Biogeochemical Cycling of Arsenic in Soil–Plant Continuum: Perspectives for Phytoremediation

Hossain M. Anawar, A. García-Sánchez and M. Zabed Hossain

Abstract The bioavailability of arsenic (As) to plants and their toxicity effect are greatly influenced by physicochemical properties of soils, leaching ability and phosphate content in soils. The dynamics of As in the rhizosphere of plants that are controlled by soil pH, redox potential, organic matter, microbes, and phosphate status (in plant and soil) have been discussed in this review study. Alteration of rhizosphere pH (higher or lower) by plant roots contributes to the mobilization of As and the consequent toxic effect on plant growth. It is clearly observed that increased As solubility under reduced conditions is associated with dissolution of Fe and Mn oxides/hydroxides. Arsenic mobility and bioavailability in soil may be influenced by the content and type of soil organic matter. The rate of As cycling depends on the microbial community present and on its metabolic activity. The predominant As forms in plant tissue are the inorganic As species (arsenate As(V) and arsenite As(III)), although there is a small portion (<1 %) of organic As species detected in the shoots. The formation of As–PCs, As–glutathione, and As–MTs complexation is a mechanism of detoxification and sequestration of As into vacuoles. Phytostabilization of contaminated soil using plant species could provide a good soil cover, prevent wind and water erosion by developing an extensive root system, and immobilize the contaminants in the rhizosphere. Phytoextraction using some plants (most notably, the *Pteris* genus and *Pityrogramma calomelanos*) has been proposed as an effective tool in stripping As from soil. Transgenic plants are being developed to improve the capacity of plants to accumulate As. Here, recent

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H. M. Anawar
School of Earth and Environment, The University of Western Australia,
Crawley, WA 6009, Australia

A. García-Sánchez (✉)
Department of Environmental Geochemistry, IRNASA-CSIC, Salamanca, Spain
e-mail: antonio.gsanchez@irnasa.csic.es

M. Zabed Hossain
Department of Botany, University of Dhaka, Dhaka 1000, Bangladesh

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research developments in the analysis of As hyperaccumulation, tolerance, metabolism, and detoxification in plants at the physiological, molecular, and genetic levels are also highlighted.

Keywords Arsenic contamination · Dynamics of arsenic · Cleanup of soil · Phytoremediation

Contents

1 Introduction

Arsenic (As) contamination in soils, water, and food chain is a global health concern due to its toxicity effect even at very low concentrations. Inorganic As was classified (IARC 2004) as group 1 carcinogenic. The World Health Organization (WHO) has set concentration limits for drinking water at 10 $\mu g\ l^{-1}$ and for foodstuffs (0.5–1 mg kg^{-1} DW) set by legislation of many countries, e.g., Germany, Australia, UK, India, and The Netherlands (FAC 1975; WHO 1989; ANFA 1993).

About 25 different As compounds have been identified in biological samples, mainly in marine ecosystems (Francesconi and Kuehnelt 2002). However, usually, only the organic species monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) are found in detectable concentrations in soils besides abundant inorganic As(V) and As(III) species (Takamatsu et al. 1982). Paddy soils typically show larger extractable concentrations of MMAA and DMAA suggesting that methylated arsenicals are produced under anaerobic conditions (Takamatsu et al. 1982). As is causing a global epidemic of poisoning, with tens of thousands of people having developed skin lesions, cancers, and other symptoms (Pearce 2003).

In fact, already many people have died and hundreds of millions are now thought to be at serious risk in countries throughout the world such as China, India, Vietnam, and Bangladesh (Rahman et al. 2001; Chakraborti et al. 2003). About 1.4 million potentially contaminated sites have been identified within the European Community that are affected to various degree by trace metal/metalloid and/or organic pollutants. What is more, pollution by As and other trace metals in water, soils, vegetables, and plants (food chain) at a large scale can still occur in different countries. The mining and smelting activities in these zones have led to contamination of the agricultural land, with top soil As values as high as several thousand mg kg^{-1} due to weathering of arsenopyrite to scorodite and hydrous ferric oxides (HFO) which attenuate the As dispersion around mine spoils (García-Sánchez et al. 1996; Anawar et al. 2006, 2010; Casado et al. 2007a, b). By contrast, As contents in noncontaminated soils are typically well below 10 mg kg^{-1} (Fitz and Wenzel 2002; García-Sánchez and Alvarez Ayuso 2003).

Given the ecological and human health hazards of As, scientists are conducting researches for development of different technologies for the in situ cleanup of arsenic-contaminated soils and groundwater (Table 1). However, most of these technologies currently available for the remediation of metal/metalloid contaminated soils are expensive, unaffordable, and not feasible and sustainable; and they can create health risks to workers and produce high volumes of toxic sludge and secondary waste. Therefore, development of phytoremediation to clean up the contaminated soils, water, leachate, municipal, and industrial wastewater with green plants for low cost has attracted considerable attention (Schneegurt et al. 2001; Krämer 2005; Fitz and Wenzel 2002; McGrath and Zhao 2003). Understanding how plants accumulate and store metal ions is relevant to two important applications: metal nutrition for humans and in agriculture; and metal detoxification using plants as biological detoxification systems for the ‘phytoremediation’ of metal contamination in the environment (Cobbett 2003).

