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Arsenic Toxicity in Plants: A Significant Environmental Problem

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ABSTRACT

Arsenic (As) contamination of soil and water is a major global problem that impacts on many areas of biological sciences. Widespread use of Arsenic as pesticides has significantly contributed to the elevation of arsenic concentration in soil. Arsenic contamination in groundwater is a sever global environmental problem. Arsenic is a widespread natural element, which is not a bioorganic element to plants. in terrestrial plants both organic and inorganic Arsenic species have been found, with the inorganic species As(V) and arsenite, As(III) being the most dominant. Arsenic availability to plants greatly influenced by its forms in the soil. The background levels of arsenic are around 5 mg per kg worldwide with substantial variation depending on the origin of the soil. The behavior of Arsenic is distinctly different under anaerobic or flooded and aerobic or non-flooded soil conditions with flooded or anaerobic conditions being likely the most hazardous in terms of uptake by plants and toxicity. Agricultural application of arsenicals has introduced many different kinds of arsenic compounds to the soil environment. Biomass production and yields of a variety of crops are reduced significantly at elevated arsenic concentrations. Arsenic concentrations are generally low in plants. In all plant species tested so far, it has been shown that arsenate is taken up via the phosphate transport systems. Arsenic toxicity in plants is now recognized as a serious threat to human health, as a consequence of consumption of contaminated plant material. This review paper attempted to summarize the incidents of arsenic contamination in the irrigation of water-soil-plant system. It poses a significant risk to public health. Therefore, the first priority to remediate the crises should be early identification of the affected sources.

Keywords: Arsenic, Groundwater, Soil Environment, Phosphorus Transport System, Public Health.

INTRODUCTION

Widespread use of arsenicals as pesticides has significantly contributed to the elevation of arsenic concentrations in soils [1]. Arsenic contamination in groundwater is a severe global environmental problem [2]. Arsenic is a heavy metal with a name derived from the Greek word *arsenikon*, meaning potent. Arsenic is ubiquitous, found in air, water, fuels, and marine life [3]. Contamination of soil and water by arsenic (As) impacts on many areas of soil biology. It is now recognised as a serious threat to human health, as a consequence of consumption of contaminated plant material [4, 5]. Furthermore, As toxicity towards plants may pose threats to plant establishment, particularly in re-vegetation of

contaminated sites. Arsenic is non-essential and generally toxic to plants. Roots are usually the first tissue to be exposed to As, where the metalloid inhibits root extension and proliferation. Upon translocation to the shoot, As can severely inhibit plant growth by slowing or arresting expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production [reviewed by 6]. At sufficiently high concentrations, As interferes with critical metabolic processes, which can lead to death. Most plants possess mechanisms to retain much of their As burden in the root. However, a genotype-dependent proportion of the As is translocated to the shoot and other tissues of the plant.

Numerous physiological processes are susceptible to As toxicity. Cellular membranes become damaged in plants exposed to As, causing electrolyte leakage [7]. Membrane damage is often accompanied by an increase in malondialdehyde, a product of lipid peroxidation, pointing to the role of oxidative stress in As toxicity. Arsenic exposure induces antioxidant defense mechanisms. The synthesis of ascorbate, the γ-Glu-Cys-Glytripeptide glutathione (GSH), and the GSH oligome r([γ-Glu-Cys]*n*-Gly) phytochelatin (PC) increases throughout the plant, but particularly in the roots [7, 8, 9, 10, 11], while anthocyanin accumulates in leaves [12]. Plant transpiration intensity can be reduced [13]. Low As burden causes the number of nitrogen-fixing root nodules to be repressed in soybean [14]. The molecular mechanisms underlying these physiological responses to As exposure are not clear, but have recently attracted increased attention.

One of the many interesting paradoxes related to As toxicity is that plant growth is stimulated at low As concentrations [15, 6]. The fact that this phenomena occurs under axenic conditions in cultured plants, such as *Arabidopsis thaliana* [16], indicates that the trait is not based on As disrupting plant-biotic interactions. Instead, it results either from a direct interaction of As with plant metabolism, or from an interaction of As with plant nutrients. While the mechanism is unknown, it has been suggested that the growth benefit arises from As stimulation of Pi uptake [17].

There are relatively few species of plants that are naturally As tolerant. Among these are a group of plants including *Pteris vittata* and other members of the Pteridaceae that hyperaccumulate As [18, 19, 20]. The growth of these plants is not compromised during times when they are accumulating extremely high levels of As. In contrast to As non-hyperaccumulating plants, hyperaccumulators tend not to restrict As to the roots, instead allowing transfer of the toxicant immediately to the shoots. This is likely to be an important aspect of the hyperaccumulation phenotype. The mechanisms by which these plants are able to hyperaccumulate As are being elucidated, but it is not entirely clear how they are able to avoid As toxicity while As is actively accumulating to extremely high levels in the leaves [21].

Forms of Arsenic in Soils: The chemistry of As in soils is complex, and As can be present in both inorganic and organic forms [22]. Availability to soil microorganisms and plants, particularly relevant to uptake, is influenced by environmental factors that include soil redox potential, pH, composition (including clay mineralogy, organic content, the presence of Feand Al-oxides and hydroxides, and other elements), and microbial activity generally [23, 24]. Importantly, addition of fertilizer-P can mobilize adsorbed As, though the extent of mobilization is again heavily dependent on underlying soil chemistry [24]. The inorganic forms of As, As(V) and arsenite (As(III)), usually dominate in As-contaminated soil, and in aerobic soils the toxic conditions favour the presence of As(V) over As(III). The As(III) in herbicides and pesticides is oxidised to As(V) [25].

