Bioremediation of Arsenic Toxicity

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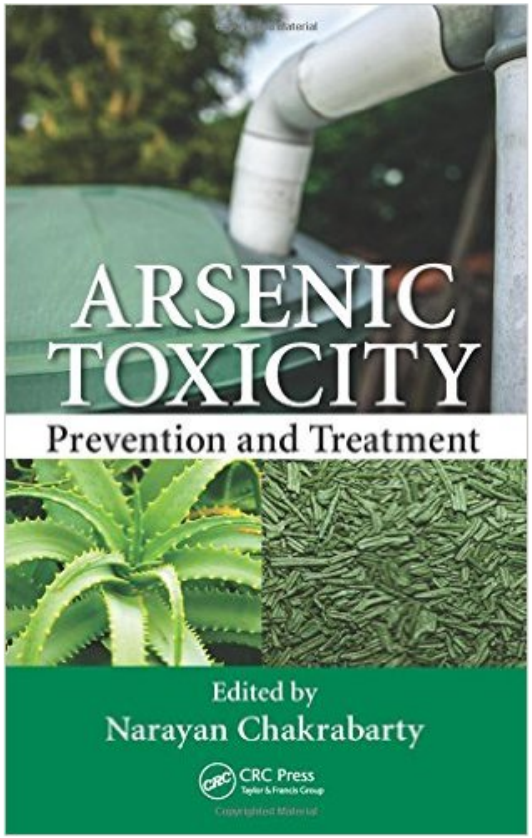

Arsenic Toxicity: Prevention and Treatment

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The most talked about metalloid in the modern world, arsenic affects the liver, kidney, and lungs; leads to cardiovascular diseases, cancer, and diabetes; and may cause blindness with long-time exposure. With naturally occurring arsenic boosted by mining and other industrial processes contaminating soil and drinking water, arsenic toxicity is a major challenge to health professionals and scientists around the world. Arsenic Toxicity: Prevention and Treatment reviews current understanding of arsenic poisoning and the health consequences resulting from exposure.The book paints a vivid picture of the sources of arsenic toxicity including ground water; food such as rice, fruits and vegetables, fish, and chicken; as well as occupational exposures from industries such as using inorganic arsenic like glass production, non-ferrous alloy, wood preservation, and semiconductor manufacturing units. It details the health hazards of arsenic toxicity and then examines removal, mainly from soil and water, highlighting eco-friendly bioremediation techniques. It discusses classical and modern treatment methods for arsenic toxicity, emphasizing the use of nutraceuticals and functional foods.With its focus on the remediation of arsenic toxicity using nutraceutical and functional food, the book provides a unique resource for combatting this global scourge. It provides strategies for defending arsenic toxicity naturally without causing any additional adverse effects.

7 Bioremediation of Arsenic Toxicity

Nguyen Van Hue

CONTENTS

7.1 CHEMICAL AND ENVIRONMENTAL PROPERTIES OF ARSENIC

Arsenic (As) is a highly toxic element that has poisoned many people by being added to their food and drink, mistakenly, unknowingly, or deliberately [1]. Arsenic occurs in trace quantities in all rock, soil, water, and air [2,3]. Naturally, total As is about 1–2 mg/kg in rock, 5–10 mg/kg in soil, and 1–3 μg/L in seawater [4]. Volcanoes and microbial activities can release As into the atmosphere as arsine gas (AsH_3) or methylated Arsine species. The atmospheric residence time of As species is relatively short and the As concentration is generally low (0.02 $\mu g/m^3$), except in the vicinity of these sources [5]. However, anthropogenic sources such as fossil fuel combustion, mining, smelting of sulfide ores, pesticide application, timber preservation, and the application of sludge and manure have elevated As levels and may cause As contamination in the food chain and drinking water [6]. As an example, past use of arsenical herbicides in sugarcane fields resulted in total As levels ranging from 50 to 950 mg/kg in some Hawaiian soils [7,8].

Although As can have –3, 0, +3, and + 5 oxidation states, the two oxidation states of As (+3) (arsenite, with ionic radius, r = 0.58 Å) and As (+5) (arsenate, r = 0.46 Å) are most important environmentally and biologically. Because of its size and polarizability, As (+3) has a relatively high affinity for softer S- and N-donating species. Thus, As (+3) can react strongly with thiols of cysteine residues and/or imidazolium nitrogens of histidine residues from cellular proteins, inactivating many enzymes [9]. In aqueous solution, it is found mainly as the neutral [$As(OH)_3$] species whose first $pK_a = 9.2$. Arsenate (+5) with its greater charge and smaller ionic radius leads to a higher stability with harder O-donating species, and its prevalence in aqueous

solution as $H_2AsO_4^-$, $HAsO_4^{2-}$ depending on pH (pK_a's = 2.3, 7.0, and 11.5), chemically, AsO_4^{3-} and PO_4^{3-} whose pK_a's 2.1, 7.2, and 12.7 are very similar. Thus, As (+5) can replace phosphate in energy transfer phosphorylation and block protein synthesis. On the other hand, like phosphate, arsenate can be adsorbed strongly on sesquioxides, particularly amorphous $Fe(OH)_3$, rendering it less bioavailable and less toxic [7,10].