Moreover, the dynamics of As in the rhizosphere are controlled by plant phosphate status, regulation of arsenate uptake via phosphate transporters, and regulating iron plaque formation. As cycling is also controlled by soil redox potential, interconversion between As species, microbial oxidation or reduction, and methylation of As, producing MMAA and DMAA (Fig. 1), which are poorly transported across the plasmamembrane of root epidermal cells (Abedin et al. 2002). Soil mineralogy will also play an important role, regulating the soil solution concentration of As species because of surface binding and precipitation of poorly soluble As salts (Fitz and Wenzel 2002).

2 Bioavailability of Arsenic to Plants

Plants possess unique system that acquire and concentrate essential and nonessential elements as well as numerous metabolic activities, all of which are ultimately powered by photosynthesis (Krämer 2005). The phytotoxicity of As is

Table 1 Methods for remediation of arsenic from soil and their advantages and disadvantages

Methods	Principle	Advantages/disadvantages
Stabilization/solidification/fixation	Involves the addition of binding agents to a contaminated material to impart physical/dimensional stability to contain contaminants in a solid product and reduces hazard potentials	Fast and easy applicability, relatively low cost. Disadvantages include high invasivity to environment, generation of significant amount of solid waste, additional tasks for by-products management and control, risk of contamination
Vitrification	High temperature treatment that reduces the mobility of metals resulting in the formation of vitreous material, usually an oxide solid that is chemically durable and leach resistant	The vitrified waste may be recycled and used as clean fill, aggregate, or other reusable materials. However, the method is expensive because of the nature of energy dependence
Soil washing/acid extraction	Involves separation of soil particles that host majority of contamination from the bulk soil and removing contaminants from the soil by aqueous chemicals	Ex situ techniques are efficient and rapid. Disadvantages include the fact that the contaminants are simply moved to a different place where they must be monitored, the risk of spreading contaminated soil, and high cost
Pyrometallurgical recovery	Conversion of contaminated waste into a product with high concentration of contaminants by applying heat	Products (pure metals) may be reused. But the method is expensive and may not be feasible for large agricultural field
In situ soil flushing	Flush away contaminants from soil by flowing flushing solution	Method is easy to apply. There is a risk of degradation of soil quality due to the application of chemicals and the method may not be feasible for soils of a large field
Phytoremediation	Hyperaccumulation and hypertolerance of plants to As, transgenic plants with As resistance and hyperaccumulation gene	Methods are environment friendly, easy to apply and control, and less expensive. Disadvantages include management cost of the As accumulated plants
Geomicrobial process	Microbial transformation of As from toxic to nontoxic form, growth stimulation, and improved nutrition of plants by microbes	Methods are environment friendly and less expensive. However, the method is difficult to be monitored for their efficiency

affected considerably by the chemical form in which it occurs in the soil and concentration of the metalloid; water soluble form being more phytotoxic than other firmly bound forms (Patra et al. 2004), and might be a good guide to assess the bioavailable As in the soils (Camm et al. 2004). As accumulation in plants is greatly influenced by a number of factors (Fig. 1) including physicochemical properties of the soil, which affect the plant growth by toxicity effect, and bioavailability of As and growth limiting nutrients (Mkandawire and Dudel 2005).

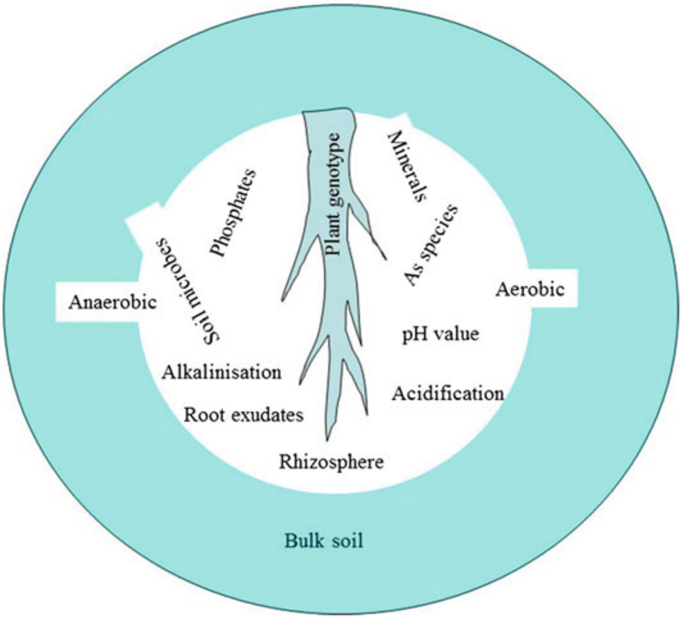

Fig. 1 Factors influencing the availability of arsenic to plant roots

Genotypic differences in plants are also likely to vary in their responses to various interactions with As and soil environment.

Different soil chemical extraction methods, including single chemical extractions and sequential extractions are available to extract various pools of soil As (Anawar et al. 2008), but their ability to quantify the amount of plant-available As from soil is still uncertain. However, a good relationship exists between water soluble As in soil and As in plants. Therefore, water-soluble As in soil solution may be considered as an indicator of the bioavailable As pool. However, the $(NH_4)_2SO_4$-extractable soil As content indicates the readily labile As amount, correlating well with As concentrations in field-collected soil solutions and in plant shoots (Wenzel et al. 2001). In mining polluted soils, the strongest relationship between As determined by single soil chemical extraction and As in plant biomass was found for sodium acetate (amorphous Fe–Mn oxides fraction) and mixed acid (0.05 N HCl and 0.025 N H_2SO_4) extractant (weakly adsorbed to surface of soil particles + water-soluble) (Anawar et al. 2008). The critical level of As for water-soluble forms is 0.04 mg kg^{-1} in soils (Bohn et al. 1985), although the concentration in soil solution (under toxic conditions) is generally very low (Anawar et al. 2008; García-Sánchez et al. 2010). However, Xu and Thornton (1985) and Cao and Ma (2004) found the maximum water-soluble As of 2.78 % of total As in polluted garden soils and 3.02–13.6 % in the industrially chromated copper arsenate contaminated soils, respectively, that far exceed the water-soluble As contents <1 % of total As found in mining sites (Anawar et al. 2008; García-Sánchez et al. 2010).

