



## *Tansley review no. 133*

# Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species

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### Summary

**Key words:** arsenic, toxicity, hyperaccumulation, arsenic resistance, metabolism, uptake, phenotypic variation.

Elevation of arsenic levels in soils causes considerable concern with respect to plant uptake and subsequent entry into wildlife and human food chains. Arsenic speciation in the environment is complex, existing in both inorganic and organic forms, with interconversion between species regulated by biotic and abiotic processes. To understand and manage the risks posed by soil arsenic it is essential to know how arsenic is taken up by the roots and metabolized within plants. Some plant species exhibit phenotypic variation in response to arsenic species, which helps us to understand the toxicity of arsenic and the way in which plants have evolved arsenic resistances. This knowledge, for example, could be used produce plant cultivars that are more arsenic resistant or that have reduced arsenic uptake. This review synthesizes current knowledge on arsenic uptake, metabolism and toxicity for arsenic resistant and nonresistant plants, including the recently discovered phenomenon of arsenic hyperaccumulation in certain fern species. The reasons why plants accumulate and metabolize arsenic are considered in an evolutionary context.

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## I. Introduction

Arsenic in the environment is often associated with other elements (Au, Ag, Cu and Sn in particular), and mining and processing of these ores has led to extensive arsenic pollution of mining regions throughout the world (Nriagu, 1994). Historically, the use of arsenic-based pesticides has led to considerable contamination of domestic and agricultural land, through their use as lawn herbicides, and insecticides for rice, orchards and cotton (Woolson *et al.*, 1971; Murphy & Aucott, 1998).

Recently, the environmental fate and behaviour of arsenic has received increasing attention due to a crisis in South-East Asia (West Bengal, Bangladesh and Vietnam). Tens of millions of people have been exposed to high levels of arsenic in ground-water, the region's primary source of drinking-water (Christen, 2001). Up to 1 000 000 tube-wells drilled into Ganges alluvial deposits may be contaminated with arsenic, with concentrations up to 1000  $\mu\text{g l}^{-1}$  in Bangladesh and West Bengal. Similarly, ground water is contaminated with up to 3000  $\mu\text{g l}^{-1}$  in Vietnam. Large areas of Bangladesh, West Bengal and Vietnam have to rely on arsenic-contaminated ground-water for irrigation of staple crops such as rice (Nickson *et al.*, 1998; Berg *et al.*, 2001; Christen, 2001; Abedin *et al.*, 2002a,b). Consequently, in addition to exposure through drinking-water, people are being exposed to arsenic through ingestion of vegetation which has been contaminated by irrigation with arsenic-contaminated water, and from livestock and their products, where livestock have been fed on arsenic-contaminated vegetation. Understanding how arsenic is taken up by plants and subsequently transformed is essential for estimating the risks posed by arsenic-contaminated soils to human and wildlife populations, in these regions and elsewhere.

Arsenic is released into the environment in both inorganic and organic forms. Arsenate [As(V)] and arsenite [As(III)] are the inorganic, phytoavailable forms of arsenic in soil solution. However, microbes, which can methylate and demethylate arsenic species in soils, may transform inorganic arsenic species to organic species and vice versa (Turpeinen *et al.*, 1999). In addition, invertebrates and mammals metabolise arsenic from inorganic to organic species (Tamaki & Frankenberger, 1992; Zaman & Pardini, 1996; Vahter, 2000). Until recently, arsenic speciation in plant tissues was poorly understood, with the majority of studies concentrating on the transformation of arsenical pesticides in crops such as rice, or uptake of arsenic species by plants (Hiltbold, 1975; Marin *et al.*, 1993; Carbonell-Barrachina *et al.*, 1998). A series of recent papers has transformed our knowledge of how arsenic is metabolised in terrestrial plant tissues. These papers fall into two classes: those that characterise the form of arsenic extracted from plant tissues without regard to whether these species were chelated in the tissues or not (Table 1); and those that consider chelation. The former have identified that a range

of arsenic compounds found in other organisms are also found in plant tissues, for example, methylated arsenic species, arsenobetaine and arseno-sugars (Table 1). The latter have shown that arsenite within a range of plant tissues is complexed with phytochelatins (PCs) (Sneller *et al.*, 1999; Pickering *et al.*, 2000; Schmöger *et al.*, 2000; Hartley-Whitaker *et al.*, 2001b,c). When these PCs are extracted in media with a pH > 7.2, the PC-complexes may dissociate to free PC and arsenate (reduction of the PCs and oxidation of the arsenite to arsenate). Thus when arsenate is detected in plant extracts (see Table 1), it may have originated from PC complexed arsenite.

Plants also vary in their sensitivity or resistance to arsenic (Meharg, 1994). Arsenate is the dominant form of arsenic in aerobic soils and is an analogue of phosphate, competing for the same uptake carriers in the root plasmalemma (Meharg & Macnair, 1992). Arsenate resistance has been identified in a range of plant species including *Holcus lanatus*, *Calluna vulgaris* and *Silene vulgaris* (Paliouris & Hutchinson, 1991; Meharg, 1994; Fitter *et al.*, 1998; Sharples *et al.*, 2000b). Resistance is generally achieved via suppression of the high-affinity phosphate/arsenate uptake system (Meharg & Macnair, 1992). It is thought that this suppression reduces arsenate influx to a level at which the plant can detoxify, presumably by constitutive mechanisms (Meharg, 1994).

Because of the interaction between phosphate and arsenate transport, mycorrhizal fungi are likely to have a strong influence on arsenate uptake and resistance, due to their role in enhancing phosphate acquisition for the host plant (Smith & Read, 1997). The relationship between arsenate and phosphate uptake and the influence of mycorrhizal fungi will be discussed later in this review.

Despite this understanding of the processes controlling decreased arsenate uptake, arsenate resistant plants can still accumulate considerable levels of arsenic in their tissues, for example, 3470  $\mu\text{g g}^{-1}$  as in *Agrostis tenuis* and 560  $\mu\text{g g}^{-1}$  as in *H. lanatus* (Porter & Peterson, 1975). The toxicity of arsenic is dependent on its speciation, with inorganic arsenicals thought to be more toxic than organic forms (Tamaki & Frankenberger, 1992). It must therefore be assumed that arsenic-resistant plants either compartmentalise and/or transform arsenic to other less phytotoxic arsenic species in order to withstand high cellular arsenic burdens (Meharg, 1994).

Genetic variation in the response of plants to arsenic, in terms of uptake and metabolism, provides the potential to devise agronomic cultivars better suited to arsenic enriched soils. For example, the development of cultivars that assimilate less arsenic, or restrict arsenic translocation to fruits/seeds would lead to reduced dietary exposure to arsenic.

