

Review of Literature

2.1 HISTORY OF AS AND ITS POSITION IN THE PERIODIC TABLE

The name 'Arsenic' is derived from the Greek word *arsenikon* meaning potent (Frost 1984). Arsenic was discovered by an Arabian alchemist, Geber during the eighth century when he heated orpiment (As_2S_3) (Mellor 1954). In 1775, a famous Swedish chemist, Scheele discovered arsine (AsH_3) (Nriagu 2002), however its deadly nature was not known until the death of a chemistry professor in Munich, who inhaled a minor quantity of AsH_3 , in 1815. The period from 1850 to 1950 is regarded as the *century* of As contamination. This was the time when human beings were affected by As in medicine, food, air, water and at work; and the world production of As trioxide (As_2O_3 [white As]) increased from 5,000 to 60,000 tons year⁻¹ during this period (Jenkins 1972; Nriagu 2002). Arsenic minerals such as realgar (AsS) and orpiment were used in pigment formation, wall paintings and as depilatories in the leather industry (Nriagu 2002). Several arsenical compounds such as sodium arsenite (NaAsO_2), calcium arsenate (CaAsO_4), and lead arsenate (PbAsO_4) were used in the manufacturing of pesticides, herbicides, wood preservatives, cotton desiccants, dyes and ceramics. During the 19th century in the UK, Fowler's Solution was the best known curing product (containing 1% As trioxide), which was mixed with animal feeds to help kill worms in animal gut (Ratnaïke 2006). It was also used for the treatment of leukaemia, psoriasis and dermatitis herpetiformis ailments (Ratnaïke 2006). From early 1900s to 1955, sodium arsenite was used to treat ticks

Boophilus microplus L.) in livestock throughout the world (e.g., the USA, South Africa, New Zealand) including Australia (Smith et al. 1998; Okonkwo 2007; Sarkar et al. 2007).

Arsenic is a metalloid (element that has properties of both metals and non-metals), and belongs to the Group VA in the periodic table of elements (Allen 1989; Emsley 1991; Smith et al. 1998). Electronegativity of As falls within the standard Pauling scale of electronegativities and enables it to characterise as a metalloid (Allen 1989). It can exist in the allotropic forms: rhombohedral (yellow, α -As) and hexagonal (black, β -As) crystal structures (Allen 1989). Arsenic has an atomic number 33, atomic mass $74.9216 \text{ g mol}^{-1}$ and an electronic configuration of $4s^2 3d^{10} 4p^3$; therefore, elemental As has 5 valence electrons (Smith et al. 1998; Grafe and Sparks 2006). It can be present in four different oxidation states: arsenide (As^{3-}), elemental As (As^0), arsenite (As^{III}) and arsenate (As^{V}). Arsenate and As^{III} are the most abundant species in the soil environment (Smith et al. 1998). Arsenite is a dominant species under anoxic soil conditions and more mobile and toxic than As^{V} , while As^{V} is commonly found in the oxidised soil environments.

2.2 DISTRIBUTION OF AS IN THE SOIL ENVIRONMENT

Arsenic is ubiquitous in nature and widely distributed in soils, sediments, water and atmosphere. In the terrestrial environment, concentration of As ranges from $1.5\text{--}3 \text{ mg kg}^{-1}$ (Lombi et al. 2000b) and is the 20th most abundant element in the earth crust (Mandal and Suzuki 2002; Smedley and Kinniburgh 2002; Mahimairaja et al. 2005). In seawater and human beings it is ranked the 14th and 12th element in abundance, respectively (Dermatas et al., 2004; Hudson-Edwards et al., 2004).