Measurements of speciation in aerobic soil (or artificial soil-like media) initially containing As(V) made after plants had been grown experimentally have shown in general that As(V) still predominates over As(III) [23, 24]. However, use of compartmented pots has shown relatively high As(III) levels in rhizospheres compared with bulk soil. [24] suggested that rhizosphere accumulation might be caused by enhanced As transformation by a relatively high density of microbes, or by mass flow of As(III) from bulk

soil associated with water uptake. The latter suggestion is feasible because the soil used was relatively high in water-soluble As(III) as well as As (V). [24] suggested that the cause might be reduction of As(V) caused by low redox potential in microsites around roots, or release of As (III) from roots. [26] showed rapid efflux of As(III) into nutrient solution from roots supplied with As(V). They suggested that this is part of a detoxification mechanism, and that roots and soil microbes are likely to be engaged in ongoing As(V)/ As(III) reduction/oxidation, with considerable As(III) cycling between roots and soil. We consider their results in more detail below

Absorption of arsenic species by plants: Uptake of As(V) and As(III) has been extensively studied in plants, and some attention has been given to the uptake of organic species. The uptake of As(III), which is predominantly undissociated below about pH 8, is believed to occur passively through membrane aquaporins [27, 20]. The suggestion that As(III) uptake into rice is active, i.e. energy-dependent, can be discounted, as it was based entirely on the fact that uptake versus concentration showed saturation kinetics [28]. Unsurprisingly, attention on uptake of As from aerobic soils has focused on As(V) rather than As(III). Physiological and electrophysiological experiments have shown that As(V) competes weakly with Pi for uptake [29, 30, 31]. These studies were made with excised roots, root pieces and (to a smaller extent) intact plants, all in solution culture. The major disadvantage of using excised roots is the disruption of the signaling from shoots to roots. This is unlikely to be a confounding factor in short-term experiments with material grown under P-starvation, but shoot/root signaling will be significant in longer-term studies. For example, [32] showed that after 12 h exposure of Arabidopsis to As(V) the biggest effect was on Pi transport to the shoot. They suggested that changes in P-related signals from the shoot might influence high-affinity Pi uptake. Such effects would also be expected in soil-grown plants. There are certainly differences in the interactions between Pi and As(V) for uptake by plants grown hydroponically compared with plants grown in soil or soil-like substrates. In soil, the interactions depend on Pi and As(V) availability, as affected by underlying soil chemistry, so that addition of fertilizer-P can increase or lower plant growth [33]. Release of adsorbed As(V) can occur following high applications of P, potentially increasing uptake of both [34]. However, because growth is often reduced in the presence of As, elevated P concentrations, whether plants are grown in solution or soil, may actually be due to the lower biomass (i.e. so-called 'tissue concentration', using 'concentration' in a different sense) that occurs in smaller plants.

Although some investigations have shown Pi/As (V) competition for uptake [35], other investigations give no evidence for competition. [36] found no effect of additional Pi on As (V) uptake by rice in flooded soil (P contents were not provided); these results contrasted with short-term uptake from nutrient solution [28]. [37] found that additional Pi ameliorated As toxicity in soil-grown Medicago truncatula (medic) and Hordeum vulgare (barley) but had no effect on the specific uptake of As (V) (i.e. uptake per g root). In this case the amelioration may have been due to the much higher tissue P concentrations at the higher external P level, with the result that P was able to outcompete As in metabolic reactions. [38] showed that increased P supply in soil had no effect on the P/As ratio in chickpea (Cicer arienatum) at low external As and only a small effect at higher As, again indicating little or no competition between As(V) and Pi for uptake from soil.

Arsenic Acquisition and Transport: In the environment, As can exist as inorganic or organic species. Of the two inorganic forms, the more highly oxidized arsenate, As(V) predominates in aerobic environments, while the more highly reduced arsenite, As (III) is the predominant form in anaerobic environments, such as flooded rice paddy fields. Microbes are able to biotransform inorganic As to organic forms [39]. The organic species of As(V) that are found at low concentrations in most soils include monomethylarsenic acid (MMA^V–super- script denotes As oxidation state), dimethylarsenic acid (DMAV) and trimethylarsine oxide (TMAOV). The concentrations of the methylated species are higher in anaerobic soils than in aerobic soils [28]. The corresponding mono-,di-,and tri-methylated derivatives of As III (MMAIII, DMAIII, TMAIII) are volatile. They are produced in the soil through processes likely to be limited by the availability of MMAV [40]. Like As V and As III, the methylated forms of As are phytotoxic [39].

As V is an analog of inorganic phosphate (Pi) and is easily trans- ported across the plasmalemma by Pi transporter (PHT) proteins [41, 42]. As V and Pi compete for uptake through the same transport systems in As hyperaccumulators [43, 17]. As-tolerant non-hyperaccumulators [44] and As-sensitive non-accumulators [28, 29].Under low Pi conditions, As V may outcompete Pi for entry into the plant, amplifying Pi deprivation symptoms. Conversely, Pi fertilization can protect plants, including the hyperaccumulator *P. vittata*, from As V toxicity [17]. Increasing or decreasing the rate of Pi and As uptake by increasing or decreasing PHT protein amount or activity at the plasma membrane through genetic means can also increase or decrease, respectively, the toxicity of As V [12, 42]. In the *Arabidopsis pht1–3* mutant, which has a compromised Pi uptake system, As accumulates without causing toxicity similar to the Pi fertilization effect in *P. vittata*. The lack of toxicity in such non-hyperaccumulating systems has been explained by postulating that as lower rate of As accumulation allows the plant to detoxify the incoming As before defense systems are overloaded and the toxicant can exert its toxic effects [12].

