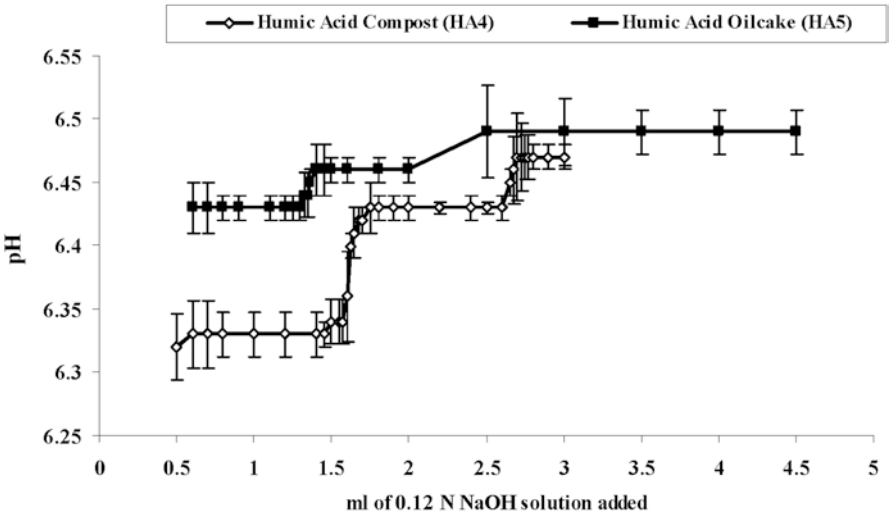

Fig. 16.4 Graphical representation of pH-potentiometric titration curves of humic acids of compost (HA_4) and oilcake (HA_5). (Source: Ghosh et al. (2012))

(derived from the viscosity study) of HA/FA were observed to rise with the rise of carbon number in acid moieties or enhance the coiling character (i.e., HA and FA of compost and oilcake), hence leading to the higher molecular weight of these samples. Spectrophotometric study through the ratio between the extinction coefficient at 465 nm (E_4) and 650 nm (E_6) of the studied HA/FA samples, which indicates an index of aliphatic/aromatic balance, also confirms these trends. The stability constant (log*K*, where *K* is the stability constant of arsenate-HA/FA complexes) of the complexes derived from the soil inherent HA/FA fractions was quite stable, while for the complexes of the HA/FA fractions of the organic manures with arsenate in aqueous phases, nature and properties of the humic polymers are of prime importance as they govern the retention/release of arsenate in soil. At pH 5.0, the stability constant values of arsenate-HA/FA complexes significantly ranged from 0.682 (with HA extracted from As-unaffected soil) to 8.32 (with FA extracted from compost), the highest log*K* and complexing fraction *x (*number of moles of HA/FA acid that becomes associated with 1 mole of arsenate) values being obtained with FA extracted from compost. Arsenic liberation from the arsenate-HA/FA complexes by soluble sulfate and nitrate salts was also tested through appropriate exchange isotherms where sulfate in general expressed a modestly greater extent of replacing ability with arsenate compared to nitrate, at elevated concentrations. Among the HA studied, arsenate complexed by compost-derived HA exhibited the least replacement by sulfate/nitrate (Fig. 16.5) in comparison with other HA samples examined. Also, the exchange of arsenate by sulfate was much less when compost was applied to the As-contaminated soil.

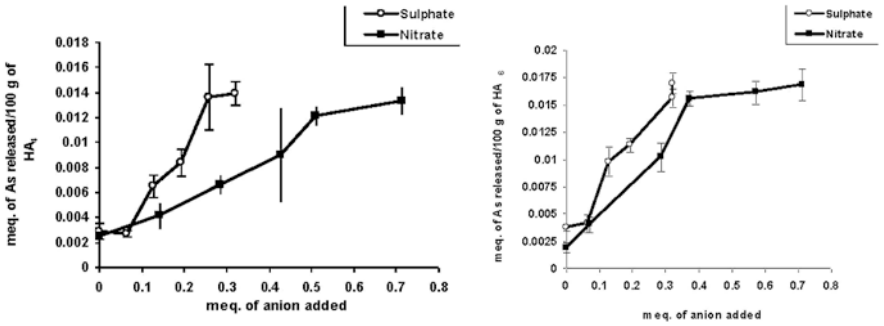

Fig. 16.5 Release (molc L^{-1}) of arsenate from humic acid of compost (HA_4) and compost-treated soil (HA_6) by the use of aqueous sulfate and nitrate (mol L^{-1}). (Source: Ghosh et al. (2012))

16.4.2.3 Arsenic Retention by Surface Charge Characteristics of Soil

There are several pieces of evidence which directly or indirectly indicates the entrapment of As in soils and sediments by oxides (e.g., of Fe, Al, Mn) via inner-sphere complexes formation or ligand exchange reactions (Woolson 1977; Livesey and Huang 1981; Majumdar and Sanyal 2003; Sanyal et al. 2015; Sanyal 2017). This is shown as follows (Hingston et al. 1974; Sanyal 2002, 2017; Ghosh et al. 2004):

$$\begin{aligned} &M-OH+H_2O \rightleftharpoons M-OH_2^{+}+OH^{-} \\ &M-OH_2^{+}+H_2AsO_4^{-} \rightleftharpoons M-O^{+}H_2.....H_2AsO_4^{-} \end{aligned} \tag{16.5}$$

$$\text{Or, } M-OH+H_2AsO_4^{-} \rightleftharpoons \left[M-O^{+}-H....H...HAsO_2^{2-}\right] \rightleftharpoons M-HAsO_4^{-}+H_2O \tag{16.6}$$

Also,

$$\left[M-OH_2^{+}\right]+\left[As^{V}O_4^{3-}\right] \rightleftharpoons \left[M-OH_2^{+}.....As^{V}O_4^{3-}\right] \rightleftharpoons \left[M-As^{V}O_4^{2-}\right]+H_2O \tag{16.6a}$$

However, the non-specific adsorption (through electrostatic mechanism) of As is also observed to a limited to pH-dependent charged surfaces at pH levels below the point of zero charges (PZC) for a given adsorbent (Sanyal 2002). Following the reaction schemes (Eqs. 16.5, 16.6, 16.6a), the abovementioned ligand exchange facilitates the rise in the negative charge of the soil colloidal fraction, as of iron oxides, and thus shifts the PZC of the As-enriched soil to lower pH. This fact findings were demonstrated by Ghosh et al. (2003) in an incubation study where As enrichment of soils from the contaminated site was observed to push the PZC to lower pH in comparison to the untreated soil accompanied with the elevation of the negative potential of the variable surface charge and the surface charge of the respective soil colloidal fraction. Nevertheless, such decrement of PZC of

noncrystalline Fe(III) oxide upon reaction with As(III) [and As(V)] was truly ascertained which provides yet another (indirect) evidence of the inner-sphere complex formation, involving specific adsorption of As(III) on the oxide surfaces (Manning and Goldberg 1996; Sanyal 1999, 2002).

16.4.2.4 Arsenic Retention by Clay Minerals

Manning and Goldberg (1997) investigated sorption behavior and durability of As(III) at the clay mineral-water phase and found that alkaline solution (pH > 9.0), devoid of clay mineral solids, created homogenous transformation of As(III) to As(V), whereas retrieve of As from As(III)-treated clay mineral solids suggested enhanced conversion of As(III) to As(V) by heterogeneous oxidation on kaolinite and illite, but not on montmorillonite surface.

Das et al. (2015) observed the variations in the As content in soil (As-affected and As-unaffected soil) which is primarily due to the clay fraction, dominant clay minerals, amorphous iron, and aluminum along with the calcium plus magnesium content of the given contaminated soils. Among the clay minerals, As is mainly adsorbed by illite and kaolinite minerals in As-affected soils. Manning and Goldberg (1996) reported that kaolinite and illite minerals have greater adsorption maxima for As compared to montmorillonitic minerals. For kaolinite, there is hardly any isomorphous substitution in either tetrahedral or octahedral layer which leads to virtual electroneutrality of these layers. Furthermore, the layers are strongly held through electrostatic hydrogen bonding which essentially leads to the lower specific surface area and nonavailability of interlayer spaces for As adsorption. In case of illite, the substitution of tetrahedral Si^{4+} ions by Al^{3+} ions leads to an excess negative charge which was further balanced by the interlayer monovalent or divalent cations. Because the 2:1 layers are held together strongly in the interlayer spaces by the exchangeable cations, namely, K^{+}, Ca^{2+}, and Mg^{2+} ions, illites are non-expanding with fewer interlayer spaces for As adsorption. So whatever As adsorption takes place, it primarily depends on surface reaction. According to Mohapatra et al. (2007), As(V) sorption in clay minerals includes inner-sphere surface complex formation and steady specific ion adsorption which is accountable for higher sorption in kaolinite/illite minerals. Due to the variations in soil pH and occupancy of these minerals, the ratio of As(V) to As(III) varied in these soils.

16.4.2.5 Arsenic Retention Under Varying Soil Conditions

Arsenic adsorption is mostly regulated by Fe (hydro) oxides whereas, comparatively less extent by Mn (hydro) oxides in soils with better aeration (Mok and Wai 1994). The following reactions show the As(III) oxidation by oxygen at high pH while that by Fe and Mn oxides in solution at lower pH values (Oscarson et al. 1981).

$$O_2 + 4OH^- + 2H_2As^{III}O_3^- = 2As^{V}O_4^{3-} + 4H_2O\ E_O = 1.18V \quad (16.7)$$

$$2Fe^{III}(OH)_3 + H_3As^{III}O_3 + 4H^+ = 2Fe^{+2} + H_3As^{V}O_4 + 5H_2O\ E_O = 0.40V \quad (16.8)$$

$$Mn^{IV}O_2 + H_3As^{III}O_3 + 2H^+ = H_3As^{V}O_4 + H_2O + Mn^{+2}\ E_O = 0.62V \quad (16.9)$$

Majumder and Sanyal (2003) studied the dependency of arsenate adsorption on the adsorbent pH. These workers (2003) noted a decrease of arsenate sorption with the rise of pH only at low levels of arsenate concentration, but the opposite trend was observed for higher arsenate equilibrium concentration. This was explained regarding the varying electrostatic potential of soil colloidal surfaces with pH, solubility product principles, and buffering action of the arsenate salt used (Majumder and Sanyal 2003). In contrast, Carey et al. (1996) reported higher arsenate adsorption at pH 6.0–7.0 than at pH 5.0 in two New Zealand soils, where the prime responsible agent for the observed rise in As sorption with pH (Carey et al. 1996) could be attributed to the application of $Ca(OH)_2$ to elevate the equilibrium pH values. The divalent Ca^{2+} ions would partially offset the abovementioned fall of charge.

Sun and Doner (1998) suggested that in addition to mineral surface type, As adsorption/desorption in soils was sensitive to pH and E_h conditions. Xu et al. (1988) also studied As(V) sorption phenomena by alumina, hematite, kaolin, and quartz as a function of pH (2–10). While adsorption on quartz was low (pH 3–9), adsorption maximum on alumina and kaolin was observed at about pH 5.0, decreasing sharply beyond pH 6.0. Hematite behaved similarly, showing, however, less pronounced decrement in As(V) sorption at higher pH. The differences in As(V) adsorption on alumina, kaolin, hematite, and quartz at pH 5.0 were roughly by the corresponding differences in anion exchange capacities.

Goldberg (2002) showed that arsenate sorption on amorphous Al oxides was 100% within pH 3–9 and then becomes lessen with a further rise in solution pH, whereas arsenite adsorption on Al oxide disclosed a parabolic sorption curve.

16.4.3 Arsenic and Other Nutrient Element Interactions in Soils

Competitive trace oxyanion interactions in soils and soil components are of importance since the same mechanism, namely, surface complexation through ligand exchange with surface functional groups (OH_2^+ and OH) of the edges of clay minerals and hydrous oxide surfaces of Fe, Mn, Al, etc., seems to be implicated in the retention of these oxyanions (Hingston 1981; Sanyal 2000). Whereas Cl^-, NO_3^-, and SO_4^{-2} ions exist at concentrations, generally observed salt-affected soils have a meager effect on As sorption in soils (Manful et al. 1989; Sanyal 1999). Arsenate, phosphate, and molybdate are tetrahedral oxyanions, estimated to occupy on goethite surface mean areas of 0.61, 0.61, and 0.31 nm^2, respectively (Hingston 1981).

In goethite, PO_4^{3-} compete with arsenate for the common surface sites in soil, and further PO_4^{3-}, like arsenate, can be sorbed over such surface as an inner-sphere complex via a ligand exchange mechanism (Persson et al. 1996; O'Reilly et al. 2001; Kuo and McNeal 1984; Mukhopadhyay and Sanyal 2004; Sanyal and Dhillon 2005). The competitive interactions of arsenate and phosphate have an important significance since, in addition to occupying (on the substrate) similar sites, they form triprotic acids characterized by similar dissociation constants. Several workers (Manning and Goldberg 1996; Mukhopadhyay et al. 2002) inferred that the influence of phosphate could decrease arsenate sorption, and the decrement was much higher than the competitive effects of arsenate on phosphate adsorption by soil minerals, though a greater fluctuation in the extent of competition between these two oxyanions has been reported (Hingston 1981; Goldberg 1986; Roy et al. 1986; Reynolds et al. 1999; Jackson and Miller 2000). Manning and Goldberg (1996) observed that the comparative attraction of As(V), P, and Mo for the goethite and gibbsite surfaces was pH-dependent and falls in the sequence of $P \approx As(V) > Mo$ at normal pH. The ligand exchange process may change the pH of the sorption system by raising the OH^- ion concentration, which, in turn, may compete with phosphate or arsenate for surface complexation sites (Melamed et al. 1995). Laboratory experiments were performed to study the As liberation in batch suspensions of five lead arsenate affected soils by Peryea (1991) where he concluded that the liberation of As was positively correlated to the applied phosphate concentration but nonsignificantly affected by phosphate source.

The interaction effect among As, farmyard manure (FYM), and phosphorus in an incubation study was investigated by Mukhopadhyay et al. (2002). The release of both As and phosphorus in the soil solution suggested that irrespective of the doses of the applied FYM and phosphorus, there was a marked increase of the extractable phosphorus over that of the control during the period of incubation, while the extractable As showed a declining trend compared to that observed with application of phosphorus alone. However, FYM application led to significant reduction in As release into the soil solution, whereas phosphorus addition, in the absence of FYM, led to an increase of extractable As (Mukhopadhyay et al. 2002). Das et al. (2005a) observed that the amount of As content in soil significantly depressed with the use of graded doses of organics. However, such decrement was observed to be more prominent with well-decomposed FYM compared to vermicompost.

Gustafsson (2001) studied the competitive adsorption of As with anions in a spodic B horizon having the predominant adsorbent minerals like proto-imogolite allophane and ferrihydrite. For the determination of the complexation constant of the corresponding reference oxides, a CD-MUSIC (charge distribution multi-site ion complexation) model was utilized where arsenate being the adsorbing ion coupled with the incorporation of a sample of small amounts of organic matter to minimize the interference effect of competing for organic substances. To describe the adsorption of added arsenate properly, the worker considered the competitive interactions of sulfate, silicic acid, and phosphate. The model was adjusted by the specific surface area of solely oriented (AlOH) allophane groups, the sulfate surface complexation constant on allophane, and the total concentration of reactive silicic

acid by keeping the other parameters fixed utilizing reference oxide values. The outcome revealed greater surface complexes on ferrihydrite compared to gibbsite or allophane were developed with arsenate, phosphate, and silicic acid; however, the opposite happened for sulfate.

Interreaction of zinc (Zn) and As and time span of submergence were examined by Garai et al. (2000) for As-contaminated West Bengal soils. Such interaction study showed that As content increased with the progress of submergence up to 35 days. However, such increase in As extractability was due to the application of graded doses of Zn. Das et al.(2005a) showed that the quantity of As content in soil diminished drastically with the use of differential doses of Zn in the form of $ZnSO_4$. This intensity of reduction varied larger (0.73–2.72 mg kg^{-1}) into the treatment of the application of Zn at 10 mg kg^{-1} and lower (0.70–1.08 mg kg^{-1}) with the application of Zn at 20 mg kg^{-1} was. A similar effect was perceived while experimenting Fe in place of Zn. Das et al. (2016), in a laboratory incubation study, observed the interaction of graded levels of zinc and As under 10 and 25 days incubation. The results of this study revealed the decrease of As liberation in the soil solution of the studied soils on use of zinc, which tends to tie As electrostatically in the soil matrix, hence facilitating to combat the As toxicity in the soil-plant system to certain levels. The As-zinc interactions were once again verified in the As-contaminated fields where the reciprocal moderating effect (as observed in the incubation study) was observed in rice-rice cropping sequence.

16.4.4 Precipitation and Coprecipitation of Arsenic

Precipitation is a process of formation of solid insoluble reaction products(e.g., calcium arsenates), formed by the reaction between two types of dissolved species (such as As(V) oxyanions with other dissolved species such as Ca^{2+}, Fe^{3+}, or manganese cations) in water or other liquids. The avenues of precipitation are evaporation, oxidation, reduction, modification in pH, or the mingling of chemicals into an aqueous solution. Precipitation is an imperative process that restricts the mobility of As in natural systems and can also be used for decontamination of water (Henke and Hutchison 2009).

The sorption of *minor* or *trace element* (such as As) or absorption of the same into the newly forming or fresh precipitates of different chemical species is called as coprecipitation. Since adsorption includes the assimilation of contaminants onto or within sorbents, it ought to be recognized separately with coprecipitation which happens as or right away after the precipitation of host solids from the solution (i.e., As coprecipitating with iron (oxy)(hydr)oxides in acid mine drainage). According to US Environmental Protection Agency, coprecipitation may likewise include entanglement of (absorption) As-bearing colloids or other fine-grained particles in the inside of precipitating substances (US EPA 2002; Yuan et al. 2003). Also, As could coprecipitate by replacing (as As(V) partially replacing carbonate) into the

precipitating crystalline compounds (in the developing crystalline structures of jarosite) (Savage et al. 2000; Savage et al. 2005; Henke and Hutchison 2009).

16.4.5 Microbial Transformation of Arsenic from Soil

The arsenite and arsenate forms are the most prevalent species in the aquatic environment. Typically, As is toxic to microorganisms and hence specific transporters for its uptake do not evolve. However, as the arsenate and arsenite forms resemble glycerol and phosphate analogues, they are assimilated by these transporters in conditions of high As concentration as these analogues form the part of bacterial nutrition (Stolz et al. 2006). The transporters facilitating the uptake of phosphate also facilitate arsenates to enter the cell and hence interfere with the energy-generating processes dependent on phosphate. Whereas, aqua-glycerol porins provide the entry of arsenite which affects a broad range of cellular processes (Lloyd and Oremland 2006). However, the microbial cells protect themselves by performing detoxification mechanisms against As toxicity. Notably, *Escherichia coli* uptake arsenate through phosphate transporters, Pit (low-affinity, high-capacity constitutive system) and Pst (high-affinity, low-capacity system induced by phosphorus starvation) system, and arsenite via the glycerol transporter GlpF (a bacterial glycerol facilitator) (Tsai and Singh 2009; Rosen and Liu 2009).

Typically for bioremediation, microbes employed can convert mobile and toxic trivalent As to less toxic and immobile pentavalent state (Jyothsna and Murthy 2016). Multifarious microbes carry out bioremediation either through detoxification, mobilization, or immobilization of As. These microbial processes operate through oxidation, reduction, biosorption, or biomethylation processes within the cell (Wang and Zhao 2009). With respect to this, several microbes along with specific bioremediation methods employed are provided by Jyothsna and Murthy (2016) where they chose specific organism performing for specific processes as for oxidation, *Sulfolobus acidocaldarius* and *Thiomonas arsenivorans*; for reduction, *Thiomonas arsenivorans*, *Desulfosporosinus* sp., *Shewanella* sp., and *Clostridium* sp.; and for biomethylation, *Penicillium* sp., *Chlorella vulgaris*, *Polyohysa peniculus*, *Fusarium oxysporum melonis*, *Closterium aciculare*, and *Methanobacterium formicicum*.

Arsenic gets mobilized through microbial transformation of inorganic As to organic forms, namely, MMA and DMA (Jia et al. 2013; Xu et al. 2016), which are more efficiently translocated in plant body through roots to the (frequently edible) aboveground parts compared to inorganic As (Carey et al. 2010, 2011, 2012); hence, microbiological transformation to organoarsenicals can boost human dietary exposure to As. Volatile As species (arsine gas and mono-/di-/trimethylarsine) may be developed either biotically – by fungi, bacteria, and algae (Turpeinen et al. 2002; Mestrot et al. 2011; Wang et al. 2014) – or abiotically (Wang et al. 2014). In typical systems of nature, arsines promptly interact with oxygen and get most quickly oxidized to form oxidation products not volatile; hence AsH_3 becomes difficult to

determine in environmental samples. The As cycle gets completed through the oxidation of the arsine gases to inorganic As species, with the return of As to the soil through rain or dry deposition (Pongratz 1998). Decreasing redox potential and organic matter application increases As methylation in soils (Frohne et al. 2011). Similar to the As methylation, As volatilization was noted to increase when rice straw and animal waste materials (Jia et al. 2013; Mestrot et al. 2013). In heavily contaminated and spiked soils, inoculation of fungi (*Penicillium* and *Ulocladium* spp.) can increase As volatilization up to eightfold (Edvantoro et al. 2004). Arsines in gas state are volatilized from As-affected soils to the atmosphere at very sluggish rates as revealed from a microcosm study where 0.50–70 μg of As kg^{-1} soil $year^{-1}$ got volatilized from a variety of soils with varying levels of As (Mestrot et al. 2011) although the field evaluation of As volatilization are 1–2 times lower compared to those observed in the laboratory mesocosms (Meharg and Zhao 2012).

16.5 The Fate of Arsenic in the Soil-Plant System

16.5.1 Arsenic Accumulation through the Soil in the Plant Body

The contribution of As to soil from different sources may demonstrate adverse to plant through its ingestion to the toxic limit, thereby entering the food chain. There thus exists a natural concern as there is the likelihood of biomagnification in the course of the travel of the toxicant up in the food web (Sanyal and Nasar 2002; Ghosh et al. 2004; Sanyal and Dhillon 2005; Sanyal 2005; Das et al. 2005a). The propensity of plants to build up and transfer this contaminant to palatable and harvested parts relies upon, as stated earlier, largely on soil and climatic factors, plant genotype, As concentration in groundwater (irrigation source), and agronomic management (McLaughlin et al. 1999). Several researchers pointed out the buildup and conversion of As by many plant species are grown in the As-contaminated areas. Among the crops, elephant foot yam, green gram, cowpea, sesame, groundnut, etc. tended to demonstrate the accumulation of As in considerable amounts in various plant organs (ICAR 2001; Sanyal and Dhillon 2005; Sanyal 2005; Sanyal et al. 2015). In fact, pointed gourd, a vegetable creeper plant, has indicated significant As stacking when cultivated in the contaminated soils of West Bengal (Panda and Das 2001). Various vegetables specifically cauliflower, tomato, and bitter gourd were found to gather As in their economic produce (Chowdhury et al. 2001; Ghosh et al. 2004; Sanyal 2005). In general, the partitioning of As in plant parts by and large followed the order: root > stem >leaf > economic produce (Ghosh et al. 2004; Sanyal 2005; Das et al. 2005a; Sanyal et al. 2015).

16.5.2 Arsenic Metabolism in Plants

According to Nissen and Benson (1982), As is metabolized from inorganic to organic species with restricted confirmation that this form develops in plant but by a variety of organisms; metabolism normally happens through biomethylation to yield monomethyl As (MMA), dimethyl As (DMA), tetramethyl arsonium ions (TETRA), and trimethylarsonium oxide (TMAO) (Cullen and Reimer 1989). In some organisms and terrestrial plants, further metabolism of As gives rise to arsenocholine, arsenobetaine, and some arseno sugars (Tamaki and Frankenberger 1992; Geiszinger 1998) though it is doubted whether these compounds metabolized in plants or are taken up from the soil. Arsenate to arsenite reduction has been convincingly exhibited in plants, yet methylation has not been illustrated (Van Den Broeck et al. 1998; Pickering et al. 2000). Organoarsenic forms in field plant samples may be withdrawn from the soil solution similar to that forms existed in soil by microbial action (Cullen and Reimer 1989; Koch et al. 1999). This theory is upheld for MMA and DMA, as their uptake has been reflected in many plant species through hydroponic solutions (Martin et al. 1992, 1993; Carbonell-Barrachina et al. 2000). However, it is possible that the plant species can methylate As themselves. Koch et al. (2000) observed that in spite of no outer methylation in neighboring soil and water, many plant species accumulated MMA, DMA, TETRA (tetramethylarsoniumion), and TMAO (trimethyl arsonium oxide). As a resistance against basic metals remaining at potentially harmful concentrations, various enzymatic activities act as stimulant in plants, for example, production of phytochelatins, glutathione, and superoxide dismutase is activated in response to Cd and Hg, whereas As may prompt such reactions enabling the plants to decontaminate lower levels of As exposure (Hartley-Whitaker et al. 2001a,b). Further in arsenobetaine and arsenocholine, As(III) acts as a nitrogen analogue (Marschner 1995), and in addition, glycine betaine and choline are osmoregulators, derived by various succulent species upon exposure to water scarcity, thereby enabling the plants to synthesize organoarsenic species in their body upon stress condition(Sakamoto and Murata 2000, Meharg and Hartley- Whitaker 2002).

16.5.3 Arsenic Toxicity to Plants

Inorganic As species are typically very harmful to plants. As discussed earlier, arsenate resembles phosphate analogue and is carried through the plasma membrane by phosphate cotransport framework (Ullrich-Eberius et al. 1989). Upon entry to the cytoplasm, As contends with phosphate and replaces it from ATP to form unstable ADP-As which resulted in the interruption of energy drift in cells (Meharg 1994). However, Bertolero et al. (1987) pointed out that arsenate will not necessarily possess high cytoplasmic concentrations to express harmful effect because it is quickly reduced to arsenite in plant tissue. Arsenite is also extremely harmful to plants as it

inhibits cellular function and death by interacting with sulfhydryl groups (-SH) of enzymes and tissue proteins (Ullrich-Eberius et al.1989). In vitro transformation of arsenate to arsenite is facilitated nonenzymatically by glutathione (Delnomededieu et al. 1994), which has greater concentrations in cells of plant tissues (Alscher 1989). Besides, microorganisms, for example, yeasts, facilitate reduction of arsenate to arsenite through arsenate reductase enzyme (Mukhopadhyay et al. 2000), which may be operative in plant tissues, although they remained unidentified to date.

Bhumbla and Keefer (1994) observed that majority of the As, ingested by crops, has a tendency to reside in the roots and is not usually carried readily to shoots. Furthermore, comparative tolerance of various crops to As was also different. Among the crop plants, apples, grapes, rye, cabbage, carrots, tobacco, tomato, potato, wheat, cotton, and peanuts are the tolerant crops, while the medium tolerant are cherries, beets, corn, squash, radish, and turnips, and further low tolerant crops are peas, onion, spinach, beans, soybeans, rice, and cucumber (Adriano 1986; Sanyal 2005).

The interconnection between soil As and plant growth relies upon the form and plant extractability of soil As. The toxicity of As varied with its form and valence, the order of its toxicity being AsH_3 > As(III) > As(V) > organic As (Wu and Xie 1990; Sanyal and Nasar 2002).

Several workers have worked on the As toxicity symptoms and critical concentrations causing such symptoms (Table 16.2).

16.5.4 Arsenic Accumulation in Crop Plants

The concentrations of As in flowering plants were found to be 0.114, 0.203, 0.214, 0.235, and 0.293 mg kg^{-1} when the levels of soil As were 0, <20, 20–30, 40–50, and > 50 mg kg^{-1}, respectively (Zhou 1986).

Table 16.2 Toxicity aspects and critical concentrations of arsenic in plant

Crop	Toxicity aspects and critical concentrations	Source
Plants in general	Plasmolysis of root and wilting of leaf, followed by root discoloration and necrosis of leaf tips upto 9 $mgkg^{-1}$ of the soluble arsenic concentration in soils	Machlis (1941)
Cotton	Vegetative growth declined by 50% at internal arsenic concentration of the plant exceeding 4.4 $mgkg^{-1}$	Deuel and Swoboda (1972)
Rice	Symptoms of straight head disease which include sterility through the application of MMA in cotton soils	Wells and Gilmour (1977)
Rice	The phytotoxicity threshold levels indicates 55.5% yield reduction in tillering and 54.8% at harvesting stages when the maximum uptake at this threshold levels at the two stages was 36.4 and 34.01 mg kg^{-1}, respectively	Das et al. (2013)

Transformation of arsenate to greater toxic arsenite is favored by bringing down of redox potential (E_h) which is experienced under oxygen-depleted soil conditions, with arsenite being more soluble and portable than arsenate, thereby inducing toxicity to rice plant (Tensho 1973; Sanyal 1999, 2005; Sanyal and Nasar 2002; Sanyal and Dhillon 2005; Saha et al. 2005). In the selected districts of Bangladesh, the total As uptake of the rice crop was 10 mg kg^{-1} and even higher at 14% moisture level (Duxbery et al. 2003). In a pot culture experiment, Xie and Huang (1998) found out critical contents of As in some paddy soils of China, leading to toxicity to rice plants. The As contents of the leaves and stem at harvest were 5.51 and 9.34 mg kg^{-1}, respectively, when potato was grown with irrigation water with an As loading of 0.22 mg L^{-1} (Adak et al. 2002).