The reasons why plants transform arsenic and how they facilitate this transformation are still major questions in arsenic research, and will be the focus of this review. For organic arsenic compounds determined in plant tissues (Fig. 1), it is not known whether the compounds are simply

**Table 1** Arsenic species detected in a range of plant species where inorganic and organic arsenic species were analysed

Arsenic species	Plant	Exposure	Ref no.
Arsenate	<i>Vigna radiata</i> (Mung bean)	As(V), As(III)	1
	<i>Daucus carota</i> (Carrot)	Field	2
	<i>Oryza sativa</i> (Rice)	Field	3
	<i>Malus domestica</i> (Apple)	Field	4
	<i>Rubus idaeus</i> (Raspberry)	Field	5
	<i>Potentilla fruticosa</i> (Shrubby cinquefoil)	Field	5
	<i>Hordeum jubatum</i> (Foxtail barley)	Field	5
	<i>Agrostis scabra</i>	Field	5
	<i>Drepanocladus</i> sp. (Moss)	Field	5
	<i>Zea mays</i> (Maize)	As(V)	6
	<i>Cucumis melo</i> (Melon)	As(V)	6
	<i>Pisum sativum</i> (Pea)	As(V)	6
	<i>Lycopersicon esculentum</i> (Tomato)	As(V)	6
	<i>Lycopersicon esculentum</i> (N and P deficient tomato)	As(V)	6
	<i>Brassica juncea</i> (Indian mustard)	As(V)	7
	<i>Dactylis glomerata</i> (Cocksfoot grass)	Field	8
	<i>Trifolium pratense</i> (Red clover)	Field	8
	<i>Plantago lanceolata</i> (Ribwort plantain)	Field	8
	<i>Asplenium viride</i> (Green spleenwort)	Field	9
	<i>Dryopteris dilata</i> (Broad buckler fern)	Field	9
	<i>Deschampsia cespitosa</i> (Tufted hair grass)	Field	9
	<i>Picea abies</i> (Norway Spruce)	Field	9
	<i>Larix deciduas</i> (European Larch)	Field	9
	<i>Alnus incana</i> (White Alder)	Field	9
	<i>Fragaria vesca</i> (Wild strawberry)	Field	9
	<i>Vaccinium myrtillus</i> (Bilberry)	Field	9
	<i>Vaccinium vitis-idaea</i> (Cowberry)	Field	9
	<i>Rubus idaeus</i> (Raspberry)	Field	9
	<i>Achillea millefolium</i> (Yarrow)	Field	9
	<i>Equisetum pratense</i> (Shady horsetail)	Field	9
	<i>Scirpus</i> sp. (Sedge)	Field	10
	<i>Thuja plicata</i> (Cedar)	Field	10
	<i>Erigeron</i> sp. (Fleabane)	Field	10
<i>Mimulus</i> sp. (Monkey flower)	Field	10	
<i>Oryza sativa</i> (Rice)	Glass House	11	
Arsenite	<i>Vigna radiata</i> (Mung bean)	As(V) As(III)	1
	<i>Daucus carota</i> (Carrot)	Field	2
	<i>Oryza sativa</i> (Rice)	Field	3
	<i>Malus domestica</i> (Apple)	Field	4
	<i>Rubus idaeus</i> (Raspberry)	Field	5
	<i>Potentilla fruticosa</i> (Shrubby cinquefoil)	Field	5
	<i>Hordeum jubatum</i> (Foxtail barley)	Field	5
	<i>Agrostis scabra</i>	Field	5
	<i>Drepanocladus</i> sp. (Moss)	Field	5
	<i>Zea mays</i> (Maize)	As(V)	6
	<i>Cucumis melo</i> (Melon)	As(V)	6
	<i>Pisum sativum</i> (Pea)	As(V)	6
	<i>Lycopersicon esculentum</i> (Tomato)	As(V)	6
	<i>Lycopersicon esculentum</i> (N and P deficient tomato)	As(V)	6
	<i>Brassica juncea</i> (Indian mustard)	As(V)	7
	<i>Dactylis glomerata</i> (Cocksfoot grass)	Field	8
	<i>Trifolium pratense</i> (Red clover)	Field	8
	<i>Plantago lanceolata</i> (Ribwort plantain)	Field	8
	<i>Asplenium viride</i> (Green spleenwort)	Field	9
	<i>Dryopteris dilata</i> (Broad buckler fern)	Field	9
	<i>Deschampsia cespitosa</i> (Tufted hair grass)	Field	9
	<i>Picea abies</i> (Norway spruce)	Field	9
	<i>Larix deciduas</i> (European Larch)	Field	9
	<i>Alnus incana</i> (White Alder)	Field	9

Table 1 continued

Arsenic species	Plant	Exposure	Ref no.
	<i>Fragaria vesca</i> (Wild strawberry)	Field	9
	<i>Vaccinium myrtillus</i> (Bilberry)	Field	9
	<i>Vaccinium vitis idaea</i> (Cowberry)	Field	9
	<i>Rubus idaeus</i> (Raspberry)	Field	9
	<i>Achillea millefolium</i> (Yarrow)	Field	9
	<i>Equisetum pratense</i> (Shady horsetail)	Field	9
	<i>Scirpus</i> sp. (Sedge)	Field	10
	<i>Thuja Plicata</i> (Cedar)	Field	10
	<i>Erigeron</i> sp. (Fleabane)	Field	10
	<i>Mimulus</i> sp. (Monkey flower)	Field	10
	<i>Oryza sativa</i> (Rice)	Glass House	11
Monomethyl- arsonic acid	<i>Oryza sativa</i> (Rice)	Field	3
	<i>Malus domestica</i> (Apple)	Field	4
	<i>Hordeum jubatum</i> (Foxtail barley)	Field	5
	<i>Agrostis scabra</i>	Field	5
	<i>Drepanocladus</i> sp. (Moss)	Field	5
	<i>Lycopersicon esculentum</i> (N and P deficient Tomato)	As(V)	6
	<i>Asplenium viride</i> (Green spleenwort)	Field	9
	<i>Deschampsia cespitosa</i> (Tufted hair grass)	Field	9
	<i>Picea abies</i> (Norway spruce)	Field	9
	<i>Alnus incana</i> (White Alder)	Field	9
	<i>Fragaria vesca</i> (Wild strawberry)	Field	9
	<i>Vaccinium myrtillus</i> (Bilberry)	Field	9
	<i>Vaccinium vitis-idaea</i> (Cowberry)	Field	9
	<i>Achillea millefolium</i> (Yarrow)	Field	9
	<i>Equisetum pratense</i> (Shady horsetail)	Field	9
	<i>Scirpus</i> sp. (Sedge)	Field	10
	<i>Thuja Plicata</i> (Cedar)	Field	10
Dimethylarsinic acid	<i>Oryza sativa</i> (Rice)	Field	3
	<i>Malus domestica</i> (Apple)	Field	4
	<i>Hordeum jubatum</i> (Foxtail barley)	Field	5
	<i>Agrostis scabra</i>	Field	5
	<i>Drepanocladus</i> sp. (Moss)	Field	5
	<i>Lycopersicon esculentum</i> (N and P deficient Tomato)	As(V)	6
	<i>Asplenium viride</i> (Green spleenwort)	Field	9
	<i>Dryopteris dilata</i> (Broad buckler fern)	Field	9
	<i>Deschampsia cespitosa</i> (Tufted hair grass)	Field	9
	<i>Picea abies</i> (Norway spruce)	Field	9
	<i>Alnus incana</i> (White Alder)	Field	9
	<i>Fragaria vesca</i> (Wild strawberry)	Field	9
	<i>Vaccinium myrtillus</i> (Bilberry)	Field	9
	<i>Rubus idaeus</i> (Raspberry)	Field	9
	<i>Achillea millefolium</i> (Yarrow)	Field	9
	<i>Scirpus</i> sp. (Sedge)	Field	10
	<i>Thuja Plicata</i> (Cedar)	Field	10
	<i>Erigeron</i> sp. (Fleabane)	Field	10
	<i>Oryza sativa</i> (Rice)	Glass House	11
Tetramethyl- arsonium ion	<i>Drepanocladus</i> sp. (Moss)	Field	5
	<i>Dactylis glomerata</i> (Cocksfoot grass)	Field	8
	<i>Trifolium pratense</i> (Red clover)	Field	8
	<i>Plantago lanceolata</i> (Ribwort plantain)	Field	8
	<i>Asplenium viride</i> (Green spleenwort)	Field	9
	<i>Dryopteris dilata</i> (Broad buckler fern)	Field	9
	<i>Fragaria vesca</i> (Wild strawberry)	Field	9
	<i>Vaccinium vitis-idaea</i> (Cowberry)	Field	9
	<i>Mimulus</i> sp. (Monkey flower)	Field	10
Trimethylarsine oxide	<i>Dactylis glomerata</i> (Cocksfoot grass)	Field	8
	<i>Trifolium pratense</i> (Red clover)	Field	8