The water solubility of As in soils is irrespective of the soil total As content, but depends on the soil characteristics, such as texture, pH, Eh, organic carbon, and minerals of the soil. If the original minerals in the deposition of mine spoils are altered little, then the solubility and bioavailability of As in contaminated soil, mine tailings, and spoils will be reduced drastically (Warren et al. 2003). The low water-soluble fraction of As indicates that As is strongly bound to a solid-phase, predominantly in the Fe–Mn oxide (FM–As) and sulfide aluminosilicate (SAlS-As) phase; and very low contents of As are present in water-soluble, exchangeable, and carbonate fraction (WEC-As) and major fractions of the As contamination is immobile in mine tailings and spoils.

Adsorption of As(V) on soil mineral surfaces is a very important process that affects its mobility and fate in the environment, as well as its bioavailability. Hydroxyl groups on the surface of many soil minerals are the most abundant and reactive adsorption sites, and consequently oxides and hydroxides (Fe, Al, Mn) have a strong affinity for As(V). This adsorption capacity is pH dependent and shows maximum values at pH $\sim$ 4–5 (García-Sánchez et al. 2002), since these adsorption reactions of As by the ligand exchange mechanism are favored by low pH, as shown by the release of OH^- into solution. In addition, low pH also causes the protonization of surface-OH groups to–OH_2^+, which facilitates the ligand exchange, since H_2O is easier to displace from metal binding sites than OH^- (McBride 1994). A large number of reported studies on the As adsorption on oxides, oxyhydroxides, and soils show great adsorption capacities of oxyhydroxides with a maximum adsorption around pH 3–5. So, because of the high As adsorption capacity of Fe oxyhydroxides in the pH range of the soils, the mobility of As around the mine spoils is low and the areas of polluted soil are limited to narrow zones. The rise of the pH values of soil due to the amendment (lime, etc.) addition may account for the reduction in the As sorption capacity of the soil and the increase of its mobility. The lower mobility of As is presumed to be the strong binding of As with the surface of oxide minerals by the formation of inner-sphere mononuclear or binuclear monodentate–bidentate complexes (Fendorf et al. 1997).

A change of pH to more alkaline conditions due to the addition of amendments such as lime or calcareous sands could also lead to increased As solubility (Masscheleyn et al. 1991). Although the extent of uptake by plants varies depending upon various factors, the literature reviews indicate that the concentrations of As in plants increase with increasing those in soils (Adriano 2001; Ullah et al. 2009). Although As toxicity effects on plants increase under more acid conditions when As-binding species become more soluble, the uptake of As by plants may be increased in higher pH soils (O’Neill 1990). As uptake by plants is largely dependent on the source, chemical speciation, pedological factors (pH, Eh, organic matter, soil texture, minerals, and drainage conditions), plant species, and age and part of plants (García-Sánchez et al. 1996). The use of P fertilizers on agricultural soils has the potential to greatly enhance the mobilization of As (Cao and Ma 2004; Szegedi et al. 2010), especially in the absence of active plant growth. Hence, attempts to use plants to remove As from soils need to take the multiple effects of phosphate into consideration.

3 Fate of Arsenic as Related to Rhizosphere pH

Factors affecting rhizosphere pH are the source of nitrogen supply (NO_3^- vs. NH_4^+ uptake), nutritional status of plants (e.g., Fe and P deficiency), excretion of organic acids, CO_2 production by roots and rhizosphere microorganisms, and the buffering capacity of the soil (Marschner 1995). Alteration of rhizosphere pH by plant roots is also known to contribute to the mobilization of As and toxic effect on plant growth (Fitz and Wenzel 2002). Most soils exhibit toxic conditions; hence an increase of rhizosphere pH could favor mobilization of labile adsorbed and exchangeable As(V)-fractions in the root vicinity and consequently enhance plant uptake. Plant growth reduced water-soluble As and increased soil pH in the rhizosphere soil (Silva Gonzaga et al. 2006). The ability of *Pteris vittata* to exude large quantities of dissolved organic carbon and to change the rhizosphere pH enhanced the As bioavailability in soils, thereby increasing its As uptake (Tu et al. 2004). Hence, fertilization of plants grown on As-contaminated soil with NO_3^- as the N source, would potentially increase rhizosphere pH, and thus possibly enhance As accumulation in plant tissues (Marschner and Römheld 1983). Rhizosphere acidification via net H^+ released by N_2-fixing symbionts (e.g., legumes and actinorhizal plants) may dissolve As sorbents such as Fe oxides/hydroxides, and would favor As(V) mobilization in soil. The As hyperaccumulator *P. vittata* was reported to prefer calcareous soils of neutral to slightly alkaline pH (Jones 1987; Ma et al. 2001). This implies that changes of rhizosphere pH would be no prerequisite for As hyperaccumulation due to the high pH-buffer power of calcareous soils. However, *P. vittata* and *Pityrogramma calomelanos* have been as well found on acidic soils and mine tailings in Thailand, respectively. The main As(V) species in soil at normal pHs are $H_2AsO_4^-$ and $HAsO_4^{2-}$, and their adsorption (by ligand exchange reaction) on oxide and oxyhydroxide surfaces is favored when the net charge of these mineral surfaces is positive, i.e., when $pH < pHpzc$ (Sadiq et al. 1983).