16.5.5 Risk Assessment of Arsenic-Contaminated Soil

Attempts had been made by Golui et al. (2017) to assess the As content in rice grain by field studies, conducted in selected blocks of Malda district of West Bengal, India, and to compare the same with that anticipated by the combined solubility-free ion activity model without accounting the free ion activity of soil solution (Hough et al. 2004; Datta and Young 2005). The free ion activity model (FIAM) indicates that plant uptake may be governed by metal/metalloid ion activity in the soil pore water. There have been several earlier attempts to undertake such risk assessment of growing food crops in metal-contaminated soils by using the FIAM. The resultant entry of the hazardous metal in human food chain was noted to depend on how one can anticipate the solubility of metals in soils. Such risk of metal-contaminated soils can also be assessed by predicting metal uptake by crops grown on contaminated soils on a routine basis (Sanyal 2017). The guiding principle depends on the premises that the response of plants and soil organisms toward metal toxicity is determined primarily by the deviation in free metal ion activity in soil pore water. Thus, Datta and Young (2005) developed the protocol for prescribing toxic limit of metals, based on extractable metals and soil characteristics, using the solubility and free ion activity models. Dissolvability of metals in soil was derived by employing the following pH-reliant Freundlich equation (Jopony and Young 1994) as per the free ion activity of metal and metalloid (FIAM):

$$\left(M^{2+}\right) = M_c \,/\left[k_M \left(H^+\right)^{-n}{}_M\right] \tag{16.10}$$

where (M^{2+}) is the free metal ion activity in soil solution in soil pore; M_C is the labile pool of soil metal, assumed to be exclusively adsorbed on humus (mol. kg carbon^{-1}); and k_M and n_M are empirical constants which express the pH dependence of the metal distribution coefficient. It follows from detailed theoretical considerations (Datta and Young 2005; Meena et al. 2016; Golui et al. 2017):

$$p\left(M^{2+}\right) = \left[p\left(M_c\right) + k_1 + k_2 pH)\right] / n_F \tag{16.11}$$

where k_1 and k_2 are empirical, metal-specific constants, expressing the pH dependence of metal distribution coefficient and n_F is the power term from the Freundlich equation. The metal transfer factor from soil solution to plant biomass is given as:

$$\text{Transfer factor} = \log\left[M_{plant} / \left(M^{2+}\right)\right] \tag{16.12}$$

where M_{plant} is the metal loading of plant biomass.

Equations 16.11 and 16.12 can be combined to lead to Eq. 16.13 as follows:

$$p\left(M_{plant}\right) = C + \beta_1 pH + \beta_2 p\left(M_c\right) \tag{16.13}$$

where C, β_1, and β_2 are empirical metal- and plant-specific constants. This model (FIAM) anticipates the free ion activity of trace metal and metalloid in soil solution as a function of labile soil extractable metal and pH with the easy presumption that the total metal (M_C) is sorbed on soil humus.

Closely related to what has stated above, the hazards to human health by consumption metal through food material has been computed regarding what is known as the hazard quotient, HQ_{gv}. The latter is given as:

$$HQ_{gv} = \left(ADD / R_f D\right) \tag{16.14}$$

where ADD = average total daily dose of metal intake through diet and drinking water (mg metal.kg body weight^{-1}. day^{-1}) and R_fD = the corresponding reference dose which is defined as the maximum permissible daily intake of the particular metal that does not lead to any hazardous health effects. Obviously, $HQ_{gv} > 1.0$ suggests hazard to human health. However, the point worth noting here is that ADD refers to the daily intake of a given metal *from all the food items and drinking water*. It is thus evident that for any one food item (e.g., a vegetable or rice), the limiting value of HQ_{gv} will be less than 1.0. In this context, Meena et al. (2016) argued that the admissible limits of metal and metalloid in soil were set up, in light (i) dissolvability of metal and metalloid in soil; (ii) metal and metalloid found in crop, as, for example, rice and wheat grain; and (iii) human health hazardous effect, linked with consumption of metal and metalloid through crop grown on metal-contaminated soils. For setting the hazardous limit of the extractable metal and metalloid in soils at a specific pH and organic carbon, the critical value of HQ_{gv} used by these authors was 0.5 for *any one given food item* (especially for a staple food item). Meena et al. (2016) developed a ready reckoner to calculate the permissible limit of the extractable metal and metalloid in soils, relied upon soil pH and organic carbon content, *corresponding to the respective HQ_{gv} values (associated with metal intake by human through food, for instance, rice grain) remaining below the critical value of 0.5*, as mentioned above. These permissible limits were derived from the predicted HQ_{gv} by

the aforesaid solubility-FIAM. Golui et al. (2017) reported $HQ_{gv}$to be 5.69 ± 5.13 for drinking water in the area of study, which is far above the safe limit of HQ_{gv}, i.e., 1. The HQ_{gv} for As in case of rice was 0.72 ± 0.72. The As content in rice was also predicted through the abovementioned solubility-free ion activity model whose result was in close agreement with the earlier one. The variation in As uptake by rice grain could be described by the given solubility-FIAM model to the extent of 78% which was considered highly satisfactory for habitual risk appraisal of As-affected soil, given easily quantifiable soil properties like extractable As, soil pH, and soil organic carbon.

The given study (Golui et al. 2017) further showed that the permissible (Olsen) extractable limit of As in soil varied widely with a change in soil organic matter content (the extractable limit increasing with the soil organic carbon status), while such variation was not evident with soil pH. The latter may be attributed to a short range of soil pH values (in the alkaline range in the study area). Such findings further strengthen the argument that total As content is not a good index of As hazard about human health. This shows the importance of fixing the permissible limit of extractable As in soil by taking due cognizance of the important soil properties. Hence appropriate management interventions (e.g., organic amendments, liming, etc.) may be designed to render an As (or, in general, metal)-contaminated soil remain within a risk-free domain for raising the crops for human consumption, *without* posing the health hazards (Sanyal 2017).

16.5.6 Phytoremediation Options of Arsenic in Plants

Among the possible mitigation options/interventions, phytoremediation (which means the utilization of green plants to expel toxicants from the environment or render them safe) tends to offer a potentially useful avenue to address the problem of contaminated agricultural soils and crops (Chhonkar 2004; Das et al. 2005b; Sanyal 2017). Among the phytoremediation techniques, hyperaccumulation may be a way of escaping contest from less metal-tolerant plants, a strategic method for achieving metal resistance, the consequences of unintentional take-up of heavy metals (Brooks 1998).

Some plant species, including the dominant wild species, growing in the As-affected areas, can accumulate As. Nevertheless, it is worth mentioning in this matter that such hyperaccumulation of As does *not* prompt its detoxification as such (Sanyal 1999; Sanyal and Nasar 2002; Das et al. 2005b). Ma et al. (2001) described the hyperaccumulation of As from the toxicant infested soils by the brake fern, *Pteris vittata*, and its successive mobility into the aboveground biomass implies that the As buildup in plant was mostly in the toxic inorganic forms, and regarding the distribution of species in the affected soil in which the fern grows, highly toxic As(III) was found with greater proportion in the plant biomass than that of the lesser toxic As(V) form (Ma et al. 2001). Certainly, the detoxification process would be aided by the metabolic transformation of the plant-gathered inorganic forms of the

toxin to non (or less)-toxic organometallic forms. In this context *Polyphysa peniculas* (Cullen and Hettipathirana 1994), a unicellular alga, is reported to mobilize As in plant body which can detoxify As also.

Pursuance from different literature unveils many plant/microbial species, acknowledged for As accumulation/bioindicator (as an option of bioremediation), which can efficiently take off As (and other heavy metals) from the water bodies, for example, water hyacinth stem and leaves can remove 170 and 340 μg As g^{-1} dry weight, respectively, when nurtured in a pond having 10 mg $Asdm^{-3}$ (Chigbo et al. 1982). As a consequence, proper protection has to be taken up while translating the As the content of aquatic environment to derive As accumulation by water hyacinth (Low and Lee 1990). Hydrilla (*Hydrilla verticillata* Casp.) (Lee et al. 1991), pointed gourd (Panda and Das 2001), and several other crops, namely, Indian mustard, sunflower, cauliflower, *Thlaspi caerulescens*, *Berkheya coddii*, etc., were also noted for their ability to accumulate As in substantial amounts in their biomass (Rio et al. 2000; Baker et al. 2000; Chhonkar 2004; Das et al. 2005b).

Arsenate tolerance was elaborated by the comparative decrement of maximum root growth (MRG) by the grassy weeds, namely, *Agrostis castellana* and *A. delicatula*, against the sensitive species when exposed to As (Koe and Jaques 1993). These results tend to propose large portion of plants (if not all), irrigated with As-tainted water, or, for example, those grown on soils, or in an aquatic system carrying elevated amounts of the pollutant metalloid, tend to gather As (Das et al. 2005b).

An experiment, conducted in Thailand, where elevated As accumulation in soil and groundwater resulted from tin mining, screened mine products for evaluation of As in fronds (Wongkongkatep et al. 2003). Two species of fern were detected to have hoisted levels of As in their fronds, namely, *Pityrogramma calomelanos* (108–1156 μg g^{-1} dried weight) and *Pteris vittata* (79 μg g^{-1} dried weight). The absorption of As in *P. calomelanos* shoot two times magnified with the application of EDTA (ethylenediaminetetraacetic acid), a well-known chelating agent (Wongkongkatep et al. 2003). The application of another chelating agent, namely, DMSA (Dimercaptosuccinic acid), exhibited a five times reduced As content in the *P. calomelanos* shoot, in comparison to control up to the span of 6 weeks of As exposure. The divergent effect of these two chelating agents may be due to the powerful bonding of As ions by the thiol group present in DMSA. This study also indicated that the given fern uptakes and carries As in the form of arsenate and arsenite comparatively than DSMA complex.

Such EDTA or DSMA complex formation is central to chemically induced phytoextraction. It was also observed that *P. calomelanos* provided the most noteworthy As phytoextraction efficiency at 6 weeks exposure to As under EDTA treatment, resulting in an efficiency of 77.8 mg As kg^{-1}, considering the whole plant biomass (Wongkongkatep et al. 2003).

A number of microbial species (e.g., the bacterial species, namely, *Proteus* sp., *Escherichia coli*, *Flavobacterium* sp., *Corynebacterium* sp., and *Pseudomonas* sp.; the fungus, namely, *Candida humicola*; the freshwater algae, namely, *Chlorella ovalis*, *Phaeodactylum tricornutum*, *Oscillatoria rubescens*) have been accounted

for having differential magnitude of As accumulating capacities (Sanyal and Nasar 2002). In a study conducted with selected As-contaminated soils of West Bengal, the As-volatilizing indigenous soil bacteria, isolated from these soils, were tested for their ability to turn the toxic indigenous inorganic As to less toxic volatile arsenicals (Sanyal 2017). Approximately 37% of As(III) (undergoing anaerobic condition) and 30% As(V) (undergoing aerobic condition) were volatilized by these bacterial isolates in 3 days. As opposed to the genetically altered organism, the native soil bacteria associated with FYM were competent to evacuate 16% of As from the polluted soil with a span of 60 days incubation (Mazumdar et al. 2013a). Further, As-oxidizing bacteria hyper-resistant to both As(V) (167–400 mM) and As(III) (16–47 mM) were isolated from the selected As-contaminated soils of West Bengal (Mazumdar et al. 2013b), which were in close association with various species of *Bacillus* and *Geobacillus*, based on their 16 s rRNA gene sequences. Higher pace of As(III) oxidation (278–1250 μM h^{-1}) and arsenite oxidase activity (2.1–12.5 nM.min^{-1} mg^{-1} protein) were perceived in these isolates.

16.6 Speciation of Arsenic in Soils and Plants

Speciation analysis is the measurement of a particular chemical or physical form of an element. From the speciation study of the total As loading in the affected soils and the crops, it was observed that arsenite and arsenate oxyanion species are important for characterizing the *net* toxicity of As in the given soil-crop systems. Masscheleyn et al. (1991), while examining the forms of As in a contaminated soil, reported that at relatively high redox level, presence of lowly soluble As(V) was 65 to 98%, while the soluble As level in soil at $E_h = -200$ mV was 13 times more than that at $E_h = 500$ mV. Naidu et al. (2000) performed speciation of As (AsO_2^-, AsO_4^{3-}, and dimethylarsinic [DMA]) in typic soil solutions from affected sites in Australia where As speciation in soil aqueous phase can be accomplished in less than 5 min with detection limits of 0.50, 0.10, and 0.10 mg L^{-1} for As(III), As(V), and DMA, respectively.

Douglas et al. (2001) reported that out of total As present in rice and vegetable crops, 95% and 5% of the compounds are present as inorganic As and organic As forms, respectively, in rice, while in vegetables, 96% and 4% of the compounds are present as inorganic As and organic As forms. Vela et al. (2001) extracted As species in freeze-dried carrot samples using LC-ICP-MS, where they found As(III) and As(V) to be the only inorganic species present in the samples. These samples contained 20 ng g^{-1}–18.7 μgg^{-1} as total As of which the abovementioned forms contain less than 400 ng g^{-1} of the total loading. In a study on chemical speciation of As in rice, D'Amato et al. (2004) detected four forms of As, namely, inorganic As(III), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), and inorganic As(V) at concentrations of 88.2 ± 7.1, 50.8 ± 5.0, 15.2 ± 1.7, and 51.2 ± 3.5 ngg^{-1}, respectively.

Das et al. (2015) studied *the* distribution (in percent) of arsenate (As^{+5}) and arsenite (As^{+3}) present in the different extracts of the selected water, soil, plant, and weed samples of the As-affected areas. Total As loading is a crude indicator of As accumulation, which does *not* give any information as to the toxicity due to the given toxin. Hence the speciation study of inorganic As is considered essential to determine the *net* toxicity of the affected samples. The water acquired from the shallow tube wells of the affected areas (used for the irrigation purpose), the average distribution of arsenate was higher (79.1%) compared to the arsenite present (20.9%), whereas in canal water, the distribution is 92.4% arsenate and 7.6% arsenite. Regarding the ratio of As(V) to As(III), all the As-affected soils had a higher ratio as the As(V) is almost 2.5–3 times higher, but the unaffected soil had a comparatively lower ratio. This might be due to the pH and clay mineralogy of the soil as As(III) is adsorbed more at a pH about 7.0 by montmorillonite which was considerably higher in the As-unaffected soil compared to the affected soils. Contrary to the findings of soil and water samples, the percent distribution of arsenite was higher in all the plant samples (extracted with methanol, 50%, w/v) examined. This is because in the interior of the root cells, As(V) is quickly transformed through reduction to As(III) and, in numerous plant species, progresses toward becoming complexes. The percent distribution of arsenite varied from 77.6 (roots of rice) to 61.8% (roots of mustard) in the plant samples, while the distribution of arsenate ranged from 22.4 (roots of rice) to 38.2% (roots of mustard). Moreno-Jiménez et al. (2012) observed that the reduction of As(V) in roots seemed to be the prime factor resulting in the hindrance on the xylem transport of As in many plants. Further Zhao et al. (2009) observed that gathering of arsenite in the vacuole might be a cause as to why As transfer into the xylem is lessened. The impact of reduced As to obstruct its movement was examined in a plant mutated to silence arsenate reductase, namely, *Arabidopsis thaliana*, which is a close relative of mustard, more specifically the *Brassicaceae* family.

16.7 Conclusions

Among the several sources of As contamination, geogenic groundwater toxicity in the Bengal deltaic region is of great concern. To address this issue, knowledge on the chemistry of As is very vital as it primarily governs the chemical or biochemical As transformations in the soil as well as in water. Furthermore, studies on As mobility, retention, and precipitation elucidate the fate of the toxin in soil along with the factors modifying these processes. A closer examination of these processes also enables the adoption of appropriate management interventions for minimizing the toxicity of As in water-soil-crop-human continuum. Another aspect of importance considers As migrating from water to soil to plant body for the latter affects several transformations in the plant biomass. Management options in soil and water obviously govern the As intake by the plant body. Finally, the speciation of total As loading in the affected soils and the crops leads to the *net* toxicity of As in the given soil-crop systems by differentiating arsenite and arsenate oxyanion species which

further calls for the remediation options to render As in the soil-crop system in less toxic forms.

Indeed, it appears that more sustained research work is necessary to portray the whole array of complexities of As toxicity purview in the water-soil-plant-animal system and also to derive efficient combating options to contain the toxin in such systems. In addition to the remedial measures briefly mentioned, these include, among others, identification of potential bio-remediating species and exploration of the genetic makeup of several important plant species, covering the varieties of such cultivars, commonly used in the As belt, vis-à-vis As accumulation and tolerance by these species for identifying the relevant DNA markers and the enzyme systems of these plant species that are affected by As (Sanyal 2016, 2017).

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Chapter 17
Mechanism of Treatment Methods of Arsenic-Contaminated Water

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Abstract Water contamination by arsenic and health issues associated with the contaminated water are worldwide problems. Arsenic contamination in drinking water is causing severe health effects leading to death. The removal of arsenic (As) can be achieved by different methods, and it depends upon the composition of contaminated water. Treatment methods either transfer the pollutants from one phase to another or chemically oxidize to less toxic form. Separation and degradation methods include adsorption, chemical coagulation, membrane processes, electrocoagulation, chemical oxidation, and advanced oxidation processes; and biological methods including biological oxidation, phytoremediation, etc. are found to be efficient for the removal of As from water medium. There are several factors which have influence on each process; the removal efficiency depends upon the optimized conditions. This chapter provides a detailed review on the existing efforts for the As removal from aqueous medium, their advantages and limitations, etc.

Keywords Arsenic · Water treatment · Arsenite · Arsenate · Drinking water

17.1 Introduction

Arsenikon is a Greek term meaning potent, and it was first discovered by Albertus Magnus in 1250 for the word "arsenic" (Cullen 2008). Arsenic (As) is an ubiquitary element, which originates from the earth's crust with 33 atomic number, 5.72 g cm^{-3}

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density, and 74.92 amu atomic mass number (Sharma and Sohn 2009; Nidheesh and Singh 2017). Arsenic [As(0)], arsine [As(−III)], arsenite [As(+III)], and arsenate [As(+V)] are four different oxidation states, and along with this, organic and inorganic are two different forms of As. In the inorganic form of As, arsenite ($NaAsO_2$) and arsenate (Na_2HAsO_4) are two predominant oxidation states, which are toxic to both flora (plants) and fauna (animals) (Choong et al. 2007). As(III) and As(V) are the inorganic hydrolysis types which are found in water at high concentration and also at circumneutral pH (Nordstrom 2000), and it affects the metabolic process of living organisms. As(III) is comparatively more poisonous than As(V). Naturally, there are 200 different mineral forms of As with varying concentrations as sulfosalts and sulfides are found to be 20%, arsenates are 60%, while 20% are silicates, arsenide, arsenite, and elemental As (Onishi 1969).

Arsenic ranks 12th, 14th, and 20th in the human body, seawater, and nature, respectively (Mandal and Suzuki 2002). Arsenic is naturally distributed everywhere in the world in a different medium (air, water, soil), in different forms such as a metalloid, chemical compounds including both inorganic and organic forms. Its distribution and forms depend upon geochemical factors, like ionic species distribution, pH conditions, aquatic chemistry, oxidation-reduction reactions, and microbial activity (Shih 2005). Rocks and sediments are some of the sources of As, and by the process of leaching, it enters into natural water bodies (Robertson 1989). Increase in anthropological activities like arsenical pesticide usage, metal ore smelting, and wood preservative agents releases As into the environment directly (Ali and Jain 2004). The burning of coal has a profound effect on contamination of As in the environment. Emission of As takes place by volatilization of As_4O_6 due to the burning of coal, which is condensed in flue system and ultimately transferred into water reservoir (Bissen and Frimmel 2003). The burning of fossil fuel, use of herbicides, agricultural insecticides, wood preservatives, and mining are the main anthropogenic sources of groundwater contamination (Nriagu et al. 2007).

Natural water contamination by As is now becoming a serious issue worldwide. Arsenic speciation decides the high range of As toxicity. For living organisms along with human beings and other animals, inorganic As species are comparatively toxic than organic forms (Ventura-Lima et al. 2011), whereas organic form As is toxic to both animals and plants. Arsenic toxicity is divided into acute and chronic toxicity. Consumption of As-contaminated food or drink leads to acute As poisoning where medical treatment is necessary (Choong et al. 2007). Symptoms of As poisoning involve mouth dryness and burning throat, abnormal pain, hematuria, dysphasia, profuse diarrhea, and projectile vomiting (Choong et al. 2007). Muscle cramp, facial edema, and cardiac diseases caused by arsenic poisoning also result in dehydration (Done and Peart 1971). Arsenic causes toxicity from skin lesions to cancer of the brain, liver, kidney, and stomach in human health (Smith et al. 1992). Most of the body system dysfunctions are due to chronic exposure of inorganic As. Other than this skin rashes, cardiovascular Blackfoot disease, encephalopathy, hepatic hepatomegaly, cirrhosis, nervous peripheral neuropathy, altered heme, hematological bone marrow depression, metabolism, renal-proximal tubule

degeneration, endocrine diabetes, and papillary and cortical necrosis are caused due to As (Hughes 2002). Toxicity of inorganic As compounds is about 100 times higher than that of organic As compounds (Li and Liu 2015).

In the world, India and Bangladesh severely suffered from As contamination. West Bengal was first reported in 1984 for the groundwater contamination (Garai et al. 1984). In most of the states connecting to Ganga and Brahmaputra plains, several cases regarding chronic As toxicity have been reported. Along with this, As poisoning also affects states of Uttar Pradesh, Chhattisgarh, Bihar, Jharkhand, Andhra Pradesh, and Assam (IARC 2004; Nickson et al. 2007). Some districts of both Bangladesh and West Bengal are severely contaminated, while some are totally free of contamination. In West Bengal, total area and population affected by As are about 38865 km^2 and 50 million, respectively (Rahman et al. 2005). For the human health effect of chronic As toxicity, the word 'arsenicosis' was discovered (Guha Mazumder et al. 1988), and in while it is used by World Health Organization for persistent disease caused due to the long-term effect of As to human health (Caussy 2006). In West Bengal, there are 60 cases of dyspepsia which have been observed out of 156 (38.4%) total cases, and it is because of chronic toxicity (Guha Mazumer et al. 1998). Long-term As exposure to the skin may lead to keratosis and pigmentation which is considered as specific characteristic of the skin disease. About 3.1% cases shows normal skin out of 36 participants and 38% cases result of unusual skin pigmentation and is considered because of chronic cough (Borgono et al. 1977). Due to arsenic-contaminated drinking water, 89 (57%) case studies show symptoms of chronic lung disorder out of 156 cases (Guha Mazumer et al. 1998). Acrocyanosis, Raynaud's syndrome, and peripheral vascular are different diseases followed by varying range of toxicity in people due to ingestion of contaminated drinking water. Also, some of the other diseases caused by arsenic toxicity are related to the nervous system, hematological effects, and cardiovascular system (Guha Mazumder and Dasgupta 2011). Chronic exposure to arsenic affects all body organs causing dysfunction, majorly the lungs (cancer), urinary bladder, and skin. In a village of West Bengal, As infected diseases like arsenicosis, skin cancer, and internal cancers were determined in a case study in various range as 4,865, 38 (0.78%) and 212 (4.35%) (Saha 2003).

Bangladesh covered a total of 11,000 m^3 per capita for surface water, and it includes wetlands, lakes, rivers, oxbow lakes, and flooded river basins, also involves annual's top rainfall about 2000 mm and it is known for worst As-contaminated area in the world (Chakraborti et al. 2010). The level of this problem is serious in Bangladesh (Chowdhury et al. 1999a, 2000; Smith et al. 2000) after West Bengal, India (Mazumder et al. 1992; Mandal et al. 1996; Roychowdhury 2010; Guha Mazumder and Dasgupta 2011; Chakraborti et al. 2013). Some studies estimate that about 200,000–270,000 deaths in Bangladesh are because of cancer due to As-contaminated drinking water (National Research Council 2001). In Bangladesh, because of the heavy concentration of As, there are carcinogenic as well as noncarcinogenic effects, reported by large-scale longitudinal studies (Wasserman et al. 2004, 2007). From an estimation of about 100,000

population of Bangladesh, 2000 individuals suffered from As-induced health illness, specifically cancer of the lung, and its range varies in male (159.1) and also in female (23.1) (Chen and Ahsan 2004). Chronic disorder in case of noncarcinogenic involves the effects like a constant cough, vascular diseases, neurotoxicity, diabetes mellitus, and liver disease. Also high exposure of As contamination to a pregnant lady can adversely affect the development of the child. High concentration of As increases the risk of developing cancer (Ahsan et al. 2006). Ingestion and persistent contact to As lead to various types of cancers, like bladder cancer and lung cancer, as it is carcinogenic in nature (Ferreccio and Sancha 2006; Milton et al. 2012). A current study by WHO in Bangladesh has reported that 0.2 million of the population are still facing the problem of cancer due to the utilization of As contaminant through water (Chakraborti et al. 2010). According to the report on As contamination in Bangladesh, 10 million individuals are at risk, as reported by the World Bank (Chowdhury et al. 1999b). Arsenic-affected areas of Bangladesh suffered from different types of cancers, and non-cancerous health effects including hypertension, diabetes mellitus, cardiovascular disorders, respiratory problems, gastrointestinal effects, obstetric effects, and nervous system disorders have been reported (National Research Council 1999; International Agency for Cancer Research 2002; IARC 2004; WHO 2011).

17.2 Methods for the Removal of Arsenic from Aqueous Solution

Methods of removal of As from water medium can be classified into three as shown in Fig. 17.1. Separation methods include water treatment methods which transfer the pollutant from aqueous phase to another phase, most probably in the solid phase. Adsorption and chemical coagulation processes are the most suitable example for the separation process. In another type of separation process like membrane process, As-free water is produced by separating As from water medium using a suitable membrane. Separation methods are recognized to be efficient for the removal of As from water medium. But, removal of arsenite [As(III)] is quite difficult in comparison with arsenate [As(V)] removal. Thus, conversion of arsenite to arsenate is required and is accomplished by oxidation methods. Oxidation methods can be subdivided into two: chemical oxidation methods and advanced oxidation processes (AOPs). In chemical oxidation methods, arsenite to arsenate conversion is accomplished by the use of chemical oxidants like hydrogen peroxide, ozone, chlorine, etc., while AOPs use hydroxyl and sulfate radicals for the oxidation of arsenite. Biological treatment methods are also found suitable for As removal from water medium.

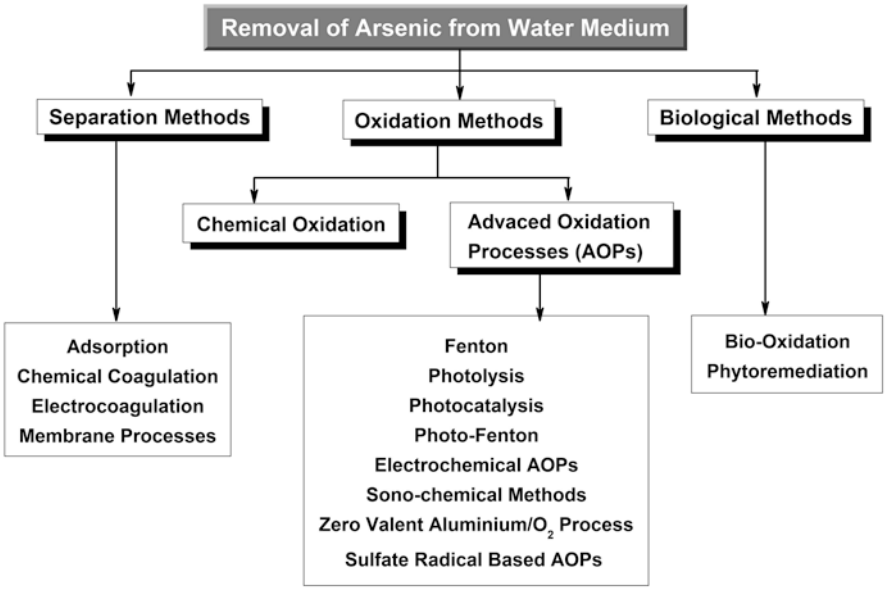

Fig. 17.1 Classification of arsenic removal methods

17.3 Separation Methods

17.3.1 Adsorption

17.3.1.1 Concept

Adsorption processes are an effective, flexible, and most extensively used method for removing organic and inorganic substances from wastewater and water. Addition of chemicals which are pollutants to water or higher cost of treatments limits the commercial application of conventional methods for As removal. The adsorption rate mostly depends on the number of active sites which are dependent on the effective surface area of the adsorbent. Arsenic removal using solid adsorbents is highly efficient and more favorable than conventional precipitation or flocculation methods. The process involves the separation of As from one phase to its concentration or accumulation on the solid surface mainly by physical forces such as electrostatic forces or van der Waals forces (Gupta and Suhas 2009).

The selection of sorbent is a tedious process; it depends mostly on the oxidation state of As in the aqueous solution. Among the four oxidation states of As species, arsenite As(III) is highly toxic and mobile in nature. Sometimes during the adsorption process involving nano-materials, the adsorption is accompanied by in situ oxidation of arsenite [As(III)] to arsenate [As(V)] (Wong et al. 2017). The active sites

of the adsorbents can change over different operational parameters and presence of other contaminants which reduces the adsorption capacity for target compound. Arsenite [As(III)] removal using zirconium polyacrylamide hybrid material was influenced by zero-point charge (pH_{PZC}); with an increase in the solution pH, the surface charge acquired negative charge (Mandal et al. 2013). The adsorption of anionic species by the adsorbent will be hindered resulting in lower removal efficiency when $pH < pH_{PZC}$ (Su et al. 2017). The sorbent selectivity based on the requirement of knowing the water profile will minimize the competitive adsorption. Researches were encouraged to use inexpensive or low-cost natural adsorbents such as red mud, bioadsorbents, blast furnace, dry plants, etc. to overcome the cost of treatment spent on other adsorbents (Ali 2014).

The adsorption capacity of the different adsorbent for As removal from water is usually represented by adsorption isotherms, indicating the adsorption capacity, molecule distribution between the solid and liquid phase in equilibrium, and energy of adsorption. Langmuir adsorption and Freundlich adsorption are mostly commonly used and can explain the adsorption efficiency of a pollutant scientifically and systematically. To describe the adsorption behavior of As into the natural systems, understanding of the point of zero charge shift, electrophoretic mobility, and ionic strength effect of the adsorbents is considerably important. These parameters govern the increasing adsorption rates with an increase in the solution ionic concentration, pH dependence, and formation of inner and outer sphere surface complex (Goldberg 2002).