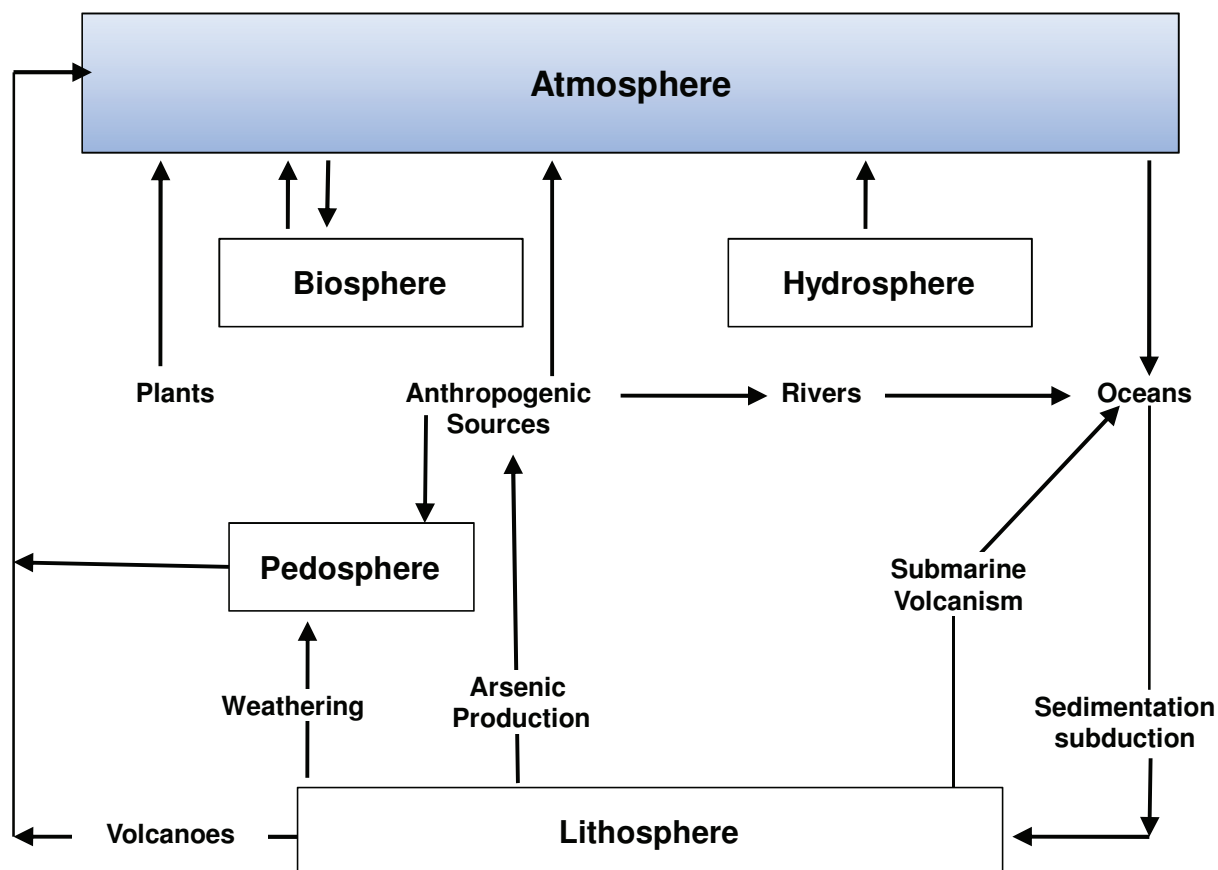


Figure 2.1 Global As cycle (adapted from Matschullat 2000).

The As cycle occurs between the lithosphere, pedosphere, biosphere, hydrosphere and atmosphere (Figure 2.1). A global atmospheric value of $73,540 \text{ t year}^{-1}$ has been estimated (Chilver and Peterson 1987). Volcanic eruptions are responsible for the deposition of As-containing particulate matter in the atmosphere which may retain for 7 to 10 days. The anthropogenic emissions account for 40% As released in atmosphere, which include wood preservation, herbicides, steel production, lead (Pb) and zinc (Zn) smelting and incineration (Matschullat 2000).

In soils, As distribution depends on the type of the parent material. Various geochemical materials containing As are listed in Table 2.1.

Table 2.1 Review of As concentrations in different materials on earth; adapted from (Smith et al. 1998; Mandal and Suzuki 2002).

Materials	Arsenic concentration (mg kg⁻¹)
(a) Igneous	
<i>Acidic</i>	
Rhyolite (extrusive)	3.2–5.4
Granite (intrusive)	0.18–15
<i>Intermediate</i>	
Latite, andesite, trachytes (extrusive)	0.5–5.8
Diorite, granodiorite, syenite (intrusive)	0.09–13.4
<i>Basic</i>	
Basalt (extrusive)	0.18–113
Gabbro (intrusive)	0.06–28
<i>Ultrabasic</i>	
Peridotite, dunite, serpentine	0.3–15.8
(b) Metamorphic rocks	
Quartzite	2.2–7.6
Slate/phyllite	0.5–143
Schist/gneiss	0.0–18.5
(c) Sedimentary rocks	
<i>Marine</i>	
shale/claystone (near shore)	4.0–25
Shale/claystone (offshore)	3.0–490
Carbonates	0.1–20.1
Phosphorites	0.4–188
Sandstone	0.6–9

Table 2.1 Continued...