Once inside the plant cell, AsV can probably move easily from one cellular compartment to another, crossing internal membranes through the various Pi transporters. For example, As V has been demonstrated to be a co-substrate for three mitochondrial dicarboxylate transporters, proteins localized to the inner mitochondrial membrane and responsible for dicarboxylate exchange with co-substrates such as Pi, between the cytosol and the organelle matrix [45]. The outcome of this rapid movement would be the rapid equilibrium of As throughout the cell, exposing all parts of cellular metabolism to the toxicant. As V can be found in the xylem, having most likely been loaded into the xylem vessels by PHT proteins [12, 39, 42]. However, roots of As non-hyperaccumulators have the ability to strongly retain As. In *Arabidopsis*, only about 3% of the As taken up by the root was translocated to the shoot [22]. Similar results have been found for other plants [46]. Of the small portion of As that is translocated, no more than 40% would be expected to be in the form of As V, based on As speciation determinations in a number of species [20]. As III is able to enter root cells through nodulin26-like intrinsic proteins [47, 27]. These proteins belong to the aquaporin family of major intrinsic proteins. In rice roots, the OsNIP2;1/OsLsi1 silicon transporter has been implicated as the major AsIII uptake protein, while AsIII efflux from rice root cells to the xylem is through the OsLsi2 silicon transporter [27]. The localization of OsLsi2 to the proximal side of epidermal and endodermal cells [48], and OsLsi1 to the distal side of the same cells is an elegant example of the heterogeneous distribution of proteins in a membrane providing directionality to solute transport across cells and tissues. Other types of proteins may facilitate the transport As III into cells. In yeast, the majority of As III uptake occurs through hexose permeases [49]. While plants have proteins with strong homology to the yeast hexose permeases, it is not known if they provide a path for As III entry into plant cells.

In As hyperaccumulating species, such as *P. vittata*, As is not immobilized in the roots, but is instead rapidly transported as As III through the xylem to the fronds [50]. In the fronds, As III is sequestered as free As III in the vacuole [19], where it accumulates to extremely high levels. It has been shown that PvACR3 is involved in the vacuolar sequestration of As III [51]. This protein is a homolog of the yeast ScACR3p protein, a plasma membrane protein responsible for the efflux of As III from the yeast cell. In *P. vittata*, the PvACR3 protein still acts to efflux As III from the cytosol, but instead of delivering the As III to the outside of the cell, PvACR3 resides on the vacuolar membrane and transports the As III into the vacuole. Single-copy *ACR3* genes are found in moss, lycophytes, ferns, and gymnosperms, but not in angiosperms, which may help explain the lack of As hyperaccumulators among the angiosperms [51].

Arsenic Metabolism in Plants: When plants were supplied As V, typically more than 90% of the As in the roots and in the shoots was found to be in the form of As III [31, 26]. Thus, As V is readily reduced to As III by plants. This reduction is accepted as the first step in the major As detoxification pathways found in plants [31]. The reduction of As V to As III occurs both enzymatically and non- enzymatically. In the non-enzymatic pathway, two molecules of GSH are able to reduce As V to As III. The oxidation of GSH is via the formation of a disulfide bond, producing a GSH dimer [52], which can be rapidly recycled to two

GSH molecules by GSH reductase [53]. The plant ACR2 protein is related to the CDC25 cell cycle dual specificity tyrosine phosphatases. Interestingly, AtACR2 has phosphatase activity, while the PvACR2 enzyme, like the yeast ScAcr2p protein, does not [18, 54]. Also like ScAcr2p, the plant ACR2 enzyme uses GSH and glutaredoxin (GRX) as electron sources, suggesting that the catalytic cycle involves the formation of a mixed disulfide between GSH and ACR2 that is resolved by GRX [55]. The *Arabidopsis* As V reductase activity has an As V-inducible component that has been attributed to *AtACR2*, as well as a constitutive component that is not diminished in *AtACR2* T-DNA insertion lines [44]. Moreover, As III has been stated to remain the predominant form of As present in *AtACR2* T-DNA insertion lines supplied with AsV [20]. Together, these results indicate that *Arabidopsis*, and thus in all likely hood other plants, possess enzymes in addition to ACR2 that have As(V) reductase activity.

Multiple enzymes from other systems have been shown to exhibit As V reductase activity. These include glyceraldehyde-3-phosphatedehydrogenase (GAPDH), polynucleotide phosphorylase, purine nucleoside phosphorylase (PNP), glycogen phosphorylase, and the mitochondrial F1Fo ATP synthase [56, 57]. Each of these enzymes can incorporate As V instead of Pi into biological molecules, forming an arsenoester that would readily undergo hydrolysis. In the presence of a suitable thiol group, for example GSH, the hydrolysis can result in the reduction of As V to As III. It is not known if the analogous plant enzymes can also reduce As V in the presence of thiols. However, one form of the plant GAPDH is known to interact with GSH [58], suggesting it as a candidate ACR. Moreover, a cytosolic triose-phosphate isomerase (PvcTPI)from *P. vittata* has also been shown to have ACR activity [59]. Since the TPI reaction does not involve the transfer of a Pi group, the mechanism by which PvcTPI promotes the production of As III is unclear. However, like the enzymes mentioned above, the plant TPI interacts with GSH [59]. The number of enzymes that could misincorporate As V for Pi, and therefore have the capacity to form arsenoesters, is large, providing many opportunities for the enzymatic reduction of As V to As III. However, it is not known whether these enzymes affect the redox status of As *in vivo*. It is likely that any contribution that they make to the reduction of As V to As III will depend on the concentrations of substrates and effectors in the cell [56, 57].