17.3.1.2 Type of Adsorbents

The conventional adsorbents for removal of As are zeolites, activated carbon, metal oxides, composite metal complexes, carbonaceous materials, different types of soil and its complexes, etc. (Ng et al. 2004). Adsorbents can be used based on their properties such as chemical composition of the elements, pore size, surface area, pore volume, and carbon and ash content (Mondal et al. 2008a; Balsamo et al. 2010). Adsorbents should have uniformly accessible pores which are interlinked, have larger surface area, and are chemically and physically stable under different conditions. Researchers are intensive to improve the properties of the adsorbents by developing novel technologies by impregnating metal ions on the adsorbent surface or modifying polymeric materials by coating with adsorbents which can be easily available and economical for As removal. Amin et al. (2006) accessed the performance of four different materials for the adsorption of arsenite [As(III)] and arsenate [As(V)] and observed that 100%, 65%, 57%, and 18% of As(III) and 100%, 75%, 58%, and 55% of As(V), respectively, are adsorbed on rice husk, tea leaves, newspaper, and straw. They also reported that adsorption onto the rice husk can occur in two main paths: (a) anion exchange which occurs between As in water and carbon surface of rice husk and (b) affinity adsorption which is related to the surface charge of rice husk.

Adsorptive filtration was an innovative approach to remove As from liquid phase by coating iron oxide on cheap polystyrene beads which avoid the higher amount of sludge production, extended the surface area for adsorption, and removed low quantities of As from contaminated groundwater (Katsoyiannis and Zouboulis 2002). Surface modification of adsorbents with living microbes such as *Alcaligenes faecalis*, *Agrobacterium tumefaciens*, *Pseudomonas putida*, etc. immobilized on a solid support such as granular activated carbon (GAC) had resulted in simultaneous adsorption and bioaccumulation of As species (Mondal et al. 2008b). The microorganisms solubilize the metal/metalloid by complexolysis or produce specific proteins which bind the ions and later get precipitated.

Activated carbon (AC) with various metal ions such as copper, silver, iron, etc. is impregnated which can improve the adsorption of arsenate [As(V)] and arsenite [As(III)] (Manju et al. 1998; Chen et al. 2007). Pretreatment with Fe and Cu resulted in higher adsorption of As than untreated carbon; there was no relationship between metal ions added and the molar ratio of As (Lorenzen et al. 1995). Surface modification with ions can significantly increase the specific surface area of the adsorptive media and increases the efficiency of pollutant removal. An amorphous layer of FeOOH is formed on the surface of an untreated GAC when impregnated with Fe^{3+} which resulted in improving the adsorption capacity as well as the net positive charge of the adsorbent surface increases (Mondal et al. 2008a). A reduction of particle size of GAC and GAC-Fe does not lead to higher adsorption of As into its surface, as the internal pore surface area is much bigger than the outer surface area of the particles. The adsorption rates in coarser adsorbents are comparatively slower due to slower diffusion of As into the irregular pore structure and a longer transport path for the pollutant. The adsorption rate of As with mesoporous alumina was found to be seven times higher than conventional activated alumina; complete adsorption took place within 5 h as compared to a conventional process to reach half of the equilibrium value in 2 days (Kim et al. 2004).

17.3.1.3 Factors Affecting the Rate of Adsorption

Solution pH The rate of adsorption of As on different adsorbates is dependent on the solution pH. The adsorption rates were reduced at lower pH which may be likely due to the adsorbed As getting released due to the solubility of the adsorbent (Zeng 2004). Arsenate [As(V)] and arsenite [As(III)] from aqueous solution depend on the surface charge of particles of the adsorbent and the degree of ionization and speciation of the adsorbate which are highly pH dependent (Altundoğan et al. 2000). Adsorption rate of arsenate [As(V)] and arsenite [As(III)] on red mud varies with pH variations; As(III) was better adsorbed in the basic medium, while acidic pH was favorable for As(V) adsorption. The lower adsorption of As(V) at higher alkaline pH is mainly due to the increased repulsion between the negatively charged adsorbent surface and negatively charged arsenate species (Zeng 2004). When iron oxide was used as the adsorbent, the solution pH has a different effect on As(III) and As(V)

adsorption. Zeng (2004) reported that As(V) adsorption was maximum at a pH of 3.1 to 5 and reduced beyond pH of 7.5, while the adsorption rate was independent of pH in the range of 3 to 9 for As(III). The speciation of As(III) and As(V) varies with different pH; As(V) are present in their anionic form above pH 3, while As(III) are exclusively in their anionic form above pH 9 (Katsoyiannis and Zouboulis 2002). As(III) exist in their neutral form at acidic pH with AC at its protonated form; at pH 7 the adsorption of As(III) on AC is maximum followed by a decrease in adsorption with further increase in pH in the basic region (Wu et al. 2008).

Contact Time The efficiency of removal of As is strongly dependent on the contact time of adsorbate and adsorbent. Zeng (2004) observed that the adsorption of As(III) required less contact time compared to As(V) when Fe(III)-silica binary oxide was used as the adsorbent. The effective removal of As is achieved within an optimum contact time; the rate of removal is higher initially due to higher concentration gradient which decreases with contact time as equilibrium concentration is achieved (Mandal et al. 2013). This phenomenon can attribute to the fact that, in the initial stages, the vacant sites available for adsorption are more which decreases gradually with time and the repulsive force between the solid adsorbent and the compound in bulk. A linear relationship was observed between As(III) adsorption and contact time for iron oxide-coated sand as adsorbent, while the rate of adsorption was very less for uncoated sand (Gupta et al. 2005).

Amount of Adsorbent A significant increase in the adsorption of As was observed till the dosage reached an optimum; the absorption capacity of aluminum-loaded Shirasu-zeolite was rapid in the initial stages and then slowed down as the reaction comes up to equilibrium condition (Xu et al. 2002). A marginal increase in As(III) removal efficiency was observed when the adsorbent dose was increased from 5 g L^{-1} to 20 g L^{-1} of iron oxide-coated sand (Gupta et al. 2005). The increase in the adsorption rate with increase in adsorbent dose can be attributed to the fact that, as the amount of adsorbent increases, the surface available of adsorption is also more to an optimum dose. The availability of more micropore volume and specific surface area increases the percentage adsorption of pollutant; higher dosage of adsorbent saturates the active sites by overlapping of active sites (Mandal et al. 2013). Mondal et al. (2008a) observed that, at a lower adsorbent dosage of GAC and GAC-Fe, the As removal due to increase in adsorbent dose was high, while at higher dosage the rate was negligible. The increase in removal rate at lower adsorbent dosage may be attributed to the fact that at a higher number of active sites per unit volume of the solution increased with increase in adsorbent dosage.

Temperature The rate of adsorption of As(III) was increased from 11.3 to 14.9 (mg g^{-1} As) and As(V) from 21.1 to 21.5 (mg g^{-1} As) on iron(III) oxide/silica binary adsorbent as the temperature was increased from 20 °C to 35 °C (Zeng 2004). With the change in temperature, the solubility of adsorbate, surface properties of the adsorbent and endothermic or exothermic nature of the adsorption changes resulting in varying the adsorption capacity. The thermodynamic properties such as $\Delta G°$,

$\Delta H°$, and $\Delta S°$ reflected the spontaneous nature of arsenic adsorption on different adsorbents and varied with a change in temperature (Zeng 2004).

Presence of Other Ions Arsenic adsorption on different materials can be reduced in the presence of competing ions; the presence of phosphate ions reduced the adsorption of As by iron oxide adsorbent (Goldberg 2002). The presence of anionic components in groundwater such as phosphates, carbonates, nitrates, etc. competes for the adsorption sites resulting in inhibiting the removal of As. Sufficient pretreatment is required to increase the removal efficiency unless theses ions are present in limited amounts (Katsoyiannis and Zouboulis 2002).

17.3.2 Chemical Coagulation

Chemical coagulation is one of the old and efficient techniques for the removal of turbidity, COD, fluoride, As, heavy metals, etc. from the water and wastewater. Chemical coagulants are used for the colloid aggregation by destroying the forces that stabilize colloidal particles in aqueous solution. Apart from As, coagulation process can also remove color, fluoride, suspended and dissolved constituents, phosphate, and notable turbidity from water (Kang et al. 2003; Song et al. 2006).

The mechanisms responsible for the destabilization of inorganic colloidal particles are more concern for the elimination of As present in the water and wastewater. Because As occurs in groundwater in two major inorganic forms, namely, trivalent arsenite [As(III)] and pentavalent arsenate [As(V)], and due to biological activities in surface water, different organic forms of As may also exist (Smedley et al. 2001; Kinniburgh and Kosmus 2002; Kinniburgh et al. 2003). The As removal using chemical coagulants involved the following three main mechanisms or steps:

(i) Adsorption: the electrostatic binding of soluble As species into the surfaces of amorphous insoluble metal hydroxide, which again is adsorbed onto the coagulated flocs.
(ii) Precipitation: leads to the formation of amorphous insoluble compounds like $Al(AsO_4)$/$Fe(AsO_4)$.
(iii) Coprecipitation: the soluble As species incorporate into the growing metallic hydroxide phase, and the trivalent arsenite species behaves like a neutral molecule under the normal pH conditions, which can be separated by the sieving effects, whereas pentavalent arsenate being negatively charged can be separated by both the electrical and steric mechanisms (Pal et al. 2014).

The speciation of the As present in the water or wastewater depends upon the pH, redox potential, microbial activity, and the types and amount of sorbents present. With most of the existing As removal technologies, the As(III) shows less removal efficiency compared to the As(V) from aqueous solutions. The chemical agents like chlorine, potassium permanganate, NaOCl, H_2O_2, dissolved oxygen, etc. are used to pre-oxidize neutral As(III) to As(V) for the better removal before coagulation

process (Mondal et al. 2006). In the process of coagulation, fine particles of As in water first aggregate into large particles with the strong reduction of the absolute value of zeta potentials of the As particles by the addition of aluminum or ferric ions. The different As ions (As(III) or As(V)) precipitates with the aluminum or ferric coagulants in the coagulation process, and then due to slow mixing the coagulates concentrate and are termed as As-borne coagulates. The metallic coagulant dissociates to form metallic hydroxide which precipitates. Coagulation of metallic hydroxide and coprecipitation of As with other minerals and metallic ions eventually bind As in an insoluble form with such coprecipitators.

Many coagulation agents involving salts of iron, aluminum, calcium, etc. can be used for the removal of As (Roberts et al. 2004; Song et al. 2006; Baskan and Pala 2009; Bilici Baskan and Pala 2010; Sullivan et al. 2010; Wang et al. 2011; Randall 2012). Commonly used chemical coagulants include aluminum sulfate, ferric chloride, ferrous sulfate, etc. because of easy handling and their relative low cost rather than calcium and titanium compounds.

Calcium Coagulant Calcium is used as a coagulant for the As removal in the form of lime, hydrated lime, and calcium carbonate leading to the formation of largely insoluble amorphous calcium-As compounds. The precipitation chemistry of arsenates and arsenite with hydrated lime ($Ca(OH)_2$) is described by the following Eqs. 17.1 and 17.2 (Dutré and Vandecasteele 1995).

$$H_3AsO_4 + Ca^{2+} \rightarrow Ca_3\left(AsO_4\right)_2\left(\text{solid precipitate}\right) \tag{17.1}$$

$$H_3AsO_3 + Ca^{2+} \rightarrow CaHAsO_3 \tag{17.2}$$

The calcium reacts with the arsenite and arsenate and forms an insoluble species of calcium As complex, which can be separated by gravity settling. The calcium generated precipitation gives removal efficiency at slightly higher pH. The efficient pH of calcium generated coagulation/precipitation is 10.5. The calcium coagulant can bring down the As concentration present in the aqueous solution below 10 μg L^{-1} (Swash and Monhemius 1995).

The addition of ferric ion to the system along with calcium ion produces Ca–Fe–AsO_4 compounds with a high degree of insolubility leading to the subsequent precipitation of the finally formed compound (Swash and Monhemius 1995). The chemical reactions involved in the formation of Ca–Fe–AsO_4 compounds are given below in Eqs. 17.3 and 17.4:

$$Fe^{3+} + H_3AsO_4 \rightarrow FeAsO_4 \text{precipitation}\left(\text{solid precipitate}\right) \tag{17.3}$$

$$Ca^{2+} + FeAsO_4 \rightarrow Ca - Fe - AsO_4 \text{complex co} \quad \text{precipitation} \tag{17.4}$$

The coprecipitation of the calcium iron and As complex efficiency increases in the acidic and near-neutral pH.

Iron Coagulant Ferric salts ($FeCl_3$ and $Fe_2(SO_4)_3$) can be used as coagulants for the precipitation of ferric arsenate with higher insolubility than the calcium arsenate. The ferric ion is more soluble in water when compared to aluminum ions. These will increase the efficiency of As removal with coagulant dosage. In As removal by using ferric ion coagulation, the chemical ions are responsible for the precipitation of an insoluble ferric solid because of the transformation of dissolved As into an insoluble ferric solid. Arsenic ions precipitate with the ferric ionic compounds on the coagulants and thus increase the concentration of the coagulates (Song et al. 2006). The formation of iron oxide-As complex is given in Eqs. 17.5 and 17.6.

$$Fe^{3+} + H_3AsO_4 \rightarrow FeAsO_4\left(\text{solid precipitate}\right) \tag{17.5}$$

$$Fe^{3+} + 3OH^- \rightarrow Fe\left(OH\right)_3 \tag{17.6}$$

Arsenic ions which are in dissolved form may also be adsorbed on the surface of the solid hydroxide sites and coprecipitated with the other precipitates. The agglomerated flocs will settle by gravity, and the fine solid flocs can be separated through sedimentation and/or by filtration. The use of Ferric chloride as coagulant leads to the following reaction as shown in Eq. 17.7.

$$FeCl_3 + 3H_2O \rightarrow Fe\left(OH\right)_3 + 3H^+ + 3Cl^- \tag{17.7}$$

In water, the dissolved Fe^{3+} ions combine with the hydroxides and form precipitates of $Fe(OH)_3$. The formed precipitates $Fe(OH)_3$ are in equilibrium with the soluble charged different species of Fe^{3+} ions (e.g., $Fe(OH)_2^+$, $Fe(OH)_4^-$, etc.) depending on pH of the solution. Those species can get adsorbed upon the surface of the metallic precipitates, giving a positive or a negative charge. At the point of absolute zero net charge (PZC), the precipitate is neutral, and at higher pH values, the precipitate has a negative charge, and at lower pH values, it is positively charged. The zero net charge is close to pH 4 for Fe species (Mustafa et al. 2008; Oehmen et al. 2011). The ferric arsenate is not stable at higher pH (8) ranges as it formed a solid layer of ferric hydroxide on the ferric arsenate precipitate. The formation of ferric hydroxide layer on the ferric arsenate reduces the precipitation efficiency. The As removal using ferric coagulants are high at pH 5 and with further increase in pH results in the decrement of the efficiency of removal of As. The As removal with ferric ion coagulant is high at the pH range of 5–8 which means nearby neutral pH.

In the natural water, high concentrations of coagulants are required for the destabilization of the low quantity of colloidal particles due to the collision between colloids. At the same time, highest removal efficiency is achieved at a low concentration of coagulants for the high quantity of colloids (Bilici Baskan and Pala 2010). The iron compound present in the groundwater will also help in coagulation of the As compounds. The presence of dissolved oxygen and chloride pres-

ent in the groundwater will enhance the reaction. The oxidation of dissolved bivalent iron present in the groundwater to iron (hydr)oxide follows the following Eqs.17.8 & 17.9.

$$4Fe^{2+} + O_2 + 6H_2O \rightarrow 4FeO(OH)\downarrow + 8H^+ \tag{17.8}$$

$$2Fe^{2+} + HClO + 3H_2O \rightarrow 2FeO(OH)\downarrow + Cl^- + 5H^{++} \tag{17.9}$$

These compounds increase the precipitant surface available for the adsorption of As. These iron (hydr)oxide precipitates make a complex with the As and sediments down. The coagulation with iron compounds can bring down the final As concentration from 100 μg L^{-1} to 7 μg L^{-1} at the pH 6.8 and coagulant dosage of 15 mg L^{-1} (Ćurko et al. 2011).

Aluminum Coagulant The aluminum sulfate is the primarily used coagulant for the As removal from contaminated water. When aluminum sulfate is added as a coagulant for the removal of As from the contaminated water, aluminum compound dissociates into an aluminum ion. The aluminum ion reacts with the hydroxide; therefore, aluminum hydroxide is formed, which coprecipitates with As as shown in Eqs. 17.10, 17.11, and 17.12.

$$Al_2(SO_4)_3\,18H_2O \rightarrow 2Al^{+3} + 3SO_4^{2-} + 18H_2O \tag{17.10}$$

$$2Al^{3+} + 6H_2O \rightarrow 2Al(OH)_3 + 6H^+ \tag{17.11}$$

$$H_2AsO_4^- + Al(OH)_3 \rightarrow Al - As\,\text{complex} \tag{17.12}$$

During coagulation with aluminum ion, As species are removed primarily by the following three mechanisms including precipitation, coprecipitation, and adsorption. The precipitation includes the formation of the insoluble compound precipitates of $Al(AsO_4)$, which removes As. In coprecipitation, the growing metal hydroxides incorporate soluble As species through inclusion and occlusion, or adsorption of As and hydroxides forms a complex which settles down. The soluble As bind to the external surfaces of the insoluble amorphous aluminum metal hydroxide electrostatically and settle down. All those three mechanisms can independently contribute toward As removal.

The removal of neutral As(III) is comparatively less compared to the removal of As(V). As(III) has a very little affinity for aluminum hydroxides; thus the oxidation of As(III) is a precondition before the coagulation process. With aluminum sulfate as coagulant, arsenate removal percentage increases with increase in the pH and the optimum pH range is 6–8 (Bilici Baskan and Pala 2010). In the As-contaminated drinking water source, the formation of $Al(AsO_4)$ solid precipitate is not that easy. Moreover, the precipitation will not contribute to the overall performance. The co-precipitation

and adsorption are the two active mechanisms for As removal in the drinking water sources. The aluminum coagulants decrease the As concentration less than 6 μg L^{-1} from 160 μg L^{-1} at pH 6 and aluminum dosage of 20 μg L^{-1} (Hu et al. 2012).

The arsenate removal efficiency decreases at the high acidic pH, and also at pH above 8, the removal efficiency decreases considerably. The increase or decrease in the arsenate removal efficiency by aluminum ions is highly affected by the solubility of the amorphous insoluble hydroxides of aluminum. The amorphous hydroxides of aluminum are stable in the pH range of 6 to 8, thus increases the arsenate removal efficiency in this pH range. And also the adsorption on the amorphous hydroxide is the major mechanism of As removal with the aluminum ion. The $Al(OH)_3$ precipitates are in equilibrium with the soluble charged different species of Al^{3+} ($Al(OH)_2^+$, $Al(OH)_4^-$, etc.), and the zero net charge is approximately pH 8 for Al (Cañizares et al. 2009; Oehmen et al. 2011). Thus, the net charge of aluminum precipitate is positive at pH 6.5 and gets repulsed by the positively charged ions. The effective pH for As removal with aluminum chloride was 5–7 with aluminum ions (Oehmen et al. 2011).

Titanium Coagulant $Ti(SO_4)_2$ is one of the best coagulants for the removal of As. $Ti(SO_4)_2$ has the potential to remove As(III) directly without the oxidation to As(V). $Ti(SO_4)_2$ could reduce As(III) from 0.20 mg L^{-1} to 7.8 mg L^{-1} at the pH 8 (Sun et al. 2013). The As(V) adsorbed on the external surface of the $Ti(SO_4)_2$ precipitate. The $Ti(SO_4)_2$ coagulation of As(III) leads to the precipitation of absorbed AsO_3^{3-}-$Ti(SO_4)_2$ precipitate with the binding energy of 44.4 eV. The As(V) removal occurs at the precipitation with $Ti(SO_4)_2$ with binding energy of 45.3 eV (Li et al. 2010; Sun et al. 2013; Wang et al. 2016). The pH range from 5 to 7 gives higher removal efficiency of As(III) because of the high negative zeta potential (0 to -49.4 mV). The $Ti(SO_4)_2$ precipitate has a highly negative zeta potential (from −49.4 to −60.7 mV) at the pH range of 7–10. At that pH range, the removal efficiency decreases. In addition, at the high pH range, the electrostatic repulsion between the primary precipitate particles of $Ti(SO_4)_2$ clearly hinders the aggregation leading to the formation of smaller precipitate particles compared to that at low pH. However, the $Ti(SO_4)_2$ gives better As removal compared to other coagulants at high pH values, because of the strong electrostatic repulsion between negatively oxidized As(V) species and the precipitate. This also happens because of the reduction in electrostatic repulsion between the precipitate and neutral arsenite (Wang et al. 2016).

Polymeric Coagulant The conventional coagulations will remove the As more effectively at the near-neutral pH; this disadvantage can be overcome by the use of polymeric coagulants. The polymeric coagulants like poly-aluminum chloride (PACl) are effective for removing concentration of arsenate both at acidic and alkaline pH as well as at neutral pH compared to that of the conventional coagulants like aluminum sulfate and chloride. Hu et al. (2012) and Mertens et al. (2012) proposed different PACls that contain the active species of e-Al_{13} polycation (Keggin-type e-Al_{13} polycation, $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$) and ϒ-$Al_{30}$ poly-

cation (Keggin-type ϒ-Al30 polycation $[Al_{30}O_8(OH)_{56}(H_2O)_{24}]^{18+}$) for the effective arsenate removal under acidic and alkaline conditions of coagulation. The PACls containing e-Al_{13} polycation give the higher efficiency of removal at the pH range of 6–8. The e-Al_{13} polycation removes the arsenate concentration more effectively than the conventional monomeric aluminum coagulants. The arsenate concentration increases above the pH of 9 with PACls containing e-Al_{13} polycation (Matsui et al. 2017).

Sulfated PACls have the capacity to suppress the charge reversal and also to accelerate the kinetics of aluminum hydroxide precipitation, thus having a wide application in the treatment of raw water (Matsui et al. 2017). The sulfated PACls give higher removal efficiencies even with the pH above 8. At pH 7–11, the adsorption of the divalent sulfate ion and divalent arsenate ion ($HAsO_4^{2-}$) (Wilkie and Hering 1996) is related to the aluminum species distribution. The three broad species responsible for aluminum coagulation are the monomeric form, polymeric form, and colloidal aluminum. The presence of polymeric and colloidal form of aluminum is responsible for the increase in the arsenate removal efficiency even in the alkaline pH (Matsui et al. 2017). At the near-neutral pH, the monomeric and polymeric pH gives similar removal efficiency.

The presence of polymers as the coagulant aid will also enhance the removal process of As by coagulation. The three different forms of polymers, organic polymers, cationic polymers, and anionic polymers, can be used as coagulant aid for increasing As removal. The use of organic polymers increases the strength of the flocs, size of the flocs, and settleability of the flocs. The increase in removal efficiency corresponding to the presence of cationic polymers, anionic polymers, and nonionic polyelectrolytes, respectively, is shown in Fig. 17.3. The cationic coagulant aids increase the removal efficiency considerably when compared to the anionic and nonionic aids. The increase in nonionic polyelectrolytic coagulant aids has not shown significant removal efficiency of As (Bilici Baskan and Pala 2010). The nonionic polyelectrolytes do not increase the arsenate removal. The cationic polyelectrolytes are more effective than nonionic and anionic ones because of the increase in the cationic character of the inorganic coagulant, which leads to the formation of amorphous solids, which gets adsorb easily (Zouboulis and Katsoyiannis 2002). Apart from the addition of polymers as the coagulant aids, the addition of coarse particles to the process will also increase the As removal efficiency. The addition of coarse particle as a supplement in the coagulation process reduces the number of fine particles in the suspension and also increases the size of coagulates. This increase in the size of the flocs and reduction of fine particles may be due to the increase of aggregation rate and also because of the high collision rate between the coarse particle and fine particle compared to the fine particle and fine particle (Song et al. 2006).

17.3.3 Membrane Processes

17.3.3.1 Concept

Membrane processes have been proven to be a reliable and effective treatment process capable of removing As from water. The process removes As as the water containing the pollutant pass through a filter medium which is capable of retaining the impurities in it. "The main characteristic of a membrane is its capacity to control or prevent the passage of some solutes in solution while permitting transport of others" (Fang et al. 2005). The process uses membranes with higher selectivity, operating under moderate pressure in the presence of an oxidizing agent or permeable membranes. Membrane processes for removal of As depend mainly on factors such as surface charge, pore size, membrane material, operating conditions like pH, applied pressure and temperature, solution concentration, etc. (Uddin et al. 2007b). Synthetic membranes with millions of microscopic holes which can act as the selective barriers for the impurities are usually employed as membrane, and the molecules move across these membranes under a driving force (Shih 2005). Pressure-driven membrane filtrations such as reverse osmosis, nano-filtration (NF), ultrafiltration (UF), and electrodialysis are the common separation processes used to remove As from water. The higher molecular weight of As species governs their separation behavior (Vrijenhoek and Waypa 2000).

Separation process of ions strongly depends on the charge of membranes and pore size; highly charged membranes are better able to remove co-ions. The pore size of the membranes also varies with the intensity of the driving force; high-pressure membranes remove impurities through chemical diffusion, while low-pressure membranes through physical sieving (Shih 2005). The charge possessed by the membranes has a significant impact on ion rejection; negatively charged membranes have a higher rejection for charged solutes than for noncharged solutes and are sensitive to operating conditions such as pH, the presence of other ions, As concentration, etc. (Ng et al. 2004). Pre-oxidation of wastewater containing As(III) is required to increase the efficiency of the process.

17.3.3.2 Type of Membrane Processes

Movement of molecules through synthetic membranes with macroscopic pores under a driving force can exclude or reject certain ions/solutes present in water. The pore size of the membranes is related to the pressure required to drive the water through it; compared to low-pressure membranes, the pore size of high-pressure membranes are smaller (Shih 2005). The efficiency of each process depends upon the pressure applied and the pore size of the membranes; smaller pore size helps to remove more amount of particulate as well as a colloidal form of As in water. Microfiltration is a low-pressure-driven process which removes particles having

size more than 0.02 μm to 10 μm from a fluid mixture (Uddin et al. 2007a). The membrane pore sizes are large, and they are effective in removing dissolved and colloidal As species. The removal efficiency of the process is increased combining the process with coagulation and flocculation so that the particle size will be more (Shih 2005).

Ultrafiltration (UF) process primarily removes the As species through physical sieving; the removal efficiency is very low because of higher pore size of membranes. A significant amount of pretreatment process is required to remove the colloidal and particulate As. UF membranes with electric repulsion have a higher efficiency of removal when compared with membranes with only pore size sieving; the presence of divalent cations in the solvents reduces the removal efficiency. Also, as the bulk solute concentration was increased, the rejection rate of As(V) by UF membranes also increased (Uddin et al. 2007a).

The nano-filtration process uses membranes with small pores and is cost-effective than reverse osmosis because of its lower operating pressure and higher membrane flux rates with higher selectivity on As(V) oxyanions. Membrane characteristics, membrane pore size, and charge are the main factors which control the rejection of solutes by nano-filtration membranes and have significant control on charge exclusion and size exclusion. The charged membranes are capable of excluding solutes by electrostatic interaction between ions from the solution and size exclusion based on the pore size and size of the permeating solutes (Košutić et al. 2005). A highly charged membrane excludes the passage of ions with the same charge as a membrane by the electrostatic interaction known as Donnan exclusion (Uddin et al. 2007a). These processes provide higher water fluxes at lower transmembrane pressure and usually are negatively charged at alkaline and neutral media which results in changing the separation rates of monovalent anions (Uddin et al. 2007a). The surface charge of membranes measured as zeta potential changes with pH, charge repulsion or electrostatic repulsion, and the separation on ionic species with Donnan exclusion become more dominant (Sen et al. 2010).

Reverse osmosis membranes contain extremely smaller pores; the free volume between the segments of the polymer of which the membrane is constituted helps in accomplishing the transport of solvent (Shih 2005). Though the flow rate of solvent through or across these membranes is comparatively low, over 90% efficiency of removal of arsenate was reported. Ning (2002) reported that the removal of As(V) is more effective than As(III) while using a RO process at higher pH.

Low-cost clay minerals such as montmorillonite, illite, and kaolinite were explored for arsenate removal to investigate their ability to retain As. Clay membranes are ideal for rejecting ionic solutes from the solution because of their porosity and surface charge density. The negative charge possessed by the surfaces repels the anions attempting to pass through the charged compacted clay membranes (Fang et al. 2005). The pressure requirement for the clay membranes was significantly higher than synthetic organic membranes (Fang et al. 2005); a greater removal efficiency of As was observed. The rate of solute rejection in clay membranes depends on increased solute concentration at the membrane surface (Fang et al. 2005), increase in total solute mass flux, and compaction of membranes as a result of overlying pressure

(Fang et al. 2005). As the membranes become more compacted, the pore size reduces and consequently increases the As rejection (Fang et al. 2005)

Among the different processes, RO and NF processes are more effective in treating dissolved As contaminants since the dissolved compounds have relatively lower molecular weights. Even though NF membranes have higher water flux, RO membranes are more reliable because of higher removal of ions and molecules (Uddin et al. 2007b). Multiple membrane units in series are required to increase the rejection efficiency of As species. The generation of negatively charged NF membranes with a higher selectivity and lower operational cost is promising for removing a combination of multivalent ions with a moderate retention of monovalent ions (Nguyen et al. 2009).

17.3.3.3 Factors Affecting the Rate of Removal

Oxidation State Species exist as divalent and monovalent anion within a pH range of 5–9 and preferentially as a monovalent anion in a pH range of 4–6. Higher removal of As at high pH occurred in an NF membrane process at lower As concentration in the permeate and can be attributed to the fact that the repulsive force between the divalent anion and negatively charged membrane surface was higher than the monovalent anion and membrane (Uddin et al. 2007a). The trivalent species are predominant under a reducing condition, while the pentavalent species exist in a non-oxidizing condition (Zaspalis et al. 2007). Sato et al. (2003) observed that the existence of As(III) in neutral molecular form, compared to negatively charged As(V), the removal efficiency of As(V) was more than As(III) in a pressure range of 0.3–1.1 MPa (Sato et al. 2003).

Solution pH The transport of arsenate and arsenite is pH dependent, and they exhibit different transport characteristics with pH (Kim et al. 2006). Optimum pH for the operation of processes has a significant effect on the removal efficiency; from pH 7 to 10 and from 3 to 5, increase in removal efficiency was observed. Optimizing the pH conditions before the treatment process can increase the performance of removal (Ng et al. 2004). At lower pH, the zeta potential of the membranes becomes less negative resulting in reducing the rejection as the charge exclusion has less impact on removal process (Vrijenhoek and Waypa 2000). The charge variation of the membranes with pH largely affects the rejection of As from feed water; the trend in the increase in As rejection relates to the degree of deprotonation of As species. In the case of As(V), the rejection was less at lower pH (pH 2) and more dominant at higher pH (pH 10), while the rejection of As increased sharply from pH change from 2 to 10 (Uddin et al. 2007b). The existence of As(III) in the neutral state within the pH range of 5–8 has no significant effect on the removal rate (Elcik et al. 2016).