4 Fate of Arsenic as Related to Rhizosphere Redox Potential

Among the chemical forms or species of As in soils such as As(V), As(III), MMAA ($(CH_3)AsO(OH)_2$), and DMAA ($(CH_3)_2AsO(OH)$), arsenate predominates under oxidizing conditions (Sadiq et al. 1983; Haswell et al. 1985). Arsenite predominates under paddy conditions, but arsenate, MMAA, and DMAA are also present in some quantities (Abedin et al. 2002). It has been known that plants adapted to grow on anaerobic (reducing) flooded soils, and oxygenate their rhizosphere resulting in the formation of an iron oxyhydroxide plaque. The redox potential significantly influences speciation and solubility of As in rhizosphere (Masschelyn et al. 1991; Marin et al. 1993; Onken and Hossner 1996). Generally, inorganic As is mainly present as As(V) in aerobic conditions (high redox potential) and as As(III) in flooded (low redox potential) soils. As is less toxic and less mobile in the +V than in the +III oxidation state. It has been repeatedly observed that increased As solubility under reduced conditions is associated with dissolution of Fe and Mn oxides/hydroxides. Significant correlations have been found between dissolved Fe and As (Masschelyn et al. 1991; Marin et al. 1993), confirming that Fe oxides/hydroxides represent the major sorbing agents for As in soils. Under oxidized conditions, soluble As concentrations were three times larger at pH 8 than at pH 5, because of the decreased positive surface charge at $pH > pH_{pzc}$. Under reducing conditions, As(III) became the major dissolved species with total soluble As being smaller at pH 8. Dissolved Fe concentrations did not significantly increase upon reduction at pH 8 (Masschelyn et al. 1991). In contrast, Marin et al. (1993), using the same experimental set up, reported increased As solubility upon pH decrease (7.5–5.5) for both reduced and oxidized conditions without providing any explanation. As concentrations in rice (*Oryza sativa* L.) increased upon decreasing redox potential (Marin et al. 1993). The oxidation of the rhizosphere is a well-known phenomenon for paddy rice as these plants are able to transport O_2 through aerenchyma to roots, which results in leakage of O_2 into the rhizosphere (Flessa and Fischer 1992). Rice roots grown in reduced suspensions were coated with Fe plaque containing As (Marin et al. 1993). Doyle and Otte (1997) found formation of Fe plaque also around roots of salt marsh plants which led to an effective fixation and consequently detoxification of As in the rhizosphere.

5 Fate of Arsenic as Related to Soil Organic Matter

As mobility and bioavailability in soil and aquatic environments may be influenced by the content and type of soil organic matter (OM) (Saada et al. 2003; Bauer and Blodau 2006). Natural organic matter (OM) contents of soil and combination of functional groups, such as carboxylic, phenolic, quinona, amino, hydroxyl, sulphydryl, etc., determine the mechanism of As association in each case. Dissolved organic matter (DOM) concentration correlated well with As concentration in aqueous extracts of soil (Kalbitz and Wennrich 1998). The DOM can facilitate As transport in soil by acting as a “carrier” through the formation of soluble As–organic complexes. The binding of As to DOM has been documented (Chen et al. 2006; Dobran and Zagury 2006; Hake et al. 2007). The formation of arsenate complexes with carboxylic groups has been proposed (Lin et al. 2004; Wang and Mulligan 2006) in spite of the negative charge of arsenate anion at circumneutral pH; complexation is possible through a metal bridging mechanism with cations such as Fe(III) and Ca(II) (Ritter et al. 2006). Arsenite could form a negatively charged complex with a carboxylic group where H-bridges stabilize it.

The phenolic groups can make a ligand exchange bonding with arsenate (Buschmann et al. 2006). Other authors (Saada et al. 2003) suggest that the major anion retention sites on DOM are positively charged amino groups, which have electrostatic attraction to arsenate ($H_2AsO_4^-$) at around pH 7. On the other hand, fulvic or humic acids form stable complexes with mineral surfaces (Kaiser et al. 1997) effectively blocking As adsorption. Therefore, competitive adsorption for binding sites on soil between arsenate and OM could inhibit the As adsorption and propitiate its mobilization. Weng et al. (2009) studied the effects of fulvic acids on arsenate adsorption to goethite, and concluded that fulvic acids reduced the arsenate retention by competition. Likewise, simple aliphatic acids with low molecular weight excreted by plant roots (acetic, fumaric, oxalic, citric, etc.) might also mobilize adsorbed As on oxyhydroxides by competing for reactive sites, or by dissolution of oxyhydroxides (Mohapatra et al. 2006; Shi et al. 2009).

6 Role of Soil Microbes

Soil microbes can methylate inorganic As to give monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) (Meharg and Hartley-Whitaker 2002). Leung et al. (2006) reported that the indigenous arbuscular mycorrhizas enhanced As accumulation in the As mine populations of *P. vittata* and also sustained its growth by aiding P absorption. For *Cynodon dactylon* (a multi-metal root accumulator), As was mainly accumulated in mycorrhizal roots and translocation to shoots was inhibited. Indigenous mycorrhizal fungi played an important role in As tolerance.