Table 1 continued

Arsenic species	Plant	Exposure	Ref no.
	<i>Plantago lanceolata</i> (Ribwort plantain)	Field	8
	<i>Asplenium viride</i> (Green spleenwort)	Field	9
	<i>Dryopteris dilata</i> (Broad buckler fern)	Field	9
	<i>Deschampsia cespitosa</i> (Tufted hair grass)	Field	9
	<i>Picea abies</i> (Norway spruce)	Field	9
	<i>Larix deciduas</i> (European Larch)	Field	9
	<i>Alnus incana</i> (White Alder)	Field	9
	<i>Fragaria vesca</i> (Wild strawberry)	Field	9
	<i>Vaccinium myrtillus</i> (Bilberry)	Field	9
	<i>Vaccinium vitis-idaea</i> (Cowberry)	Field	9
	<i>Rubus idaeus</i> (Raspberry)	Field	9
	<i>Achillea millefolium</i> (Yarrow)	Field	9
	<i>Equisetum pratense</i> (Shady horsetail)	Field	9
Arsenobetaine	<i>Dactylis glomerata</i> (Cocksfoot grass)	Field	8
	<i>Trifolium pratense</i> (Red clover)	Field	8
	<i>Plantago lanceolata</i> (Ribwort plantain)	Field	8
	<i>Deschampsia cespitosa</i> (Tufted hair grass)	Field	9
Arsenocholine	<i>Plantago lanceolata</i> (Ribwort plantain)	Field	8
Glycerol ribose	<i>Asplenium viride</i> (Green spleenwort)	Field	9
	<i>Dryopteris dilata</i> (Broad buckler fern)	Field	9
	<i>Deschampsia cespitosa</i> (Tufted hair grass)	Field	9
	<i>Picea abies</i> (Norway spruce)	Field	9
	<i>Alnus incana</i> (White Alder)	Field	9
	<i>Fragaria vesca</i> (Wild strawberry)	Field	9
	<i>Vaccinium myrtillus</i> (Bilberry)	Field	9
	<i>Vaccinium vitis-idaea</i> (Cowberry)	Field	9
	<i>Rubus idaeus</i> (Raspberry)	Field	9
	<i>Achillea millefolium</i> (Yarrow)	Field	9
	<i>Equisetum pratense</i> (Shady horsetail)	Field	9

Ref nos. 1. Van den Broeck *et al.* (1998); 2. Helgesen and Larsen (1998); 3. Heitkemper *et al.* (2001); 4. Caruso *et al.* (2001); 5. Koch *et al.* (2000); 6. Nissen and Benson (1982); 7. Pickering *et al.* (2000); 8. Geiszinger (1998); 9. Kuehnelt *et al.* (2000); 10. Koch *et al.* (1999); 11. Abedin *et al.* (2002b).

taken up from soil, or whether transformation takes place within the plant. However, all plants so far investigated, from both arsenic-contaminated and uncontaminated sites, have more than one arsenic species in their tissues (Table 1). The speciation of arsenic in plant tissues also has to be considered in an evolutionary context. Where plants have evolved resistance to elevated arsenic in soils, transformation of arsenic may be a constitutive or adaptive mechanism of withstanding high levels of arsenic (Meharg, 1994). The reason why plants that have not evolved arsenic resistance, transform arsenic is another matter, as typical levels of arsenic present in unenriched soils are low (Nriagu, 1994).

## II. Toxicity of arsenic species to plants

Arsenic is toxic to a wide range of organisms including plants. Early studies of the toxicity of arsenic species to terrestrial plants focused on the effects of arsenical pesticide residues on rice (*Oryza sativa*) (Wells & Gilmour, 1977). In the USA, rice production was increased in the 1970s using land previously

planted with cotton (*Gossypium* spp.). Most of the cotton soils had had repeated application of monomethylarsenate (MMA), and rice grown in these soils exhibited symptoms of straighthead disease, which included increased sterility (Wells & Gilmour, 1977). Investigations revealed that exposure to MMA did cause straighthead symptoms, that MMA was accumulated by rice, and that cultivars varied widely in their resistance to MMA (Wells & Gilmour, 1977; Gilmour & Wells, 1980; Frans *et al.*, 1988).

Studies on arsenate (the dominant form of arsenic phytoavailable in aerobic soils) toxicity have shown that plant species not resistant to arsenic suffer considerable stress upon exposure, with symptoms ranging from inhibition of root growth through to death (Macnair & Cumbes, 1987; Meharg & Macnair, 1991; Paliouris & Hutchinson, 1991; Barrachina *et al.*, 1995). There is significant evidence that exposure to inorganic arsenic species results in the generation of reactive oxygen species (ROS) (Hartley-Whitaker *et al.*, 2001a). This probably occurs through the conversion of arsenate to arsenite, a process which readily occurs in plants, and leads to the