The As (+3)/As (+5) two-electron redox potential ($E^{0\prime}$ = +140 mV at pH 7.0, 25°C vs. normal hydrogen electrode) is significantly higher than that of phosphate ($E^{0\prime}$ = −690 mV), resulting in the existence of both As (+3) and As (+5) in environmental and biological conditions [5]. In addition, As can form stable bonds with carbon, yielding compounds such as mono-[CH_3AsH_2], di-[$(CH_3)_2AsH$], tri-methyl arsines [$(CH_3)_3As$], arsenobetain [$(CH_3)_3$–As^+–CH_2COO^-], arsenocholine [$(CH_3)_3$–As^+–CH_2–CH_2–OH], and arsenosugars [5,6].

7.2 BIOLOGICAL PROPERTIES OF ARSENIC AND ITS TOXICITY

Due to its chemical similarity to phosphate, As (+5) is taken up by microorganisms, plant roots, and animal (intestinal) cells by two pathways used for phosphate [11]. The low-affinity P inorganic transport (Pit) pathway uses energy from the transmembrane proton gradient, while the high-affinity P specific transport (Pst) pathway has certain selectivity for phosphate over arsenate with a periplasmic phosphate binding protein and an ATP-hydrolyzing membrane transporter [12,13]. Neutral arsenite [$As(OH)_3$], on the other hand, diffuses through membrane-spanning channels created by aquaglyceroporin proteins, which allow the diffusion of water, glycerol, $Si(OH)_4$, and other neutral species [12,14]. The properties of organoarsenic species are modulated by their organic substituent(s), and this affects their uptake by these or other pathways.

Normal human blood levels of As are 0.3–2 μg/L but can be one to two orders of magnitude higher when elevated levels of As are consumed with drinking water or food [5]. The overall half-life of As in humans is about 10 h, and 50%–80% of absorbed As is excreted in about 3 days [15]. Most excretory As is in urine [16].

Ingestion of inorganic As (60–120 mg as As_2O_3) would result in acute toxicity characterized by vomiting, abdominal pain, bloody diarrhea, which lead to dehydration, convulsion, coma, and death [17]. Chronic As exposure leads to skin lesions, hyperpigmentation, keratosis, diabetes, and cardiovascular disease [18]. "Black foot" disease, which shows a discoloration and blackening of the extremities, especially the feet, in Southwestern Taiwan was caused by drinking water from deep artesian wells high in As [19]. Millions of people in Bangladesh have been poisoned by consuming groundwater contaminated with high levels of As, sometimes as high as 800 μg/L [18,20]. The World Health Organization (WHO) and the United States (U.S.) Environmental Protection Agency have set 10 μg/L As as the maximum concentration for drinking water [21]. Rice grown in the southeastern United States is of concern to human health because of measured As levels of over 300 μg/kg due to past use of Ca-arsenate as a cotton defoliant [22]. Although currently there are no national standards for As in food, previous WHO guidelines established a provisional tolerable weekly intake for inorganic As of 15 μg/kg body weight, but these

are currently being reconsidered [21]. Inorganic As species are of most concern to human health, with As (+3) being more toxic than As (+5) [23–25]. Organic As species, particularly arsenobetain and arsenocholine are generally considered less or nontoxic, though some debates remain [26].

7.3 BIOREMEDIATION OF ARSENIC

Bioremediation is the use of microorganisms or plants to detoxify an environment (mainly soil or water) by transforming or degrading pollutants. In case of diffused pollution, *in situ* bioremediation is better adapted for treatments of large areas. Such treated land becomes available for less risky uses at economically acceptable cost.

Arsenic being a metalloid, unlike many organic pollutants, cannot be converted to CO_2 and dissipated into the atmosphere (although the release and capture of the toxic arsine species are possible). The likely strategy would be oxidation of the more toxic As (+3) to the less toxic As (+5), methylation of inorganic species, and/or extraction (uptake) by plants and then disposing of the high-As plant biomass [27,28].

7.3.1 Arsenite Oxidation

Although pH and concentration dependent, the redox potential of As (+3)/As (+5) is about +140 mV at pH 7, making both species exist in environments that support many microbial growth and activities [29]. Arsenite itself can serve as an electron (e^-) donor for microbial respiration processes, oxidizing to As (+5) with e^- being passed to suitable e^- acceptors, such as oxygen (O_2) or nitrate (NO_3^-) under aerobic conditions. A wide array of microorganisms have evolved an energy requiring detoxification process catalyzed by the *ars* operon, linked to the intracellular reduction of As (+5) by the ArsC protein and its efflux as As (+3), as shown in Figure 7.1.