Presence of Co-occurring Inorganic Solutes The rejection of As(V) of using nanofiltration 45 (NF45) membrane increased by 50% in the presence of Cl^- ions, at lower bulk concentrations the rejection rate was higher (Vrijenhoek and Waypa 2000). In a

multiple solute solution containing chloride and arsenate, the presence of a more permeable ion of like charge increases the rejection of arsenate due to its larger molecular weight and less concentration (Fang et al. 2005). The removal efficiency of UF processes can be extremely influenced by counter ions or organic matter which locally neutralizes the membrane charges (Uddin et al. 2007a).

Applied Pressure and Temperature The driving force which allows the separation of constituents present in a solvent comes from the applied pressure. High-pressure methods remove a wider range of pollutants at higher energy and cost of operation than low-pressure methods. During removal of As(III), with an increase in applied pressure, dilution effect was found more and lowered the As(III) concentration in the permeate flux (Uddin et al. 2007b). The increase in transmembrane pressure increased the flux through the membranes which resulted in increasing the rate of As removal efficiency (Elcik et al. 2016). Increase in temperature of the feed solution increased the diffusive As transport across the membrane and decreased the rejection rate (Uddin et al. 2007b; Figoli et al. 2010).

17.3.4 Electrocoagulation

The electrocoagulation (EC) process has been practiced since the last century. It is a very effective, economic, fast, and eco-friendly technique for water as well as wastewater treatment (Holt et al. 1999, 2005; Bouhezila et al. 2011). Electrocoagulation resembles with coagulation in which without the manual addition of coagulant the hydroxides and coagulants are produced in situ. The amount of the solid sludge generation is minimized through controlling the rate of generation of hydroxides by the applied charge which results in lucrative technology for the treatment of water as well as wastewater (Balasubramanian et al. 2009). In electrocoagulation, the mechanisms for removal involve deposition, decomposition, oxidation, reduction, absorption, adsorption, coagulation, precipitation, and flotation (Parga et al. 2005).

The mechanism for electrocoagulation process is shown in Fig. 17.2. In electrocoagulation process, soluble metallic electrodes are used like zinc, aluminum, iron, etc., which in situ generate metallic coagulants. After application of direct current (DC) or alternate current (AC), the electrolytic oxidation of the anode occurs. At metallic anode di- or trivalent metallic ions dissociate and release equivalent electrons due to the application of the electric potential. Various reactions which occur at the anode and cathode are shown below (Eqs. 17.13, 17.14, 17.15, 17.16 and 17.17).At the anode:

$$\mathrm{M_{(s)} \rightarrow M^{n+}_{(aq)} + ne^-} \tag{17.13}$$

$$\mathrm{2H_2O \rightarrow 4H^+_{(aq)} + O_{2(g)} + 4e^-} \tag{17.14}$$

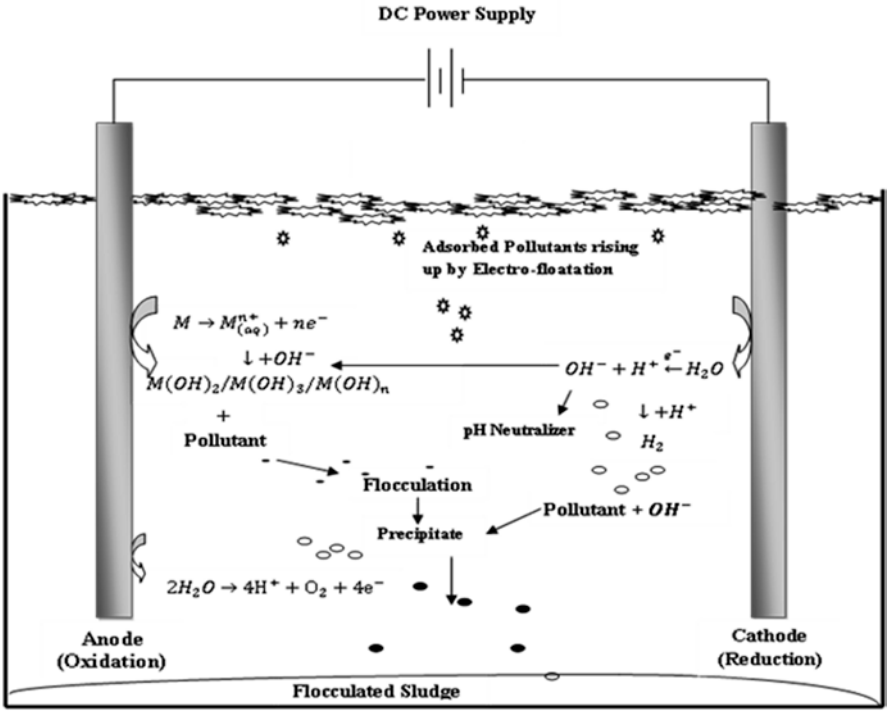

Fig. 17.2 Schematic representation of electrocoagulation mechanism. Reprinted with permission from Ref. (Nidheesh and Singh 2017). Copyright 2017 Elsevier

At the cathode:

$$nH_2O + ne^- \rightarrow (n/2)H_{2(g)} + nOH^-_{(aq)} \tag{17.15}$$

$$M^{n+}_{(aq)} + ne^- \rightarrow M_{(s)} \tag{17.16}$$

The amount of generated metallic ions is directly proportional to the applied current density (Faraday's law; Eq. 17.17); as the current density increases, the dissociation of metallic ions increases (Anantha Singh and Ramesh 2014).

$$w = \frac{itM}{ZF} \tag{17.17}$$

where w is the anode dissolving (g cm^{-2}), i the current density (A cm^{-2}), t the time (s), M the molecular weight of anode material, Z the number of electrons involved in the oxidation/reduction reaction, and F Faraday's constant (96,487 C eq^{-1}).

In charge neutralization, the metallic ions migrate to oppositely charged ions, and aggregation occurs (Nidheesh and Singh 2017). In an aqueous system, hydrogen and oxygen are released through water reaction/oxidation at the anode. Due to

electron transfer, the water reaction occurs in the surface of the electrode at cathode regime (Nidheesh and Singh 2017). Because of molecules of the water, electron interaction occurs; hydrogen and hydroxide ions are dissociated at the cathode surface. H_2 gas is generated as the released hydrogen ions again combine with the free hydrogen ions which help in electro-floatation of the floc particles. While at anode formed metallic ions combine with the hydroxides which are generated at the cathode which forms metallic hydroxides which are very good adsorbents for the pollutant removal (Nidheesh and Singh 2017). The generation of monomeric or polymeric as well as soluble or insoluble metallic hydroxide depends on the pH of the aqueous solution (Sass and Rai 1987; Anantha Singh and Ramesh 2013). The formed amorphous $M(OH)_n$ metallic hydroxides have large surface areas having the potential of the very high capacity of adsorption, forming a bond with the pollutants forming flocs (Sánchez Calvo et al. 2003). With pollutant materials, hydroxides form precipitate and settle down as sludge because the self-settling size is attained by metallic flocs as it increases the size from micro- to macro-flocs (Nidheesh and Singh 2017). Micro-flocs are taken up by a generated gas called electro-floatation as it floats on the top of the aqueous solution, forming a foam-like phase which can be removed by skimming (Nidheesh and Singh 2017). Toxic species are converted to nontoxic species through oxidation as oxygen generated at anode leads to the formation of hydrogen peroxide (intermediate).

On the basis of initial pH, an excess of hydrogen and hydroxide ions generated in the EC process helps in buffering the pH of the aqueous solution and maintains neutral pH (Nidheesh and Singh 2017). The oxygen level increases due to slow mixing during EC process in the system which oxidizes the pollutants and mixing of pollutants with metallic flocs (Nidheesh and Singh 2017). The corrosion of metallic species is increased by the ions or electrolytes like chloride in the anode regime (Chen 2004; Cañizares et al. 2005, 2007).

Trivalent As(III) and pentavalent As(V) are the predominant species in a water medium (Nidheesh and Singh 2017) among all oxidation states of As. The As(III) is difficult to remove as it is stabilized; therefore it is needed to oxidize, whereas As(V) species can be easily removed from the system (Nidheesh and Singh 2017). The oxidization of As(III) to As(V) has to be done for effective As removal. But EC process is effective in oxidizing As(III) to As(V) and improves its removal without separate oxidation process (Ratna Kumar et al. 2004). The EC process can remove As from aqueous solution up to the level of 5 μg L^{-1} (Mohora et al. 2012).

17.3.4.1 Arsenic Removal by Electrocoagulation Process

Iron Electrodes For As removal iron and aluminum are broadly used as electrode materials in electrocoagulation process. Iron or stainless steel electrode is widely used because of its low cost, easy availability, and higher efficiency of these materials for the removal of As (Nidheesh and Singh 2017). The iron dissociates into Fe^{2+}, and the formed Fe^{2+} reacts with the dissolved oxygen present in the system or oxygen supplied for mixing and oxidized to Fe^{3+}. The generated oxygen by the anode

water reaction plays an important role in the conversion of Fe^{2+} to Fe^{3+}. This reaction contributes to the unstable negatively charged oxygen. The Fe^{2+} in the presence of unstable negatively charged oxygen and H^+ give Fe^{3+} and H_2O_2. The H_2O_2 is known for its high oxidant capacity. The Fe^{2+} present in the system reacts with the H_2O_2 generated from the last reaction and gives a highly unstable intermediate component Fe(IV). This intermediate species of iron Fe(IV) is responsible for the oxidation of arsenite(As(III)) to arsenate (As(V)). In EC process iron is used as the sacrificial anode; the removal of As(III) was facilitated by the presence of Fe(II) along with Fe(III), which in turn turns to reactive Fe. As(III) is oxidized to As(V) and As(V) adsorbed to the iron oxides $Fe(OH)_{n(s)}$ (Parga et al. 2005; Amstaetter et al. 2010; Wan et al. 2011). The oxidation of As(III) to As(V) has also been proposed to occur with soluble species of Fe(II) oxidation in the presence of dissolved oxygen (Sahai et al. 2007; Ciardelli et al. 2008; Lakshmanan et al. 2010). The As(V) combines with the amorphous solid species like $Fe(OH)_2/Fe(OH)_3$ or higher polymeric species and settles down.

The significant mechanisms of removal of pollutant in an EC process system for the removal of As are sweep floc coagulation and adsorption and charge neutralization (Nidheesh and Singh 2017). The performance of EC and its pollutant removal mechanism are affected by the pH of the solution which is an important factor (Nidheesh and Singh 2017). The speciation of iron (ferrous (Fe^{2+}) and ferric (Fe^{3+}) ion) in the aqueous solution depends mainly on the solution pH (Eqs. 17.18, 17.19, 17.20, 17.21, and 17.22). Ferrous ions present in the aqueous solution take the forms of Fe^{2+}, $Fe(OH)^+$, $Fe(OH)_2$, and $Fe(OH)_3^-$ (Nidheesh and Singh 2017). At pH below 10, all the ferrous ions are in the form of Fe^{2+} (Nidheesh and Singh 2017). Similarly, $Fe(OH)^+$, $Fe(OH)_2$, and $Fe(OH)_3^-$ predominate at pH below 11, below11.5, and above 1.5, respectively (Nidheesh and Singh 2017). The Fe^{2+} present in the aqueous solution below pH 10 have an important role in the formation of the intermediate product Fe(IV) when compared to the other species of ferrous ion. The ferrous ions also lead to the formation of insoluble species like FeOOH which acts as a strong adsorbent for the removal of As.

$$\mathrm{Fe}_{(s)} \rightarrow \mathrm{Fe}^{2+} + 2\mathrm{e}^- \tag{17.18}$$

$$\mathrm{Fe}^{2+} + 1:5\mathrm{H_2O} + 0:25\mathrm{O_2} \rightarrow \Upsilon - \mathrm{FeOOH}_{(s)} + 2\mathrm{H}^+ \tag{17.19}$$

$$2\mathrm{Fe}^{2+}{}_{(aq)} + 4\mathrm{H_2O}_{(l)} + \mathrm{O_2} \rightarrow 2\mathrm{Fe(OH)}_{3(s)} + \mathrm{H}_{2(g)} \tag{17.20}$$

$$4\mathrm{Fe}^{2+}{}_{(aq)} + 10\mathrm{H_2O}_{(l)} + \mathrm{O_2} \rightarrow 4\mathrm{Fe(OH)}_{3(s)} + 8\mathrm{H}^+{}_{(aq)} \tag{17.21}$$

$$\mathrm{Fe}^{2+}{}_{(aq)} + 2\mathrm{OH}^-{}_{(aq)} \rightarrow \mathrm{Fe(OH)}_{2,(s)} \tag{17.22}$$

The pH below 3, 3–4, 4–6, and 6–9.5 and above 9.5 ferric ions, Fe^{3+}, $Fe(OH)^{2+}$, $Fe(OH)_2^+$, $Fe(OH)_3$, and $Fe(OH)_4^-$, respectively, predominates in the water medium

which can be seen with their equilibrium constants in Eqs. 17.23, 17.24, 17.25, 17.26, 17.27, 17.28, 17.29, and 17.30 (Benefield et al. 1982). At pH 5, ferrous hydroxide starts to precipitate. The concentration of ferrous hydroxide increases with increase in pH. After 2 min of mixing in the aqueous solution, the concentration of soluble ferrous iron (Fe^{2+}) and insoluble iron oxide/hydroxide ($Fe(OH)_{3(s)}$/$FeOOH_{(s)}$ as Fe^{3+}) is formed (Lakshmanan et al. 2010). At pH 12, The concentration of insoluble ferrous hydroxide is maximum (Nidheesh and Singh 2017) and decreases as the pH of solution increases. Similarly, the minimum pH 4 and 1 are required for the precipitation of aluminum hydroxide and ferric hydroxide, respectively (Nidheesh and Singh 2017).

$$Fe^{3+} \Leftrightarrow FeOH^{2+} + H^{+} \tag{17.23}$$

$$Fe^{3+} \Leftrightarrow Fe(OH)_2^{+} + 2H^{+} \tag{17.24}$$

$$2Fe^{3+} \Leftrightarrow Fe_2(OH)_2^{4+} + 2H^{+} \tag{17.25}$$

$$Fe^{3+} \Leftrightarrow Fe(OH)_3^{0} + 3H^{+} \tag{17.26}$$

$$Fe^{3+} \Leftrightarrow Fe(OH)_4^{-} + 4H^{+} \tag{17.27}$$

$$Fe^{2+} \Leftrightarrow FeOH^{+} + H^{+} \tag{17.28}$$

$$Fe^{2+} \Leftrightarrow Fe(OH)_2^{0} + 2H^{+} \tag{17.29}$$

$$Fe^{2+} \Leftrightarrow Fe(OH)_3^{-} + 3H^{+} \tag{17.30}$$

The other hydroxides of iron reported for the removal of As are iron oxides (magnetite (Fe_3O_4), lepidocrocite (FeO(OH)), iron oxide (FeO)) (Gomes et al. 2007). At pH 6.5 and 7.5, arsenate (As(V)) removal efficiencies were similar compared to the removal efficiency at pH 7.5 and 8.5. The efficiency of arsenate (As(V)) removal decreased with the increase in pH. The formed iron flocs remove As by precipitation and/or adsorption (Balasubramanian et al. 2009) (Eq. 17.31):

$$Fe(OH)_{3(s)} + AsO_4^{3-}{}_{(aq)} \rightarrow \left[Fe(OH)_3^{*} AsO_4^{3-}\right](s) \tag{17.31}$$

Most of the iron present is soluble Fe^{2+}, which would not adsorb As(V) at pH 6.5 (Lakshmanan et al. 2010). The pH of the aqueous solution shows less significance in the removal of arsenite (As(III)) and arsenate (As(V)) in 5 to 8 pH range. The removal of As(III) shows higher oxidation in the lower neutral pH than the higher neutral pH. At low pH, the Fe(II) oxidizes the As(III) to As(V) (Su and Puls 2001; Ratna Kumar et al. 2004). The percent of removal of As(III) and As(Total) removal with the iron electrode is similar excluding at high pH. At high pH, there can be a

small difference due to the effect of temporary pH increase which affects the As(III) oxidation (Lakshmanan et al. 2010). The air or the oxygen or dissolved oxygen in the EC process enhances the oxidation of Fe(II) which helps in oxidizing As(III) to As(V) and the adsorption on iron hydroxides (Ratna Kumar et al. 2004; Parga et al. 2005).

Aluminum Electrode The mechanism of the aluminum electrode for As removal is similar to that of iron except for the formation of the intermediate product. The main floc formation $Al(OH)_3$ also depends on the pH conditions of the solution. For As(III), the capacity of adsorption of hydrous aluminum oxide is much lower in comparison with hydrous ferric oxides (Hering Janet et al. 1996; Ratna Kumar et al. 2004). The normal aluminum oxides absorbed in the As adsorption are aluminum oxides (bayerite ($Al(OH)_3$), diaspore (AlO(OH)), and mansfieldite ($AlAsO_4{\cdot}2(H_2O)$) (Gomes et al. 2007). The removal efficiency of As(III) with the aluminum electrode is around 50% at the neutral pH, which is due to the less adsorption capacity of As(III) in aluminum hydroxides (Hering Janet et al. 1996). At the low pH range of 2.5, aluminum species can bring down the As concentration from 13.4 mg L^{-1} to 0.09 mg L^{-1} (Gomes et al. 2007). Among different species of As present with respect to pH, the negatively charged species by the insoluble aluminum hydroxides can be easily removed and the aluminum species at low pH $Al(OH)_2^+$, $Al(OH)^{2+}$ (Song et al. 2014). In the As removal, adsorption and charge neutralization mechanism (Nidheesh and Singh 2017) play a major role at this condition. At the high alkaline pH, due to the same charge of As species and the aluminum species formed, the removal efficiency is very low.

Various researchers also explained As removal mechanism, based on zeta potential studies. In the presence of iron electrodes, formed flocs are negative, while that of aluminum electrode is positive (Kim et al. 2014). Therefore, for the removal of cations via sorption process, sludge formed in the presence of iron electrode is more favorable than that of aluminum electrode (Nidheesh and Singh 2017).

Other Electrodes In the EC reactor with titanium electrodes, the TiO_2 formation takes place, which partially oxidizes the As(III) to As(V) (Ratna Kumar et al. 2004). The As(V) then gets adsorbed in the hydroxides of titanium. That partial oxidation of As(III) might be occurring, and the oxidized As(V) might be getting adsorbed on to hydroxides of titanium (Ratna Kumar et al. 2004). Gomes et al. (2007) used a combined electrode of Al-Fe combination for the As removal. It was reported that the formation of bayerite, diaspore, iron oxide, lepidocrocite, magnetite, and mansfieldite occurs with the Al-Fe electrode combination when compared to the aluminum hydroxide, aluminum oxyhydroxide in the Al-Al combination (Gomes et al. 2007).

17.3.4.2 Inference of Other Ions in Electrocoagulation of Arsenic

The oxidation of As(III) to As(IV) is an important aspect which decides the efficiency of EC process. The oxidation of As(III) species to As(V) species during EC process occurs due to the presence of Cl_2, Cl_2 produced or by redox reaction at the

anode surface, which will improve the removal efficiency of arsenic (Tong et al. 2014). The presence of phosphates, sulfates, and silica will not influence the oxidative removal efficiency of As(III), whereas adsorption of As(V) had a greater influence from all ions. The presence of phosphate competes the surface adsorption of As in iron hydroxide (Meng et al. 2002; Wan et al. 2011). The presence of 10–50 mg L^{-1} SO_4^{2-} did not affect the removal of As because sulfate did not affect lepidocrocite formation as well as doesn't adsorb as strongly as As(V) and phosphate (Wan et al. 2011). Moreover, sulfate did not affect the performance of the electrocoagulation process (Meng et al. 2002; Wan et al. 2011). The significant effect of As removal in the presence of silica up to a concentration of 36 mg/L was not reported (Davis et al. 2001; Meng et al. 2002; Wan et al. 2011), beyond that the increase in concentration silica affects the formation of lepidocrocite (Wan et al. 2011). In the presence of ions like Na^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, HCO_3^-, SiO_3^{2-}, K, Cl^-, F^-, and PO_4^{3-}, the EC process can bring down the concentration of As from 600 μg L^{-1} to 10 μg L^{-1} (Martínez-Villafañe et al. 2009; Gadgil et al. 2010). The increase in the As(V) concentration decreases the removal efficiency significantly (Balasubramanian et al. 2009). The liquid motion or liquid + air/oxygen attributes to the better removal of As from the water by EC process. The entity of air combined with water circulation educed the detention time required by less than 50% compared to the conventional flow (Martínez-Villafañe et al. 2009; Sharma et al. 2014).

The effect of initial dissolved oxygen in the bulk solution which promotes the oxidation of arsenite [As(III)] to arsenate [As(V)] has very less effect on the As removal from aqueous solution by EC process (van Genuchten et al. 2016). It may be the concern that the anode oxidation in EC process generates oxygen due to water oxidation (Parga et al. 2005; Anantha Singh and Ramesh 2013) which helps in the oxidation process.

The presence of Cl^- concentration in water reduces the effect of passivation in the process of As removal by EC (Mohora et al. 2012). Arsenic removal by EC process increases the effluent pH to the alkaline range (from 7.2 to 9.5) at steady-state, which may require neutralization before using for drinking purpose (Kumar and Goel 2010).

17.4 Oxidation Methods

17.4.1 Chemical Oxidation

Several conventional methods like coagulation, precipitation, adsorption, etc. are applied to As removal from water medium. Unfortunately, these methods are not much efficient for the removal of arsenite, which is more carcinogenic than arsenate. At the same time, conventional methods are highly effective for the removal of arsenate. Thus, removal of arsenite from water medium can be accomplished by the conversion of arsenite [As(III)] to arsenate [As(V)] (oxidation) and subsequent removal of arsenate by conventional methods.

Aeration is one of the low-cost methods of oxidation and is useful for the oxidation of iron, manganese, etc. Oxidation in the presence of dissolved oxygen is found effective for the arsenite oxidation as compared to the oxidation of iron or manganese. For example, arsenite oxidation in groundwater with the purging of air and pure oxygen is only in between 54% and 57% after 5 days (Kim and Nriagu 2000). Therefore, researchers used other chemical oxidants like ozone, chlorine, and hydrogen peroxide for the oxidation of arsenite and found higher oxidation rates than aeration. Ozone is able to oxidize arsenite within 20 min (Kim and Nriagu 2000) from groundwater with an initial concentration in the range of 46–62 μg L^{-1}. Arsenite oxidation is highly effective using ozone microbubbles for a wide pH range (4–9) (Khuntia et al. 2014). Hydrogen peroxide is another oxidant which is more efficient at the alkaline condition, and the arsenite oxidation rate increases with increase in ionic strength of water (Pettine et al. 1999). Similar to ozone and hydrogen peroxide, potassium permanganate, chlorine, hypochlorite, monochloramine, and chlorine dioxide were also found as effective arsenite oxidants (Sorlini and Gialdini 2010). Hypochlorite and hypochlorous acid are the active agents for the arsenite oxidation by chlorine and hypochlorite. Vasudevan et al. (2006) electrolytically generated hypochlorite using metal oxide-coated titanium anode, stainless steel cathode, and sodium chloride electrolyte for the oxidation of arsenite. The authors observed complete oxidation of arsenite at neutral pH. Arsenite oxidation reactions in the presence of these oxidants are given below (Eqs. 17.32, 17.33, 17.34, 17.35, 17.36 and 17.37).

$$2H_3AsO_3 + O_{2(aq)} \rightarrow 2H_2AsO_4^- + 2H^+ \tag{17.32}$$

$$H_3AsO_3 + OCl^{\bullet} \rightarrow H_2AsO_4^{\bullet} + 2H^+ + Cl^{\bullet} \tag{17.33}$$

$$3H_3AsO_3 + 2MnO_4^{\bullet} \rightarrow 3H_2AsO_4^{\bullet} + H^+ + 2MnO_2 + H_2O \tag{17.34}$$

$$H_3AsO_3 + O_3 \rightarrow H_2AsO_4^{\bullet} + H^+ + O_2 \tag{17.35}$$

$$H_3AsO_3 + 2ClO_2 + H_2O \rightarrow H_2AsO_4^- + 2ClO_2^- + 3H^+ \tag{17.36}$$

$$H_3AsO_3 + NH_2Cl + H_2O \rightarrow HAsO_4^{2-} + NH_4^+ + Cl^- + 2H^+ \tag{17.37}$$

Iron and manganese ions and their compounds, oxides, etc. are also found to be very effective oxidants. Arsenite concentration was reduced to 25 μg L^{-1} from 200 μg L^{-1}, with potassium permanganate oxidation (Viraraghavan et al. 1999). Bordoloi et al. (2013) found that 0.5 mg L^{-1} of potassium permanganate is sufficient to remove As in the range of 1.33–6.67 μM (WHO guideline value). Manganese oxide is also efficient to oxidize arsenite, and the contact time required for the oxidation is very less (Bajpai and Chaudhuri 1999). Ociński et al. (2014) prepared MnO_2 supported over a poly(styrene-divinylbenzene) copolymer containing -SO_2NBrNaoxidative functional groups and used for arsenite oxidation. Manganese oxide was dispersed uniformly over the surface of the polymer and

found rapid arsenite oxidation for a broad range of pH. Ferrous ion in the presence of dissolved oxygen is also able to oxidize arsenite in a water medium. Arsenite oxidation in this system is accomplished mainly by the generation of ferrate ions (Bisceglia et al. 2005; Shao et al. 2016). This process is efficient in the presence of ferrous ion, iron oxides, and hydroxides (Bisceglia et al. 2005; Ona-Nguema et al. 2010; Shao et al. 2016). Since ferrate is responsible for the arsenite oxidation, a few researchers checked the arsenite oxidation in the presence of ferrate ions and found it very effective. Arsenite to ferrate stoichiometric ratio is found as 3:2, and a minimum of 2 mg L^{-1} of ferrate is required to bring down As content in river water from 517 μg L^{-1} to below 50 μg L^{-1} (Lee et al. 2003).

17.4.2 Advanced Oxidation Processes

Advanced oxidation process (AOP) is an efficient tool for the removal of non-biodegradable organic compounds present in the water medium. In all the AOPs hydroxyl radicals, which have high oxidation potential after fluorine, are generated and are responsible for the oxidation of organic contaminants. These radicals are also efficient for arsenite oxidation. AOPs used for the oxidation of arsenite are described below.

17.4.2.1 Fenton Process

Fenton process is a widely accepted advanced oxidation process, in which hydroxyl radicals are generated in an aqueous medium by the reaction between the ferrous ion and hydrogen peroxide as in Eq. 17.38 (Nidheesh et al. 2013; Nidheesh 2015). This reaction is optimal at pH near 3, and both reactants are more stable in this pH condition. Hydroxyl radicals generated via Fenton reactions are very efficient for the oxidation of arsenite. Hug and Leupin (2003) observed complete oxidization of 6.6 μM arsenite by externally added 20 μM hydrogen peroxide and ferrous ion. At the same time, arsenate production was not at all observed only in the presence of oxygen, hydrogen peroxide, iron, and iron oxides. This indicates that hydroxyl radicals are fully responsible for arsenite oxidation. Hydroxyl radical quenching study (using 2-propanol) carried out by the authors also revealed the role of hydroxyl radical on the oxidation of arsenite.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^{\bullet} \tag{17.38}$$

Transition metals other than iron and having two oxidation states also undergo Fenton-like reactions in the presence of hydrogen peroxide (George et al. 2014; Nidheesh and Gandhimathi 2014a). Pettine and Millero (2000) studied the arsenite oxidation by hydroxyl radical generated in water medium by the reaction between hydrogen peroxide and metal ions like Fe^{2+}, Fe^{3+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, and Mn^{2+}. Among

these metals, arsenite oxidation rate was higher in the presence of copper and iron. The authors also checked the arsenite oxidation in freshwater, NaCl solution, and seawater. The rate of arsenite oxidation in freshwater is two times higher than that in NaCl solution and seawater.

17.4.2.2 Photolysis

Photolysis of hydrogen peroxide leads to the generation of hydroxyl radicals in a water medium, and thus the process is considered as an AOP for wastewater and water treatment. This process is found efficient for the oxidation of arsenite (Lescano et al. 2011; Sorlini et al. 2014). But, the oxidation of arsenite occurred only in the presence of hydrogen peroxide and UV light.

17.4.2.3 Photocatalysis

Photocatalysis is an advanced oxidation process used in water and wastewater treatment with wide applications. Illumination of light energy over semiconductor excites electrons in the conduction band to valence band, with the generation of holes in conduction band. Both electron and hole migrate to the surface of semiconductor and undergo redox reactions with other ions present in the water medium. Hydroxyl ions and water molecules are oxidized by holes and dissolved oxygen are reduced by electrons. These reactions produce hydroxyl radicals and superoxides in water medium. TiO_2, ZnO, CuO, etc. are the well-known photocatalysts which have the potential to generate hydroxyl radicals and superoxides in water medium with the illumination of light energy. Based on the radical generation ability of photocatalysts, researchers checked its performance for arsenite oxidation and found significant arsenite removal within a few reaction time. Arsenite oxidation by UV/TiO_2 was rapid and the reaction follows zero-order kinetics (Dutta et al. 2005). López-Muñoz et al. (2017) observed complete oxidation of arsenite at pH 9 within 35 min and at pH 3 within 10 min. These results also show that UV/TiO_2 process is pH independent as reported by Dutta et al. (2005). ZnO is capable of removing both organic and inorganic As from water medium (Rivera-Reyna et al. 2013). Arsenate produced by the oxidation of arsenite is also adsorbed effectively on the surface of ZnO, and it is capable of producing water with As concentration less than 10 μg L^{-1} from water contaminated with a higher amount of As.