Endophyte fungi can positively affect germination performance of *Festuca rubra* up to an As concentration of 25 mg l^{-1} in solution (Vazquez de Aldana et al. 2013). A strain of the species *Rahnella aquatilis* (c-Proteobacteria class) exhibited strong endurance to As in polluted mining soils in Spain (Valverde et al. 2011). Arsenic-resistant bacteria with strong M(III) oxidizing ability can have the potential to improve bioremediation of As(III) contaminated water using *P. vitatta* (Wang et al. 2002). Rhizobacteria increased significantly the biomass and As content of *P. calomelanos* (Jankong et al. 2007).

In soils, some parts of total As is present as organic bound form, rather than inorganic ions. Plant mineral nutrition depends primarily on the uptake of inorganic minerals. However, these organic matter-bound soil As is also plant-bioavailable, probably due to the interconversion of carbon-bonded As into inorganic ions by soil microbes. The rate of As cycling depends on the microbial community present, and on its metabolic activity, though it is not yet known if specific microbial species or genera control this process.

7 Arsenic–Phosphorus Interaction

If the ligand exchange theory and share charge hypothesis for oxyanion sorption on soil mineral surfaces is considered (McBride 1994), the anion $HAsO_4^{2-}$ adsorption is disfavored by competition with anion phosphate. So, As might be mobilized in presence of phosphate in aerobic soils. However, as As uptake by plants is associated with the phosphate uptake mechanism (Pickering et al. 2000), phosphate inhibits efficiently As uptake by plants due to the competition between them (Meharg et al. 1994). In addition, the ability to accumulate more P under As stress may contribute to the efficiency of hyperaccumulator plants in As accumulation (Silva Gonzaga et al. 2009). Due to the existence of high- and low-affinity Pi transport systems, plants adapt their growth to conditions of limited Pi supply through the activation of high-affinity Pi transport, which enhances Pi acquisition (Rausch and Bucher 2002). The arsenate uptake is increased through Pi transporter overexpression, and As tolerance is enhanced through YCF1-mediated vacuolar sequestration (LeBlanc et al. 2013). As and P concentrations in fronds and roots in Chinese brake fern were negatively correlated and phosphate uptake was significantly inhibited by As(V) (Lou et al. 2010). Use of a synchrotron X-ray microprobe showed that As(V) and P were co-transported in *P. vittata* and that this process could be enhanced by As(V) exposure or P deficiency (Lei et al. 2012).

It has been reported that P-deficient plants show an enhanced exudation of carboxylic acids, such as oxalic, citric, and malic acid (Neumann and Römheld 1999). This response is thought to change soil pH, to displace P from sorption sites, resulting in enhanced availability of P (Kirk et al. 1999). Hence, it is reasonable to assume that carboxylate exudation could play a role in the mobilization of As in the rhizosphere and enhance As uptake by plants. In conclusion, it seems that P, Fe, and As uptake by As hyperaccumulator species may be related to each other. Reductive dissolution of Fe oxyhydroxide minerals inevitably dissolves Fe-bound As; therefore, root exudates enhancing P mobilization are likely to desorb As as well. Besides rhizosphere processes, As hyperaccumulator most likely possess a particular As uptake mechanism whereas suppression of the high-affinity phosphate uptake system is involved in adaptive tolerance of plants to As. The phosphate ion plays a prominent role in anion–As interactions due to its physicochemical similarity to As (Adriano 2001). In a hydroponic experiment, increasing the phosphate supply decreased the As uptake in *P. vittata* markedly, with the effect being greater on root As concentration than on shoot concentration. Wang et al. (2002) concluded that As(V) is taken up by *P. vittata* via phosphate transporters, reduced to As(III), and sequestered in the fronds primarily as As(III). At neutral pH, arsenite is uncharged and behaves as a water analog with respect to plasma membrane transport, previously observed in yeast (Wysocki et al. 2001).

8 Arsenic Accumulation in Crops

The concentrations of As in edible plants, especially crops are generally low, follow the gradient: roots > stems > leaves, even when the crops are grown on contaminated land (O'Neill 1990; Ullah et al. 2009). It is generally accepted that concentrations in edible plants grown on uncontaminated or unmineralized soils are 0.01–1.5 mg As kg^{-1} dry weight (DW) (Bowen 1979). Xu and Thornton (1985) reported that the concentrations of As in vegetables grown on contaminated soils by mining and smelting activities averaged 0.85 mg kg^{-1} (DW) in lettuce, 0.21 in carrot, 0.20 in onions, and 0.04 in pea and bean. Elevated As contents of 3.9–5.4 mg kg^{-1} (DW) were reported in root samples of sugar beet and potatoes (Moyano et al. 2009), 3–7 mg kg^{-1} in leaves and roots of carrots (Mayorga et al. 2013), and up to 0.3 mg kg^{-1} in Bangladesh market vegetables (Anawar et al. 2012) when they were grown on land irrigated with As-rich groundwater. These As contents were higher than the limits for foodstuffs (0.5–1 mg kg^{-1} DW) set by legislation of many countries, reflecting the risk of food chain contamination by As. Grains of wheat, rye, and barley in Europe contain <1 mg As kg^{-1}; albeit it was reported that As content in rice globally ranges between 0.03 and 1.83 mg kg^{-1} with the higher contents in samples from Bangladesh, India, and USA. The higher accumulation in rice could be due to the soil anaerobic conditions and consequently higher fraction of As(III) whose uptake occurs via aquaporin channels (Ma et al. 2008). Rice is a more efficient accumulator of As than any other cereal crops. Recent findings from the FDA (USA Food and Drugs Administration) found the average levels of inorganic As up to 3.5 mg kg^{-1} in rice (Hide 2013).