Arsenic Toxicity in Plants: The results from a number of hydroponic experiments agree that As phytotoxicity depends on the chemical species supplied to the plant, but disagree on the identity of the most phytotoxic form of As [60]. These hydroponic experiments provide the clearest insights into the potency of externally supplied As on whole plant growth because they eliminate the complex and confounding phyto availability issues that arise from differences in the mobility of various As species through the diverse growth substrates. The studies generally agree with the hydroponic survey of 46 different plant species [61] that the uptake of As by plants has the order As III>As V>MMAV >DMAV, while translocation from the roots to the rest of the plant has the order DMAV >MMAV >As V≥As III. However, no one As form appears to be consistently most phytotoxic. In two *Spartina* species, where the order of uptake was As III>As V≈MMAV>DMAV, the order of phytotoxicity was DMAV≈MMAV>As III≈As V [62]. This would suggest that DMAV, with lowest uptake and high phytotoxicity, exerted the most highly toxic effects within the plant. In contrast, the uptake order in rice was As III>MMAV >As V>DMAV, an order that is similar to the order of phytotoxicity, which was MMAV >As III>As V=DMAV [63]. Finally, the order for phytotoxicity in maize, a species with the typical order for uptake [61], was As V>As III>DMAV [60].

a) Disruption of Phosphate Metabolism: An important mode of action of As V toxicity may be the replacement of Pi in critical biochemical reactions. Substitution of Pi by As V has been demonstrated to occur in numerous biochemical reactions, and any reaction with Pi or a Pi-ester as a substrate is a potential target for As V disruption [64]. Potential As V-sensitive reactions would include those central to cellular metabolism (i.e., glycolysis, oxidative phosphorylation) and biosynthesis (i.e., phospholipid metabolism), information storage and retrieval (DNA, RNA metabolism), and cellular signaling (i.e., protein phosphorylation/ dephosphorylation). When As V comes into contact with the surface of a cell within the

plant root, it is probable that a Pi transporter will be the first enzyme where As V will compete with Pi. Plants have both low-and high-affinity Pi transport systems. High-affinity transport is mediated by PHT1 proteins. The protein responsible for the low-affinity transport is unknown, although some PHT1 proteins also have a low-affinity activity. Competition by As V with Pi for entry in to the cell through both of these transport systems has been demonstrated in numerous plants, both monocots and dicots, and both As-hyperaccumulators and non- hyperaccumulators [43, 44, 29, 17] Since Pi competes with As V for uptake, As V toxicity is lower under high Pi conditions. On the other hand, As V may outcompete Pi for uptake under low Pi conditions, exacerbating Pi deprivation [17]. Other transporters besides PHT1can also be fooled in to utilizing As V instead of Pi. For example, As V is able to move across the plant inner mitochondrial membrane through the Pi translocator [65] and the dicarboxylate carrier (Palmieri et al.,2008). The toxicant can also pass through the *Arabidopsis* AtPHT4 family of Pi transporters localized to the plastid and golgi [66]. Relatively few enzymes use Pi as a substrate due to the irreversible nature of most Pi-liberating reactions. Therefore, few enzymes are expected to use AsV directly as a substrate [67]. Perhaps the predominant Pi-requiring reaction is the phosphorylation of ADP to ATP by the F1Fo-type ATP synthases found in the mitochondrial inner membrane and the plastid thylakoid membrane. The mitochondrial enzyme uses As V in a reaction that produces ADP-As V [64]. The *K*M and $\square_{max}$ of this reaction are remarkably similar for both Pi and As V [68] demonstrating that atleast some enzymes are capable of recognizing and reacting equally well with As V and Pi. These characteristics are most probably shared with the plastid F1Fo-type ATP synthase [69], although this has not been demonstrated directly.

Other Pi-dependent enzymes that are able to use As V include the glycolytic enzyme GAPDH. Like the ATP synthase reaction, the GAPDH reaction where As V replaces Pi has remarkably similar kinetic constants to the Pi-dependent reaction [70]. Aspartate-β-semialdehyde dehydrogenase has a critical role in the biosynthesis of essential amino acids in plants, catalyzing the reversible reductive dephosphorylation of β-aspartylphosphate to l-aspartate-β-semialdehyde. This enzyme, too, is able to use As V nearly as efficiently as Pi, judged from the *K*M and *k*cat values [71]. PNP catalyzes the phosphorolysis of various nucleosides, producing the free nucleotide base and ribose-1-phosphate. The substitution of AsV for Pi in the PNP reaction releases ribose-1-AsV in an arsenolysis reaction with a *K*M that is again quite similar to that of the Pi-dependent reaction [72]. While much of the above information comes from non-plant systems, there is little reason to believe that the behavior of homologous plant enzymes would be substantially different.