17.4.2.4 Photo-Fenton

Photo-Fenton process is an extended Fenton process (Nidheesh et al. 2013), in which hydroxyl radicals are generated in the aqueous medium via Fenton reactions and photooxidation of hydrogen peroxide. Arsenite oxidation is occurring in the photo-Fenton system by hydroxyl radicals generated in the system and

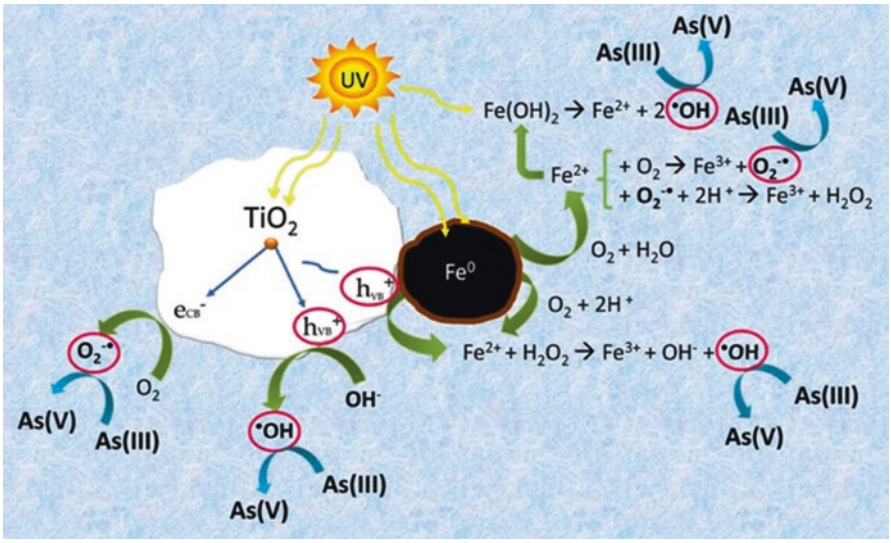

Fig. 17.3 Arsenite oxidation reaction during photocatalysis and photo-Fenton processes. Reprinted with permission from the Ref. (López-Muñoz et al. 2017). Copyright 2016 Elsevier

externally added hydrogen peroxide. López-Muñoz et al. (2017) studied the photo-Fenton oxidation of arsenite in the presence of zerovalent iron as a catalyst. Arsenite oxidation was optimal at pH 3, which is the optimal pH of Fenton reactions. At that condition, first-order kinetic rate for arsenite oxidation was recorded as 0.0608/min. Almost complete removal of arsenite was noticed after 150 min. Various reactions occur during photo-Fenton and photocatalytic oxidation of arsenite is shown in Fig. 17.3.

17.4.2.5 Electrochemical Advanced Oxidation Processes

Electrochemical advanced oxidation processes are widely accepted as a superior water and wastewater treatment due to its higher mineralization potential, low cost, and simplicity (Nidheesh and Gandhimathi 2012; Nidheesh et al. 2013; Nidheesh 2015). Electro-Fenton (EF) process is one of such process widely used for the degradation of non-biodegradable organic contaminants from water medium (Brillas et al. 2009; Nidheesh and Gandhimathi 2014b; Nidheesh et al. 2014). Hydrogen peroxide is generated in situ on cathode surface in EF process by the two-electron reduction of oxygen (Eq. 17.39) at the acidic condition. In situ generated hydrogen peroxide reacts with externally added ferrous ion and generates hydroxyl radicals in aqueous solution. These hydroxyl radicals and hydrogen peroxide are responsible for the oxidation of arsenite in EF process.

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2 \tag{17.39}$$

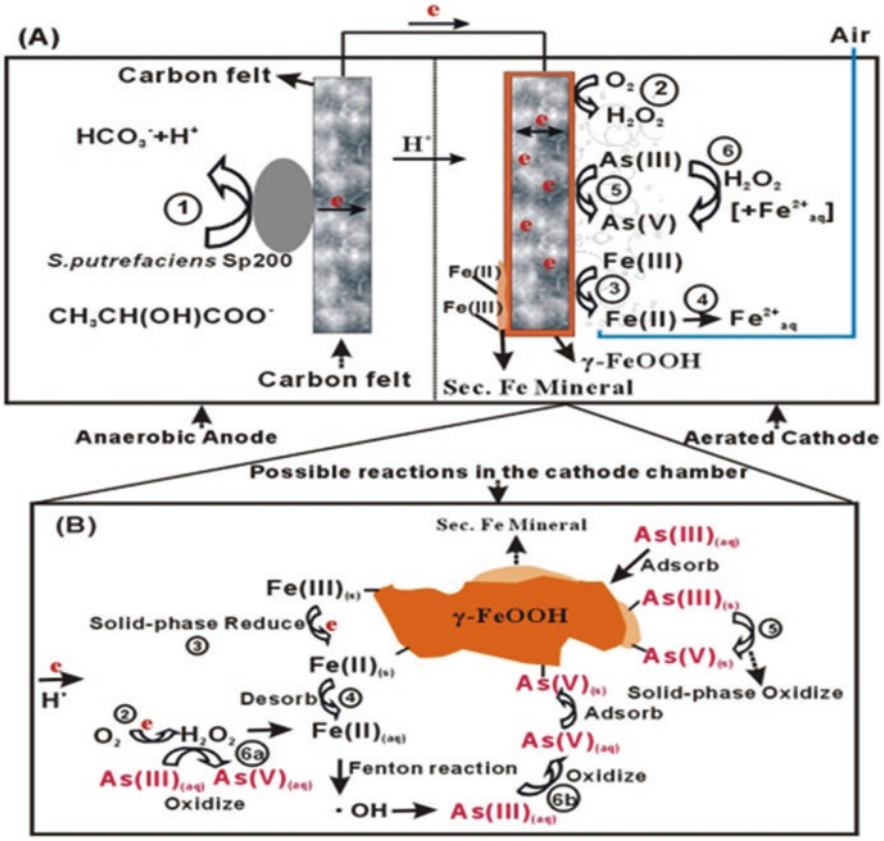

Fig. 17.4 Arsenite oxidation mechanism of bio-EF process. Reprinted with permission from the Ref. (Wang et al. 2014). Copyright 2014 Elsevier

Wang et al. (2014) investigated the performance of bio-EF process on arsenite oxidation. In bio-EF process, the electricity required for the EF reactions is generated in situ by biochemical reactions. Carbon felt was used as cathode and anode for the entire reactions. Anode chamber was operated in anaerobic conditions while the cathodic chamber was operated in aerobic condition. Both chambers are separated by a cation exchange membrane. Lactate was used as electron donor in the anodic chamber. The cathode was covered with γ-FeOOH, which acts as an iron source for Fenton reactions. The overall arsenite oxidation reactions in the bio-EF system are shown in Fig. 17.4. Initially, electrons and protons are generated in the anode chamber with the oxidation of lactate by *Shewanella putrefaciens* SP200 pure microbial culture. Protons generated in the anodic chamber pass through the membrane, while electrons though externally connected wire. The electricity generated in the system helps to generate hydrogen peroxide with aeration and subsequent generation of hydroxyl radicals in the cathodic chamber. These in situ generated oxidants oxidize arsenite present in the cathodic chamber.

Anodic oxidation is another type of electrochemical advanced oxidation process, which can generate hydroxyl radicals in a water medium. In anodic oxidation process, water oxidation at oxygen overvoltage anode occurs which results in the generation of hydroxyl radicals (Eq. 17.40).

$$M + H_2O \rightarrow M\left(^{\bullet}OH\right) + H^{+} + e^{-} \tag{17.40}$$

with M, anode material.

Zhang et al. (2014) used SnO_2 loaded onto Ti-based TiO_2 nanotubes for the generation of hydroxyl radicals and subsequent oxidation of arsenite. The authors observed efficient oxidation of arsenite by the anodic oxidation process. Complete oxidation of arsenite within 60 min of electrolysis at 50 mA was observed by the authors. Since an iron plate was used as a cathode in the anodic oxidation process, the authors also studied arsenate removal by electrocoagulation process with reversing electrode polarity of the anodic oxidation process. Ferrous ion generated from the anode surface is responsible for the complete removal of arsenate (generated in the electrolytic cell by the oxidation of arsenite) within 10 min of electrolysis at 10 mA. Thus, total As was removed from the solution with 70 min of electrolysis in sequential anodic oxidation-electrocoagulation process (Fig. 17.5).

17.4.2.6 Sonochemical Methods

Introducing ultrasound energy for enhancing the chemical reaction rate is a new area in the field of water and wastewater, known as sonochemistry. Addition of ultrasound in aqueous medium improves results in the generation of radicals including hydroxyl radicals, hydrogen radicals, superoxides etc. in water. These radicals are capable of degrading organic pollutants including non-biodegradable and persistent organic pollutants. Hydroxyl radicals are generated in the system by the

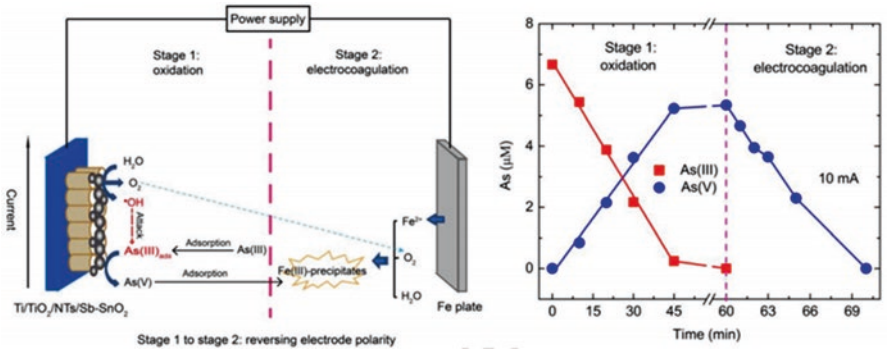

Fig. 17.5 Arsenite oxidation and subsequent arsenate removal by sequential anodic oxidation-electrocoagulation process. Reprinted with permission from the Ref. (Zhang et al. 2014). Copyright 2014 Elsevier

thermal decomposition of water. Acoustic cavitation, that is, the generation of microbubbles, its growth and explosion, accompanied with high temperature (> 5000 °C) and pressure (c.a. 5×10^7Pa) is responsible for radical generation.

Simple ultrasound addition itself is able to oxidize arsenite from aqueous solution (Neppolian et al. 2009), and hydroxyl radicals generated by the water decomposition are responsible for the oxidation. The authors concluded this by measuring hydrogen peroxide concentration in water. Hydrogen peroxide is generated in the system during the propagation of chemical reactions in a sonicator. Hydroxyl radicals generated in the system reacts together and forms hydrogen peroxide. Hydrogen peroxide concentration (generated by sonication) in water containing arsenite was substantially low compared to that in distilled water. This indicates that hydroxyl radicals generated with sonication are completely used for arsenite oxidation other than scavenging reactions.

Arsenite oxidation by sonication is independent of solution pH, but dependent on dissolved oxygen concentration (Neppolian et al. 2009). As(IV), which is the oxidized form of arsenite, is highly unstable and undergoes further oxidation in the presence of oxygen and hydroxyl radicals. This is the main reason behind dissolved oxygen requirement in a sonicator during arsenite oxidation.

External addition of ferrous ion to this system improved the performance of sonication significantly (Cui et al. 2011). Arsenite oxidation also occurs in the system with the hydroxyl radicals generated via Fenton reaction. Externally added ferrous ion reacts with in situ generated hydrogen peroxide and produces hydroxyl radicals in the water medium. Apart from that, arsenate generated by the oxidation of arsenite also is removed from the aqueous phase by coprecipitation and adsorption over the surface of ferric hydroxides.

17.4.2.7 Zerovalent Aluminum/O_2 Process

Application of zerovalent aluminum (Al^0 or ZVAl) for the water and wastewater treatment is an emerging technology and is found very efficient for the removal of various pollutants via reduction and adsorption. Like zerovalent iron, ZVAl is also found very efficient to produce hydrogen peroxide in water medium in the presence of dissolved oxygen as in Eq. 17.41 (Bokare and Choi 2014). Hydrogen peroxide generated in the aqueous medium undergoes further reaction with ZVAl and generates hydroxyl radicals as in Eq. 17.42. ZVAl/O_2 advanced oxidation process is highly efficient for the oxidation of arsenite (Hsu et al. 2016). This higher oxidation rate is mainly by hydrogen peroxide and hydroxyl radicals as in Fenton processes. With the external addition of ferrous ion into ZVAl/O_2 system, the efficiency of the process increased significantly, and the improved system is very efficient for arsenite oxidation even from acid mine drainage (Hsu et al. 2016). This improved performance with ferrous ion addition is mainly attributed by the additional hydroxyl radical generation via Fenton reactions. Neutralizing the solution after arsenite oxidation removed all the arsenate generated in the water medium.

$$2Al^0 + 3O_2 + 6H^+ \rightarrow 2Al^{3+} + 3H_2O_2 \quad (17.41)$$

$$Al^0 + 3H_2O_2 \rightarrow Al^{3+} + 3OH^- + 3HO^{\bullet} \quad (17.42)$$

Like ferrous ion, external addition of polyoxometalate also improved the performance of ZVAl/O_2 process significantly. Additional hydrogen peroxide production in the presence of polyoxometalate and increased hydrogen peroxide generation by the amplified dissolution of the aluminum oxide layer are responsible for the enhanced arsenite oxidation (Wu et al. 2013).

17.4.2.8 Sulfate Radical-Based AOPs

Sulfate radical-based AOPs is an emerging field in water and wastewater treatment. Sulfate radicals can generate in the water medium by using persulfate or peroxymonosulfate as parent oxidants. Decomposition of these parent oxidants is a slow process, and an initiator such as heavy metals, UV light, ultrasound, heat, etc. is required for the effective generation of sulfate radicals. Sulfate radicals are found as more powerful than hydroxyl radicals for the degradation of dyes from water medium (Nidheesh and Rajan 2016). Similar to this study, sulfate radicals are highly efficient for the oxidation of arsenite. UV/persulfate process was found to be a very effective process for arsenite oxidation and the performance of the process unaltered for solution pH in the range of 3 and 9 (Neppolian et al. 2008). Similar results were observed for ultrasound/persulfate process (Neppolian et al. 2010). The rate of arsenite oxidation was around ten times higher than the concentration of persulfate. The authors found that hydroxyl and sulfate radicals are responsible for arsenite oxidation in ultrasound/persulfate process. A similar result was reported by Zhou et al. (2013) for ferrous-activated persulfate process. Dissolved oxygen plays an important role in arsenite oxidation by sulfate radical-based AOPs (Neppolian et al. 2008, 2010). Arsenite oxidation was only 40% in the absence of dissolved oxygen, while it was recorded as 80% in the presence of 10 mg L^{-1} dissolved oxygen and after 5 min of reaction time, in ultrasound/persulfate process (Neppolian et al. 2010). Based on the results, Neppolian et al. (2008) proposed arsenite oxidation mechanism by sulfate radical-based AOP in Eqs. 17.43, 17.44, 17.45, 17.46, and 17.47:

$$As(III) + SO_4^{-\bullet} \rightarrow As(IV) + SO_4^{2-} \quad (17.43)$$

$$As(IV) + SO_4^{-\bullet} \rightarrow As(V) + SO_4^{2-} \quad (17.44)$$

$$As(IV) + O_2 \rightarrow As(V^{**}) + O_2^{-\bullet} \quad (17.45)$$

$$SO_4^{-\bullet} + H_2O \rightarrow HO^{\bullet} + H^+ + SO_4^{2-} \quad (17.46)$$

$$As(III) + HO^{\bullet} \rightarrow As(IV) + HO^- \quad (17.47)$$

17.5 Biological Methods+

17.5.1 Bio-oxidation

Microbes conduct the process of oxidation such that values of metals get maintained in the solid phase (remain enriched). In bio-oxidation, microbes sustain metal values in solid phase, and the remaining solution is discarded. The substances which are harmful to the environment, as well as human health, can be detoxified with the help of naturally occurring fungi, bacteria, or flora. In 1974 the first patent of biological remediation agent has been registered, being a strain of *Pseudomonas putida* (Prescott et al. 2002). Microorganisms which are used for performing the function of bioremediation are termed as bioremediators. When oxidation-reduction potential changes take place, it is considered as primary principle of the remediation. The main purpose of bio-oxidation is for extracting metals from low-quality, low-grade, as well as complex ores. These ore concentrates such as As contain refractory gold concentrates (Jaatinen 2011).

Microorganisms have developed different mechanisms like arsenite methylation, arsenite oxidation, etc., to transform the more toxic form of As, i.e., arsenite, to less toxic form arsenate (Qin et al. 2006). A special type of enzyme is present in the protoplasm of As-oxidizing bacteria, As oxidase. With the help of this enzyme, the bacteria oxidize arsenite to arsenate (Andreoni et al. 2012). Chowdhury et al. (2009) isolated a novel strain, KRPC10YT from As-contaminated borewell of West Bengal, India, which can tolerate up to 30 mM arsenate and 20 mM arsenite.

For the treatment of water, autotrophic bacteria with a low nutritional requirement are more exciting. Some studies have reported that indigenous microorganism uses As(III) from groundwater for oxidation process (Hambsch et al. 1995); other than this, it can be used as a source of energy by two chemolithotrophs (Ilyaletdinov and Abdrashitova 1981). In the year 1981, oxidation of As by using bacteria was first discovered in the cattle-dipping tank. There is one arsenate reducer bacteria named as *Bacterium arenreducens*, and the other is arsenite oxidizer, *B. arsenoxydans*, given by Green. Arsenate-reducing bacteria have been separated from As-rich soil, whereas by the use of anaerobic media, sediments get isolated; in this situation, As acts like a sole terminal acceptor (Nicholas et al. 2003). As(III)-oxidizing process has been studied by Mokashi and Paknikar (2002) by using bacteria *Microbacterium lacticum* for the treatment of As-contaminated groundwater. Ninety-two percent of As(III) oxidation is gained in constant flow bioreactor with immobilized oxygenated As(III)-oxidizing bacteria when it is applied for pretreatment of As removal from groundwater (Ito et al. 2012). For removal of As, a combination of distinct bacterial metabolisms and adsorbents has been used (Corsini et al. 2014).

It is estimated that for arsenite, microbial oxidation is more eco-friendly as compared to chemical method. In the presence of oxygen, arsenite is converted to arsenate by various species of bacteria, while in the absence of oxygen arsenite acts as an electron donor. Bacteria which are involved in arsenite-oxidizing process are both heterotrophic and chemoautotrophic. These kinds of bacteria are known as

arsenite-oxidizing bacteria (AOB) which have the ability to utilize CO_2 as a carbon source. Also, there are some bacteria which oxidize arsenite, in the anaerobic situation by the help of nitrate (Rhine et al. 2006; Zhang et al. 2015) or chlorate (Sun et al. 2010). In this case, nitrate or chlorate acts as a terminal electron acceptor while arsenite as an electron donor. A recent study suggests that in biofilm reactor, some of immobilized AOBs are used for oxidizing arsenite. About 1100 μg L^{-1} As(III) from artificial groundwater source were converted to As(V) by the use of this bioreactor within 10 min without adding any chemical or any nutrient (Li et al. 2016). Large quantity of exopolymers have been produced in the occurrence of As, by the use of bacteria *H. arsenicoxydans*. These exopolymers may be used to detoxify As contaminant from natural water (Muller et al. 2007). Arsenate reduction into arsenite has been described by two major biological mechanisms. In the first mechanism, detoxification of cells was carried out. There is a structural similarity in between phosphate ion and arsenate ion, so arsenate ions enter the cells through phosphate transporter. The cell now entered into the cytoplasm where enzyme Ars C reduced As(V) to As(III) (Mukhopadhyay and Rosen 2002). In the second step of mechanism, the process involves dissimilatory reduction, which is conducted through bacteria of varying phylogenetic groups together with facultative anaerobic microbes or obligate microbes (Páez-Espino et al. 2009). In this case, bacteria inhale As (termed as respiratory arsenate reductase) and gain energy for metabolic activity, where As(V) is used as an electron acceptor (Macy et al. 2000).

17.5.2 Arsenic-Resistant Microorganisms

High As concentration can be tolerated easily and also take part in the vital functioning of the cells which has been reported by the study on bacterial growth at high phosphorus-As ratios (Rosen et al. 2011). The presence of naturally occurring arsenate and arsenite in water and soil environment which could enter the cells by the phosphate transport system has given pressure for microorganisms to maintain their As detoxification systems for surviving purposes. One of the commonest forms of As resistance in microorganisms is by detoxification operons, which are encoded on genomes or plasmids (Musingarimi et al. 2010). The mechanism for As detoxification can be divided into four; first is As(V) uptake in the form of arsenate and is mediated by phosphate transporter. Second is the use of aquaglyceroporins for the uptake of As(III) in the form of arsenite (Rosen 2002). Next to this is a reduction of As(V) to As(III) by arsenate reductases (Rosen 2002), and last is extrusion or sequestration of As(III) (Rosen 2002). Microbial consortium oxidizes As(III), result into increase in sediment as the concentration of the downstream of the hydrothermal source and considerable decrease in dissolved As concentration. The microbiological molecular analysis had verified that As(III)-oxidizing groups are present in the system (aroA-like genes) and also for As oxidation there is a need of organic action demonstrated by in situ oxidation experiments (Lim et al. 2014). Arsenate reduction to arsenite by using microbes is carried out by dissimilatory reduction

mechanism that could be conducted in the presence of strict or facultative anaerobic condition where, the terminal electron acceptor is arsenate. Microorganisms have the ability to oxidize inorganic (sulfide and hydrogen) and organic (e.g., formate, aromatics, and lactase acetate) as electron donors which will lead to the production of arsenite. Liao et al. (2011) reported that 11 As-reducing bacteria strains from seven different genera (i.e., *Pseudomonas*, *Psychrobacter*, *Citrobacter*, *Bacillus*, *Bosea*, *Vibrio*, and *Enterobacter*) were isolated from environmental groundwater samples collected from well AG1 in southern Yunlin County (Liao et al. 2011), west-central Taiwan. Other bacteria with the ability of reducing arsenate to arsenite are *Sulfurospirillum barnesii* and *Sulfurospirillum arsenophilum* from the *ε-proteobacteria* as well as *Pyrobaculumarsenaticum* from *Thermoproteales* order and *Chrysiogenes arsenatis*. *Archaebacterium*, *Sulfolobus acidocaldarius* strain BC, *Alcaligenes faecalis*, *Shewanella algae*, *ε-proteobacteria* strain UPLAs1, *Alcaligenes faecalis*, *Comamonas terrae* sp. nov, some heterotrophic bacteria (*Herminiimonas arsenicoxydans*), and chemoliautotrophic bacteria are reported to have the ability to oxidize arsenite to a less toxic arsenate (Oremland and Stolz 2005). For the conversion of arsenite into a lesser poisonous form or for the production of secondary metabolites which bind to arsenite, several researchers have used bacteria like *Hydrogenobaculum* strain and fungi like *Penicillium* sp., *Gliocladium roseum*, and *Scopulariopsis brevicaulis* (Donahoe-Christiansen et al. 2004). For the elimination of arsenite from the As-contaminated water, bacteria *Lactobacillus acidophilus* has been used, even it stay alive in higher arsenite concentration. In nature, microorganisms are capable of carrying out the oxidation of As(III) with the enzyme As(III) oxidase, a member of the DMSO reductase family (Ellis et al. 2001). A number of microbial isolates such as *Ancylobacterdichloromethanicum* strain As 3-1b were able to oxidize arsenite.

Polluted environment isolates a broad variety of bacteria, which have a capacity of synthesizing and oxidizing arsenite enzymatically. It consists of chemoautotrophic as well as heterotrophic bacteria where As(III) acts as an electron donor by reducing nitrate or oxygen. In this condition, energy production takes place for the fixation of CO_2 and gives it to bacteria for development. There is a presence of both chemoautotrophic and heterotrophic bacteria in a population where, in case of heterotrophs, As(III)-oxidizing bacteria grow up by the use of biological substances made up by chemoautotrophic bacteria (Battaglia-Brunet et al. 2002).

17.5.3 Phytoremediation

In 1983 the use of different plant species which are able to remove heavy metal contaminants or other compounds has been first proposed; however, around 300 years ago, this concept has been applied to wastewater discharge (Dwivedi et al. 2015). In 1996 at the University of Georgia, researchers first used phytoremediation technology (Dwivedi et al. 2015). A few years back, the use of green flora for treating contaminated soil as well as water received a lot of consideration (Vasavi et al. 2010). The

term phytoremediation is formed from the Greek prefix "phyto-" which means flora or plant and comes from Latin suffix "remedium" which means restore or clean (Cunningham and Ow 1996). This term is basically used for various groups of plant-dependent technology, which can either be genetically engineered or naturally occurring plants for clearing contaminants from the environment (Flathman and Lanza 1998). Phytofiltration or rhizofiltration, phytoextraction, phytodegradation, and phytovolatilization are four different steps involved in phytoremediation (Fig. 17.6) (Long et al. 2002; Jadia and Fulekar 2009). For the primary treatment of extracted low concentrate contaminant from the surface, ground, and waste water, phytofilteration or rhizhofilteration has been used (Khan et al. 2009). In this technique Cr, Ni, Cd, Zn, Pb, and Cu are some metal contaminants which are mainly retained within the roots (Khan et al. 2009). It includes use of flora to clean various aquatic environments. Spinach, tobacco, sunflower, corn, rye, and Indian mustard are some of the studied plant species for their maximum capacity for the eradication of lead from water (Jadia and Fulekar 2009). In phytostabilization technique, plant roots acts as boundary pollutant mobility as well as bioavailability in the soil. In the same process and by the help of plant, percolation of water within the soil medium takes place, which results in harmful leachate production and is also acts as a barrier. Because of this, the direct contact with contaminated soil again disturbance in soil erosion, which results into the transfer of hazardous metal to another site (Khan et al. 2009). In phytovolatilization technology, green plants remove the volatile toxins (Se and Hg) from contaminated soil medium and release them into the air (Karami and Sahmsuddin 2010).

In phytodegradation, both plants and microorganism uptake, mobilize and degenerate the biological contaminant. In this case of detoxifying soil pollutant with

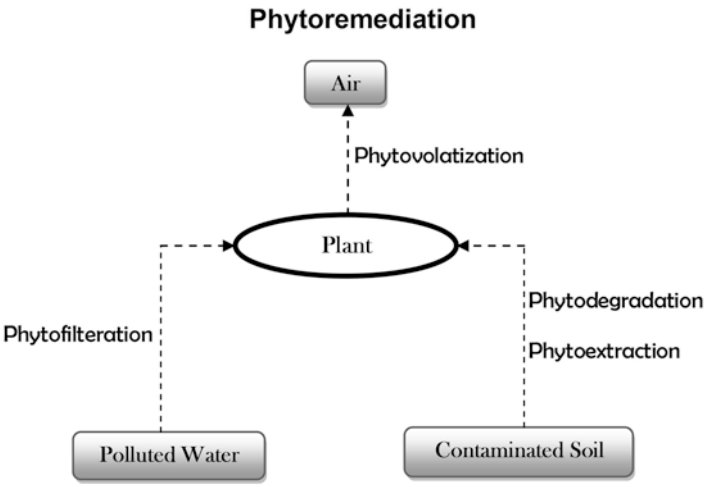

Fig. 17.6 Pollutant removal mechanisms of phytoremediation

biological compound, plant roots are organized with microbes (Garbisu and Alkorta 2001). In phytoextraction technology, plant species take up metals from soil and transfer them toward shoot where it gets accumulated. Now for the exclusion of contaminants from soil, both root and shoot are consequently harvested.

Testing of aquatic as well as terrestrial flora was carried out for the remediation of contaminated water and soil. There are some aquatic floras that are known for the accumulation of As in high percentage from water medium and used for phytoremediation. These aquatic floras are water spinach (*Ipomoea aquatica*) (Lee et al. 1991; Rahman and Hasegawa 2011),water fern (*A. filiculoides* and *A. pinnata*) (Rahman et al. 2008; Rahman and Hasegawa 2011), water hyacinth (*Eichhornia crassipes*) (Alvarado et al. 2008), water cabbage/water lettuce (*Pistia stratiotes*) (Lee et al. 1991), hydrilla (*Hydrilla verticillata*) (Rahman and Hasegawa 2011), duckweeds (*Lemna gibba*, *L. minor*, *Spirodela polyrhiza*), *Azolla pinnata* (Rahman and Hasegawa 2011), *Lepidium sativum* L. (Rahman and Hasegawa 2011), watercress (*Nasturtium officinale*, formerly *Rorippa nasturtium-aquaticum*), needle spikerush (*Eleocharis acicularis*) (Ha et al. 2009), waterweed/pondweed (*Elodea canadensis*), curly waterweed (*Lagarosiphon major*), Brazilian waterweed (*Veronica aquatica*), water pepper (*Polygonum hydropiper*) (Robinson et al. 2005), and miriophyllum (*Myriophyllum propinquum*) which have been reported for the uptake of As from water.

Aquatic macrophytes or other floating flora can gracefully achieve toxic agent during the process of phytoremediation; also bioaccumulation, as well as biosorption process, takes place for accumulation of bioavailable agents from water medium (Brook and Robinson 1998). Two kinds of plants are involved in aquatic phytoremediation systems which are submerged in water type, and another is floating on surface water. In case of floating aquatic flora, roots suck up or accumulate toxicant while in case of submerged flora the accumulation of contaminant by their whole part takes place (Rahman and Hasegawa 2011). For the uptake of As species in aquatic macrophytes, there are three mechanisms which have been proposed (Tripathi et al. 2007; Zhao et al. 2009): first is active uptake, second is passive uptake, and third is physicochemical adsorption.

Active uptake process is carried out with the help of phosphate transporter which is present in plasmalemma of the plant. In this case, both As(V) and phosphate compete with each other as these are chemical analog, for intake of a contaminant into plasmalemma (Mkandawire et al. 2004). Therefore the more the phosphate concentration, the more As(V) is desorbed (Smith and Read 2008). As(V) enters into the plant via phosphate transporters (Tripathi et al. 2007; Zhao et al. 2009). The aquatic plants use the same mechanism.

Passive uptake process is carried out with the help of aquaglyceroporins. In this process, As species are transported (which is reported by physiological studies), although exact mechanism in higher flora for As(III), organoarsenicals, monomethylarsonic acid (MMAA), and dimethyl As acid (DMAA) is yet to be studied

(Abedin et al. 2002). According to current molecular study, with the help of intrinsic membrane protein (NIPs) nodulin26, As(III) travels into rice roots. NIPs is a subfamily of aquaporins transporter, which transports neutral molecules like urea, glycerol, and water (Ma et al. 2008). Aquaglyceroporins and aquaporins are two out of three subfamily of water channel proteins (WCPs). In transmembrane proteins, there is a three-dimensional structure and a pore through which neutral molecules, water molecules, and other small molecules are permeable (Benga 2009). Arsenic species by the help of aquaporins/aquaglyceroporins get to transfer through plasma membrane when there is a struggle between As(III) and glycerol for entering into the rice (*Oryza sativa* L.) (Meharg and Jardine 2003). But rice is a wetland plant so that it can be removed from the above concept.