9 Coordination Environment of Arsenic in Plant Tissue

Speciation of As in tissues of tolerant and nontolerant clones of *Holcus lanatus* revealed the predominant As forms as the inorganic As species (arsenate As(V) and arsenite As(III)), although small portion (<1 %) of organic As species were detected in shoot material (Quaghebeur and Rengel 2003). The X-ray absorption spectroscopy (e.g., XANES and EXAFS) study on the coordination environment and speciation of As indicated that As is predominantly accumulated as As(III) in the fronds of *P. vittata* (L.), and As(V) can be reduced to As(III) in both roots and shoots (Webb et al. 2003; Kertulis et al. 2005). At extremely high As concentrations (ca. 1 % As DW), As in the fern fronds is coordinated to a significant degree by sulfur in addition to oxygen indicating that thiol-rich compounds are implicated in the biochemical transformations of As within the plant. As is compartmentalized mainly in the upper and lower epidermal cells, probably in the vacuoles in *P. vittata* (Lombi et al. 2002).

Based on the results of X-ray absorption spectroscopy, Pickering et al. (2000) reported that As was taken up by roots as oxyanions [As(V) and As(III)], and a small fraction was exported to the shoot via xylem in Indian mustard (*Brassica juncea*). As was identified as As-III-tris-thiolate and As-III-tris-glutathione complex in shoot and root, where the thiolate donors were probably either glutathione or phytochelatins. Raab et al. (2004) reported that As preferred the formation of arsenite [As(III)]–PC3 complex over GS–As(III)–PC2, As(III)–(GS)3, As(III)–PC2, or As(III)–(PC2)2 (GS: glutathione bound to As via sulfur of cysteine) in *H. lanatus*, while *P. cretica* formed dominantly the GS–As(III)–PC2 complex. In both plant species, As was dominantly in nonbound inorganic forms, with 13 % being present in PC complexes for *H. lanatus* and 1 % in *Pteris cretica*.

10 Detoxification of Arsenic in Plants

Plants can respond to As(V) stress (ATP, oxidative stress) by stimulating the antioxidant system and to As(III) stress by thiol metabolism (Mishra et al. 2008). Synthesis of low molecular weight thiol compounds was related to As exposure in *P. vittata* (Cai et al. 2004). The *Cicer arietinum* L. and soybean plant tolerated and detoxified As through chelation with GSH, PCs, and hPCs (Gupta et al. 2008; Vazquez et al. 2009). In addition to PCs, metallothioneins (MTs) have the capacity of binding As through the thiol group of cysteine (Chakrabarty et al. 2009). Duan et al. (2005) reported that an arsenate reductase (AR) in the fern (*P. vittata*) showed a reaction mechanism similar to the previously reported Acr2p, an AR from yeast (*Saccharomyces cerevisiae*), using glutathione as the electron donor and AR plays an important role in the detoxification of As in the As hyperaccumulating fern.

Indriolo et al. (2010) characterized two *P. vittata* genes, ACR3 and ACR3;1, which encode proteins similar to the ACR3 arsenite effluxer of yeast, and showed that ACR3 localizes to the vacuolar membrane in gametophytes, indicating that it likely effluxes arsenite into the vacuole for sequestration. In addition to complexation (glutathione, PCs, and MTs) as a mechanism of detoxification, plants can restrict translocation from root to shoots and sequestration of As into vacuoles in the form of complex or ions (Gupta et al. 2011).

11 Phytoremediation by Hyperaccumulating Plants

Phytoremediation, that is a cost effective, promising and environment friendly technology, is gaining a lot of importance in recent times, and it will be an useful weapon in the fight against the trace elements and organic chemical contamination in soil and water.

In soil phytoremediation, there are several strategies such as, phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization (Salt et al. 1998; Raskin and Ensley 2000). Phytostabilization aims to provide a good soil cover and prevent wind and water erosion by developing an extensive root system, and immobilize the contaminants in the rhizosphere. In this way, transpiration and root growth help to immobilize contaminants, including As by reducing leaching, controlling erosion, creating an aerobic environment in the root zone, and adding organic matter to the substrate that binds As. Phytostabilization of mining sites is a well-established environmental compliance using plant species that adapt different strategies such as metal tolerance, metal accumulation in roots, and metal exclusion in aerial parts avoiding risk for fauna and cattle (Dahmani-Muller et al. 2000; Otones et al. 2011a, b).

The phytoextraction involves the cultivation of hyperaccumulator plants that can tolerate and accumulate very high concentrations of soil and water contaminants in their above-ground tissues (Krämer 2005). The concentrations of trace elements in dry leaf biomass of metal hyperaccumulator plants are usually up to 100-fold higher than the concentrations in the soil (McGrath and Zhao 2003). Worldwide metal hyperaccumulating plant species have been identified in at least 45 plant families and 400 plant species (Kraemer 2003). The minimum concentration of As required for a plant to be classified as a hyperaccumulator of As was set at 1,000 mg kg^{-1} (0.1 %) on a dry weight basis (Ma et al. 2001). While some plants can survive in an environment containing extremely high concentrations of metals, they do not show a high ability of accumulating metals. *Agrostis tenuis* growing on As mine wastes contained 3,470 mg kg^{-1} As, although As concentration was as high as 26,500 mg kg^{-1} in the corresponding soils (Benson et al. 1981). It seems that this species is a "hypertolerant" species, rather than a true hyperaccumulator.