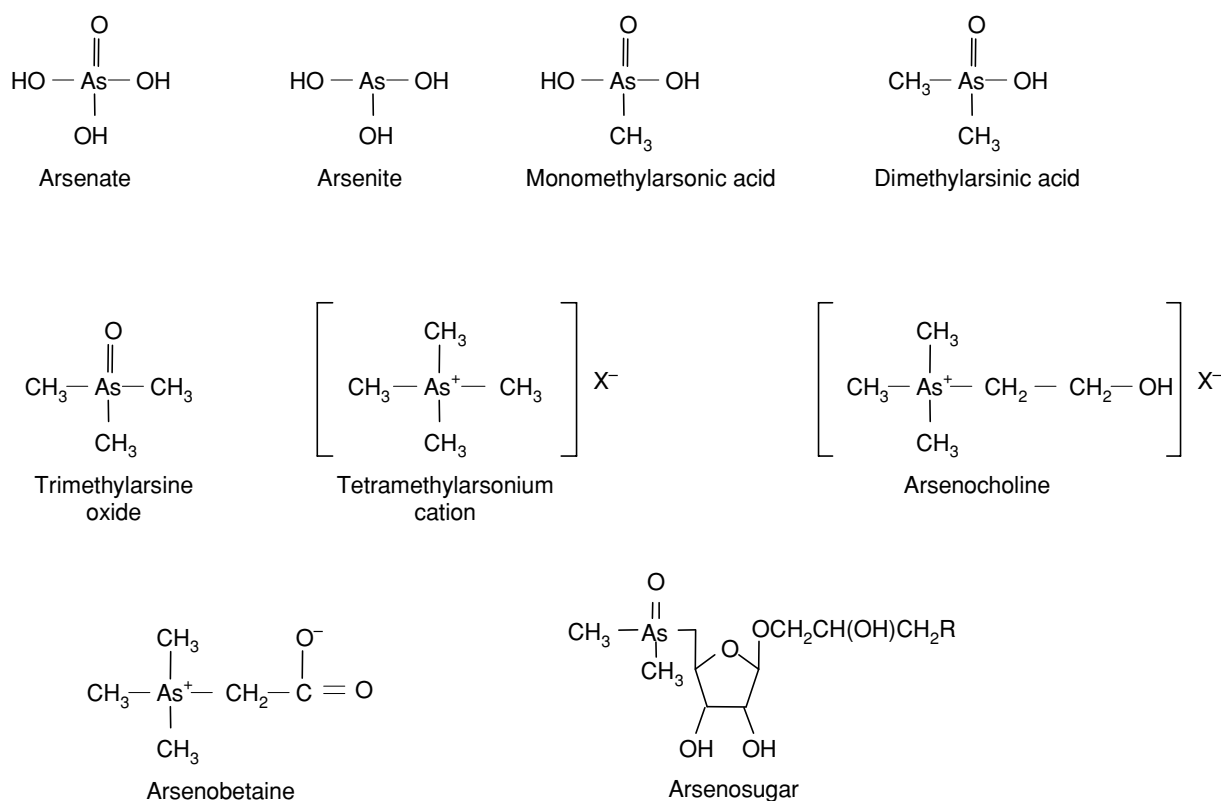


Fig. 1 Diagrammatic representation of arsenic species detected in terrestrial plants.

synthesis of enzymatic antioxidants such as superoxide dismutase (SOD), catalase and glutathione-*S*-transferase, and nonenzymatic antioxidants, for example glutathione and ascorbate (Alscher, 1989; Mylona *et al.*, 1998; Dat *et al.*, 2000; Hartley-Whitaker *et al.*, 2001a). In addition to glutathione's role as an antioxidant, it is also the precursor of phytochelatins (PCs) ( $[\gamma\text{-glutamate-cysteine}]_n\text{-glycine}$ ), which are synthesised upon exposure to inorganic arsenic. Synthesis of PCs can therefore result in glutathione depletion, reducing the amount of antioxidant available for quenching ROS (De Vos *et al.*, 1992; Sneller *et al.*, 1999; Hartley-Whitaker *et al.*, 2001b). The role of PCs in arsenic speciation in plants will be considered in more detail in section V.

Following the reduction of arsenate to arsenite in plants, arsenic may potentially be further metabolised to methylated species leading to further oxidative stress (Zaman & Pardini, 1996). Methylation in other organisms is thought to be redox driven and such reactions could give rise to ROS. Methylation of arsenic has been observed in the filamentous fungus *Scopulariopsis brevicaulis* (Cullen & Reimer, 1989), in cell suspensions of *Catharanthus roseus* (Cullen & Hettipathirana, 1994) and in phosphate-starved tomato plants (Nissen & Benson, 1982). Furthermore, methylated arsenic species are present as a minor fraction of the arsenic burden in a wide range of plants (Table 1). Arsenic metabolism will therefore be discussed in more detail later.

Inorganic arsenic species are generally highly toxic to plants. Arsenate acts as a phosphate analogue and is transported across the plasma membrane via phosphate cotransport systems (Ullrich-Eberius *et al.*, 1989). Once inside the cytoplasm it competes with phosphate, for example replacing phosphate in ATP to form unstable ADP-As, and leads to the disruption of energy flows in cells (Meharg, 1994). However, Bertolero *et al.* (1987) point out that because arsenate is rapidly reduced to arsenite in plant tissue, arsenate will not normally have high enough cytoplasmic concentrations to exert toxicity. Arsenite is also highly toxic to plants as it reacts with sulfhydryl groups ( $-\text{SH}$ ) of enzymes and tissue proteins, leading to inhibition of cellular function and death (Ullrich-Eberius *et al.*, 1989). This reduction of arsenate to arsenite, observed *in vitro*, is brought about nonenzymatically by glutathione (Delnomdedieu *et al.*, 1994), which has high cellular concentrations in plant tissues (Alscher, 1989). Also, microorganisms such as yeasts reduce arsenate to arsenite via an arsenate reductase enzyme (Mukhopadhyay *et al.*, 2000). Such reductases may also be in operation in plant tissues, though they have not been identified to date.

Mechanisms of arsenite uptake by plant roots are not clearly understood. Uptake into the ericoid mycorrhizal fungus *Hymenoscyphus ericae* is passive (Sharples *et al.*, 2000a). It has recently shown that arsenite uptake in rice is active, described by two additive hyperbolic functions over high

affinity (low concentration) and low affinity (high concentration) ranges (Abedin *et al.*, 2002a). Arsenite was as effectively transported across the plasmalemma as arsenate, although via a different uptake mechanism. An investigation into the molecular biology of arsenite transport in the yeast *Saccharomyces cerevisiae* showed that arsenite uptake was mediated by a glycerol channel (Wysocki *et al.*, 2001). The mechanistic basis for arsenite transport in rice has still to be determined. Also, it is not known if other plant species actively transport arsenite.

Organic arsenic species are generally considered to be less toxic than inorganic species to a wide range of organisms, including aquatic plants, animals and humans (Tamaki & Frankenberger, 1992). It has been presumed that this is also the case for terrestrial plants, however, research using a range of plants including *Spartina* sp., rice (*O. sativa*) and radish (*Rhaphanus sativus* L.) has reported varied results. The phyto-availability (measured as long-term arsenic uptake) of four arsenic species in hydroponic systems, to *Spartina patens* followed the trend: dimethylarsinic acid (DMA) < MMA  $\approx$  As(V) < As(III), however, the order of phytotoxicity was the reverse with As(V)  $\approx$  As(III) < MMA < DMA. This would suggest that organic arsenicals were more toxic than inorganic arsenic species (Carbonell-Barrachina *et al.*, 1998), and was confirmed in studies of radish where DMA was also identified as the most toxic arsenic species (Carbonell-Barrachina *et al.*, 1999). The uptake of arsenic by rice in long-term hydroponic culture was DMA < As(V) < MMA < As(III), however, in contrast to the *Spartina* sp. study the order of phytotoxicity was the same as the order of phytoavailability (Marin *et al.*, 1992). Differences in translocation of the four arsenic species to the shoots were also observed in both studies, with a greater proportion of MMA and DMA translocated to the shoots compared with As(V) and As(III). It has recently been shown for rice that the short-term uptake of MMA and DMA is considerably less than that of arsenate and arsenite (Abedin *et al.*, 2002a).

In soil-based studies, redox conditions and pH significantly affected the availability and consequent phytotoxicity of inorganic and organic arsenic species; however, these studies have been conducted only in wetland soils (rice paddies) where redox conditions are very different to nonwetland soils (Marin *et al.*, 1993). The redox state and pH of the soil has a major influence on arsenic speciation and solubility (Carbonell-Barrachina *et al.*, 2000). It is therefore not surprising that soil parameters influence the toxicity of arsenic species due to altered availability (solubility or mobility).