Materials	Arsenic concentration (mg kg ⁻¹)
<i>Non-marine</i>	
Shales	3.0–12
Claystone	3.0–10
<i>Recent sediments (marine)</i>	
Muds	3.2–60
Clays	4.0–20
Stream/river	5.0–4,000
Lake	2.0–300
Soils	< 0.1–97

Soils developed from sedimentary rocks have a higher (1.7–400 mg kg⁻¹) background As concentration than soils which originate from igneous rocks (1.5–3.0 mg kg⁻¹) (Kabata-Pendias and Pendias 1984; Nriagu and Pacyna 1988). In various countries, including India, Bangladesh, Argentina, China, France, Germany, Italy, Japan, Mexico, South Africa, Switzerland and the USA, As concentrations in soils range from 0.1–50 mg kg⁻¹ (mean = 5 mg kg⁻¹). Arsenic has been reported in the range of 1–40 mg kg⁻¹ in uncontaminated soils, with the lowest concentrations found in sandy soils and those derived from granites, while higher concentrations are reported in alluvial and organic soils (Mandal and Suzuki 2002). In Australia, the average As concentration in the continental earth crust ranges between 1 and 2 mg kg⁻¹; however, reports up to 40 mg kg⁻¹ As are not unusual (Smith et al. 2003).

Naturally occurring As sulfide minerals include arsenopyrite (FeAsS), realgar (AsS) and orpiment (As₂S₃). Hydrothermal and magmatic ore deposits are the natural habitat of these compounds. Other transition metals such as copper (Cu), cobalt (Co) and nickel (Ni) also

form a variety of sulfides and sulfosalts in combination with As and sulfur. Various minerals of As occurring in soil are presented in Table 2.2.

Arsenate and As^{III} are the two main inorganic As species present in soil and monosodium methyl arsenate (MSMA) and disodium methyl arsenate (DSMA) are the most abundant organic species. Inorganic compounds are more toxic than organic compounds and As^{III} is more toxic and mobile than As^{V} (Masscheleyn et al. 1991; Smith et al. 1998; Mahimairaja et al. 2005; Al-Abed et al. 2007; Ascar et al. 2008). Under reduced soil conditions (redox potential; $E_h < -200$ mV), As^{III} is predominant but in oxic ($E_h > 200$ mV) environments As^{V} is dominant (Masscheleyn et al. 1991; Marin et al. 1993; Ascar et al. 2008). Various factors, such as sorbing components of the soil, pH, E_h affect the forms of As in soil. Arsenic compounds which are more important in the environment are shown in the Table 2.2.

Inorganic As species. Arsenate and As^{III} are the two main inorganic As species present in soil depending on the pH and E_h conditions (Masscheleyn et al. 1991; Marin et al. 1993; Smith et al. 1998; Al-Abed et al. 2007; Ascar et al. 2008). Under normal pH range (4–8) the most thermodynamically stable As species present in soil are H_3AsO_3 (As^{III}) and H_2AsO_4^- and HAsO_4^{2-} (As^{V}) (Masscheleyn et al. 1991). Arsenate is a weak triprotic acid with acid dissociation constants (pka) values ranging between 2.20 and 11.53 (eqs. 1–3) (Smith et al. 1998; Grafe and Sparks 2006). Arsenate acts as a proton (H^+) acceptor as well as donor within the pH range of 2.23–11.50. Arsenate may therefore behave both as Bronsted acid and Bronsted base. Arsenate can also accept a pair of electrons (Lewis acid) as well as donate a pair of electrons (Lewis base), since H^+ is considered an electron pair acceptor and its conjugate base is an electron pair donor (H^+ versus AsO_4^{3-}) (Grafe and Sparks 2006). Arsenate is chemically analogous to phosphate, and thus classified as a border line hard base; therefore, preferentially reacts with other hard or borderline hard acids (Fe^{3+} , Al^{3+} or $\text{Mn}^{2+/4+}$).

Table 2.2. Arsenic minerals in the soil environment; adapted from Mandal and Suzuki (2002).

Mineral	Composition	Occurrence in the soil
Native arsenic	As	Hydrothermal veins
Proustite	Ag ₃ AsS ₃	Generally one of the late Ag minerals in the sequence of primary deposition
Rammelsbergite	NiAs ₂	Commonly in mesothermal vein deposits
Safflorite	(Co,Fe)As ₂	Generally in mesothermal vein deposits
Seligmannite	PbCuAsS ₃	Occurs in hydrothermal veins
Niccolite	NiAs	Vein deposits and norites
Realgar	AsS	Vein deposits, often associated with clays and limestones, deposits from hot springs
Orpiment	As ₂ S ₃	Hydrothermal veins, hot springs, volcanic sublimation products
Cobaltite	CoAsS	High-temperature deposits, metamorphic rocks
Arsenopyrite	FeAsS	The most abundant arsenic mineral, dominantly mineral veins
Tennantite	(Cu,Fe) ₁₂ As ₄ S ₁₃	Hydrothermal veins
Enargite	Cu ₃ AsS ₄	Hydrothermal veins
Arsenolite	As ₂ O ₃	Secondary mineral formed by oxidation of arsenopyrite, native As and other As minerals
Claudetite	As ₂ O ₃	Secondary mineral formed by oxidation of realgar, arsenopyrite and other As minerals
Scorodite	FeAsO ₄ .2H ₂ O	Secondary mineral
Annabergite	(Ni.Co) ₃ (AsO ₄) ₂ .8H ₂ O	Secondary mineral

Table 2.2 Continued...