As concentration in terrestrial plants rarely exceeds 1 mg kg^{-1} DW. The ''As reference plant value'' is 0.1 mg kg^{-1} DW (Markert 1997). Some grass species and a few fern species are known to accumulate or hyperaccumulate high As contents in their biomass. Some species of the *Agrostis* genus growing on contaminated soils have been found to accumulate and tolerate high As concentrations up to 6,640 mg kg^{-1} (DW) in the old leaves of *Agrostis canina* and *Agrostis tenuis* (Porter and Peterson 1975), 1,350 mg kg^{-1} in *Agrostis stolonifera* (Porter and Peterson 1977), 1,900 mg kg^{-1} in *Agrostis castellana,* and 1,800 mg kg^{-1} in *Agrostis delicatula* (de Koe 1994), 3,475 mg kg^{-1} in *A. castellana* (García-Sánchez et al. 1996). *Pseudosuga taxifolia* growing on soils of contaminated areas was able to accumulate As concentration up to 8,200 mg kg^{-1} (Warren et al. 1968). Aquatic plants such as *Ceratophyllum demersum*, *Egeria densa* and *Potamogeton pectinatus* accumulated As up to 1,160, 1,120, and 4,990 mg kg^{-1}, respectively, without any apparent damage (Dushenkov and Kapulnik 2000). *Scirpus holoschoenus* L. highlights for its important phytostabilization potential since it accumulates As up to 3,164 mg kg^{-1} in roots, maybe in iron plaque (Otones et al. 2011a). *Rumex acetosella* L. could behave as an index plant, reaching above-ground As concentrations up to 218 mg kg^{-1} in polluted mining soils (Otones et al. 2011b).

Chinese brake (*P. vittata* L.) fern accumulated up to 23 g kg^{-1} when grown on an As-spiked soil (Ma et al. 2001). Another fern [*P. calomelanos* (L.)] has also been reported to hyperaccumulate As up to 8,350 mg kg^{-1} from soil containing 135 mg kg^{-1} of As. Both these species are grown in tropical and subtropical regions of the world (Francesconi et al. 2002) and have the potential for phytoremediation of As-contaminated soil. Zhao et al. (2002) identified that, in addition to *P. vittata,* three more new species such as *P. cretica, Pteris longifolia,* and *Pteris umbrosa* in the *Pteris* genus also hyperaccumulate As to a similar extent. They found that there was little difference between *Pteris* species, or between different accessions of *P. vittata* reflecting As hyperaccumulation as a constitutive property in *P. vittata*. (Meharg and Hartley-Whitaker 2002) addressed the evolutionary basis of As hyperaccumulation in ferns, outlining that primitive life forms, including early land flora, could have evolved in an As rich environment. However, Meharg (2003) identified two fern species (*Pteris straminea* and *P. tremula*) belonging to the *Pteris* genus as nonhyperaccumulator for As also.

Gumaelius et al. (2004) reported that gametophytes of *P. vittata* hyperaccumulate As in a similar manner to that previously observed in the sporophyte. Gametophytes are able to grow normally in medium containing 20 mM arsenate and accumulate >2.5 % of their dry weight as As. This contrasts with gametophytes of the related nonaccumulating fern *Ceratopteris richardii*, which die at even low (0.1 mM) As concentrations. Interestingly, gametophytes of the related As accumulator *P. calomelanos* appear to tolerate and accumulate As to intermediate levels compared to *P. vittata* and *C. richardii.* Analysis of gametophyte populations from 40 different *P. vittata* sporophyte plants also revealed the existence of natural variability in As tolerance but not accumulation. Such observations should open the door to the application of new and powerful genetic tools for the dissection of the molecular mechanisms involved in As hyperaccumulation in *P. vittata* using gametophytes as an easily manipulated model system.

12 Novel Transgenic Strategies for Phytoremediation

Although metal hyperaccumulator plants, therefore, appear to have ideal properties for phytoextraction, most of these plants produce little biomass; and are thus primarily used as model organisms for research purposes. The plants are under improvement for phytoremediation traits by both conventional methods and transgenic technology. Dhankher et al. (2002) used the combined expression of bacterial enzymes involved in glutathione biosynthesis and arsenate reduction significantly to enhance both As accumulation and tolerance. The root systems of the As hyperaccumulating fern *P. vittata* possess a higher affinity for arsenate uptake than those of a related nonaccumulator fern species. A suppression of endogenous arsenate reduction in roots may serve to enhance root-to-shoot translocation of As (Pickering et al. 2000; Dhankher et al. 2002), and the overexpression of a glutathione-conjugate pump in the leaves could increase the capacity for detoxification of As III–glutathione complexes in the vacuole. Finally, phytochelatins synthesized by the ubiquitous plant enzyme phytochelatin synthase (Clemens et al. 1999; Vatamaniuk et al. 1999)—are known to contribute to As detoxification in all types of plant species (Schmöger et al. 2000; Hartley-Whitaker et al. 2001; Zhao et al. 2003; Raab et al. 2004).