Robinson and others reported that for the accumulation of As(V) into water flora, the process of physiological adsorption has been conducted as an optional mechanism (Robinson et al. 2006). In this mechanism, by use of floated oxides of iron (Fe plaque) which are present on aquatic floral surface, adsorption and accumulation of As species take place. Rahman et al. (2008) reported and studied that when the plant was exposed to As(V), there is a considerable relationship between iron and As. This is because of the precipitated iron oxides found in roots/fronds of S. polyrhiza L. where As species get absorbed. From this study, it was concluded that As(V) accumulation is carried out by the mechanism of adsorption through the surface of the aquatic plant as well as on precipitated iron oxides on the roots. It has been noticed that, upon introduction of As(V), the quantity of As and iron in *S. polyrhiza* L. was not significantly interconnected in phosphate-sufficient condition, while in case of phosphate-deficient condition, it is highly connected. This can be recognized that in phosphate-deficient solution, from the surface of the plant, there is high adsorption of As on iron plaque, whereas in phosphate-sufficient solution, it was blocked by phosphate (Rahman and Hasegawa 2011).

From phytoremediation, phytomining also emerged as an environment-friendly technology that utilizes plant species for uptake of heavy metal contaminant (Brook and Robinson 1998). *Wolffia globosa* SS tolerates up to 400 mg As kg^{-1} dry weight (dw), and accumulation is about >1000 mg As kg^{-1} dw (Zhang et al. 2009). *Spirodela polyrrhiza* has As removal efficiency of 40.0%, whereas *Echhornia crassipes* has 80% (Mishra et al. 2008). When evaluation of *water hyacinth* and *Lemna minor* was conducted, it has been found that water hyacinth shows 18% removal recovery and removal rate is 600 mg As ha^{-1} d, whereas in case of *Lemna minor*, removal recovery is 5% and 140 mg As ha^{-1} d^{-1} is the removal rate, respectively (Alvarado et al. 2008). *S. americanus* tolerated As concentrations up to 4 mg L^{-1} in the hydroponic medium for a period of 10 weeks (Alarcon-Herrera et al. 2013). A similar study was carried out by Alvarado et al. (2008) on *E. crassipes*, and it shows removal recovery of 18% which is similar to water hyacinth, while removal rate is of 600 mg As $ha^{-1}d^{-1}$ under field condition.

17.6 Conclusions and Perspectives

Arsenic has been a dangerous and severe pollutant of water from early days and has a serious human impact at some locations. Several technologies were developed over the years for As removal from water. Advantages and disadvantages of various treatment technologies are given in Table 17.1. The removal efficiency of each process varied and depends upon the matrix in which As contamination occurred.

Table 17.1 Advantages and disadvantages of various arsenic treatment techniques

Process	Advantages	Disadvantages
Adsorption	Use of low-cost materials	Higher cost for regeneration of adsorbent
	Agricultural and industrial waste can be used as adsorbents	Difficult to find inexpensive and effective sorbents
	Easy operation and less maintenance cost	Lack of excellent physical and chemical durability for adsorbents
	Minimum sludge production	Contaminants are separated from aqueous solution, but not destroyed
	Safer to handle	
	Lower energy and chemical requirement for the process	
Membrane processes	Removal efficiency less affected by pH and chemical composition of the feed	Posttreatment required to provide adequate minerals for water supply
	Less addition of chemicals	Disposal of sludge or wastewater
	Disposal of used membranes is simple	Loss of 10–15% feed water after treatment
	Operational requirements are minimal	Higher operating cost
	Scaling up of processes is easy	Membrane fouling and corrosion
	No sludge production	Limited lifetime of membranes
Chemical coagulation	Cost-effective process for arsenic removal	Direct addition of the coagulant to the water leads to increase in residual levels of iron or aluminum, which is undesirable to the consumers
	The solid flocs generated can be easily removed through sedimentation or filtration	Adsorption by iron oxides is highly pH sensitive
		The pre-oxidation of arsenite to arsenate is required for effective removal
		Sludge generation with chemical coagulation is higher

(continued)

Table 17.1 (continued)

Process	Advantages	Disadvantages
Electrocoagulation	Low cost, compared to other methods	Requires a large capital investment and the expense of electricity supply
	Highly efficient to remove arsenic	EC is a pH-dependent process which affects the removal of arsenic
	High conductivity favors high process performance and low operating cost	The results obtained in laboratories under controlled conditions cannot always be extended to real field environments
	EC brings in the advantages of higher adsorption capacity, no manual chemical addition, less area requirement, and less coagulant and produces less sludge compared to chemical coagulation	
	EC process is easy to control	
Chemical oxidation	Increases the removal of arsenic species by oxidizing to arsenate	Higher use of chemicals
	Process in pH dependent	Formation of secondary pollutants when chemicals are used
Advanced oxidation processes	Does not create sludge as with physical chemical process or biological processes	High operating cost
	Rapid reaction rate	High energy requirement
	Installation cost is less	
	Can be used for water disinfection	
Bio-oxidation	Low operation cost	Optimum conditions are required for growth of microorganisms
	Less sludge production	Longer duration of the process
	Less consumption of chemicals	
Phytoremediation	This technique has received much attention lately as a cost-effective alternative to the more established treatment methods used at hazardous waste sites	As phytoremediation is plant-based systems of remediation, it is not possible to completely prevent the leaching of contaminants into the groundwater
	Phytoremediation technology is highly promising because it is cheaper and is able to minimize the contaminating levels of arsenic	Phytoremediation site should be large enough to grow plants
	In phytoremediation, the use of plant species to clean up soil and water, since it is a cost-effective, eco-friendly technology	It is a slow process and hence more time-consuming
		The survival of the plants is affected by the toxicity of the contaminated land and the general condition of the soil

Adsorption is an effective and well-versed technology for removing As from aqueous solutions as it stabilizes the pollutants to a solid surface in a less toxic form. The rate of adsorption of As depends on many of the factors such as the type of adsorbent, pH, temperature, contact time, etc. The higher effluent quality, high efficiency, flexibility, and easy operation make membrane processes more promising for As separation. Pretreatment of the solution and controlling the oxidation state of As species present can increase the degree of As rejection. The technology further faces the issues associated with charge interaction and membrane fouling which can substantially reduce the efficiency of the process.

Coagulants such as calcium salts, iron salts, aluminum salts and titanium salts are more effective for the removal of As from aqueous solution. The initial pH of the solution has a significant impact on the removal efficiency. The application of polyelectrolytes as coagulant aids results in the enhanced As removal. Aluminum, iron, titanium, etc. were used as electrodes for a potential electrochemical method like EC process for As removal from water medium. The initial pH of the solution has a significant effect on the process and also controls redox reactions occurring in the electrolytic cell. The electrocoagulation with different electrodes follows the following order of removal efficiency, iron> titanium>aluminum. Chemical oxidation was more effective, as it oxidizes As species to less toxic form. The higher oxidation potential of hydroxyl radicals and sulfate radicals rapidly oxidizes As present in water. Biological methods and plant-based methods are also found effective for As decontamination from water medium. But, the major concern on these processes is the further disposal of phytoremediating aquatic macrophytes. This chapter would be helpful in understanding the effects on As contamination, various treatment technologies developed used over the years, and their potential to remove the contaminant.

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Chapter 18
Status of Arsenic Toxicity in the World

Mustafeez Mujtaba Babar and Aneela Tariq

Abstract Arsenic (As) is a major environmental contaminant that affects the plant, animal, and human life. It is obtained from the earth's crust and finds its use in industrial, cosmetic, agricultural, and health sectors. Though due to the medical concerns, its use has decreased, yet it continues to be bioavailable in different forms. Released through geological and anthropogenic activities, it presents to be a major risk to the human population. The current chapter reviews the role of As as a potential environmental contaminant and its effect as a carcinogen and toxin to the humans. Various sources of introduction of As into the environment are then discussed. A comprehensive review of the geographical patterns of As occurrence in environmental samples has then been provided along with the interventional strategies that can be employed to decrease the effects of As toxicity.

Keywords Arsenic toxicity · Global perspective · Geographical distribution · Arsenicosis · Arsenic poisoning

Abbreviations

µg	Microgram
As	Arsenic
As (III)	Trivalent arsenic
As (V)	Pentavalent arsenic
ATSDR	Agency for Toxic Substances and Disease Registry

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Ca	Calcium
CDC	Centers for Disease Control and Prevention
CDK	Cyclin-dependent kinases
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
Co	Cobalt
Cr	Chromium
Cu	Copper
DEC	Department of Environment and Conservation
DMAsV	Dimethylarsinic acid
DNA	Deoxyribonucleic acid
EIL	Ecological Investigation Levels
Fe	Iron
GDP	Gross domestic product
JECFA	Joint FAO-WHO Expert Committee on Food Additives
kg	Kilogram
L	Liter
LOD	Limits of detection
MAPK	Mitogen-activated protein kinases
MCL	Maximum Contaminant Level
Mg	Magnesium
MSMAsV	Monosodium methanearsonate
P	Phosphorus
Pb	Lead
PC	Phytochelatin
ppb	Parts per billion
PTE	Potentially toxic elements
ROS	Reactive oxygen species
UAE	United Arab Emirates
USA	United States
WHO	World Health Organization
Zn	Zinc

18.1 Introduction

Arsenic (As) is a metalloid that is one of the twenty most abundant components in the earth's crust. It is released into the environment through various geological as well as man-made procedures. Naturally, it is found in the volcanic ash, in sulfide-containing hot springs, and in groundwater. Due to human activities like mining, excessive irrigation, and environmental pollution, As has been brought into continuous circulation. It is now found in wells, dust, air, vegetation, and animal meat. Significantly higher levels are also found in the human hair, nail, and urine samples. The source of As in all these sections is considered to be water. Over the past several decades, due to an improvement in the screening techniques, concerns related to

increased amounts of As have been raised across the world (Mukherjee et al. 2006). The levels of As in the Indian subcontinent have been elevated in the order that the researchers are calling it as the greatest calamity in the history of Asia. Millions of people around the globe are exposed to very high levels of As on a daily basis due to their exposure to As-rich water and the presence of As in food.

As, in its organic and inorganic forms, is absorbed by the plant and animal body. It is then converted to forms that can accumulate in the cell for a long period, thereby, causing significant adverse events. These effects are mediated by damage to the cellular organelles, nucleus, cell membrane, and various components of the biomachinery (Bánfalvi 2011). At the organ level, these effects are manifested as damage to cardiovascular, nervous, integumentary, gastrointestinal, muscular, and skeletal systems (Abernathy et al. 1999). As toxicity not only causes damage to various organs in the body but has been implicated to be responsible for causing toxicity across various geographical regions. The highest number of cases of As toxicity in recent history has been reported from Asia. Bangladesh, India, China, Mongolia, Pakistan, and Iran have reported cases from a variety of regions. Similarly, Romania, Croatia, and Bulgaria in Europe have also indicated a high level of As contamination in groundwater. Though due to regulatory control, the number of cases of As toxicity is decreasing in the developed world including the United States (USA), yet it has been found that the concentration of the metalloid is on the rise (Welch et al. 2000a).

This chapter initially provides an introduction to As as environmental and clinical toxin. Thereafter, the geographical burden of the As toxicity is provided by discussing all the continental regions separately. At the end of the chapter, various mitigation strategies that employed throughout the world to decrease the As load have been discussed.

18.2 Arsenic as an Environmental Toxin

Arsenic is present in different concentrations throughout the ecosystem. It is introduced into the environment through natural as well as due to human intervention. It also forms an important component of many medicinal agents. However, as defined by the basic rules of pharmaceutical sciences, it is the concentration of a substance that makes it a medicine or a poison; As is also identified as a hazardous substance. As per the Centers for Disease Control and Prevention (CDC) guidelines, As is an important means of causing toxicity. It is on the first position of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Priority List of Hazardous Substances published by the Agency for Toxic Substances and Disease Registry (ATSDR) (Richardson 2017). As, a metalloid by nature, is present in both organic and inorganic forms. It is present in elemental (0), trivalent (III), and pentavalent (V) form. Humans are generally exposed to As (III) and As (V) which are collectively referred to as the arsenicals (Yang et al. 2018). The arsenite, As trioxide, monomethylarsonous acid, and dimethylarsinous acid contain As (III), while

arsenate, As pentoxide, monomethylarsonic acid, dimethylarsinic acid, trimethyl arsine oxide, arsanilic acid A, and arsenobetaine possess As (V) (Hoffmann et al. 2018). Toxicologically, As (III) forms are more hazardous for human health as it possesses the capability to generate oxidative stress and cause cellular toxicity.

Due to the highly potent nature of As compounds, they are used in pesticides. In the form of Paris green, it has been used for killing potato beetles and mosquitoes (Casagrande 2014). Similarly, lead arsenate has also been used as a pesticide for fruits. However, due to an increase in the understanding of the potential risks of As compounds, their use in this form has decreased. However, due to the long persistence of As, it is thought that many hectares of land still contain As in various forms. These long-lived species tend to adversely affect the human health as the scope of urbanization is increasing and many farmlands are now being converted to residential areas. Though the use of As as pesticide has decreased, various preparations of As are still in use. Chromated copper arsenate, for instance, is still being used to preserve wood especially of marine facilities and on roads (Kramb et al. 2016). Among the organic arsenicals, the pentavalent form of As is still being used as monosodium methanearsonate (MSMAsV) and dimethylarsinic acid (DMAsV). These variants are not readily accumulated within the cells. The US Environmental Protection Agency (EPA) does not classify DMAsV as a carcinogen due to its low toxicity (Hughes and Kitchin 2006). As is also used in the metal extraction industry. It is used for copper, gold, and iron smelting. However, due to the increased awareness of occupational hazards of As, its use in the mining industry has now significantly reduced.

As is introduced into the environment through water, soil, air, and agricultural processes. Generally, inorganic form of As, as arsenite and arsenate, is present in water. Estimates have established the median groundwater concentration of As to be around 1 μg/L in the USA (Focazio et al. 2000). However, in the underdeveloped and the developing world, it is thought that this concentration is significantly higher. A number of studies have reviewed the adverse effects of inorganic As on the human health which have been reviewed in the subsequent section. Due to the As-contaminated water in the environment, it is readily taken up by the plants. Dietary vegetation has, hence, become the major source of introduction of As into the animal and human diet. Rice, beans, and legumes generally contain high levels of As (Meharg 2004; Silva et al. 2014). Humans, being omnivorous, not only directly feed on these plants but while consuming meat are also subjected to As toxicity. The dietary intake of As varies in different regions of the world. As discussed, the biologically transformed variety of As obtained from the plants and animals are relatively less toxic. Arsenolipids, arsenosugars, arsenobetaine, and arsenocholine have been isolated from various varieties of fish, crabs, and mollusks. Among these arsenobetaine is the least toxic form of the metalloid. Arsenosugars are currently being studied to establish their toxicological profiles. Similarly, humans may be exposed to As through soil as well. On average the soil contains 0.01–600 mg/kg of As (Yan-Chu 1994). Incidental ingestion, dust, and contamination of water samples with soil particles form the major sources of introduction of As through soil. It can also be absorbed through the skin and through the wind-blown dust. As can also be introduced into the human body through the air. However,

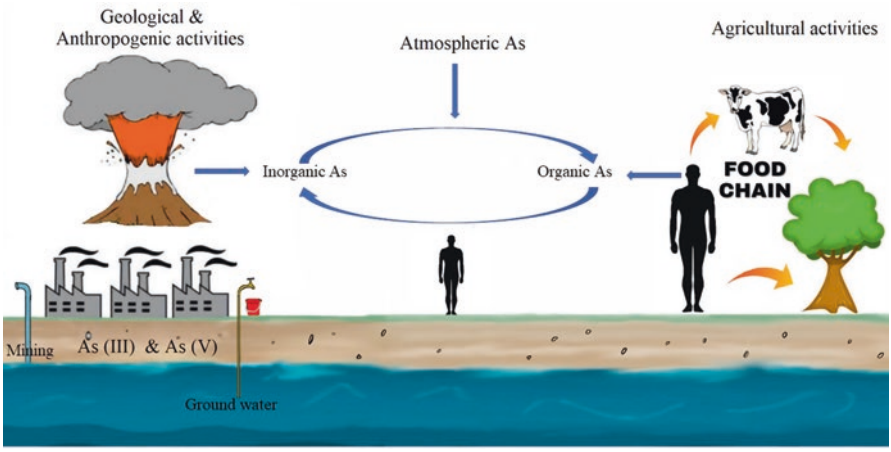

Fig. 18.1 Regulation of environmental arsenic

it is the least effective means to introduce As into the body. Various studies have related the proximity of industrial areas to the higher chance of being affected by air. Figure 18.1 presents the various sources that can potentially serve as means to introduce As to humans.

Various regulatory agencies have specified values for categorizing the soil, air, and water samples safe. The US Environmental Protection Agency (EPA) specifies 10 ppb of As in potable water to be safe for human consumption (Nachman et al. 2017). Similarly, the World Health Organization (WHO) establishes 10 μg/L of As as the Maximum Contaminant Level (MCL) for As (World Health Organization 2004). The US EPA does not provide any specific limit for As in soil. However, it is considered that a 0.4 mg As/kg of soil corresponds to a risk of one in one million. Similarly, European Commission and other international authorities have established specific maximum limits of As in food and beverages (Tóth et al. 2016). A compliance to these values ensures that the human health is not adversely affected. However, even a slight increase can result in long-term chances of developing pathological effects.

18.3 Pathological Aspects of Arsenic Toxicity

Arsenic, once entered into the body through various routes, can directly be taken up by the cells or can be biotransformed to form other viable forms (Skröder Löveborn et al. 2016). It is converted from the pentavalent form to the trivalent form followed by its oxidative alkylation to form trivalent arsenicals. These alkylated arsenicals are released via human urine into the environment and, hence, form one of the mechanisms to identify the level of As toxicity in the human body. The enzyme mainly involved in the process is methyltransferase (Engström et al. 2015). It is also

noted that the genotypic and epigenetic difference in this enzyme results in a variable chance of causing various diseases.

Once the toxic form of As is available in the body, it can cause morbidity through various mechanisms. It has the capability to alter various genomic, transcriptomic, proteomic, and metabolic steps of the cells. It has been observed that As binds to the sulfhydryl groups in the proteins. This resulted in the formation of As-S bonds causing the enzymes to be dysfunctional. Similarly, As also affect the binding with P that results in the formation of arsenoesters (Finnegan and Chen 2012). These entities result in the inactivity of various phosphorus containing moieties like adenosine diphosphate and adenosine triphosphate resulting in an alteration in the energetics of the cell. Increased cellular levels of As also cause mitochondrial damage followed by the oxidative burst resulting in the release of reactive oxygen species (ROS) (Ellinsworth 2015). The ROS secondarily causes the damage of the lipid bilayers, the endoplasmic reticulum, and the enzymes within the cell. It has been reported that As changes the activity of more than 200 different enzymes (Ratnaike 2003). As, also, passes the nuclear membrane and causes genotoxicity resulting in chromosomal abnormalities and genomic instability. Due to the dysfunctional enzymes, ligase which is involved in DNA repair also malfunctions resulting in the karyolysis mechanism. Secondarily, the cyclin-dependent kinases (CDKs) are also inhibited resulting in continuous cellular proliferation and, hence, the formation of neoplasms (Muenyi and Ljungman 2015). Moreover, the mitogen-activated protein kinases (MAPK) are also modified resulting in the alteration in the signal transduction pathways (Hou et al. 2014). All these subcellular events result in a variety of cellular presentations depending upon the organ type. These changes may be manifested as apoptosis, necrosis, transformation, or adaptation.

Arsenic toxicity is presented in various forms in the human body. The integumentary system presents hyperkeratosis and hyperpigmentation (Niedzwiecki et al. 2018). This may be represented as dark warts in the surface of the skin especially on the palmer and plantar surface of the hands and feet. Back is also affected by arsenicosis. Due to lack of the CDK-based inhibition, the cell cycle keeps on producing a large number of cells which are transformed tissue types and may be presented in the form of lung cancer, kidney cancer, hepatocellular carcinoma, and bladder cancer (Kuo et al. 2017). Both central and peripheral nerves also weakened. This weakness is presented in the form of stocking and glove neuropathy which might progress to paralysis (Abdul et al. 2015). Headache, delirium, and coma are also observed in such individuals. There is also a constant sensation of pins and needles in the periphery. Nearly all the rapidly dividing cells are affected as a result of As poisoning. The gastrointestinal tract epithelium is also necrotized resulting in malabsorption of food, generalized body weakness, and muscle fatigue. Cardiotoxicity has also been reported in a number of cases of As poisoning (Alamolhodaei et al. 2015). Cardiac failure and anemia are common presentations of chronic toxicity. In men, arsenicosis leads to decreased libido and impotency. In a number of studies, there have been reports of low birth weights and miscarriages in women who consume As-rich water or As through other sources. Chronic exposure to As also leads to vitamin A deficiency, sometimes, even resulting in night blindness (Mazumder 2015). It also

affects the respiratory epithelium resulting in lower respiratory tract disorders including alveolitis and pneumonia. Diabetes has also been related to As toxicity (Kuo et al. 2015). As can be isolated from hair samples, blood, urine, nails, and skin exudates. In case of plants, the molecular mechanisms are nearly the same. There is a decrease in shoot size and diminished fruit and seed output. In case of very high levels of As, the plant dies. In response to As toxicity, the antioxidant pathways are activated (Farooq et al. 2016). Chief among these is the glutathione model. Similarly, the DNA repair mechanisms are also activated, and proapoptotic signals are also generated to prevent the cells from being transformed.

As toxicity is, hence, one of the major health risks due to environmental and occupation exposure. It has been found that more than 140 million people are dependent upon As-contaminated water with concentration significantly greater than that prescribed by the WHO (Gadd and Ravenscroft 2009). Currently, the regions of South Asia, Latin America, and Africa have been mainly identified as the high-risk regions of As poisoning. In Bangladesh alone, in 2012, more than 39 million people were exposed to As-contaminated water (Flanagan et al. 2012). Keeping in view the diverse sources of poisoning and the seriousness of the adverse effects, it is necessary to employ ways and means to identify the risk and develop adequate interventional strategies. A thorough understanding of the geographical distribution of As in the world would facilitate the employment of adequate mitigation strategies.

18.4 Continent-Wise Distribution of Arsenic Toxicity

The As toxicity is highly prevalent throughout the world which can be described with reference to clinical, environmental, or geographical aspects. A direct relationship has been established between the geographical abundance and clinical aspects of the condition. This review, hence, describes the status of As toxicity in terms of geographical regions, i.e., on the basis of continents. Figure 18.2 presents the As toxicity in various continents.

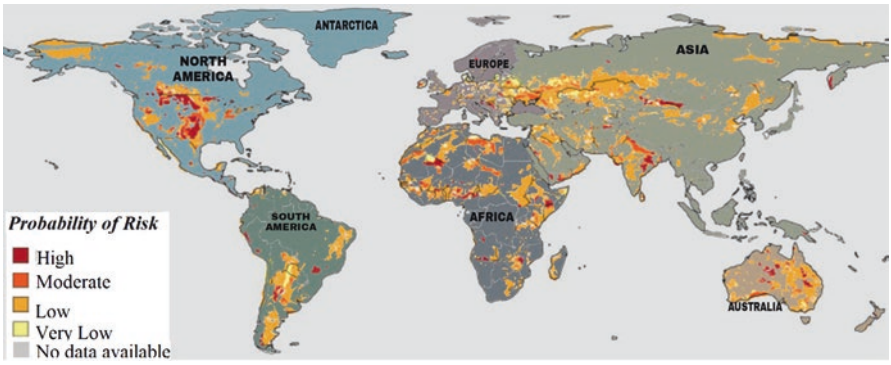

Fig. 18.2 Potential risk of arsenic toxicity around the globe. (Adapted from Schwarzenbach et al. 2010)

18.4.1 Asia

Asia is the most populous and geographically the largest continent of the world. It comprises of 48 sovereign states which are grouped into 4 regions depending upon their geographical locations. North Asia mainly comprises of Russia and the surrounding regions. East Asia consists of the countries like China, Japan, Korea, and Taiwan among others. Bangladesh, India, Pakistan, Afghanistan, Sri Lanka, Nepal, and other countries of the region form South Asia. West Asia is also sometimes referred to as the Middle East as it bridges Asia to Europe and Africa. It comprises of countries like Iran, Iraq, Saudi Arabia, UAE, and Syria.

Arsenic toxicity has been reported throughout Asia. In the north, however, its occurrence in groundwater is not considered a major source of toxicity. In surface water in Russia, a higher concentration of As has been observed (Amini et al. 2008). Similarly, in the rivers and streams in the Siberian region, As levels are elevated (Gordeev et al. 2004). Consequently, soil, vegetation, fish, and dust particles also possess a higher concentration of the metal (Allen-Gil et al. 2003). The human population is, however, protected from the metal toxicity as their dependence on this water is minimum. Among the countries of East Asia, some states of mainland China, Shanxi and Xinjiang, have been reported to have the highest concentration of As in drinking water. Moreover, significantly higher levels of As species have been found in the crop samples from various regions of the Guangdong Province. On average the As consumption through ingested rice was found to be around 2 μg/kg of body weight (Lin et al. 2015). In another study carried out in the Jianghan Plain, it was found that the amount of As in the groundwater was of the order of 0.01 target cancer risk, where the acceptable limit is 0.0001, exhibiting an elevated risk of development of pathological conditions (Li et al. 2018). Zhang and colleagues have recently reviewed the distribution of As in the southwestern region where As has contaminated soil, vegetation, and surface water (Zhang et al. 2017). In another study carried out in Shunde, near the Pearl River Delta, around 238 topsoil samples were evaluated for detecting the presence of As and other metalloids. It was found that more than 2% of the soil samples had significantly higher levels of As (Cai et al. 2015). This increased concentration was attributed to soil contamination due to industrial and agronomic practices of the region. The much similar situation has been observed in Japan, Korea, and other countries of East Asia. In Japan, it has been reported that around 12,000 infants were subjected to As toxicity with 100 fatalities due to its presence in the formula milk in 1955 (Nakagawa and Iibuchi 1970). Moreover, as high as 300 μg/L of As in water from wells has been reported (Kondo et al. 1999). However, due to the industrial development and adherence to international regulations, there has been a major decrease in the introduction of As through the anthropogenic activities, and, now, the major source of contamination is marine water. In Korea, the major source of As is the abandoned mining sites and seawater. The results of a study are carried out on the gold mines; it was found that nearly 63–99% of the leachate of the ores contained high concentrations of As (Kim et al. 2002). The water around these sites is used for agricultural purposes and

causes significant human morbidity. It has been found that the average concentration of As in urine was 7.10 μg/L (Bae et al. 2013). Another study evaluated the concentration of As and other metals in various species of seaweeds generally utilized as a food source. Though higher concentrations of As were found yet, they lied within the limits specified by the Joint FAO-WHO Expert Committee on Food Additives (JECFA) (Khan et al. 2015). It should, however, be specified that these As concentrations in Korea are not the representative of the whole geographical region and higher concentrations are generally reported in the areas near ores and in the populations mainly depending upon marine food.

The South Asian region is the most populous among the geographical regions of Asia. In nearly all the countries of South Asia, As toxicity has been reported. Bangladesh, for instance, has the highest reported cases of the menace. A number of research studies have reported the As concentration of more than 800 μg/L in many regions of Bangladesh and the neighboring West Bengal regions of India. Similarly, it has been reported that water from more than 46% of the tube wells has a significantly higher concentration of As (Kinniburgh and Smedley 2001). In another study, it has been observed that only 1% of the population in the affected area has access to As-clean water (Gadgil et al. 2012). In India, higher incidence of As has been reported mainly in the Ganges Delta. More than 57% of the wells in the West Bengal region contains As levels of greater than 50 μg/L. More than 66% of these wells have a concentration exceeding 300 μg/L (Rahman et al. 2001). The incidence of As contamination in water has resulted in higher incidence of mortality and morbidity (Abdul et al. 2015). Similarly, in Pakistan, many water sources contain levels greater than the Maximum Contaminant Limit (MCL) specified by the WHO. The average concentration of As in groundwater in Southern Punjab in Pakistan has been observed to be 37.9 μg/L (Shakoor et al. 2015). In Sindh, it was found that the As levels were 2.6–230 times greater than the maximum specified limit (Brahman et al. 2016). The concentration of As is not only higher in the water samples but also in the dust (Alamdar et al. 2016). Moreover, these sources serve as a means for regulating the concentration of As throughout the environment from where the metalloid is taken up by the plants and then ingested by humans and animals as well.

In the West Asia or the Middle East, As has not been reported in the groundwater. The main reason may be the scarcity of water and lack of studies carried out in the region. However, As has been identified in other food sources and environmental samples. In a study carried out in Arabian Gulf, on the marine biota, it was found that clams, pearl oyster, shrimp, cuttlefish, and other finfish species all contained a higher concentration of organic and inorganic forms of As (Krishnakumar et al. 2016). In the findings of another project that aimed at reviewing the studies carried out to determine the concentration of As in breast milk, it was reported that the average concentration of the metalloid was more than 149 μg/L (Rebelo and Caldas 2016). As levels were found to be significantly higher in soil samples from Baghdad, Iraq. The concentration was found to be 36 mg/kg (Hamad et al. 2014). The concentration of As was found to be elevated in the stems, leaves, and pods of *Vicia faba* growing the Kurdistan region of Iraq (Sadee et al. 2016). In a similar study, carried out to determine the effect of As-rich smoke emitted from oil combustion

on workers' health, it was found that the levels of glutathione were significantly higher. The results indicated that the anti-oxidative compensatory metabolic processes of the body were activated due to an increased exposure to As (Mahmood et al. 2014). Based on these reports, it is imperative that nearly all the regions of Asia are exposed to high levels of As toxicity through various geological, agricultural, and anthropogenic sources.