The ArsC is an As (+3)-specific exporter, which removes As from the cell. This process can be passive or active, which the latter case involves an associated ATPase. The ArsC is a cytoplasmic protein of 13–15 kDA related to tyrosine phosphate phosphatases facilitates the reduction of As (+5) when a suitable e^- donor, such as reduced thioredoxin or glutaredoxin, is provided. The genes involved are clustered in an *ars* operon that is located on plasmids or chromosomes of a diverse group of organisms, including Archaea, Bacteria, and yeasts [9,30,31].

The microbial oxidation of As (+3), a recognized detoxification process, involves two enzymes: Aso (also called Aox) and Arx [9,32].

$$As(+3)O_2^- + 2H_2O \xrightarrow{Aso} As(+5)O_4^{3-} + 4H^+ + 2e^-$$

The reaction sequence is completed once the electrons generated are passed to a physiological e^- acceptor, such as a *c*-type cytochrome [33,34]. Aso, normally located in the periplasm (Figure 7.1), has been isolated from a variety of organisms,

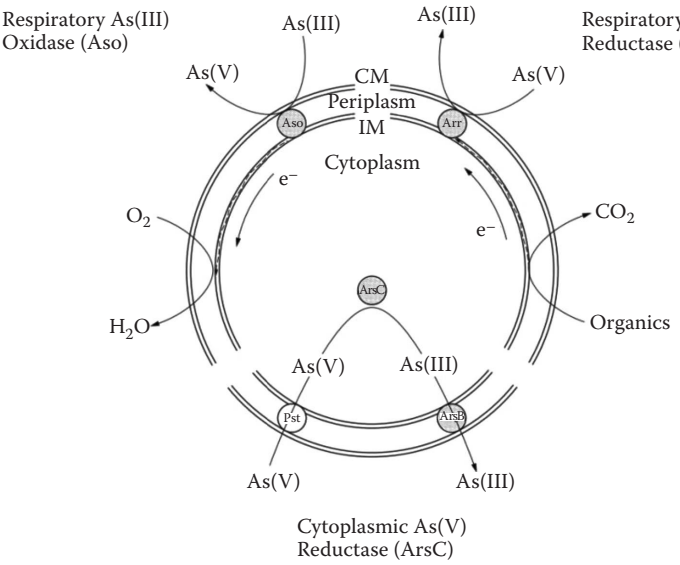

FIGURE 7.1 Biochemical transformation of inorganic As species by microbial cells. (Adapted from Lloyd, J.R. et al., Microbial transformations of arsenic in the subsurface, in: *Microbial Metal and Metalloid Metabolism: Advances and Applications*, ASM Press, Washington, DC, pp. 77–90, 2011.)

including *Rhizobium* sp. Str. NT-26, *Hydrogenophaga* sp. Str. NT-14, *Alcaligenes faecalis*, *Arthrobacter* sp. Str. 15b, and *Ralstonia* sp. Str. NT-14 [34]. Aox consists of two heterologous subunits: AoxA and AoxB. The large catalytic AoxA contains a molybdenum (Mo) atom coordinated by two pterin molecules and a [3Fe-4S] cluster; the smaller AoxB subunit contains a [2Fe-2S] cluster, as illustrated in Figure 7.2 [32]. The As (+3) oxidase, Arx, usually operates under anaerobic conditions and is distantly related to Aso, but has not been fully characterized.

Oremland et al. [35] isolated a facultative chemoautotrophic bacterium, strain MLHE-1, from arsenite-enriched bottom water from Mono Lake, California, that oxidized As (+3) anaerobically to As (+5) using nitrate as terminal e^- acceptor [35]. This organism was also able to grow heterotrophically with acetate as carbon and energy sources and oxygen (aerobic growth) or nitrate (anaerobic growth) as terminal e^- acceptors. Phylogenetic analysis based on its 16S rDNA places this organism with the haloalkaliphilic *Ectothiorhodospira* of the γ-*Proteobacteria*.

Mateos et al. [9] proposed that *Corynebacterium glutamicum* (a member of the genera *Corynebacterium* of biotechnological importance for the large-scale production of amino acids, such as L-glutamate and L-lysine), which is gram-positive with a thick cell wall, be used to accumulate/sorb As (3+) (and As (+5) after it is reduced to As (+3)) as a means to clean up (bioremediation) As in water [9]. The authors showed that *C. glutamicum* can tolerate up to 12 mM As (+3) and more than 400 mM As (+5) [9].

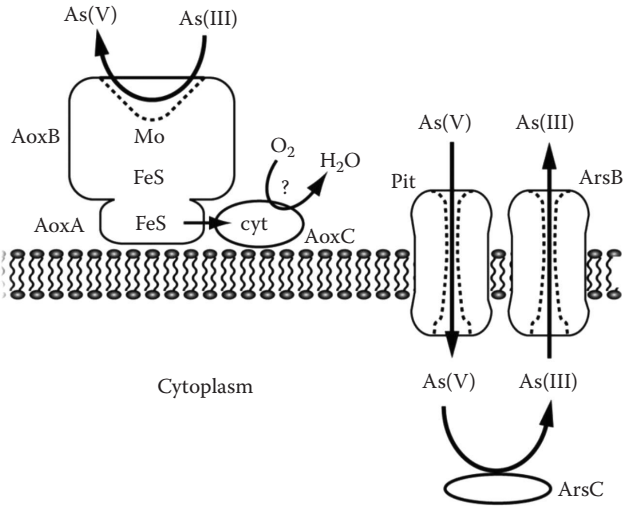

FIGURE 7.2 Model of arsenite oxidase (AoxA and AoxB) and arsenate reductase (ArsC). (Adapted from Saltikov, C.W., Regulation of arsenic metabolic pathways in prokaryotes, in: *Microbial Metal and Metalloid Metabolism*, ASM Press, Washington DC, pp. 195–210, 2011.)