b) Binding Thiols: The mode of action of As III differs substantially from that of As V. As III is a thiol reactive compound that can bind up to three sulfhydryl groups [73]. This allows As III to act as a cross-linking agent by binding up to three monothiol molecules, such as the antioxidant GSH. Alternatively, it could bind to a single molecule of a poly-thiol compound, such as PC, the Cys-rich polymerization product of GSH. As III can also bind to thiol-containing proteins and co-factors. Dihydrolipoamide, which in plants is a co-factor associated the mitochondrial and plastid pyruvate dehydrogenase complexes (mtPDC, ptPDC), the 2-oxoglutarate dehydrogenase complex (OGDC), the Gly decarboxylase complex (GDC) and the branched-chain 2-oxoacid decarboxylase complex (BCOADC), has been long-thought to be an important cellular target for As III binding [74]. The stability of As III complexes increases with the number of bonds formed. The half-life of an As III-monothiol peptide complex is only about1–2s. The half-life increases to about1.3 and 155min when two or three intramolecular thiols are bound [75]. The high stability of As III-trithiol complexes is supported by the finding that As III preferentially binds zinc-fingerproteins containing three or more Cys residues in the zinc-finger motifs. This study did not find As III binding to zinc-finger motifs with only two Cys residues, possibly due to the time needed to process the samples [76]. Complexes where As III forms intramolecular links between peptides are more stable than those with intermolecular bonds [75]. The binding of As III to dithiols is enhanced when the sulfhydryl groups are in close proximity to one another [73], but the optimal spacing for trithiols is unknown. The binding of As III to proteins can have profound effects on their folding [77, 78]. More than 100 enzyme activities that were sensitive to As compounds were identified by 1966 and that number will have grown

considerably. In various systems, proteins that are known to bind As III include transcription factors, signal transduction proteins, proteolytic proteins, metabolic enzymes, redox regulatory enzymes, and structural proteins. Among the 35,386 predicted translation products from the *Arabidopsis* genome sequence (TAIR10 release), there are 64,335 dithiols with optimal spacing for As III binding [73] on 23,578 proteins. About one-third of these dithiols, residing on 11,559 proteins, form part of a trithiol that may be optimally spaced for As III binding, assuming that the optimal sulfhydryl spacing for As III binding to trithiol groups is symmetrical with the dithiol spacing ($CX_{0-14}CX_{0-14}C$). This analysis ignores the potential for intramolecular cross-links, but raises two intriguing questions: What types of proteins in *Arabidopsis* are among the 2123 proteins lacking a Cys residue? Are there evolutionary pressures for these proteins in particular to lack the ability to interact with As III? One conclusion to be drawn from these values is that As III has the capacity to interact with a large proportion of any cellular proteome and it will be a large task to identify which proteins among the As III targets are most critical to cell survival. While As III is an inhibitor of many enzymes, the recent finding of methylated forms of As III in plant cells [79, 80, 46] has important implications in this respect. Half maximal inhibition of pyruvate dehydrogenase was found to occur at about115μM As III, while two-to six-fold less of several methylated As III derivatives was need for similar inhibition [81]. Compared to As III, MMAIII is a more potent inhibitor of other enzymes, including GSH reductase [82] and thioredoxin reductase [83]. MMAIII and DMAIII in the low to mid micromolar range were able to displace Zn^{2+} from a zinc-finger protein [84], an important class of proteins involved in gene expression and DNA repair. Both methylated forms were also more potent inhibitors of zinc-finger protein activity than As III, again highlighting the necessity to critically evaluate the ability of plants to methylate inorganic As into more toxic forms or reduce methylated-As V compounds to their As III counterparts.

c) Oxidative Stress: It is well documented that exposure of plants to As III and As V induces the production of ROS, including superoxide ($O2^{\bullet-}$), the hydroxyl radical (•OH), and H_2O_2 [7, 85, 86]. ROS can damage proteins, amino acids, purine nucleotides and nucleic acids and cause peroxidation of membrane lipids [87]. Lipid peroxidation not only compromises cellular function, but leads to the production of lipid-derived radicals [87, 88]. Induction of lipid peroxidation by As V was also observed in the As hyperaccumulator *P. vittata* [7], indicating that ROS production is a feature of the general plant As response and that the magnitude of the redox imbalance in the cell may be an important determinant of ROS-induced toxicity. Although the mechanism of the As-induced production of ROS is not well understood, it has been proposed that As detoxification processes, including the reduction of As V to As III and the induction of PC synthesis [89], have roles to play in ROS production. The molecular targets that are most sensitive to the ROS produced by As exposure are not yet clear, although there are many candidates [87]. Under normal cellular conditions, ROS homeostasis is delicately balanced. Relatively small changes in nutrient availability or environmental conditions such as temperature and light can cause small ROS imbalances that act as signals of cellular status and are easily managed by pre-existing antioxidant defense mechanisms [87, 88, 53]. However, under stronger stresses, such as As exposure, where ROS generation increases, these defense mechanisms may be overwhelmed, leading to cellular damage. This damage can lead to cell death [88]. Unless the cell death is part of a developmental program, cellular responses must seek to restrict ROS-mediated damage or the survival of the organ or individual will be jeopardized. Several enzymes are involved in ROS defense strategies. Highly reactive superoxide can be converted to less active but longer-lasting H_2O_2 through the action of superoxide dismutase (SOD). SOD activity in plants varies quite widely with As treatment. In some plants, like *Zea mays*, As-sensitive clones of *H. lanatus*, and the As-hyperaccumulator *P. vittata*, the enzyme is induced by low As exposure, and either stays at the same level or decreases in activity at higher As levels [90]. One explanation put forward for this variation in activity is that SOD is a metallo-enzyme [89]. However, part of the explanation may also be at the level of gene expression. In *Arabidopsis*, genes encoding the three classes of SOD (FeSOD, MnSOD, Cu/ZnSOD) responded to As V differentially at the transcript level [91]. Transcripts for genes encoding a chloroplastic and a cytosolic Cu/ZnSOD were induced more than two-fold by As V exposure, while transcripts for an FeSOD were down-regulated about five-fold [91]. These

observations raise the question of what effects these changes in the SOD transcript pool have on the characteristics of the SOD activity, and, if the characteristics of the SOD activity changes, what are the adaptive advantages, if any, and the underlying mechanisms, of those changes.