18.4.2 Europe

The As concentration in Europe has been reported to be higher in some countries of Europe. However, the effect of the toxicity is negligible mainly because of the better socioeconomic conditions and access to clean drinking water. Moreover, the screening mechanisms for testing As toxicity are in place that ensure that the European population is minimally affected by the exposure. Most of the cases have been reported in Hungary and Romania. Cases of As toxicity were reported in soil samples in Hungary in 1991 which were associated with an increased risk of developing cancer (Varsányi et al. 1991). In another study carried out in Pannonian Basin, Hungary, the significantly higher concentration of As was found in the soil and groundwater (Varsányi and Kovács 2006). A positive correlation was established between the As concentration in groundwater and the incidence of Basal Cell Carcinoma (BCC) in a study carried out in Hungary, Romania and Slovakia (Leonardi et al. 2012). The soil levels of As and other metals were found to be elevated at a longtime mining site in Zlatna region of Romania (Weindorf et al. 2013). Moreover, in the water samples obtained from drinking water wells in Timis-Bega area of West Romania, it was found that even after classical filtration process, the amount of As was 0.10–168 μg/L which exceeded the guideline limit (Senila et al. 2017).

A study carried out to determine the As concentration in drinking water and urine samples collected from Cornwall region of England found that nearly 5% of the water samples contained more than the permissible amount of As. Similarly, at least 10% of the urine samples had higher levels of As (Middleton et al. 2016). A study carried out around a historical mining site in the UK reported that even after the mining operations have stopped in the area, As continues to be present and is continuously being released in the environment (Rieuwerts et al. 2014). Higher levels of As have also been found in the seaweeds obtained from Ireland (Ronan et al. 2017). In a similar study carried out in Saxony, Germany, it was found that As was accumulated in the *Lemna gibba* species grown in the tailing waters of mining sites (Mkandawire and Dudel 2005). As, as discussed, is not contained within the water sources. The elevation of water table along with the anthropogenic activities causes the mobility and transformation of various As species to the soil service. Though there is variation in the amount of As isolated from various regions over different periods of time, it has been observed that as high as ten times elevated quantities were found in porewaters through a study carried out in Germany (Huang and Matzner 2006). The forest ecosystem is also involved in the accumulation and

cycling of As. In a study carried out in Bavaria region of Germany, it was found that the annual runoff of various organic species of As was around 0.08 g/ha/yr (Huang and Matzner 2007). Though the levels are not in propensity with the other values, it indicates that the forests are also involved in regulating the As levels in the European region. Based on the distribution of the As, in a study carried out on children from Amrum, Germany, it was found that the weekly intake of As was around 2.31 μg/kg of body weight which lied within limits established by the international health agencies identifying the effectiveness of the As mitigation strategies (Wilhelm et al. 2003).

In Greece, similarly, in a study performed to identify the bioaccumulation of As in various plant species from Attica, a positive correlation was found in the As levels in water and the amount present in plants (Kampouroglou and Economou-Eliopoulos 2017). The levels of Cu, Zn, Co, Ca, Mg, Fe, and P were also found to be significantly higher. Antoniadis and colleagues performed a study to determine the level of As and other metals in the soil samples isolated from roadsides and plant from 31 industrial sites in Greece (Antoniadis et al. 2017). It was found that the levels of As, Cu, Cd, Cr, Ni, Zn, and Pb were above the maximum permissible amounts. Elevated As amounts are not only limited to the soil and water samples. In the Oropos-Kalamos region of Greece, the rock and sediments also had a high elemental contamination. A study to quantify the level of potentially toxic elements (PTE) was performed in the region in which it was observed that the concentration of As, Sb, Pb, and Hg was above the internationally accepted limits (Alexakis and Gamvroula 2014). Similarly, higher concentrations of As have been reported from other countries including Italy, Bolivia, Spain, and Poland (Sánchez-Rodas et al. 2007; Acosta et al. 2015; Barbieri et al. 2016; Michalski et al. 2016).

18.4.3 Africa

The cases of As toxicity reported from the African continent are relatively low. However, it should be mentioned that it is not the lack of As toxicity but also the unavailability of data that can be related to the fewer reported cases. In the streams of Asante Akim in the northern region of Ghana, the concentration of As has been found to be around 0.15 mg/L that exceeds the limits set by the WHO and the Ghana Environmental Protection Agency (GEPA) (Gyasi et al. 2014). Similarly, the significantly higher concentration of As has been found in the shellfish as well as the finfish samples from Ankobra, Densu, and Volta basins of Ghana (Gbogbo et al. 2017). It was found that the As concentration ranged between 0.2 and 2.8 mg/kg of the fish in 15 out of 17 species of fish. In correspondence with the higher level of As in water and soil samples, it was found the As concentration was significantly higher in the nail and skin samples of inhabitants of Rift Valley in Ethiopia (Merola et al. 2014). Water samples from the same region were evaluated for their compliance with the quality of the European and international standards. It was, however, observed that of the 138 collected samples, not even a single sample met the established criteria (Reimann et al. 2003). Higher levels of pesticide poisoning have also been reported

in the Ethiopian region which might be one of the reasons for increased As toxicity (Teklit 2016). The meat of snoek and yellowtail from South Africa was also found to be contaminated with As (Bosch et al. 2017). Similarly, among other animals, a higher concentration of As was found in the liver of *Lichia amia*, *Argyrosomus japonicus*, and *Pomadasys commersonnii* isolated from South Africa (Nel et al. 2015). Eggs of *Larus dominicanus*, the sea gull, were also found to possess a significantly higher concentration of As.

In North Africa, a number of cases of As toxicity have been reported in Egypt. Ghani et al. reported that the concentration of As was significantly higher in the samples collected from the Egyptian Mediterranean coast. The average concentration of As was 29.9 mg/kg of the sample (Abdel Ghani et al. 2013). The chief reason for the contamination was pointed out to be the offshore oil fields and industrial wastes. In another study, high concentration of As was also found in the water samples from Nile (Shaheen et al. 2017). A significant correlation was found between the water and soil samples and the bioavailability of As in human body. In the Egyptian population, 0.04–1.04 mg As was found in every kg of human hair (Saad and Hassanien 2001). It was also found that 60% of the smokers and 66.6% of passive smokers had more than 0.25 mg As/kg of hair. In studies carried out by Lawgali in Maknwessa, Aril, and Taswaa regions of Libya, the As concentration was two- to threefold lower than the global average. However, the irrigation water did contain a high level of As (Lawgali and Meharg 2011). In another study, the As levels in the groundwater in Tripoli, Libya, were found to be as high as 31 ng/ml (Etorki et al. 2013).

Among the East African countries, a study carried out in Kenya showed the presence of 8.93 mg As per kg of soil (Mungai et al. 2016). Similarly, in a study carried out in Migori, Southwest Kenya, very high level of As, ranging between 29.3 and 8246.5 mg/kg of soil samples, was reported (Odumo et al. 2011). In correspondence with the higher level of As in water and soil samples in the region, it was found the As concentration was significantly higher in the nail and skin samples of inhabitants of Rift Valley in Ethiopia (Merola et al. 2014). Water samples from the same region were evaluated for their compliance with the quality of the European and international standards. It was, however, observed that of the 138 collected samples, not even a single sample met the established criteria (Reimann et al. 2003). Higher levels of pesticide poisoning have also been reported in the Ethiopian region which might be one of the reasons for increased As toxicity (Teklit 2016).

In the western part of Africa, the highest incidence of As toxicity has been reported in Nigeria. Recently, Izah and colleagues reviewed the studies carried out to identify the level of As in soil and water in Nigeria (Izah and Srivastav 2015). The authors reported that the As levels in the northern and southwestern regions were usually above the prescribed limits of WHO. As was also found in the rice samples obtained from Akure, Ore, Ondo, and Ikare (Adeyemi et al. 2017). However, the As levels were below the maximum levels set by the WHO. Industrial and occupational exposure of As in Nigeria has also been reported. In an oil-producing region in Niger Delta, it was found that the As concentration was significantly higher in water samples (Ejike et al. 2017). Similarly, workers in crude electronic waste manage-

ment were found to possess higher levels of As in blood (Igharo et al. 2014). The levels were nearly tenfold higher than the unexposed individuals. The As concentration was found to be as high as 1.35 mg/L of drinking water in the areas surrounding the oil depot regions in Aba, Nigeria (Akakuru et al. 2017). Arsenic levels were also found to be significantly higher in Algeria, in the northwestern part of Africa. The principal component analysis of water and sediment samples from Seybouse Wadi showed a very high concentration of As (Khaled-Khodja et al. 2018). Al, Se, Zn, Cu, Cr, Pb, and Cd levels were also reported to be raised in the area. Similar results were also obtained from cultivated and uncultivated soil samples from Constantine region of Algeria (Naili et al. 2016).

18.4.4 The Americas

The As toxicity in the Americas is mainly attributed to the industrial development in the region. As per the data provided by the Centers for Disease Control and Prevention (CDC), anthropogenic activities result in nearly three times greater release of As in comparison to the natural sources. The major sources of As are the landfills and the ores. Among the natural sources, the groundwater is the major source of contamination especially in the southwest, northeast, and northwest Alaskan regions (Atsdr 2007). As per the US Environmental Protection Agency (EPA), the MCL for As in groundwater is 10 ppb. However, nearly 2% of the water supplies have 20 ppb which significantly exceed the prescribed limits. Though As is still found in elevated amounts in the identified regions of the USA, the efficient and effective control mechanisms have resulted in a significant decrease in the incidents of As toxicity. Many of the reports available are, hence, of a few decades back. In California (CA), for instance, the As levels have been found to decrease over the past several decades. The concentration of As carried out on the soil samples isolated from Wasco, Fallbrook, and Wyo showed a low level of As contamination (Manning and Goldberg 1997). In another study carried out on water samples from Owens Lake, CA, the dissolved As amount was found to be up to 96 mg/L which was higher in the central region as compared to the shoreline (Ryu et al. 2002). In a review of various research studies focused on the western part of the USA, it was found that the As concentration in groundwater was not above the MCL (Welch et al. 1988). Conversely, it has also been reported that the As concentration above than 10 μg/L was found in New England, Minnesota, Wisconsin, South Dakota, Oklahoma, and Michigan (Welch et al. 2000b). In a study carried out in Hayden and Winkelman, Arizona, it was reported that the residents living near the copper mining and smelting towns had increased exposure to As which was proved by the release of As in the urine samples (Hysong et al. 2003). In Maine and New Hampshire, it was observed that nearly 30% of the drinking water wells had elevated levels of As (Ayotte et al. 2003). In these areas, up to 40 mg As was found per kg of the rock samples. The concentration was especially higher in case of water with a higher pH. In a recent study carried out in New Hampshire, it was found that

there was a significant risk of DNA methylation following As toxicity (Green et al. 2016). This may, ultimately, cause congenital or chronic defects. In Alaska, it was found that the aquatic organisms were significantly affected by the high levels of As in the Red Devil Creek region (Matz et al. 2017). The tissue concentration of As in the area showed that the consumers of the fish captured from the area are at a higher risk of developing toxicity. A recent study carried out on drinking water samples from across the USA established that the As III was mostly present in the midwest region, while both As III and As V are present in the east, west, and far west regions of the country (Sorg et al. 2014). In Canada, similarly, As concentration of as high as 970 μg/L has been found in the surface water near the mining sites in Ontario (Sprague and Vermaire 2018). Similarly, around 430 μg/L of As was found in the lake water in comparison to 2.2 μg/L in control water. High concentration of As was found in the bedrock samples in Quebec implicating that the private well water users are affected by As toxicity (Bondu et al. 2017). In a study carried out on 2000 pregnant Canadian women, it was found that around 90% had As levels more than the limits of detection (LOD) (Ettinger et al. 2017). In a risk assessment study carried out in Nova Scotia, it was observed that due to As exposure, the bladder cancer risk was up to 18% higher than the control group (Saint-Jacques et al. 2018). These properties, therefore, imply that due to the presence of elevated levels of As, there is a significant health risk in individuals living in and around As-rich areas.

In South America, nearly all the countries have reported cases of As toxicity. The Minas Gerais region of Brazil, for instance, is a rich source of gold, lead, and zinc. Moreover, due to irrigated systems and sulfide-rich water, there is a greater prevalence of As toxicity. In a study carried out in 2010–2011, it was found that all the samples of sediment and more than one-third of water samples had higher than permissible As levels (Rezende et al. 2015). Gao and colleagues reported the detection of a high concentration of As in fish from the North Sea and Port Acu, the coastal regions of Brazil (Gao et al. 2018). As was found in the liver as well as muscle samples of dogfish, lemon sole, whelks, and scallops. It was also established in another study carried out in the Southeast Brazil that rice and beans, being the staple food of the country, contribute to nearly 66–90% of the dietary As intake (Ciminelli et al. 2017). The situation is not quite different in other countries of the continent. Groundwater samples from El Divisorio brook, Argentina, revealed the presence of toxic levels of As indicating that the population around the area is at a risk of arsenicism (Díaz et al. 2016). In another study, it was found that the women in the Puna region of the Argentinian Andes had higher blood values of As and a number of protective gene expression mechanisms within the body were altered (Broberg et al. 2016). In the western Amazonian region of Peru, hydrogeochemistry of groundwater sources revealed the presence of As in concentrations that were harmful for human consumption (de Meyer et al. 2017). A positive correlation between the groundwater As levels and occurrence of cardiovascular and respiratory diseases has also been reported in Chile (Steinmaus et al. 2016; Hall et al. 2017). Similarly, toxic concentrations of As have been found in the maternal and

cord blood of expecting mothers in the mining cities of Bolivia (Barbieri et al. 2016). In general, it is estimated that the nearly 4 million people are affected by the increased As concentration in Chile, Argentina, Peru, and Mexico (Bundschuh et al. 2009). Studies suggest that the As toxicity in the Latin American region is much higher in reality than the reported cases.

18.4.5 Oceania and Other Regions

As toxicity has also been reported in a number of countries in the Oceanic region. In a study carried out in the State of Western Australia, it was found that the concentration of As exceeded the Ecological Investigation Levels (EIL) set by the Department of Environment and Conservation (DEC) especially around the mining areas (Abraham et al. 2018). In addition to the naturally occurring As in water, it was found that certain species of seaweeds on the shores of Lake Macquarie, Australia, were also involved in the biomagnification process, thereby, resulting in higher concentration of As (Barwick and Maher 2003). Phytotoxicity in various regions of Australia has also been reported (Smith et al. 2008; Kader et al. 2016). Bioaccumulation and biomagnification in many plant species have also been reported in the Lake Murray, Papua New Guinea (PNG) (Bowles et al. 2001). Higher concentrations have also been reported in the mining regions and shallow water regions of PNG (McKinnon 2002; Price and Pichler 2005). In a recent study carried out at a mining site in New Zealand, it was reported that the As levels were directly related to the distance from the mining center (Malloch et al. 2017). As was also isolated in various forms from the acid-sulfide-rich hot spring Champagne Pool located in Waiotapu, New Zealand (Hug et al. 2014).

The anthropogenic cause of As is not limited to the heavily inhabited regions. Recently, As was found in Antarctica as well. Studies suggested that the As originated from the copper mines in Chile (Schwanck et al. 2016). As was also isolated from the King George Island, Antarctica. Among the concentrations of various metals screened for the study, it was found that As had the most relevant enrichment rate (Ribeiro et al. 2011). Bioaccumulation of As has also been established in the same region (Trevizani et al. 2016; Espejo et al. 2017). Using a variety of analytical techniques on a snow-pit in Dome Argus, Antarctica, Rong and colleagues found that there has been a gradual rise in the concentration of As from the mid-1980s till the end of the century (Rong et al. 2016). Similarly, high levels of inorganic As were found in East Antarctica attributed primarily to the ornithogenic activities (Lou et al. 2016).

Higher concentrations are, hence, observed in nearly all the continental regions. Based on the diverse geographical distribution of As toxicity, it is imperative that effective yet efficient mitigation strategies may be formulated to address the problem.

18.5 Mitigation Strategies Against Arsenic Toxicity

The vast geographical distribution of As toxicity necessitates the development of effective and efficient measures to address it. Screening of water for the presence of high concentration of As is necessary (Keil et al. 2015). For the purpose, water sources should be tested on an annual basis. Similarly, the soil and air quality also need to be monitored. The amount of As in crops and other food sources of the affected areas should be detected. Once the risk has been identified, the affected population should be prevented from any further exposure. Adequate means to supply safe drinking water, clean food sources, and water for irrigation purposes should be established. WHO recommends the classification of water sources on the basis of their As content and then employs the low As water for cooking, drinking, and irrigation purposes. Conversely, the high As water should be used for cleaning purposes. In case of scarcity of water, blending of low and high As content water can be done in order to achieve a minimum acceptable average concentration of As that is fit for human consumption. Moreover, occupational exposure to As should be minimized (Clewell et al. 2015). The individuals living in the area should be educated and trained about the adverse effects of As toxicity. They should, hence, be taught that the consumption of As through water, food, or direct contact is harmful for them as well as their next generations. People living in the affected areas should be monitored on a regular basis to detect any presentations of As poisoning.

The oxidative stress in As affected plants causes the activation of antioxidant system in the plant body. An increase in the As levels has been associated with a rise in the levels of thiols in the plant body (Mohan et al. 2016). Chief among these are the phytochelatins (PCs) (Anjum et al. 2015). In addition, arsenate reductase and the enzymes of thiolic metabolism are also affected. These mechanisms aid the detoxification of plants by the complexation process. A simultaneous rise in the level of antioxidant enzymes is also observed. Higher levels of superoxide dismutase, catalase, and ascorbate peroxidase have been reported in various plant species affected by As toxicity (Gill et al. 2015; Tripathi et al. 2014). In certain plant species, a rise in the guaiacol peroxidase and glutathione reductase is also reported (Singh et al. 2017). Hyperaccumulation of the metalloids has also been reported in few plant species. These species depend mainly on absorption and storage of As in various forms within the plant body (Souri et al. 2017). This mechanism, therefore, serves as an ideal mechanism to decrease the level of As in the environment. However, ingestion of the same crops can result in the introduction of the metalloid in the human food chain. A number of biotechnological approaches have been developed to address the effects of As toxicity. Molecular techniques to enhance the production of secondary metabolites like PCs and antioxidants have proved to be effective in a number of plant species including chickpea, rice, and leguminous plants. The overexpression of hyperaccumulation process can also serve as an ideal means to contribute to the phytoremediation process and, hence, decrease As toxicity.

Apart from the biological approaches to control the menace, a number of physical and chemical methods can be applied as well. Generally, a combination of both physical and chemical methods helps in the efficient removal of As from the water. In the process, the water is first pre-treated to remove any traces of As (V) by using bleaching powder, chlorine, or hydrogen peroxide. Afterward, one or a series of steps are employed to purify the water from As. These steps include oxidation, filtration, coprecipitation, adsorption, and ion exchange. In oxidation, generally through a photocatalytic process, the water is treated. The treatment results in the removal of the oxidized form of the metals that is now present in the form of complexes. Once the metal complexes are formed, the water is passed through membranes. These membrane filters act as sieves for the removal of metalloids (Bolisetty et al. 2017). Coprecipitation works on the principal of common ion effect in which the As is deposited at the bottom of the vessel due to the complex formation (Ociński et al. 2016). Pure water from the top is collected and utilized. In case of adsorption and ion exchange, physical methods are employed to release salt form of the metalloids from the As-rich water (Ali 2014). These techniques have proved to be effective in decreasing the concentration of As in toxic water.

Various techniques are, hence, employed in order to purify the water from harmful As species. The selection of method varies with the geographical location and socioeconomic conditions. However, any effective mitigation strategy employs a series of preventive and interventional techniques to decrease the level of As in the sample. It is then that the procedures can be accepted for broader application.

18.6 Conclusion

The chapter has reviewed the geographical pattern of As toxicity throughout the world. Based on the discussion, it can be concluded that mainly due to human intervention, elevated levels of As are found in all the continents. Though no cases have been reported from a few countries, due to the development of the world as a global village, it appears to be just a matter of time that those areas would be affected as well. It can, hence, be concluded that As remains to be present in different forms in different geographical as well as climatic conditions. Unfortunately, the most adversely affected areas belong to the developing world. With low GDPs and population explosion, As toxicity presents another menace. Therefore, concerted efforts need to be made by the international community to address the issue. Availability of safe, As-free drinking water should be established as one of the short-term goals with the amelioration of the toxicity as a long-term aim. Moreover, scientists, engineers, researchers, and innovators should work on methods to develop efficiently and cost-effective As mitigation strategies that can be utilized across different regions and different social strata.

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Chapter 19
Arsenic Toxicity: A South Asian Perspective

Sahar Iftikhar, Zeeshan Ali, Duaa Ahmad Khan, Najam-us-Sahar Sadaf Zaidi, Alvina Gul, and Mustafeez Mujtaba Babar

Abstract Arsenic (As) toxicity has become one of the most significant abiotic threats to agriculture and human health. Owing to various natural and anthropogenic activities, the circulation of As among various reservoirs has increased over the past several decades. Though present throughout the world, South Asian region is particularly affected by it. A major portion of the people living in the region is dependent upon agriculture for their livelihood, and they utilize untreated water for dietary consumption. Moreover, the ability of As to accumulate in the plant body increases the chances of the urban and rural populations to be exposed to the metalloid. The metalloid uses a number of molecular mechanisms which causes adverse reactions in plants and animals. In order to control the detrimental effects of As, effective interventional strategies need to devised and implemented. Various physical, chemical, and biological processes can be employed for the purpose. This chapter reviews the geographical patterns of As toxicity in South Asia. The adverse aspects of As toxicity have then been provided followed by the proposed interventional strategies that can be employed for decreasing the As-associated toxicity in South Asia.

Keywords Arsenic toxicity · South Asia · Geographical distribution · Arsenicosis · Interventional strategies

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Abbreviations

ACR	Arsenate reductase
AMF	Arbuscular mycorrhizal fungi
As	Arsenic
ATF	Activating transcription factor 6
CHO	Chinese hamster ovary
CKD	Chronic kidney disease
CO_2	Carbon dioxide
DMA	Dimethylarsinic acid
DNA	Deoxyribonucleic acid
EPA	US Environmental Protection Agency
FAO	Food and Agriculture Organization
FTCD	Forminidoyl transferase cyclodeaminase
GLUT	Glucose transporters
GSH	Glutathione
GWAS	Genome-wide association study
IARC	International Agency for Research on Cancer
IRE	Inositol-requiring enzyme
MAO	Trimethylarsine oxide
MCL	Maximum contaminant limit
mg	Milligram
MMA	Monomethylarsonic acid
NAC	N-Acetylcysteine
NAPDH	Nicotinamide adenine dinucleotide phosphate
NIPs	Nodulin26-like intrinsic proteins
P	Phosphorus
PERK	PKR-like endoplasmic reticulum kinase
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
SNP	Single nucleotide polymorphism
tHcys	Total homocysteine
UNICEF	United Nations Children's Fund
UPR	Unfolded protein response
WAT	White adipose tissue
WHO	World Health Organization
Zn	Zinc
μg	Microgram

19.1 Introduction

Human activities have changed the global cycle of heavy metals and contributed a lot in heavy metal and metalloid toxicity. The human body is prone to serious harmful effects due to toxic substances like heavy metals and metalloids that are present or released into the environment. Arsenic (As) is found in nature and can enter the human body through a number of pathways including food, water, air, and soil. It also forms an important component of the occupational hazardous agents. The earth crust is the abundant source of As. The main form in which it is present in the earth is arsenopyrite. Human activities contribute nearly 52,000–112,000 tons of As to the soil (Li et al. 2008). The contributing factors include the use of As-rich pesticides and fertilizers in the agricultural sector. As enters the human body directly (through drinking As-contaminated water) or indirectly by consumption of As-contaminated food sources, for instance, the rice grain grown in As-contaminated groundwater and soil. The metalloid alters the physicochemical properties of soil and enters the farming systems through various means like geochemical processes. It, thereafter, elicits a series of reactions leading to inhibition of plant growth. Consequently, the photosynthetic and metabolic processes are disrupted resulting in a decrease in the agricultural output. These plant responses are mainly mediated by the oxidative stress within the plant body. The reactive oxygen species (ROS) produced during the As toxicity cause damage to biological molecules including proteins and lipids. Interaction of arsenic III sulfhydryl groups with the plant enzymes ultimately results in the death of the plants. The adverse effects of As toxicity are not limited to plant body only. In humans, exposure to As causes DNA hypomethylation resulting in carcinogenesis. It also causes activation of proto-oncogene c-myc that can induce chromosome abnormalities by acting synergistically with other toxic substances. Inorganic As also causes skin and lung cancer in a human. A number of other pathological and pathophysiological conditions are also reported.

South Asia is the most populous region in the world. The total population of India, Bangladesh, and Pakistan accounts for nearly a quarter of the world's population. This region is geographically the most diverse region as well. A number of recent cases of As toxicity have been reported in South Asia. The high density of population, poverty, unavailability of As-treated water, adverse socioeconomic conditions, monetary dependence on agriculture, and a number of other factors make the people of this region at the highest risk of As-induced adverse effects (Brammer and Ravenscroft 2009).

The chapter introduces the concept of As toxicity followed by its geographical distribution in South Asia. The adverse effects of As toxicity in plants and humans are then presented. Various molecular mechanisms that are disturbed and the manipulation of which can help in addressing As toxicity are then presented. Toward the end of the chapter, various biological, physical, and chemical methods for addressing the As toxicity that can be beneficial for reducing As toxicity in the affected areas have been presented.

19.2 Arsenic as an Environmental Contaminant

Arsenic is abundant in earth's crust and is present in about 200 different minerals most important being arsenopyrite. Apart from the natural sources, human activities like use of fertilizers and pesticides also contribute to high As levels in soil and underground water. Drinking water contains an inorganic form of As. The uptake of this As-rich water by the plants results in its constant presence in the plant body where it is present in organic and inorganic forms. Studies have revealed that Asian region suffered the most with As toxicity compared to the rest of the world (Nordstrom 2002; Brammer and Ravenscroft 2009). In Asia, the highest number of cases of As toxicity has been reported in Bangladesh. Other countries including India, Pakistan, Iran, Afghanistan, Cambodia, Sri Lanka, and Nepal have also reported several incidents of As toxicity (Jain and Singh 2012).

The most important problem occurs due to leaching of As from environmental sources into drinking water sources or the seepage of the same into the underground water table. The World Health Organization (WHO) sets the maximum limit of 10 μg/l as permissible in the potable water. The dependence of the human and animal population on this water and the unavailability of water treatment options in most of the impoverished regions of the world lead to the entry of this As into the food chain. Moreover, the uptake of As by the crops also causes an additional source of entry of the metalloid into the body. Rice is a staple food in most parts of the world. When grown in As-contaminated water, it serves as an important source of As in human body contributing, thereafter, to the human disease (Sinha and Bhattacharyya 2015; Shraim 2017). Rice intake in Bangladesh, sub-Saharan Africa, and Latin America has been found to be significantly higher than the rest of the regions of the world. As per the Food and Agriculture Organization (FAO) statistics, the annual rice consumption in Bangladesh is 173 kg/capita/year (Muthayya et al. 2014). Therefore, an increased dietary intake of rice leads to elevated amounts of As in blood. In addition, cereals like wheat, corn, vegetables, and meat products are also source of As exposure to humans (Singh et al. 2015).

As is available in four oxidation states: −3, 0, +3, and +5. The variation in these oxidation states is responsible for their widespread occurrence throughout the world but also their ability to cause intracellular accumulation and pathogenesis. Many of the water bodies contain As in amounts that are greater than those prescribed by the WHO. Arsenite (+3) and arsenate (+5) are considered to be the most toxic forms of the metalloid. Naturally, As concentration varies from 10 mg/kg to 30,000 mg/kg of soil (Smith et al. 2003). The use of black shales and the release of sediments in to the running water result in the introduction of As from areas containing high levels of the metalloid to the areas where relatively lesser amounts are present. Similarly, geothermal processes including volcanoes and geysers also introduce the underground As into the soil. A number of anthropogenic activities including mining and digging cause an elevation of As levels in the environment. Constant flooding of irrigated soil is key factor for As uptake in plants. They increase the mobilization of As especially As III in soil resulting in accumulation in rice grain, while on the other

hand anaerobic cultivation of rice reduces As transport from soil (Azam et al. 2016). Similarly, food additives, pesticides, and insecticides also cause a significant rise in the level of As. Due to the variety of agricultural and other human activities being carried out by the rich population base in South Asia, it is imperative that there is going to be a significant rise in the As levels in soil and groundwater.

19.3 The Geographical Pattern of Arsenic Toxicity in South Asia

Arsenic toxicity is one of the major causes of botanical and zoological pathology. High levels of As are found in soil water throughout the world. With the major dependence of many countries of South Asia on agriculture, As toxicity of plants translates to major pathological, financial, and food security issues. Tens of As toxicity incidents have been reported throughout the world including in India, Bangladesh, Pakistan, Myanmar, Nepal, Cambodia, and China (Rahman et al. 2001). Chronic elevation of As levels has been reported in many countries of Asia that has resulted in dermatological, respiratory, urinary, hepatic, and hematopoietic presentations in humans. The plants and animals are also adversely affected as well.

The major source of As toxicity is the groundwater that seeps into the drinking water and food sources. As per World Health Organization's (WHO) recommendation, the maximum contaminant limit (MCL) of As in potable water should be 10 μg/l. Research studies have shown that As levels as high as 800 μg/l have been found in various areas of Bangladesh and West Bengal, India. In another study, it was found that more than 46% of shallow tube wells had As levels significantly higher than the WHO recommendations. Similarly, the water from around 5% deep tube wells exceeded the recommended amounts of As (Kinniburgh and Smedley 2001). Though there is a significant difference between the As levels in different wells, it has been estimated that less than 1% of the population has access to As-cleaned water. Moreover, As toxicity has been associated with more than 20% of all adult deaths in Bangladesh (Gadgil et al. 2012).

The situation is not very different in India. Though mainly associated with the areas located in the eastern part of India, the presence of As in groundwater has been referred to as "the biggest As calamity in the world." The Ganges Delta has mainly been associated with high levels of As. An analysis of the As content of tube wells of West Bengal have shown that nearly 57% of the wells contained more than 50 μg/l. Out of these more than a third had As concentrations exceeding 300 μg/l (Rahman et al. 2001). The increased concentrations were clinically correlated with increased neurological and dermatological symptoms. An increase in the incidence of miscarriages and premature deliveries has also been reported in the areas affected with As toxicity (Abdul et al. 2015).