### III. Arsenic resistance in plants

#### 1. Arsenic resistance mechanisms

Although the physiological basis of metal(loid) resistances has been investigated in some detail, it is only for arsenate

resistance that a full mechanistic understanding is available (Meharg & Macnair, 1992). Arsenic is somewhat unusual compared with transition metals and other metalloids for which plant resistances have been investigated. Other elements studied have been either nonessential or micronutrients, with nonessential elements sometimes behaving as analogues of micronutrients (Marschner, 1995). For these elements, plants have evolved homeostasis mechanisms to maintain low cellular concentrations. Consequently, detecting gross physiological changes in uptake and metabolism/complexation/intracellular compartmentalisation has been difficult, although it is increasingly clear that intracellular compartmentalisation has a major role to play in adaptation for some elements (Clemens, 2001). Arsenate is, in contrast, an analogue of the macronutrient phosphate. Thus plants growing on arsenate-contaminated soils will assimilate high levels of arsenate unless they have altered phosphate transport mechanisms (Meharg & Macnair, 1992; Sharples *et al.*, 2000a). As one of arsenate's primary modes of toxic action is competing with phosphate (such as in ATP formation), cytoplasmic concentrations of arsenate (and arsenite which is readily formed from arsenate in the cytoplasm) must be kept low to maintain cellular function (Meharg, 1994).

Arsenate resistance has been identified in a number of species on arsenic-contaminated soils including: *Andropogon scoparius* (Rocovich & West, 1975), *Agrostis castellana*, *A. delicatula* (De Koe & Jacques, 1993), *A. capillaris*, *Deschampsia cespitosa* (Meharg & Macnair, 1991), *H. lanatus* (Macnair & Cumbes, 1987), *S. vulgaris* (Paliouris & Hutchinson, 1991), *Plantago lanceolata* (Pollard, 1980), and *C. vulgaris* (Sharples *et al.*, 2000b). In populations of *H. lanatus* from uncontaminated soils across the UK, approx. 45% of seeds gave rise to arsenic-resistant plants (Meharg *et al.*, 1993), whilst individuals of *S. vulgaris* (Paliouris & Hutchinson, 1991) and *P. lanceolata* (Pollard 1980) from uncontaminated soils also exhibited resistance to arsenic.

Arsenate sensitivity is intimately linked to phosphate nutrition, with increased phosphate status leading to reduced arsenate uptake, through suppression of the high-affinity phosphate/arsenate uptake system (Meharg & Macnair, 1991, 1992). Nonresistant plants can be made more resistant to arsenate by raising their phosphorus status, which in turn leads to lower levels of arsenate accumulation through suppression of phosphate/arsenate uptake (Meharg *et al.*, 1994b). The phosphate/arsenate transporter has a higher affinity for phosphate, and if external phosphate status is high, phosphate will be taken up more effectively compared to arsenate (Meharg & Macnair, 1994). However, in most arsenate-resistant plants the high-affinity uptake system is always suppressed and is insensitive to plant phosphorous status (Meharg & Macnair, 1992). Thus decreased sensitivity in resistant plants with high phosphate status is not due to a difference in arsenate influx, but is presumably a result of higher cytoplasmic phosphate status. This may enable phosphate to

compete more effectively with arsenate for ATP, decreasing arsenate toxicity within the cell (Meharg, 1994).

The strategy generally utilised by plants to obtain arsenate resistance contrasts with that of bacteria and yeasts. Bacteria and yeasts reduce arsenate to arsenite, and then efflux the arsenite from their cells through arsenite transporters (Rosen, 1999). *Saccharomyces cerevisiae*, as well as exhibiting arsenite extrusion, can complex As(III) with glutathione to give As(GS)<sub>3</sub> which is then actively transported into the vacuole through a As(GH)<sub>3</sub> transporter (Rosen, 1999).

## 2. Arsenate resistance polymorphism

Arsenate resistance is under single gene control (Meharg *et al.*, 1992). It is unique amongst metal(loid) resistances in plants in that, at least for *H. lanatus*, it is polymorphic (i.e. that both resistant and nonresistant plants are present in populations at frequencies that cannot not be explain by mutation rate alone) in all populations from uncontaminated sites so far investigated (Meharg *et al.*, 1993). This polymorphism for arsenate resistance also appears to occur in other plant species (Fitter *et al.*, 1998). The polymorphism implies that the cost of resistance is low or that there is a counteracting benefit (Meharg *et al.*, 1993). Arsenate-resistant plants differ from nonresistant phenotypes in their reproductive behaviour, with resistant phenotypes flowering earlier and investing more in their reproductive biomass (Fitter *et al.*, 1998; Wright *et al.*, 2000). In addition, arsenate-resistant plants have higher shoot P status, despite exhibiting suppressed phosphate uptake, and it has been suggested that suppressed uptake is a feedback mechanism in response to high shoot P (Meharg *et al.*, 1994b; Wright *et al.*, 2000). It has also been observed that the frequency of resistant individuals is higher in seed than in adult plants, implying that selection is operating between these two stages (Fitter *et al.*, 1998). We postulate that – as *H. lanatus* occurs in soils that range from being phosphorus deficient to phosphorus sufficient, and that this species rapidly colonises disturbed sites yet is also found in stable pasture communities – that the 2 phenotypes endow the species with the attributes required to colonise a broad range of niches.

Plants with high shoot P status and suppressed uptake allocate more resources into seed production, and less into vegetative growth, thus affecting their competitive ability and survival. This in turn maintains the polymorphism, with arsenate resistance a coincidental benefit of down-regulation of the high affinity phosphate transporter (Fitter *et al.*, 1998). As the rate limiting step in phosphate acquisition is diffusion of phosphate to the root surface, and not transport of phosphate across the plasmalemma (Nye, 1977), having suppressed high affinity phosphate transport may have little consequence for the plants ability to acquire P. This raises the question as to why plant roots have high affinity transport in the first place. Perhaps it is to exploit nutrient flushes, or it may be an evolutionary carry over from aquatic environments

were phosphate uptake would not have the diffusion limitations observed in soil.

Whilst these pleiotrophic effects (including arsenate resistance) have been characterized in *H. lanatus*, they also appear to be more widespread. A mutant of *Arabidopsis thaliana*, *pho2*, which accumulates high P concentrations in its shoots (Delhaize & Randall, 1995), flowered earlier than a wild-type strain and invested more in its reproductive biomass, duplicating results reported for *H. lanatus* (Fitter *et al.*, 1998). This implies that the polymorphism for high and low shoot P could have evolved in a range of plant species as a trigger for flowering, although the precise mechanisms are still unclear.

## 3. Mycorrhizal fungi and arsenic resistance

Plants growing on arsenic contaminated soils tend to be mycorrhizal (Meharg & Cairney, 1999; Gonzalez-Chavez, 2000; Sharples *et al.*, 2000a,b). When considering the toxicity of arsenic to plants, the role of mycorrhizal associations must also be considered. As one of the principal roles for mycorrhizal fungi in associations is obtaining phosphorus for their hosts (Smith & Read, 1997), this may provide a problem on arsenate contaminated substrates as enhanced acquisition of phosphate may also lead to enhanced acquisition of arsenate. Gonzalez-Chavez (2000) reported that infection of both tolerant and nontolerant plants by AM fungi from arsenic contaminated sites further enhanced resistance to arsenate. However, the mechanism by which AM fungi influence arsenate resistance and its interrelationship with arsenate/phosphate uptake is yet to be elucidated.

Arsenate-resistant phenotypes of *H. lanatus* with suppressed phosphate/arsenate uptake had significantly higher arbuscular mycorrhizal fungal (AMF) infection levels than nonresistant phenotypes (Meharg *et al.*, 1994a) on uncontaminated soils. On uncontaminated sites, AM plants with suppressed uptake had lower shoot P than nonmycorrhizal (NM) plants and invested less in their reproductive biomass (Wright *et al.*, 2000). This correlation between low shoot P and lower investment in reproduction agrees with the hypothesis described above (section 2), but how AMF affect plants in this way is unclear.