d) Effect on Carbon Metabolism: A main effect of As V on plant carbon metabolism is in stimulating the accumulation of ascorbate [7, 11] presumably to bolster protection against ROS. The effects of As on primary carbon metabolism in plants is largely unknown. However, the transcriptional profiles of genes encoding proteins involved in carbon metabolism are largely unaffected both in *Arabidopsis* and rice [91, 92, 93]. Proteomic studies in the non-hyperaccumulating plants rice and maize [85] have shown that there are some changes in the abundance of proteins that participate in glycolysis and the citric acid cycle, but the changes are not consistent or systemic throughout either pathway. There are too few studies to fully understand the relevance of the changes that are observed. However, it appears that As does not have strong effects on gene expression related to carbon metabolism. This suggests that plant metabolism has sufficient plasticity to maintain adequate carbon flow without the need to adjust enzyme amounts in these central pathways. Robust assessment of the metabolite pools associated with primary metabolism is needed in plant tissues exposed to As to address this point directly. On the carbon fixation side of photosynthesis, the Rubisco large subunit content of rice leaves decreased with As V treatment [94]. The Rubisco large subunit is encoded by the plastid DNA [95]. Therefore, the decreased abundance of this protein not only indicates that As interferes with carbon fixation capacity, but raises the question of whether As also interferes with chloroplast DNA gene expression. However, in contrast to the decrease in Rubisco large subunit amount in rice, Rubisco small subunit transcripts increased in As V-treated *Arabidopsis* [91]. Whether the increased transcript abundance results in more Rubisco small subunit, or is a response to a decrease in active Rubisco is not yet known. As III inhibits the light activation of photosynthetic CO_2 fixation in isolated pea chloroplasts [96]. The inhibition is at the level of the light activation of enzyme activities associated with the reductive pentose phosphate pathway. While the extent of the effects of As on photosynthetic carbon metabolism are not fully understood, it appears likely that the toxicant decreases the amount of carbon available to the plant through decreased CO_2 fixation.

Photorespiration is a prominent path of carbon flow in most plants and includes the activity of the lipoamide-containing GDC. The dithiol group of dihydrolipoamide is a well known target for As III binding in animals [97]. Addition of this co-factor to animal cells is able to ameliorate As III toxicity [98]. In plants, lipoamide is found not only in GDC, but also in the four enzyme complexes mtPDC, ptPDC, OGDC, and BCOADC. All five lipoamide-containing complexes contain lipoamide dehydrogenase (LPD), an enzyme that catalyzes the transfer of electrons from the reduced dihydrolipoamide co-factor to NAD^+ as part of the enzymatic reaction cycle of the complex. Like the lipoamide co-factor, the plant LPD enzymes are in activated by As III, but not As V [16], presumably because of the binding of As III to the absolutely conserved dithiol present in LPD that takes part in the reaction cycle. Knock-outlines of *Arabidopsis* with decreased levels of mtLPD were more sensitive to As V treatment and produced much higher levels of Gly when exposed to As V than wild-type lines), indicating that LPD is an important target for As toxicity in plants. LPD increased in amount in leaves of As V-treated rice [94], perhaps in response to the inhibition of one or more of the LPD containing complexes. Higher plants are likely to have photorespiratory pathways that are independent of the main GDC-dependent pathway [99]. These alternative pathways are likely to come into play under various stress conditions. One of these pathways involves the non-enzymatic oxidative decarboxylation of glyoxylate to formate in the presence of H_2O_2 [100]. The formate can then be oxidized to CO_2 by NAD^+ formate dehydrogenase, which has been found among both plastid and mitochondrial proteomes [101]. The NADH that is produced is then available to donate electrons to the mitochondrial electron transport chain, linking formate oxidation to ATP production. While this pathway is not prominent under most conditions, it may become more important when GDC is inhibited [100], such as during As exposure. Not only is it possible that this pathway may be driven by ROS

produced by As exposure, but the NAD^+ formate dehydrogenase protein accumulates in leaves of rice exposed to As V [94]. During daylight hours, a large proportion of newly fixed car- bon is stored as starch. Starch hydrolysis to glucose, maltose, and malto-oligosaccharides, followed by phosphorylation of glucose by hexokinase is most likely the main path for the entry of glucose into glycolysis [102]. In wheat, it has been found that As III, and to a lesser extent, As V, are able to decrease the liberation of maltose from starch by inhibiting amylolytic activity [103]. Phosphorolysis is reaction where Pi is the attacking group to cleave a covalent bond may also contribute to the break-down of starch, maltose, or malto-oligosaccharides [102], liberating glucose-1-phosphate. As V can substitute for Pi in this phosphorolysis reaction, yielding glucose-1-AsV that quickly hydrolyzes to glucose [104]. Before this free glucose can enter glycolysis, it would need to be phosphorylated by hexose kinase, at the expense of ATP, decreasing the energetic yield of glycolysis.

Dark respiration is inhibited in alfalfa by As V exposure, but this process is more resistant to the toxicant than photosynthesis [105]. Marin et al, 1993 also found that respiratory O_2 consumption was more resistant to AsV supply, in the form of DMAV, than photosynthetic O_2 evolution in a study that did not differentiate photorespiration from mitochondrial respiration. The main effect of As on plant respiration may be the As V-dependent uncoupling of ATP synthesis from electron transport [106] that results from the synthesis of highly unstable ADP-As V [68]. The decrease in ATP synthesis brought about directly by the futile cycling of ADP through ADP-As V and indirectly by the decrease in the proton motive force by the uncoupling reaction would be expected to decrease the energy status of the cell. The finding that the *K*M and □max of the mitochondrial F1Fo ATP synthase are remarkably similar for both Pi and As V [68] indicates that uncoupling may well occur *in vivo*. Moreover, it is likely that glycolysis and the citric acid cycle would process substrates more rapidly in an attempt to maintain the proton gradient at a sufficient level. The overall expected outcome would be a cellular energy crisis.