7.3.2 Methylation of Inorganic As Species

Methylation is another mechanism that can confer As resistance and detoxification. The methylated As species include monomethyl arsonate [MMA (+5)], monomethylarsonite [MMA (+3)], dimethylarsinate [DMA (+5)], dimethylarsenite [DMA (+3)], and trimethylarsine oxide (TMAO), as well as several volatile arsines, including mono-, di-, and tri-methyl arsines (TAMs). Gosio (1897, as cited in Cullen and Reimer [36]) was the first to establish that fungi could generate methylated As. Challenger proposed a scheme in which As (+5) was eventually transformed to TMAs [37]. In this scheme, As (+5) is first reduced to As (+3) then methylated, and each methylation step results in the reoxidation of the As, thus requiring a reductive step to As (+3) prior to further methylation as shown in the equation [36]:

$$\mathrm{As(+3)O_3^{3-}} \xrightarrow{\mathrm{RCH3}} \underset{\text{methylarsonic acid}}{\mathrm{CH_3As(+5)O(OH)_2}} \xrightarrow{2e^-} \underset{\text{methylarsinous acid}}{\mathrm{CH_3As(+3)(OH)_2}} \xrightarrow{\mathrm{RCH3}}$$

$$\rightarrow \underset{\text{dimethylarsinic acid}}{\mathrm{(CH_3)_2As(+5)O(OH)}} \xrightarrow{2e^-} \mathrm{(CH_3)_2As(+3)(OH)} \xrightarrow{\mathrm{RCH3}}$$

$$\rightarrow \underset{\text{TMAO}}{\mathrm{(CH_3)_3As(+5)O}} \xrightarrow{2e^-} \underset{\text{Trimethylarsine}}{\mathrm{(CH_3)_3As(+3)}}$$

The methyl donors (RCH_3) in these reactions can be a form of methionine [36]. Several different enzymes have been identified with the methylase activity, such as S-adenosine methyltransferase in *Rhodobacter sphaeroides* [38,39].

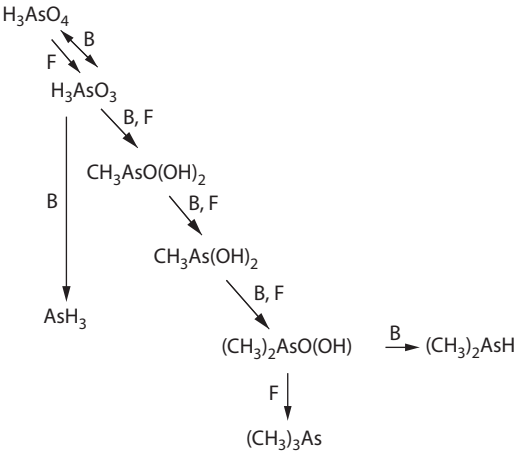

FIGURE 7.3 Summary of observed microbial interactions with arsenic species performed by (B) bacteria and (F) fungi.

Fungi, such as *Scopulariopsis brevicaulis*, *Aspergillus*, *Mucor*, *Fusarium*, *Peacilomyces*, and *Candida humicola*, have been found to be active in such As methylation [27,40,41]. Huysmans and Frankenberger [42] isolated a *Penicillium* sp. from an agricultural evaporation pond in California capable of producing trimethylarsine from methylarsonic acid and dimethylarsinic acid. The transformations of arsenic species by bacteria and fungi are summarized in Figure 7.3 [42].

The methylation of arylarsonic acids (e.g., Roxarsone) is important because their wide use as food supplements for swine, turkeys, and poultry. Methylphenylarsinic acid and dimethylphenylarsine oxide are reduced to dimethylphenylarsine by *C. humicola*. These arsines species are volatile and can be captured by activated carbon traps as illustrated in Figure 7.4 for As-contaminated water [27].

7.3.3 Arsenic Extraction by Plants (Phytoremediation)

There are two basic strategies by which higher plants can tolerate elevated levels of toxic metals, including As [43]: (1) exclusion, whereby transport of As is restricted, and low, relatively constant As concentrations are maintained in the shoot or grain over a wide range of soil concentrations, and (2) accumulation, whereby As is accumulated in nontoxic form(s) in upper plant parts at both high and low soil concentrations.