The incidence of elevated amounts of As in groundwater is widely spread among all the countries of the Indian subcontinent. In addition to Bangladesh and India,

many water sources in Pakistan also possess As above the MCL. In a study carried out in the central region of Pakistan, southern Punjab, it was found that the mean As concentration in the groundwater was 37.9 μg/l (n = 62) and a total of 53% water sources exceeded the limits provided by the WHO (Shakoor et al. 2015). Similarly, in another study carried out in Southern Pakistan, Sindh, it was found that the concentration of As in the sampled area was around 2.6- to 230-fold higher than the acceptable limit. The study also detected the As concentration in the scalp hair of boys between the age of 5 and 14 and found a positive correlation between the As concentration in their drinking water source and their hair (Brahman et al. 2016). Though groundwater is the main source of As toxicity in humans, As-rich dust can also cause significant health risks. In a study carried out in various regions of Pakistan, it was observed that among the areas with higher levels of As in dust, proportionately elevated concentration was found in human nails indicating dust as a vital source of As contamination (Alamdar et al. 2016).

In the northern part of South Asia, recent studies have indicated an increase in the incidence of As toxicity. The first case was reported in 2004 after a countrywide investigation of groundwater in a study supported by United Nations Children's Fund (UNICEF) (Ahuja 2008). It was, however, found that most of the areas of Afghanistan did not contain high amounts of As. However, there were nearly half a million people at risk of As toxicity due to rising levels in groundwater (Mukherjee et al. 2006). In Nepal, the major source of drinking water is groundwater. In a number of studies, it was found that nearly 23% of the water samples from tube wells exceeded the WHO guidelines resulting in significant health risks among the individuals living in affected areas (Shrestha et al. 2003). More than a quarter of the population was found to be exposed to elevated levels of As in the northern region of Nepal (Maharjan et al. 2006). In the same region, arsenicosis was reported to have negative effects on the physical and mental health of the people (Maharjan et al. 2007). Elevated levels of As in groundwater have been related to human disease in many regions of Sri Lanka. It has been established as one of the possible causes of chronic kidney disease and other forms of nephropathy among the individuals living in the affected areas (Redmon et al. 2014; Jayasumana et al. 2015). Figure 19.1 represents the geographical pattern of As toxicity in South Asia.

Nearly all the countries of South Asia are affected by As toxicity which results in significant agricultural, human, and veterinary losses annually. With a major portion of the South Asian population living in poverty-stricken conditions, an understanding of the mechanism of toxicity can help in providing means to eradicate the adverse aspects of As toxicity.

19.4 Mechanism of Arsenic Toxicity

Arsenic toxicity has serious effects on human health, directly through contaminated water and indirectly through the ingestion of food. The dependence on plants as a food source results in significant medical presentations. Owing to a number of

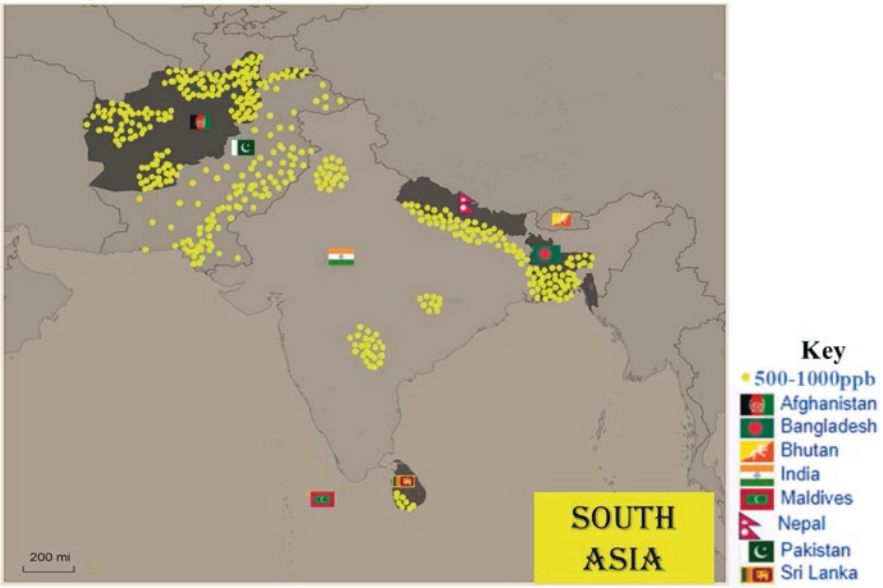

Fig. 19.1 Geographical pattern of arsenic toxicity in South Asia. Most of the regions of Bangladesh, Eastern India, Central Pakistan, and Central Afghanistan have been found to contain elevated levels of arsenic in groundwater (Figure adapted from data by (Kinniburgh and Smedley 2001; Redmon et al. 2014; Mukherjee et al. 2006; Shakoor et al. 2015; Brahman et al. 2016))

factors including organic carbon content, pH, ionic strength of soil, cation exchange capacity, and presence of iron oxides, the elevation in the amount of As is often reported in many regions of South Asia.

19.4.1 Arsenic Toxicity in Plants

In plants, As mainly accumulates in roots. However, smaller amounts also are found in the shoots. After entering the plant body, it affects the physiological processes, inhibits the growth, interferes with metabolic processes, and, ultimately, decreases the crop productivity. Arsenic disrupts the energy flows in a cell by competing with phosphorus in ATP forming an unstable product adenosine diphosphate As-V. Excess As results in oxidative stress as it generates reactive oxygen species (ROS) and free radicals (Finnegan and Chen 2012). This accumulation results in molecular perturbations and cellular disturbances. The most common forms of organic species of As found in soil include dimethylarsinic acid (DMA), trimethylarsine oxide (MAO), and monomethylarsonic acid (MMA). MMA and DMA are collectively known as cacodylic acid (Singh et al. 2015). As V is analog of inorganic phosphate and forms the major component of inorganic As. Both the forms are

transported into the cell by Pi transporter (PHT) proteins. The cross membrane transport results in the exposure of multiple organs of the plant body to the metalloid.

Once within the cells, As damages the chloroplast membrane, reduces CO_2 fixation, alters the functions of photosynthetic systems, and reduces plant growth. It mainly affects the chloroplasts of the cells which are not only involved in the photosynthesis but also serve as the site for the ROS generation. These ROS species are produced either by oxygen reduction in Mahler reaction or from chlorophyll due to the direct transfer of energy leading to the formation of atomic oxygen. Moreover, mitochondria, protein oxidation, and various metabolic pathways in peroxisomes also contribute in oxidative stress phenomenon in plants.

At the cellular level, it is observed that the membranes are most vulnerable to be damaged by As. This results in an impairment in the water and nutrient uptake by cells. Membrane stability index also decreases in As-treated plants probably due to overproduction of malondialdehyde (by-product of lipid peroxidation) which may cause electrolyte leakage in roots. Stomatal conductance is also affected. As and P have the same transporters in the plant. So, As competes with phosphorus and affects the uptake of micro- and macronutrients in plants. These effects ultimately result in the alteration of plant growth and reduction in biomass of the plants. All these pathological features have been presented in a number of crop species in South Asia including rice, barley, and wheat (Brammer 2009; Bhattacharya et al. 2010; Ahmed et al. 2011; Islam et al. 2016).

The As uptake from soil depends on different factors. Arsenic uptake in rice occurs at varying rates in different plant parts. As III is unable to enter roots through nodulin26-like intrinsic proteins (NIPs) (Finnegan and Chen 2012). In rice As III uptake is carried out by OsNIP2, OsLsi1 silicon transporter, while its efflux from root to xylem is carried out using silicon transport protein called OsLsi2 silicon transporter. In fronds, i.e., *P. ensiformis*, As is immobilized in roots and transferred as As III through xylem to fronds and PvACR3 (Finnegan and Chen 2012). Methylated forms of As V can be reduced to As III and are more cytotoxic at low concentration than inorganic As due to a better bioavailability. The protonated, uncharged forms of methylated As (MMA-V and DMA-V) enter rice roots through aquaporin channel OsLis1. Similar, transport mechanisms are also present in animals (Azam et al. 2016).

Arsenic V is reduced to As III by plants by enzymatic and nonenzymatic pathways. Among the enzymatic pathways, arsenate reductase (ACR) is of chief significance. Moreover, Pi-dependent enzymes that are capable of metabolizing As V include GADPH, PNP, aspartate-B-aldehyde dehydrogenase, etc. (Finnegan and Chen 2012). Nonenzymatic pathways are mediated by GSH molecules. At the molecular level, the As-sensitive reactions in plants include those of biosynthetic processes like metabolism of phospholipids, glycolysis, oxidative phosphorylation genetic metabolism, and signaling transduction pathways. The structure of proteins is disrupted when thiol groups on proteins provide an attachment site to As. Arsenic III, for instance, binds to thiol-containing proteins of proteins involved in signal transduction, structural proteins, proteolytic proteins, and regulatory enzymes.

MMA III and DMA III are able to displace Zn^{2+} from a number of enzymes resulting in alteration of gene expression and DNA repair.

As has a negative effect on the metabolism of various organic and inorganic species in the plant. As, for instance, reduces carbon availability in plants by decreasing carbon fixation. Studies suggest that As decreases the electron flow from thylakoid membranes decreasing ATP and NADPH production that are needed as fuel for carbon fixation reactions (Engström et al. 2009). In rice, As was found to reduce large subunit content of RuBisCO which is encoded by plastid DNA (Hajduch et al. 2001). On the other hand, As V stimulates the accumulation of ascorbates, to support protection against ROS. In wheat varieties, As III inhibits amylolytic activity and prevents the liberation of maltose from starch (Finnegan and Chen 2012). Plant carbon metabolism is highly concerned with efficient energy shuttling across molecular membranes. In As V-treated *Arabidopsis*, the fermentative capacity was increased due to increased transcripts of alcohol dehydrogenase suggesting blocks at the level of carbon flow from pyruvate to citric acid cycle. In addition to the alteration of carbon metabolism in the plant, the nitrogen and sulfur metabolism is also affected.

Intense changes in pools of amino acid were reported in As-treated plants. Regulation of transporters of amino acids was shown in roots and seedlings of As V-treated plants. Moreover, decrease in carbohydrate metabolism and low protein concentration can also affect synthesis of amino acid in As-stressed plants (Finnegan and Chen 2012). Sulfur metabolism is critical in determining the survival of plants grown in As-contaminated soil. The biosynthesis of PC and GSH requires cysteine, glutamine, and glycine as their building blocks. In rice, As III and As V cause upregulation of sulfate transporter gene resulting in an exaggerated As uptake from the soil (Finnegan and Chen 2012). It is, therefore, evident that As toxicity results in a significant alteration in the physiological, biochemical, and metabolic processes in plants.

Plants have devised a number of mechanisms to survive in As-stressed conditions. A number of defense mechanisms play their role in developing tolerance in plants including hyperaccumulation, phytochelation, and antioxidant defense mechanisms. Plants grown in metalliferous soils yet possessing the capability to tolerate the phytotoxicity are referred to as hyperaccumulators. A number of plant varieties develop this strategy to sustain the negative aspects of the environments. The process of hyperaccumulation involves an increase in uptake of As V, efflux of As III to an external medium, sequestration of As III at a vascular level in fronds, and decrease in complexation of As III-thiol (Clemens 2006). Similarly, phytochelation involves the production of biomolecules known as phytochelatins that cause the complexation of metals resulting in their inability to affect the plants. The upregulation of phytochelation production is also a major mechanism adopted by plants to strive in As-stressed conditions (Tripathi et al. 2007). The radical-scavenger system in plants is used to protect them from harmful effects of oxidative stress through antioxidant enzymes which include glutathione reductase, peroxidase, and superoxide dismutase. Other than enzymes, compounds like glutathione, ascorbates, and carotenoids are also of great importance. Plants grown in As-containing water have

been found to possess a highly efficient antioxidant system to curb the secondary effects of the metalloid toxicity (Dixit et al. 2015; Armendariz et al. 2016). A number of external factors also aid the survival of As-stressed plants. Arbuscular mycorrhizal fungi (AMF), for instance, are evident to improve As tolerance in plants. Rice inoculated with AMF under aerobic conditions improve soil nutrients like nitrogen and phosphorus and, ultimately, the plant growth. Similarly, certain bacterial species have also been found to be responsible for As methylation in rice rhizosphere (Garg and Singla 2011). Plants have evolved the process of osmolyte accumulation as another tolerance mechanism. The different metabolites act as organic osmolytes during As exposure and help to maintain water balance in the cells. These metabolites include sulfonium and ammonium compounds (Rascio and Navari-Izzo 2011).

The pathological effects of As toxicity in plants may vary in intensity and diversity among various plant species. Rice plants, for instance, represent symptoms of As toxicity by the delay in the emergence of seedling, wilting of leaves, reduction in the growth of the plant, yellowing of leaves, and reduction in grain yield. These negative effects of toxicity are not limited to the food crops only. Consumption of these food sources results in significant health risks in humans as well resulting in the presentation of conditions referred to as arsenicosis and As poisoning.

19.4.2 Effect of Arsenic Toxicity in Humans

Arsenic is an environmental toxin that has been classified as class I human carcinogen by International Agency for Research on Cancer (IARC) and as class A by US Environmental Protection Agency (EPA) (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2004; U.S. Department of Health and Human Services 2006). The use of As-contaminated drinking water has been associated with a wide range of diseases including those affecting the skin, kidney, lung, bladder, and blood and cardiovascular diseases (Argos et al. 2012).

The mechanism of disease initiation and progression due to As in humans is not fully understood, but the correlation between As exposure and generation of ROS, DNA damage, and tumor production is evident. Using the omics-based approaches, the underlying mechanisms of As toxicity are now under investigation. Recent studies in Thailand showed that As-exposed newborns possess high As levels in finger, nail, and hair and cord blood. The cord blood lymphocytes have increased promoter methylation of p53 (Intarasunanont et al. 2012). In another study in Bangladesh, it was confirmed that significant amount of As was present prenatally in babies born to mother exposed to As (Rahman et al. 2007). The gene transcripts of prenatal exposure showed upregulation of molecular networks related to inflammation, metal exposure, stress, and apoptosis. A genome-wide association study (GWAS) of As-related metabolism and toxicity experimented using urinary As metabolite and single nuclear polymorphism for Bangladeshi population. Studies showed an independent relationship for total MMA percent and DMA percent and also showed that skin lesions were associated with one SNP known as rs9527 (Argos et al. 2012; Pierce et al. 2012). Skin lesions were most commonly observed signs of As exposure.

Among Asians, Bowen disease is the most common form of As-induced skin cancer. In India, it was reported that drinking As-contaminated water caused polyneuropathy, peripheral vascular disease, lung diseases, and portal fibrosis (noncirrhotic) (Das et al. 1994). These clinical manifestations are not only related to the consumption of contaminated water but also due to As-containing food sources.

In a market survey (in Bangladesh, India, Spain, Italy, and the USA) by Williams et al., 6–65% As was present in rice (Williams et al. 2005). Studies show that inorganic form of As was mainly present in Basmati rice (Azam et al. 2016). In vivo As bioavailability studies in immature swine model showed that organic As is poorly absorbed orally, but rice cooked in contaminated water had a high bioavailability of As (Juhasz et al. 2006). Moreover, rice husk and straw are also used as animal fodder, and consuming meat from these animals also results in As toxicity (Azam et al. 2016). Figure 19.2 presents the common adverse effects presented as a result of As toxicity.

Inorganic As has been hypothesized to alter the methionine metabolism. Studies show that free radicals are generated during As metabolism in cells. The reactive oxygen (ROS) and nitrogen species (RNS) play a role in mutagenesis and carcinogenesis. They are involved directly in oxidative damage to DNA, proteins, and lipids in cells leading to cell death. The generation of O_2 and H_2O_2 generation has been observed in human keratinocyte cell line (HaCat) with the help of fluorescence and EPR techniques. The production of H_2O_2 is believed to be involved in the induction of apoptosis by arsenite in NB4 and CHO-K1 cells. It has also been suggested that As-induced apoptosis in CHO-K1 is probably initiated by H_2O_2 production which activates protein kinases through the de novo synthesis of macromolecules (Shi et al. 2004).

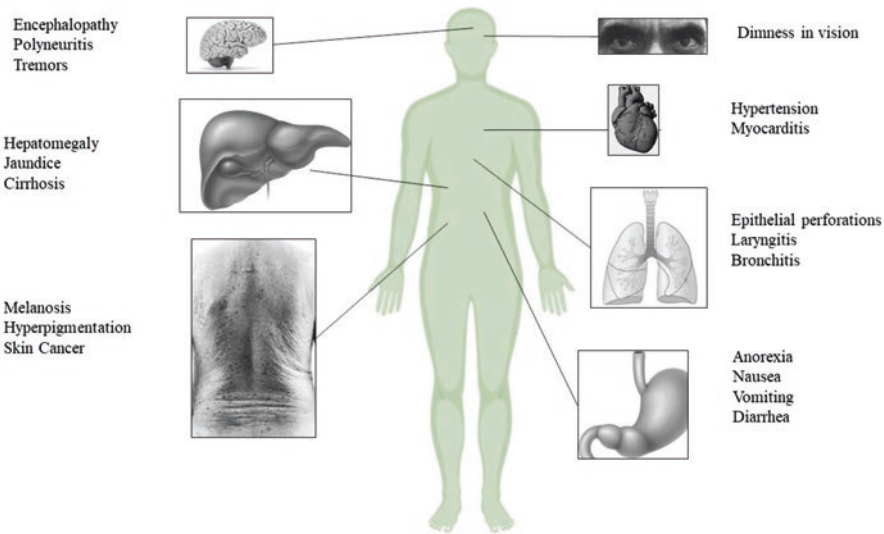

Fig. 19.2 Clinical presentations of arsenic poisoning in humans

Arsenate is converted to arsenite in blood. As has no direct interaction with the DNA that can cause mutations in the gene. It was found that it increases gene amplification and chromosomal damaging by affecting DNA repair. Arsenite causes aneuploidy and accelerates the process of microtubule polymerization. Arsenite plays its carcinogenic role by causing DNA hypomethylation which leads to aberrant gene expression (Abernathy et al. 1999). However, it has opposite effect in human lung A549 carcinoma cells. P53 gene is a tumor suppressor gene and plays a role in controlling DNA repair. Chronic arsenite exposure causes a progressive increase of CpG methylation in p53 promoter, which probably blocks transcription of p53 gene. Research showed that in cultured human alveolar L132 cells, dimethylarsinic acid (DMA) caused cross-linking of proteins and DNA and breaks in DNA resulting in the cytotoxic presentation (Abernathy et al. 1999).

Skin cancer and other cutaneous manifestations are observed as a result of As toxicity. Precancerous keratosis and melanosis are induced by As which can cause squamous cell and basal cell carcinoma in human (Williams et al. 2005). One of the major underlying mechanisms of As carcinogenicity is thought to be due to ER stress which is a cause of multiple disease conditions. Biosynthesis, folding, assembly, and maturation of membrane-bound and secretory proteins occur in the endoplasmic reticulum. Homeostasis of ER is disturbed due to increased demand of folded proteins and accumulation of unfolded or misfolded proteins. Unfolded protein response (UPR) signaling restores the protein-folding capacity by involving transcriptional and translation activities engaging the ER membrane proteins like inositol-requiring enzyme-1 (IRE1), PKR-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) (Srivastava et al. 2013). Activation of UPR signaling pathway is involved in As-induced cutaneous inflammation which is triggered by ROS (Yen et al. 2012). UPR signaling pathway activation alters differentiation and facilitates cutaneous inflammation which ultimately increases the risk of cancer. Research shows that N-acetylcysteine (NAC) blocks As-mediated ROS species as well as caused associated weakening of UPR, MAPK, and other pro-inflammatory (chemokine and cytokine) signaling pathways (El-Saad et al. 2016).

Arsenic-induced IRE1 phosphorylation results in increased splicing of X-box binding protein 1 (XBP-1), and as a result transcription of downstream target proteins is activated. Hyperphosphorylation of PERK increases ATF6 proteolytic activation and causes translation of ATF4. Increased ATF6, ATF 4, and XBP-1 regulate the downstream activity of chaperones GRP94 and GRP78. p38/ MAPK, APK-2, MAPK signaling, and alterations in cytokines/chemokine and their receptors are also induced by As (Lu et al. 2014; Huang et al. 2017). Suppression of oxidative stress in individuals affected by As toxicity is, hence, the mainstay of therapy as has been observed in a recent clinical trial in Bangladesh (Li et al. 2011).

Arsenic trioxide (As_2O_3) acts as an effective apoptosis inducer in a number of cell types. The exact role of As in immunomodulation is yet unknown. As has multiple effects on the immune system which tend to decrease the immune surveillance system and increase the rate of cancer, infection, autoimmune disease, and other immune-mediated problems. Oxidative stress, impaired lymphocyte activation, and

inflammation are generally observed in exposed individuals. The immune modulatory effect of As on T cell population is mainly due to altered expression of key immune regulator molecules, impaired T cell functions, cytokine production, induction of apoptosis, and oxidative stress induction in T cells (Haque et al. 2017).

Chronic exposure to As due to the use of contaminated drinking water and food is observed in the Asian region. Methylation of inorganic As to MMA and DMA in the human body depends on folate as a source of methyl groups. Methylation of inorganic As is thought to be a detoxification pathway because methylated forms are readily excreted compared to the inorganic As. Dietary folate deficiency decreases urinary As excretion, and as a result, As is retained particularly in liver and lung tissues (Gamble et al. 2005). Total homocysteine (tHcys) in human blood showed a positive relation with %MMA and negative association with %DMA in As-exposed population which was thought to be due to inhibition of the second step of methylation by SAH (S-adenosylhomocysteine). SAH is the product of methylation reactions in one-carbon metabolism and is a potent inhibitor of most transmethylation reactions (Gamble et al. 2005). This observation has been confirmed in a number of individuals exposed presenting hyperhomocysteinemia in Bangladesh (Argos et al. 2012).

After absorption of inorganic As, it is methylated into MMA and DMA in the liver and is excreted through the kidney along with inorganic, unmethylated As (Kuo et al. 2017). A number of liver biomarkers are altered in response to As toxicity including Apo-A1, A2ML, and Wap 65 (Banerjee et al. 2017).

Epidemiological studies in As-exposed population showed that low concentrations of methylated species of As (MMA^{III}) could inhibit sensory neuron and skeletal muscle formation. This has been attributed as a major cause of neurological dysfunction (Singh et al. 2015). Case-control studies in Asian populations enrolling infants with myelomeningocele showed that As is also a risk factor for neural tube defects. As affects folate and glucose metabolism in mother which causes neural tube defects in neonates (Mazumdar 2017).

In addition, inorganic and methylated As forms in urine are associated with nephrotoxicity. Forminidoyl transferase cyclodeaminase (FTCD) expression in liver is higher in As-affected individuals than in any other human tissue. rs61735836 is a missense variant in exon 3 of FTCD and has been associated with liver toxicity. In a longitudinal study carried out in Bangladesh, rs61735836 showed significance in percentages of all three As metabolites ultimately contributing to hepatotoxicity and nephropathy (Pierce et al. 2017). In vitro studies show that MMA has a most potent cytotoxic effect on urothelial cells in human (Khairul et al. 2017). Similarly, a lower percentage of MMA and a higher percentage of DMA have been associated with metabolic syndrome and diabetes (Kuo et al. 2017).

Consumption of As-contaminated drinking water and food is also a factor contributing in hyperglycemia that chronically develops into diabetes. Arsenic and its metabolites can affect glucose metabolism. Arsenite is capable of binding covalently with sulfhydryl groups present in molecules of insulin and insulin receptors, enzymes, and transporters including pyruvate dehydrogenase, glucose transporters (GLU-T), and alpha ketoglutarate dehydrogenase, possibly resulting in resistance

of insulin. Arsenate contains AsO3-4 ion which can affect insulin secretion by substitution of phosphate group through ATP pathway (Tseng 2004). A number of studies have related As toxicity with the prevalence of diabetes in the South Asian region including Bangladesh, India, and Pakistan (Bahadar et al. 2014; Chakraborti et al. 2016; Hassan et al. 2017).

Arsenic is also considered to be a potential obesogenic. Arsenic causes impaired metabolism of white adipose tissue (WAT), thereby promoting obesity. It increases mature adipocytes size by diminishing pre-adipocyte adipogenesis. Basal lipolysis is increased, and mRNA expression of adiponectin is downregulated. Basal and insulin-stimulated glucose uptake is also reduced. Transgenerational effects due to adipose tissue metabolism are also induced by As (Ceja-Galicia et al. 2017).

Arsenic trioxide (As_2O_3) possesses significant cytotoxic activities and has been associated with a number of cancer types. Conversely, As-containing compounds are now being recognized as one of the most effective drugs for the treatment of acute promyelocytic leukemia, solid tumors, and malignancies. However, cytotoxic mechanisms of As and methylated metabolites of As in removing cancer are not clear. Arsenic compounds in clinical trials of FDA for treatment of cancers are darinaparsin (DAR) and Trisenox (As_2O_3) (Khairul et al. 2017). Based on the significant plant and human health concerns, it is imperative that strategies may be designed to decrease the levels of As in soil and water. Moreover, effective interventional and preventive measures should be developed to protect the human population from the ill effects of As.

19.5 Strategies for Combating Arsenic Toxicity

Arsenic exposure in humans is either directly through water or other food sources. Authorities are in the process of identifying and developing means to alleviate the effects of As toxicity. Pertaining to its public health aspect, generally, two strategies are employed: primary prevention and secondary prevention. In primary prevention mitigation efforts regarding As exposure through drinking water include switching the wells and As removal strategies. Secondary prevention focuses on preventing harms of chronic As exposure. Investigating genetic susceptibility of an individual toward As toxicity helps in secondary prevention and intervention of exposed population (Argos et al. 2012).

Various technological options are available for removal of As in groundwater. These strategies employ biological, physical, or chemical treatment of As-rich water (Singh et al. 2015). Before employing any strategy, the toxic water is pretreated for the removal of pentavalent forms of the metalloid. Chlorine, bleaching powder, hydrogen peroxide, and potassium permanganate are used in pretreatment. However, the use of chemicals in drinking water is not useful as it leads to the formation of undesirable products. Moreover atmospheric oxygen is the best source of oxidation process, but it is a very slow process. Some bacteria also catalyze As oxidation and hence can be employed as an alternative (Jain and Singh 2012). After the

pretreatment, one or more of the physicochemical procedures are employed to ensure the complete removal of As from water. These processes include oxidation, filtration, coprecipitation, adsorption, ion-exchange catalysis, and membrane filtrations.

The process of oxidation involves removal of naturally occurring iron and manganese from water by oxidation of their soluble forms into insoluble forms and ultimate removal by filtration. Iron and As are first oxidized, and then As V is adsorbed on iron hydroxide precipitates and is filtered out (Singh et al. 2015). Oxidation may be brought about by the chemical treatment of water or by the photocatalysis. Solar oxidation employs photo-induced redox reactions for the removal of As (III, V). In this case, drinking water is placed in polyethylene bottles and exposed to sunlight. Citrate is added as a Fe III complexing agent that accelerates the As III oxidation. UV radiation catalyzes the oxidation process of oxidants like molecular oxygen (Singh et al. 2015). In rural settings, it has been observed that the storage of water in pitchers also results in a significant reduction of As concentration (Jain and Singh 2012).

The processes of coprecipitation involve treatment of water with coagulants like alum, ferric chloride, and ferric sulfate due to which As is adsorbed on the coagulated flocs (Jain and Singh 2012). This process is generally employed in case of treatment of water obtained from tube wells, hand pumps, or domestic water storage units. Adsorption, much similar to coprecipitation, depends upon the physical interaction of the metalloid with an adsorptive base. In this case an adsorption media is packed into columns and water is passed through it. The columns include activated alumina, iron-based adsorbents, and indigenous filters and cartridges. Once the media is fully consumed, it may be replenished by new material within the same column (Singh et al. 2015). Similarly, ion-exchange catalysis involves the movement of As between solution phase and resin phase which is three-dimensional hydrocarbon network. The water is passed through one or two columns packed with exchange resins. This process is carried out under pressure (Lee et al. 2017).

Another method employs the use of physical membranes to decrease the concentration of As by at least 50 μg per liter. There are four types of membrane processes: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). The first three processes involved the passage of water through filters of varying pore size. However, in case of reverse osmosis, the pressure is applied against the concentration gradient to ensure the removal of the metals and metalloids.

Biotechnological approaches are also employed to produce cultivars that possess a lesser ability to accumulate As. Studies have also recommended the use of quality checks to ensure the absence of As in water provided to the domesticated animals in order to block the circulation of As among various sources (Azam et al. 2016). Among the South Asian countries, Bangladesh has the highest contribution to contributing to the knowledge base and devising and implementing interventional strategies for the containment of the adverse effects. The only cross-country project referred to as Bangladesh Water Supply Program Project (BWSPP), among the countries of the regions, has been successfully implemented by Bangladesh (Sambu and Wilson 2008). The program employs various screening and mitigation strategies

for the purpose. These include the use of kits for testing, identification of wells free from As-contaminated water, surface water treatment, and piped water supply. These strategies have yielded significant positive outcomes resulting in a decrease in the incidence of reported cases of toxicity. No other country of the region has a comprehensive program for addressing the issue. However, the UNICEF, WHO, and FAO are continuously in the process to aid and facilitate the governments to protect their population from the adverse aspects of As toxicity.

19.6 Conclusion

It is evident that more than a million people of South Asia are continuously being exposed to high levels of As exposure by direct consumption of As-contaminated drinking water or indirectly through daily intake of As-contaminated food which is fatal. Treatment of As-contaminated water and soil is the most important strategy to minimize the health hazard. Biotechnological procedures to limit the As uptake and accumulation may also be employed to protect the human health. Surveys are needed to identify sites where soil and rice grains are already contaminated. A single technological program is not effective. There is a need to develop new techniques that are feasible especially in areas of South Asia. The objectives of As removal technological programs should be it should decrease the As levels below MCL. Additionally, the strategies should be cost-effective, user-friendly, and easily manageable. Moreover, it is imperative that based on the Bangladeshi model, similar programs may be devised in all the countries of the region to ensure a transcontinental control of the As toxicity. In addition to the technological expertise, diplomatic and regulatory aspects should also be addressed so that the countries of the region can learn from the experiences of other countries.

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