Plant carbon metabolism relies on efficient shuttling of molecules across cellular membranes. In As V-treated rice seedlings, a triose-phosphate/Pi translocator gene was transcriptionally up-regulated [93]. This protein would be expected to transport Pi and As V across the plastid inner membrane in exchange for triose-phosphate. In As V-treated *Arabidopsis*, transcript abundance for a mitochondrial substrate carrier protein identified as dicarboxylate carrier 2(DIC2) by Palmieri et al. (2008) was repressed [91]. DIC2 is located in the inner mitochondrial membrane and is likely to exchange Pi, sulfate, or As V for a number of dicarboxylates [45]. Malate/oxaloacetate exchange catalyzed by DIC2, coupled with cytosolic and mitochondrial NAD^+-dependent malate dehydrogenase activities, allow the flow of redox equivalents from one compartment to the other [45]. Thus, As V interacting with DIC2 may have a negative impact on the redox balance between the mitochondrial matrix and the cytosol by inhibiting efficient malate/oxaloacetate exchange. While decreases in carbon metabolism would have negative impacts on cellular energy flow and the production of biosynthetic intermediates, a decrease in leaf carbon metabolism may be an adaptive response to minimize As toxicity. The As hyperaccumulator *P. vittata* has a pronounced decrease in proteins associated with carbon metabolism in its ariel tissues when challenged with As. These proteins include enzymes involved in energy conversions (an organellar DNA-encoded subunit from each the chloroplast and mitochondrial F1Fo ATP synthase), carbon fixation (Rubisco large and small subunits, seduheptulose-1,7-bisphosphatase) and carbohydrate metabolism (malate dehydrogenase, triose-phosphate isomerase, a subunit of pyruvate dehydrogenase) Bona et al.,2010. The As-tolerant monocotyledon *A. tenuis* also had decreased amounts of Rubisco large and small subunits and a subunit on the mitochondrial ATP synthase when exposed to As V or As III [107]. The possible value of such an adaptive response is not clear.

e) Effect on Nitrogen Metabolism: Biological nitrogen fixation, including the contribution made by symbioses in the root nodules of legumes, supplies a large proportion of the nitrogen in biological systems. The exposure of alfalfa root systems supporting well-established N_2-fixing symbioses with rhizobia to As V demonstrated that symbiotic N_2 fixation is sensitive to As toxicity [105]. Moreover, alfalfa either grown

in As-contaminated soil or exposed to As III had less than half of the total number of root nodules formed in the absence of added As [108, 109]. Under controlled conditions, this reduction was due to a 90% decrease in the number of rhizobial infections [109]. The use of an As-resistant strain of rhizobia in these experiments demonstrated that the decreased establishment of the symbiosis was not due to bacterial death. Rather, plant traits including root necrosis, root hair damage, and a shorter length of the root zone that was subject to infection were implicated [109]. Transcript analysis indicated that As III exposure interferes with the expression of genes involved in early nodule development [110]. Together, these results suggest that As contamination of soil has the potential to strongly decrease N_2 fixation in ecosystems involving legume-rhizobium symbioses. As V also seems to disrupt N assimilation. Non-legumes obtain N from the soil predominantly as nitrate (NO^{-3}) or ammonium (NH^{+4}). Inorganic N in the form of NH^{+4}, is assimilated by the combined action of glutamine synthase (GS) and glutamate synthase (GOGAT) for entry into the organic molecule pool as glutamate [53]. Roots of As V-treated rice had decreased amounts of transcripts for a NO^{-3} transporter and for an NH^{+4} transporter . Another experiment using whole rice seedlings also found a decrease in transcripts for a different NH^{+4} transporter. The amount of nitrate reductase was repressed in whole seedlings of rice [93], but induced in whole seedlings of *Arabidopsis* [91]. Nitrate reductase, in combination with nitrite reductase, supplies NH^{+4} to GS for N assimilation. GS protein amount was lower in rice roots treated with As V [85]. Although it is not clear if it was the cytosolic or plastid isoform that was reduced, the chloroplastic form was sensitive to oxidative fragmentation by hydroxyl radical [111], a ROS species produced during As V exposure. While the details are far from clear, it appears that As V interferes with both the supply of inorganic N to the assimilation pathway and the activity of the pathway itself. Arsenic exposure has been reported to cause dramatic changes in amino acid pools [112]. A key question is the extent to which the changes in the amounts of these amino acids is due to changes in amino acid biosynthesis or changes to protein metabolism. Exposure to As caused decreases in total plant protein abundance in *P. ensiformis* and *P. vittata* [7], in total shoot protein abundance in red clover [113] and insoluble protein in maize [13]. Proteomic studies demonstrated that Rubisco, with its high capacity due to its great abundance to store N in the form of amino acids, can be targeted for destruction in As V treated plants [94, 114]. In *Lemna minor*, total protein increased at low As V supply, but decreased at high As supply [115], a relationship that may be linked to the stimulation of growth at low As supply that has been observed for numerous plants [116, 16]. Protein degradation has been recognized as an important source of respiratory carbon when carbohydrate levels are low [117]. Thus, the lower protein abundance that generally accompanies As exposure, coupled with a likely As-induced decrease in carbohydrate metabolism that would hinder the biosynthesis of amino acids, suggests that any changes in the size of amino acid pools would be due to amino acids flowing from protein degradation.