Most plants do not accumulate As, their As concentrations in leaves or seeds are often below 1 mg/kg [44]. The As hyperaccumulator fern, *Pteris vittata*, was discovered by Ma et al. [45] by screening many plant species growing at an As-contaminated site in Florida. Its fronds can contain in excess of 1% As (or 10,000 mg/kg dry weight) [45,46]. The ability of this fern to translocate As from the roots to the fronds and accumulate it was due in part to its ability to maintain high phosphate in its roots [47]. In fact, once entering the roots, through P transporters (Pit and Pst proteins), As (+5) is reduced to As (+3) before being expelled to cell vacuoles.

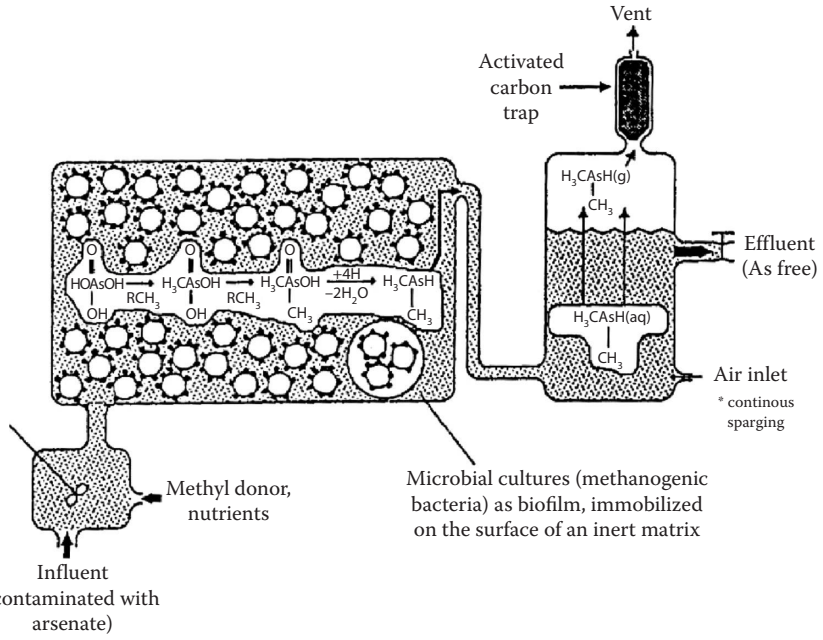

FIGURE 7.4 Bioreactor design for treatment of As-contaminated water. (Adapted from Frankenberger, W.T. and Losi, M.E., Applications of bioremediation in the clean up of heavy metals and metalloids, in: *Bioremediation: Science and Applications*, American Society of Agronomy, Madison, WI, Soil Science of Society of America Special Publication No. 43, pp. 173–210, 1995.)

Cytosolic arsenite, whether as a product of arsenate reductase or from uptake via an aquaglyceroporin, is detoxified by removal from the cytosol [48]. It is rather counterintuitive that As (+5), which is less toxic, should be converted to the more toxic As (+3) before removal from the cell interior. Perhaps, arsenate efflux would have caused phosphate efflux as well, a detrimental consequence that no living cells can afford, or perhaps it is an accident of evolution as speculated by Rosen [12]:

> Since the primordial atmosphere was not oxidizing, most As would have been in the form of As (+3), and early organisms would have evolved detoxification mechanisms to cope with As (+3), not As (+5). [*Furthermore, As (+3) can be chelated/detoxified by phytochelatins and other SH-containing proteins*] [49,50]. Once the atmosphere became oxidizing, most As (+3) in the environment would have been oxidized to As (+5). The mechanisms to cope with As (+5) make use of existing As (+3) extrusion systems. Besides, the conversion of a phosphatase to a reductase is relatively facile (at least easy in laboratory conditions), its evolution during the formation of an oxygenic world would have been rapid.

In addition to *P. vittata*, a few other fern species, such as *Pteris cretica*, *Pteris longifolia*, and *Pteris umbrosa*, also hyperaccumulate As [51]. Except for *Pityrogramma calomelanos*, all known As hyperaccumulators are ferns in the *Pteris* genus.

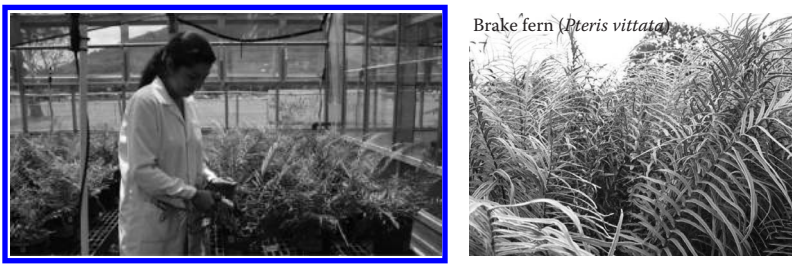

FIGURE 7.5 **(See color insert.)** Chinese brake fern (*P. vittata* L.) grown in the greenhouse (left) and in the field. (NV Hue's personal images.)