In *Calluna vulgaris*, a plant species that forms an ericoid mycorrhizal association, Sharples *et al.* (2000b) showed that the fungal symbiont *Hymenoscyphus ericae* played a major role in the colonisation of arsenic-contaminated mine-spoil by reducing arsenic exposure of the host. The plant host and mycorrhizal symbiont had coevolved to obtain phosphate and exclude arsenate. This was achieved, as resistant *H. ericae* was capable of enhanced efflux of arsenite, following reduction of assimilated arsenate. This is the mechanism that bacteria and yeasts use to achieve arsenic resistance (Silver & Phung, 1996; Rosen, 1999).

It is generally considered that arsenite is more toxic than arsenate, but this is the reverse for *H. ericae* (Sharples *et al.*,

2000a). Caution should be taken in generalising the relative toxicities of arsenic species, as it appears that this varies interspecifically, with inorganic and organic arsenic species having varied relative toxicity to a number of plant species (see section II).

#### IV. Complexation of arsenic

Synthesis of PCs is induced by a range of cations such as  $\text{Ag}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  and the oxy-anions arsenate and selenate (Grill *et al.*, 1985). PCs are synthesised from reduced glutathione (GSH) by the transpeptidation of  $\gamma$ -glutamyl-cysteinyl dipeptides, through the action of the constitutive enzyme PC synthase (Schmöger *et al.*, 2000; Vatamaniuk *et al.*, 2000). Detailed kinetic studies of intact *A. thaliana*, *S. vulgaris* and *H. lanatus* (Sneller *et al.*, 1999; Schmöger *et al.*, 2000; Hartley-Whitaker *et al.*, 2001c), cell cultures of *Rauvolfia serpentina* and *S. vulgaris* (Schmöger *et al.*, 2000), root cultures of *Rubia tinctorum* (Maitani *et al.*, 1996) and enzyme preparations of *S. vulgaris* (Schmöger *et al.*, 2000), have shown that PCs are induced on exposure to inorganic arsenic. Size exclusion chromatography indicates that arsenic is associated with PCs in cell extracts of *S. vulgaris* (Schmöger *et al.*, 2000), whilst X-ray absorption spectroscopy (XAS) of *Brassica juncea* has determined that arsenic is present in the As(III) valence state, co-ordinated with three sulphur groups (Pickering *et al.*, 2000). Further evidence of the existence of As-PC complexes was reported when a *cad1-3 A. thaliana* mutant that does not produce PCs, was shown to be considerably more sensitive to arsenate than the wild-type (Ha *et al.*, 1999), whilst *R. serpentina* cell cultures grown in the presence of  $\gamma$ -glutamylcysteine synthase inhibitor, buthionine sulfoximine (BSO), are increased in their sensitivity to arsenite (Schmöger *et al.*, 2000). In addition, the intimate involvement of PC production in arsenate resistance of *H. lanatus* was demonstrated, as higher concentrations of PCs were produced in arsenate-resistant phenotypes, and their arsenic resistance was completely removed by the presence of the inhibitor BSO (Hartley-Whitaker *et al.*, 2001b,c).

There is considerable evidence that at least part of the arsenic accumulated by plants is co-ordinated with glutathione and/or PCs, and that complexation is independent of the inorganic arsenic species originally present (Delnomdedieu *et al.*, 1994). *In vitro* nuclear magnetic resonance (NMR) studies have demonstrated that two molecules of GSH are required to reduce arsenate to arsenite, with GSH oxidized via the formation of a disulfide bond to give oxidised glutathione (GSSG) (Delnomdedieu *et al.*, 1994). This in turn is rapidly recycled to two GSH molecules (Cobbett, 2000). A ratio of 1 : 1 of arsenate to GSH only resulted in the formation of GSSG, with no formation of complexes. This demonstrates that arsenate must be reduced to arsenite before formation of GSH complexes can occur, with three GSH molecules

required to complex arsenite (Schmöger *et al.*, 2000). GSH can also reduce pentavalent dimethylarsinic acid to trivalent dimethylarsonic acid, with subsequent GSH complexes having a 1 : 1 stoichiometry (Delnomdedieu *et al.*, 1994). As DMA has been found in a wide range of plant species (Table 1), it is interesting to speculate that DMA, observed in extracts performed under nonphysiological conditions, may be co-ordinated with GSH and/or PCs in plant tissues. Although the pentavalent form of the dimethyl species has been determined in extracts, the trivalent form is unstable and will oxidise to the pentavalent form.

The effect of arsenic exposure on GSH levels in plant tissue is still unclear due to the small number of studies. Concentrations appear to stay relatively constant in *H. lanatus* and *S. vulgaris* over a range of arsenate exposure concentrations and over time courses (Sneller *et al.*, 1999; Hartley-Whitaker *et al.*, 2001a). This is despite dramatic increases in PC concentrations on arsenate exposure, and the fact that PCs are present at much greater concentrations than GSH in plants exposed to relatively high levels of arsenate. The study of Sneller *et al.* (1999) on *S. vulgaris* found that over a range of arsenate exposures the ratio of PC-SH to As was 2–5 : 1. In *H. lanatus* this ratio varied from 1 : 1 to 1.7 : 1 in arsenic resistant and nonresistant phenotypes exposed to their arsenate EC50 levels (concentration that will inhibit growth by 50%) (Hartley-Whitaker *et al.*, 2001b). As GSH is a substrate for PC synthase, it might be expected that GSH depletion would occur due to PC production. This depletion has previously been demonstrated in response to Cu, although other studies have reported an increase or no change in GSH concentrations in response to copper and cadmium exposure (De Vos *et al.*, 1992; De Knecht *et al.*, 1995; Galli *et al.*, 1996).

The localisation of As-PC complexes within plant tissue is still unknown. As-PC complexes are not stable at neutral or alkaline pHs, but are stable under acidic conditions present in the vacuole (Schmöger *et al.*, 2000; Sneller *et al.*, 2000). If As-PC complexes are also transported into the root vacuole, then under the acidic conditions present, they might remain stable preventing re-oxidation of As (III) and allowing accumulation of high concentrations of As-PC complexes in arsenate-resistant plants. The yeast *S. cerevisiae* and the protozoan parasite *Leishmania tarentolae* both form and sequester As-thiol complexes in intracellular compartments (Mukhopadhyay *et al.*, 1996; Ghosh *et al.*, 1999). In nonresistant plants, vacuolar localisation of PC-complexes would also be possible, however, differential arsenate influx and feedback regulation would prevent the formation of high levels of As-PC complexes due to the toxic effect of arsenate in nonresistant plants.