Dhankher et al. (2002) developed transgenic *Arabidopsis* plants which could transport oxyanion arsenate to aboveground, reduce to arsenite, and sequester it to thiol peptide complexes by transfer of *Escherichia coli* arsC and γ-ECS genes. *Arabidopsis* plants transformed with ArsC gene expressed from a light-induced soybean rubisco promoter (SRSIp) strongly expressed ArsC protein in leaves, but not in roots and were hypersensitive to arsenate. Arabidopsis plants expressing *E. coli* gene encoding γ-glutamyl cysteine synthetase (γ-ECS) with actin promoter was moderately tolerant to As compared to control plants. Plants expressing SRSIp/ArsC and ACT 2p/γ-ECS together showed higher tolerance to As. These transgenic plants accumulated 4- to 17-fold greater fresh shoot weight and accumulated 2- to 3-fold more As per gram of tissue than wild plants or transgenic plants expressing γ-ECS or ArsC alone.

Sauge-Merle et al. (2003) have described a different approach, expressing the *Arabidopsis thaliana* gene encoding phytochelatin synthetase (AtPCS) in *E. coli*. Similarly, Nie et al. (2002) tested the ability of transgenic canola (*Brassica napus* cv. Westar) plants that constitutively express the *Enterobacter cloacae* UW4 1-aminocyclopropane-1-carboxylate deaminase gene (thereby produce lower levels of ethylene) to grow in the soil and to accumulate more As in plant tissues than nontransformed canola (Nie et al. 2002). Transgenic *A. thaliana* were developed to increase tolerance for and accumulation of heavy metals and metalloids by simultaneous overexpression of *AsPCS1* and *YCF1* (derived from garlic and baker's yeast). Dual-gene transgenic lines accumulated over 2- to 10-folds cadmium/arsenite and 2- to 3-folds arsenate than wild type or plants expressing *AsPCS1* or *YCF1* alone (Guo et al. 2012). The increase in As tolerance and accumulation (*Nicotiana tabacum*) due to *AtPCS1* and *CePCS* overexpression, observed at the As(V) concentrations similar to those found in As-contaminated soils, makes these genes promising candidates for plant engineering for phytoremediation (Wojas et al. 2010).

13 Conclusions

As accumulation in plants and phytotoxicity depends on a number of factors including physicochemical properties of the soil (pH, Eh, organic matter, soil texture, minerals and drainage conditions), bioavailable form of As, growth limiting nutrients, and genotypic differences in plants. Water-soluble form is more phytotoxic than other firmly bound forms in the soils. In mining polluted soils, the strongest relationship is found between As extractable by sodium acetate (amorphous Fe–Mn oxides fraction) and As in plant biomass. As is predominantly bound in the Fe–Mn oxide phase with very low contents of As in water-soluble, exchangeable, and carbonate fraction. The rise or decrease of the pH values of soil/rhizosphere due to the amendment addition, fertilization or root exudates may account for the reduction in the As sorption capacity of the soil, reductive dissolution, and the increase of As mobility. The redox potential significantly influences speciation and solubility of As in rhizosphere: increased As solubility under reduced conditions is associated with dissolution of Fe and Mn oxides/hydroxides, while iron oxide coating around roots under toxic conditions can reduce the availability of As to plants. As mobility and bioavailability in soil and aquatic environments may be influenced by the content and type of soil organic matter through the formation of soluble As–organic complexes. The functional groups of soil organic matter, such as carboxylic, phenolic, quinona, amino, hydroxyl, sulfhydryl, etc., determine the mechanism of As association in each case. Soil microbes can methylate inorganic As to give MMAA and DMAA. The indigenous arbuscular mycorrhizas and microbes can enhance As accumulation in plants due to interconversion of carbon-bonded As to inorganic ions by soil microbes. As might be mobilized in presence of phosphate in aerobic soils. However, phosphate can also inhibit As uptake by plants in a hydroponic system due to competition between them. The predominant As forms in tolerant and nontolerant plants are the inorganic As species (As(V) and As(III)) with a small portion of organic As species. As is coordinated as As-III–tris–thiolate and As-III–tris–glutathione complexes in shoot and root, where the thiolate donors were probably either glutathione or phytochelatins. Although As is mainly detoxified through complexation with glutathione, PCs, and MTs, plants can restrict translocation from root to shoots and sequestration of As into vacuoles in the form of complex or ions.

Out of the four main strategies for phytoremediation such as, phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization, phytostabilization can provide a good soil cover, prevent wind and water erosion by developing an extensive root system, and immobilize the contaminants in the rhizosphere. Although there are a plenty of As-tolerant plant species such as *A. canina*, *A. tenuis*, *A. stolonifera*, *A. castellana*, *A. delicatula*, *P. taxifolia, C. demersum*, *E. densa*, *P. pectinatus, S. holoschoenus*, *R. acetosella*, etc., that can accumulate As in some degree, a few plant species like *Pteris vittata*, *P. cretica, P. longifolia, P. umbrosa*, and *P. calomelanos* can hyperaccumulate As. The root systems of the As hyperaccumulating fern *P. vittata* possess a higher affinity for arsenate uptake than those of a related nonaccumulator fern species. Dual-gene transgenic lines can accumulate over 2- to 10-folds cadmium/arsenite and 2- to 3-folds arsenate than wild type or plants expressing *AsPCS1* or *YCF1* alone. The increase in As tolerance and accumulation (*N. tabacum*) due to *AtPCS1* and *CePCS* overexpression makes these genes promising candidates for plant engineering for phytoremediation.

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