However, not all *Pteris* ferns hyperaccumulate As [47,51,52]. Wang et al. [53] examined variation of As accumulation by ferns collected at different locations in south China (Guangxi Province) and found genotypic variations within *P. vittata* that could be useful in breeding improved cultivars. Ma et al. [45] found that fronds of *P. vittata* were able to accumulate as much as 15,000 mg/kg As in 2 weeks of growth in a sandy soil spiked with 1500 mg/kg As. Kertulis-Tartar et al. [54] implemented a field trial in a copper–chromium–arsenate (CCA)-contaminated soil in Florida, with soil As averaging 278 mg/kg in the 15–30 cm depth. After 2 years of cropping, soil at that depth averaged 158 mg/kg, a 43% depletion. The bioconcentration factor (BF), the ratio of As concentration in the plant to the total As concentration in soil, ranged from 10 to 100, depending on total As levels, soil characteristics, and growing environments [7,55]. McGrath and Zhao [56] calculated that a harvest of 10 tons biomass per hectare (ha) with a BF of 20 could reduce soil As in the top 20 cm depth by 50% after 10 harvests (*P. vittata* could be harvested every 3 months; Figure 7.5).

While *P. vittata* is capable of producing substantial biomass under favorable conditions as discussed in detail by Cai and Ma [57] and others [45,56], field results have been suboptimal [55]. Cai and Ma [57] could obtain only about 1 ton of frond biomass/ha/year, regardless of harvesting procedure and frequency (although better soil and plant management could surely improve the fern biomass). It also should be noted that *P. vittata* does not tolerate frost, is hard to germinate from spores, and only grows best in the tropics and subtropics. Its introduction into nonnative locations may also pose ecological dangers.

Genetic manipulation has the potential to transfer the ability to hyperaccumulate As to desired species. Chen et al. [58] demonstrated the principle by transferring the PvARC3 gene, a key As (+3) antiporter in *P. vittata* to *Arabidopsis thaliana*. The resulting transgenic plants had an increased ability to tolerate and accumulate As. Further work is needed to develop As hyperaccumulating capacities in other plant species that could produce more biomass, be more easily harvested, or more ecologically appropriate.

7.4 FUTURE TREND IN BIOREMEDIATION

The biological treatments, using microorganisms and plants, of As-contaminated systems are making significant progress toward practical technologies that are

applicable to different agroecological regions. Recent developments in genomics and proteomics have led to the identification and characterization of As-resistance/tolerance genes and proteins, such as *ars* operon, *aio* gene in As (+3) oxidation and ArsC protein in As (+5) reduction. Such As involving genes could be engineered into other bacteria and plants that are adapting well to local environmental conditions, having high As tolerance, or increasing As uptake capacity, thus improving the efficiency of bioremediation. Applying the knowledge of many interdisciplinary sciences (e.g., microbiology, chemistry, hydrology, geology, engineering, soil and plant sciences, as well as biotechnology), bioremediation will probably become more effective, eco-friendly, socially acceptable, and profitable: the sustainable technology it should be.

REFERENCES

1. Emsley, J. *The Elements of Murder*, pp. 93–193. Oxford, U.K.: Oxford University Press, 2005.
2. WHO. *Environmental Health Criteria: Arsenic and Arsenic Compounds*, p. 224. Geneva, Switzerland: World Health Organization, 2001.
3. Ortiz-Escobar, M.E., Hue, N.V., and Cutler, W.G. Recent developments on arsenic: Contamination and remediation. In *Recent Research Developments in Bioenergetics*, Vol. 4. Kerala, India: Transworld Research Network, 2006, pp. 1–32.
4. Matschullat, J. Arsenic in the geosphere—A review. *Sci. Total Environ.* 249 (2000): 297–312.
5. Wilcox, D.E. 2013. Arsenic: Can this toxic metalloid sustain life. In *Metal Ions and Life Sciences*, pp. 475–498. Berlin, Germany: Springer, 2013.
6. Frankenberger, W.T. *Environmental Chemistry of Arsenic*. New York: Marcel Dekker, 2002.
7. Hue, N.V. Arsenic chemistry and remediation in Hawaiian soils. *Int. J. Phytoremed.* 15 (2013): 105–116.
8. Cutler, W.G., Brewer, R.C., El-Kadi, A. et al. Bioaccessible arsenic in soils of former sugar cane plantations, Island of Hawaii. *Sci. Total Environ.* 442 (2013): 177–188.
9. Mateos, L.M., Ordonez, E., Letek, M., and Gil, J.A. *Corynebacterium glutamicum* as a model bacteria for the bioremediation of arsenic. *Int. Microbiol*. 9 (2006): 2007–2015.
10. Cutler, W.G., El-Kadi, A., Hue, N.V. et al. Iron amendments to reduce bioaccessible arsenic. *J. Hazard. Mater.* 279 (2014): 554–581.
11. Willsky, G.R. and Malamy, M.H. Effects of arsenate on inorganic phosphate transport in *Escherichia coli*. *J. Bacteriol.* 144 (1980): 366–374.
12. Rosen, B.P. 2002. Biochemistry of arsenic detoxification. *FEBS Lett.* 529: 86–92.
13. Rao, N.N. and Torriani, A. Molecular aspects of phosphate transport in *Escherichia coli*. *Mol. Microbiol.* 4 (1990): 1083–1090.
14. Liu, Z., Shen, J., Carbrey, J.M. et al. Arsenite transport by mammalian aquaglyceroporins AQP7 and AQP 9. *Proc. Natl Acad. Sci.* 99 (2002): 6053–6058.
15. Liu, J., Goyer, R.A., and Waalkes, M.P. Toxic effects of metals. In *The Basic Science of Poisons*, pp. 931–979. New York: McGraw-Hill, 2008.
16. Basta, N.T., Rodriguez, R.R., and Casteel, S.W. Bioavailability and risk of arsenic exposure by the soil ingestion pathway. In *Environmental Chemistry of Arsenic*, pp. 117–139. New York: Marcel Dekker, 2002.
17. Ravenscroft, P., Brammer, H., and Richards, K. *Arsenic Pollution: A Global Synthesis*. New York: John Wiley & Sons, 2009.