#### V. Arsenic metabolism

Arsenic is metabolised from inorganic to organic forms by a wide range of organisms, with limited evidence that this occurs in plants (Nissen & Benson, 1982). In a range of

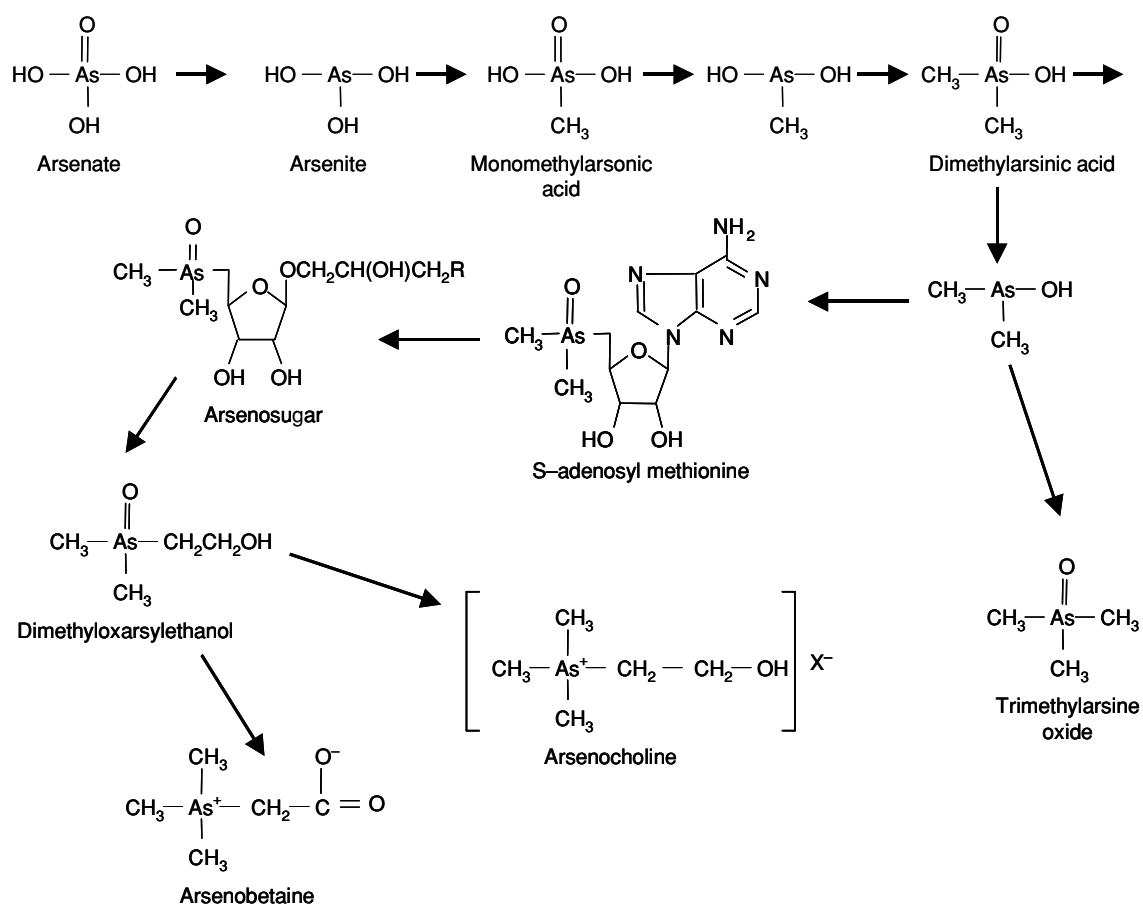


Fig. 2 Potential pathways for the reduction and methylation of arsenic by terrestrial plants based on pathways for aquatic organisms and fungi (Philips, 1990; Tmaki & Frankenberger, 1992).

organisms, metabolism typically occurs through biomethylation to give MMA, DMA, tetramethylarsonium ions (TETRA) and trimethylarsonium oxide (TMAO) (Fig. 1 and 2; Cullen & Reimer, 1989). Further metabolism to form arsenocholine, arsenobetaine and arseno-sugars occurs in a number of organisms, and these compounds have been measured in some terrestrial plants (Fig. 1; Tamaki & Frankenberger, 1992; Geiszinger, 1998; Kuehnelt *et al.*, 2000). However, it has not been proven whether these compounds are actually metabolised by the plant (Fig. 2), or simply taken up in those forms from soil solution. Factors which can influence the arsenic species present in a plant are: arsenic species present in the soil; the ability of the compounds to enter the plant, actively or passively; the ability of the plant to synthesise arsenic species; and the presence of arsenic species adsorbed to the outside surface of the plant roots.

In terrestrial plants it is not clear which of these factors influence the arsenic species identified. Reduction of arsenate to arsenite has been conclusively demonstrated in plants but methylation has not (Van den Broeck *et al.*, 1998; Pickering *et al.*, 2000). Organic arsenic species present in field samples of plants may have been taken up from the soil solution in that

form, as they can be present in soil through microbial activity (Cullen & Reimer, 1989; Koch *et al.*, 1999). This hypothesis is supported, for MMA and DMA, as their uptake from hydroponic solutions has been demonstrated in a number of plant species (Marin *et al.*, 1992, 1993; Carbonell-Barrachina *et al.*, 1998, 1999; Abedin *et al.*, 2002a), including the arsenic hyperaccumulator *Pteris vittata* (Ma *et al.*, 2001). However, it is also possible that the plants themselves are methylating arsenic. Alternatively, microbial endophytes could potentially transform arsenic species. In the study of Koch *et al.* (2000), no evidence of external methylation was found in the surrounding soil or water, and yet a number of plant species contained MMA, DMA, TETRA and TMAO. TMAO was only found in *Mimulus* sp. and the authors hypothesised that the plant may have been growing in a specific microenvironment where this compound was available. However, no evidence of TMAO was found in the surrounding environments indicating that methylation could have taken place in the plant. These methylated compounds (MMA, DMA, TETRA and TMAO) have been identified by other researchers (Kuehnelt *et al.*, 2000). In addition a small number of samples have contained arsenobetaine, arsenocholine and the arseno-sugar,

glycerol-ribose (Table 1). The authors concluded that glycerol-ribose had not been previously recorded in plants due to the very low concentrations present, requiring extremely sensitive analysis (Kuehnelt *et al.*, 2000), but whether these compounds had been taken up or metabolised internally is unknown.

An early study suggested that P – stressed tomato could methylate arsenate (Nissen & Benson, 1982). Plant-mediated methylation of arsenic has also been investigated in rice. Plants were exposed to MMA amended nutrient solution for 7 d, after which time the majority of arsenic in the plant tissues was present as monomethyl-arsenic compounds, that is, no methylation or demethylation appeared to have taken place (Odanaka *et al.*, 1985). However, when the nutrient solution was analysed, dimethyl- and trimethyl-arsenic compounds were present and comprised 18% of the total arsenic budget. This would suggest that MMA was being methylated by microorganisms in the nutrient solution or that di- and trimethyl-species were being excreted or exuded from the roots following methylation. The former explanation is more likely.

As described in section III, the ability of plants to suppress arsenate uptake is a coincidental benefit of down regulation in the P inefficient phenotype of *H. lanatus*, due to a polymorphism for P use efficiency (Fitter *et al.*, 1998). Likewise, it is also possible that the ability to metabolise inorganic arsenic is coincidental, occurring through induction of constitutive enzymes. A range of enzymatic processes have stimulated in plants as a defence against essential metals present at potentially toxic concentrations. Production, for example, of PCs, glutathione, and superoxide dismutase is stimulated in response to Cd and Hg (Hartley-Whitaker *et al.*, 2001a,b) and arsenate may also fortuitously induce such responses, allowing the plants to constitutively detoxify low levels of arsenate contamination (Hartley-Whitaker *et al.*, 2001a,b). Enzymes potentially involved in the methylation of arsenic are constitutive in plants as they are involved in necessary enzymatic processes such as polyamine and ethylene synthesis (Tamaki & Frankenberger, 1992; Ravanel *et al.*, 1998; Edmonds, 2000; Kakkar *et al.*, 2000). As(III) acts as a nitrogen analogue, notably in arsenobetaine and arsenocholine (Marschner, 1995), and in addition, glycinebetaine and choline are osmoregulators, produced by many succulent species on exposure to water stress (Sakamoto & Murata, 2000). These findings suggest that the ability of plants to synthesise organic arsenic species may again be fortuitous utilization of constitutive enzymatic processes.