f) Effect on Sulfur Metabolism: The central role played by the binding of As III to sulfhydryl groups in GSH and PC in the detoxification of the metalloid indicates a critical importance for sulfur metabolism in determining plant survival in As-contaminated soils. The biosynthesis of GSH and PC that is typically induced by As exposure requires adequate supplies of the GSH-building blocks Glu, Cys, and Gly. In both shoots and roots of wild-type *Arabidopsis*, the mass ratios of free Glu:Gly:Cys were about 20:3:1[118]. Thus, at least in *Arabidopsis*, Cys is by far the limiting substrate for GSH biosynthesis. Plants that over express enzymes involved in GSH and PC biosynthesis have higher levels of non-protein thiols than wild-type lines [119]. However, other studies indicate that As V exposure can decrease cellular Cys pools [120] and that under some growth conditions it is possible that the synthesis of PC can deplete GSH pools, decreasing the antioxidant capacity of the cell [121, 122]. These observations, combined with the possible limiting availability of Cys, suggests that increased Cys biosynthesis to support GSH and PC production would add to the effectiveness of approaches designed to increase non-protein thiols with in plants, a process that would also require inputs from sulfur metabolism. The first step that would be necessary to support increased biosynthesis of GSH and PC is the acquisition of sulfur from the soil. The main form of sulfur available to plants is sulfate. In AsV-treated rice, upto five sulfate transporter genes are unregulated in roots [92], and at least one sulfate transporter is up-regulated in *Arabidopsis* [120]. As III also induces a

sulfate transporter gene in rice and *B. juncea* seedlings [93]. It is not yet clear whether As V and As III affect the expression of these transporters equally, although at least one of the transporter genes is induced by both forms of As [93]. The up-regulation of this small number of transporters may be enough to move sulfate from the soil solution throughout the plant. The efflux of sulfate from cells that is required for transport to the tissues is likely to be down a concentration gradient and powered by the positive-outside membrane potential of the plasma membrane [123]. Before sulfate acquired from the soil can be used for the biosynthesis of Cys, and thus the biosynthes is of GSH and PC, it must be reduced via sulfite to sulfide [123]. The reduction of sulfate to sulfite is a two step pathway. The second step is catalyzed by 5'-adenylylsulfate reductase. Transcripts of a 5'-adenylylsulfate reductase gene were elevated in *Arabidopsis* in response to As V supply [91], suggesting that the sulfate assimilation pathway is induced by As V in plants as it is by As III in yeast [124]. The 5'-adenylylsulfate reductase reaction uses GSH as a reductant. It would be expected that As V exposure would lead to a lowering of GSH availability as it is diverted to As detoxification, with unknown consequences to the sulfate reduction pathway.

In rice, several methyl transferase genes are induced by As V-treatment [92]. Two of these are homocysteine S-methyltransferases, which catalyze the formation of *S*-adenosyl-l-homocysteine and Met from *S*-adenosylmethionine and L-homocysteine. The enzyme is involved in the synthesis of *S*-methylmethionine [125], and may play a role in maintaining a pool of soluble Met, in the cycling of methyl groups within cells, or as a phloem-mobile form of Met that can be used to translocate sulfur derived from protein degradation [126]. In the context of the As response, it is tempting to speculate that the translocation of Met as *S*-methylmethionine from remote sites of protein degradation (i.e., the leaves), aided by the action of homocysteine S-methyltransferases, can be used to increase the availability of Cys at sites where GSH biosynthesis is required for the binding of As III (i.e., the roots).

CONCLUSIONS

Much excellent work has been done on the metabolism of As in plants: how it is acquired and moved through the plant; how it is reduced, detoxified, and sequestered; how it mimics Pi, binds sulfhydryl groups, and causes oxidative stress. We have a fairly firm grasp of the mechanism used by As hyperaccumulators to accumulate large amounts of the toxicant without poisoning. These plants take up the metalloid more quickly than non-hyperaccumulators, do not sequester it in the root, but rather transport it quickly to the aerial tissues where it is sequestered in the vacuole as As III. The rapid rate of uptake and translocation to the frond and a higher antioxidant capacity to maintain lower ROS levels (Cao et al., 2004; Zhao et al., 2009), perhaps coupled with relatively rapid dilution in the bulk of the aerial tissues, together seem to provide the hyperaccumulators with adequate time and resources to neutralize the toxic effects of As. An interesting question that remains is how the cells in As-hyperaccumulators are physically capable of keeping As III away from vital metabolic targets during the translocation and sequestration process? Or is the As translocation rate so rapid that intracellular concentrations of biologically active As III are never high enough to exert a negative effect?

Despite a firm knowledge of the interactions between plant cells and As, we still do not have a good understanding of the exact nature of why As is toxic: Which combination of mechanisms for toxicity, Pi replacement, sulfhydryl binding, or ROS production, is the most damaging in the short and long terms to plant growth and productivity? Which parts of plant metabolism are most vulnerable to As toxicity and why? What are the most critical molecular targets for As and can we do anything to protect these targets through breeding or direct engineering? The combination of detailed physiological and biochemical studies will continue to give us great insights into the mode of action of As in plants. The recent addition of global transcript analyses and proteomics approaches has added important new dimensions to our understanding of plant responses to As exposure. The combination of these methods with ever more sensitive and informative physical and biochemical assays and transcriptome and proteome analyses are likely to provide answers to some of the critical questions raised here.

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