18. Smith, A.H., Lingas, E.O., and Rahman, M. Contamination of drinking water by arsenic in Bangladesh: A public health emergency. *Bull. WHO* 78 (2000): 1093–1103.
19. Chiou, H.-Y., Chiou, S.T., Hsu, Y.-H. et al. Incidence of transitional cell carcinoma and arsenic in drinking water: A follow-up study of 8,102 residents in an arseniasis-endemic area in Northeast Taiwan. *Am. J. Epidemiol.* 153 (2001): 411–418.
20. Anawar, H.M., Akai, J., Mihaljevic, M. et al. Arsenic contamination in groundwater of Bangladesh: Perspective on geochemical, microbial and anthropogenic issues. *Water* 3 (2011): 1050–1076.
21. WHO. *Exposure to Arsenic: A Major Public Health Concern*. Geneva, Switzerland: World Health Organization, 2010.
22. Consumer Reports. 2012. Arsenic in your food, November issue, http://www.consumerreports.org/cro/magazine/2012/11/arsenic-in-your-food/index.htm. Accessed on March 24, 2013.
23. Hughes, M.F. Arsenic toxicity and potential mechanisms of action. *Toxicol. Lett.* 133 (2002): 1–16.
24. Cohen, S.M., Arnold, L.L., Beck, B.D., Lewis, A.S., and Eldan, M. Evaluation of the carcinogenicity of inorganic arsenic. *Crit. Rev. Toxicol.* 43 (2013): 711–752.
25. Contreras-Acuna, M., Garcia-Barrera, T., Garcia-Sevillano, M.A., and Gomez-Arina, J.L. Arsenic metabolites in human serum and urine after seafood (*Anemonia sulcata*) consumption and bioaccessibility assessment using liquid chromatography coupled to inorganic and organic mass spectrometry. *Microchem. J.* 112 (2014): 56–64.
26. Watanabe, T. and Hirano, S. Metabolism of arsenic and its toxicological relevance. *Arch. Toxicol.* 87 (2013): 969–979.
27. Frankenberger, W.T. and Losi, M.E. Applications of bioremediation in the clean up of heavy metals and metalloids. In *Bioremediation: Science and Applications*, pp. 173–210. Madison, WI: American Society of Agronomy, Soil Science Society of America Special Publication No. 43, 1995.
28. Chaney, R.L., Broadhurst, L.C., and Centofanti, T. Phytoremediation of soil trace elements. In *Trace Elements in Soils*. Oxford, U.K.: John Wiley & Sons, 2010.
29. Lloyd, J.R., Gault, A.G., Hery, M., and Mackae, J.D. 2011. Microbial transformations of arsenic in the subsurface. In *Microbial Metal and Metalloid Metabolism: Advances and Applications*, pp. 77–90. Washington, DC: ASM Press.
30. Stolz, J.F. Overview of microbial arsenic metabolism and resistance. In *The Metabolism of Arsenite*, pp. 55–60. Leiden, the Netherlands: CRC Press, 2012.
31. Saltikov, C.W. Regulation of arsenic metabolic pathways in prokaryotes. In *Microbial Metal and Metalloid Metabolism*, pp. 195–210. Washington, DC: ASM Press, 2011.
32. Heath, M.D., Schoepp-Cothenet, B., Osborne, T.H., and Santini, J.M. Arsenite oxidase. In *The Metabolism of Arsenite*, pp. 81–97. Leiden, the Netherlands: CRC Press, 2012.
33. Santini, J.M., Kappler, U., Ward, S.A. et al. The NT-26 cytochrome C552 and its role in arsenite oxidation. *Biochim. Biophys. Acta* 1767 (2007): 189–196.
34. Vanden Hoven, R.N. and Santini, J.M. Arsenite oxidation by the heterotrophy *Hydrogenophaga* sp. Str. NT-14: The arsenite oxidase and its physiological electron acceptor. *Biochim. Biophys. Acta* 1656 (2004): 148–155.
35. Oremland, R.S., Hoeft, S.E., Santini, J.M. et al. Anaerobic oxidation of arsenite in Mono Lake water and by a facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1. *Appl. Environ. Microbiol.* 68 (2002): 4795–4802.
36. Cullen, W.R. and Reimer, K.J. Arsenic speciation in the environment. *Chem. Rev.* 29 (1989): 713–764.
37. Challenger, F. Biological methylation. *Adv. Enzymol.* 12 (1951): 429–491.
38. Qin, J., Rosen, B.P., Zhang, Y. et al. Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. *Proc. Natl. Acad. Sci.* 103 (2006): 2075–2080.