If arsenic metabolism of organic species does occur in plants, there are significant implications as arsenic speciation can significantly affect toxicity, as discussed above. This is certainly true for the formation of As-PC complexes. The presence of a range of arsenic species in plants suggests, at least for plants that have evolved arsenic resistance, that metabolism of arsenic is linked to resistance. As argued in this review,

metabolism appears constitutive to a wide range of plant species, not just those that have evolved resistance to arsenic. Adaptive resistances normally involve alteration of arsenic transport (Meharg, 1994), but these adaptive mechanisms are dependent on constitutive metabolism of arsenic (Hartley-Whitaker *et al.*, 2001b,c).

## VI. Arsenic hyperaccumulation

The exception to the rule that plants down regulate their arsenate uptake to tolerate high levels of arsenate is a plant that exhibits arsenic hyperaccumulation. The Brake fern, *Pteris vittata*, has the remarkable ability to hyperaccumulate arsenic in its shoots, with shoot concentrations reaching levels ~100-fold higher than soil concentrations (Ma *et al.*, 2001). This ratio is held for uncontaminated soils (6 mg kg<sup>-1</sup> As) and highly contaminated soils (1500 mg kg<sup>-1</sup> As). The fern is capable of taking up a range of inorganic and organic arsenic species including arsenate, arsenite and MMA, with up to 93% of the arsenic concentrated in the fronds (Ma *et al.*, 2001). This capability to hyperaccumulate arsenic is, to date, unique.

A wide range of plant species have been identified on arsenic-contaminated land. In a detailed study of gold mines in Zimbabwe, 150 arsenic-tolerant indigenous species were recorded, with woody species and grasses predominating (Wild, 1974). Whether any of these species are hyperaccumulators is unknown, as arsenic analysis was only performed on a limited number of species. However, many of the species were identified as occurring on nickel, copper and serpentine anomalies in Zimbabwe (Wild, 1974) and interestingly the fern, *Pteris vittaria*, was identified on one of the gold mines studied.

Arsenic is phytotoxic at elevated concentrations, as we have discussed in this review, so it is intriguing to know how arsenic is metabolised, transported and stored in *P. vittata*. This arsenic is present in inorganic forms, with 47–80% of the arsenic present as arsenite in the fronds. This would suggest that complexation with phytochelatins is occurring (see section 4), although this has not been tested for to date.

In addition to the remarkable ability of *P. vittata* to tolerate high internal arsenic burdens, the extraction of low levels of arsenate from soil is extraordinary, especially considering that arsenate mobility in soil is limited. A number of plant species have evolved mechanisms to mobilise phosphate from soils, such as the production of proteoid roots or acidification of the rhizosphere (Marschner, 1995). Similar adaptations in root morphology/rhizosphere chemistry may be deployed by *P. vittata* to mobilise arsenate, although the mechanisms used to mobilise arsenate will also mobilise phosphate. There are a number of mechanisms by which *P. vittata* could assimilate high concentrations of arsenic. For example, the phosphate nutrition of this plant has not been investigated. If the plant

is also able to hyperaccumulate phosphate, this may totally or partially explain its ability to tolerate very high concentrations of arsenate as non-resistant *H. lanatus* can become arsenate-resistant if it has a high phosphorous status (Meharg *et al.*, 1994b). However, *P. vittata* can also hyperaccumulate arsenite and MMA, suggesting that alteration of phosphate transport is not involved in hyperaccumulation, or perhaps that the speciation of arsenic is altered in the rhizosphere before uptake occurs.

A number of reasons have been put forward to explain why plants hyperaccumulate metals for which there is no metabolic need, and these hypotheses could also apply to hyperaccumulation of arsenic. Hyperaccumulation may be a means of avoiding competition from less metal-tolerant plants; a strategy for attaining metal resistance; the result of inadvertent uptake of heavy metals (i.e. potentially *P. vittata* accumulates arsenate as a consequence of phosphate acquisition); or a defence strategy to deter herbivores or kill pathogens (Brooks, 1998).

## VII. Evolutionary context

Terrestrial life appears to have evolved around subaerial hot springs, with some of the earliest known examples of land plants from Rhynie, Scotland having high levels of arsenic associated with them (Rice *et al.*, 1995). Many analogous modern hot springs also have high arsenic levels with four principal types of arsenic occurrence found: active hot springs; layered surface deposits (sinters) deposited by hot springs; highly fractured rock zones formed immediately beneath hot springs; and chemically altered and mineralized rock from the deeper roots of hot spring systems (Koch *et al.*, 1999; Webster, 1999; Craw *et al.*, 2000). Consequently, early terrestrial plant forms may have had to cope with high levels of arsenic in their environment, with this capability conferred on modern plants. Furthermore, it has also been postulated that life itself has evolved in deep sea hot springs (McClendon, 1999). Such hot springs are also enriched in arsenic and the biota present, at least the microorganisms, in such environments speciate inorganic arsenic to organic forms (Larsen *et al.*, 1997).

In photosynthetic microorganisms, exemplified by marine and freshwater algae, arsenic can reach high concentrations and is metabolised into organic arsenic species such as arsenosugars (Benson, 1989; Phillips, 1990). Arsenate is an analogue of phosphate for marine plants (as in terrestrial plants) and it is thought that low phosphate availability in seawater leads to arsenate being present at similar concentrations to phosphate (Benson, 1989). Plants will accumulate high levels of arsenate in an attempt to obtain phosphate, resulting in the need to detoxify inorganic arsenic by metabolism or arsenate exclusion. As terrestrial life evolved from marine life forms, the ability to metabolise arsenic may be an evolutionary carry over from such antecedents.

## VIII. Conclusions

Many sites are contaminated with organic arsenic compounds from the now banned application of organic arsenic pesticides and through natural transformation of inorganic arsenic to organic arsenic in soils. Plants can accumulate both inorganic and organic forms of arsenic. However, it is still not certain if plants can convert inorganic forms of arsenic into organic forms. It is clear that phytochelatin complexation is deployed by a wide range of plant species to detoxify inorganic, and potentially organic arsenic species. Even though this complexation of arsenic appears to be constitutive to all species so far investigated, those species that have evolved arsenate resistances (usually achieved by suppressing arsenate/phosphate uptake) still require this constitutive complexation. While it has long been recognised that arsenite and arsenate can exert toxicity by reacting with –SH groups and competing with phosphate in cellular process, respectively, redox stress caused by the cellular reduction and oxidation must now also be considered as a major toxic action of these ions.

It has been argued here that in general, the speciation of arsenic in plants is governed by enzyme systems which process arsenic as analogues of phosphate and nitrogen, for As(V) and As(III), respectively, or by enzyme systems which generally respond to oxidative stress and to maintaining toxic ion homeostasis in the cytoplasm. However, plants may have evolved from progenitors which lived in environments more enriched in arsenic than present on most soils today. Thus the enzyme systems which coincidentally appear to regulate arsenic speciation may have evolved in an arsenic rich environment.

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