39. Jie, Q., Lehr, C.R., Yuan, C. et al. Biotransformation of arsenic by a Yellowstone thermoacidophilic eukaryotic alga. *Proc. Natl. Acad. Sci.* 106 (2009): 5213–5217.
40. Martin, A. *Introduction to Soil Microbiology*. New York: John Wiley & Sons, 1977.
41. Pickett, A.W., McBride, B.C., Cullen, W.R., and Manji, H. The reduction of trimethylarsine oxide by *Candida humicola*. *Can. J. Microbiol.* 27 (1981): 773–778.
42. Huysmans, D.K. and Frankenberger, W.T. Evolution of trimethylarsine by a *Penicillium* sp. Isolated from agricultural evaporation pond water. *Sci. Total Environ.* 105 (1991): 13–28.
43. Baker, A.J., McGrath, S.P., Reeves, R.D., and Smith, J.A.C. Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In *Phytoremediation of Contaminated Soil and Water*, pp. 85–107. Boca Raton, FL: Lewis Publishers, 2000.
44. Schat, H., Llugany, M., and Berhard, R. 2002. Metal-specific patterns of tolerance, uptake, and transport of heavy metals in hyperaccumulating and non hyperaccumulating metallophytes. In *Phytoremediation of Contaminated Soil and Water*, pp. 171–188. Boca Raton, FL: Lewis Publishers.
45. Ma, L., Komar, K.M., Tu, C. et al. A fern that hyperaccumulates arsenic—A hardy, versatile, fast-growing plant helps to remove arsenic from contaminated soils. *Nature* 409 (2001): 579.
46. Tu, C. and Ma, L. Effects of arsenate and phosphate on their accumulation by an arsenic hyperaccumulator *Pteris vittata* L. *Plant Soil* 249 (2003): 373–382.
47. Luongo, T. and Ma, L. Characteristics of arsenic accumulation by *Pteris* and non-*Pteris* ferns. *Plant Soil* 277 (2005): 117–126.
48. Rensing, C., Ghosh, M., and Rosen, B.P. Families of soft-metal-ion-transporting ATPases. *J. Bacteriol.* 181 (1999): 5891–5897.
49. Zhao, F.J., Wang, J.R., Barker, J.M.A. et al. The role of phytochelatins in arsenic tolerance in the hyperaccumulator *Pteris vittata*. *New Phytol.* 159 (2003): 403–410.
50. Yong, C., Su, J., and Ma, L.Q. Low molecular weight thiols in arsenic hyperaccumulator *Pteris vittata* upon exposure to arsenic and other trace elements. *Environ. Pollut.* 129 (2004): 69–78.
51. Zhao, F.J., Dunham, S.J., and McGrath, S.P. Arsenic hyperaccumulator by different fern species. *New Phytol.* 156 (2002): 27–31.
52. Meharg, A.A. Variation in arsenic accumulation-hyperaccumulation in ferns and their allies. *New Phytol.* 157 (2003): 25–31.
53. Wang, H.B., Wong, M.H., Lan, C.Y. et al. Uptake and accumulation of arsenic by 11 *Pteris* taxa from Southern China. *Environ. Pollut.* 145 (2007): 225–233.
54. Kertulis-Tartar, G.M., Ma, L., Tu, C., and Chirenje, T. Phytoremediation of an arsenic-contaminated site using *Pteris vittata* L.: A two-year study. *Int. J. Phytoremed.* 8 (2006): 311–322.
55. Zhao, F.J., McGrath, S.P., and Meharg, A.A. Arsenic as a food chain contaminant: Mechanisms of plant uptake and metabolism and mitigation strategies. *Annu. Rev. Plant Biol.* 61 (2010): 535–559.
56. McGrath, S.P. and Zhao, F.J. Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotechnol.* 14 (2003): 277–282.
57. Cai, Y. and Ma, L.Q. Metal tolerance, accumulation, and detoxification in plants with emphasis on arsenic in terrestrial plants. In *Biogeochemistry of Environmentally Important Trace Elements*, pp. 95–114. Washington, DC: American Chemical Society, 2003.
58. Chen, Y.S., Xu, W.S., Shen, H.L. et al. Engineering arsenic tolerance and hyperaccumulation in plants for phytoremediation by a PvACR3 transgenic approach. *Environ. Sci. Technol.* 47 (2013): 9355–9362.