Mineral	Composition	Occurrence in the soil
Hoernesite	$Mg_3(AsO_4) \cdot 2.8H_2O$	Secondary mineral, smelter wastes
Conichalsite	$CaCu(AsO_4)(OH)$	Secondary mineral
Adamite	$Zn_2(OH)(AsO_4)$	Secondary mineral
Domeykite	Cu_3As	Found in vein and replacement deposits formed at moderate temperatures
Loellingite	$FeAs_2$	Found in mesothermal vein deposits
Pharmacosiderite	$Fe_3(AsO_4)_2(OH)_3 \cdot 5H_2O$	Oxidation product of arsenopyrite and other As minerals

Arsenic acid

Arsenite is a weak (hydroxo) acid with three pKa values ranging from 9.22–13.40 (eqs. 4–6) (Smith et al. 1998).

Arsenous acid

Organic As species. Organic chemistry of As is also very rich and similar to nitrogen and phosphorus. Arsenic makes bonds to a variety of organic ligands with different type of coordination geometries (Smith et al. 1998; Mahimairaja et al. 2005).

Where pH exceeds 9.22, As^{III} acts both as an amphiprotic and a polyprotic acid. It behaves as a Lewis base and oxidises to As^{V} by donating an electron pair located on the As^{III} atom (Grafe and Sparks 2006); As^{III} is considered to be a soft base.

Microbes play a very important role in the methylation-demethylation of As oxyanions. Microbial methylation results in the formation of methyl As compounds including monomethyl arsonics, dimethylarsines and trimethylarsines and ultimately leads to the formation of AsH_3 (gas), which is highly toxic (Smith et al. 1998). Fungi and bacteria are involved in the reduction As^{V} to the volatile methylarsines. Marine algae converts As^{V} into non-volatile methylated As compounds such as monomethyl arsenicacid [MMAA, $(\text{CH}_3\text{AsO}(\text{OH})_2]$ and dimethyl arsenicacid [DMAA, $(\text{CH}_3)_2\text{AsO}(\text{OH})]$ in sea water (Smith et al. 1998; Mahimairaja et al. 2005; Escobar et al. 2006).

2.3 SOURCES

Arsenic in the soil enters from various sources classified as either natural or anthropogenic, and both type of sources are responsible for the distribution of As in the soil environment (Smith et al. 1998; Mandal and Suzuki 2002; Mahimairaja et al. 2005). The toxic nature of As in soils present substantial environmental risks to plants, animals and most crucially human beings. In human beings, As may cause short term diseases such as hypertension or cardiovascular disease or long-term ailment such as skin, lung or bladder cancer (Ratnaike 2006). Given the toxic and carcinogenic nature of As, United States Environment Protection has listed As as the number one toxin of prioritised contaminants.

2.3.1 Natural sources

Arsenic release from natural sources is attributed to the weathering of parent material and volcanic eruptions (Smith et al. 1998). For instance, geogenic As is commonly associated

with drinking (ground)water supplies throughout South East Asia. As a result, millions of people have been exposed to As placing their health at risk (Nriagu et al., 2007; Rahman et al., 2006). It has been reported that approximately 42 million people are exposed to As containing potable water with a concentration of more than $50 \mu\text{g L}^{-1}$ and more than 100 million people worldwide are affected by As contaminated water with a concentration of more than $10 \mu\text{g L}^{-1}$ (Nriagu et al., 2007; Rahman et al., 2006). Arsenic contamination in Bangladesh and India is severe with several recent reports indicating approximately 6 million people in the 74 As-affected blocks in West Bengal, India were at risk to As exposure and more than 9% of residents suffered from arsenicosis (Mandal and Suzuki 2002). The origin of As is related to the naturally bound As to amorphous iron (Fe) oxyhydroxides. Under reduced conditions, amorphous Fe oxides bound As is released in groundwater, and after pumping the As-contaminated water has been depositing on the surface layers of soil, resulting in a serious threat of As contamination of food crops (e.g. rice) (Naidu et al. 2006).

2.3.2 Anthropogenic sources

Human beings have disturbed nature and exploited natural resources to accomplish their needs. Consequently, anthropogenic activities have also contributed in contaminating the soil environments with As. Anthropogenic sources of As include mining and smelting operations, refining of metalliferous ore including by-products such as slag, emissions from industrial manufacturing processes including electroplating, energy and fuel production, copper chromium arsenate (CCA) treatment of wood timber, and agricultural inputs such as the application of fertilizers, pesticides, herbicides, fungicides and municipal sludges to land (Smith et al. 1998; Mandal and Suzuki 2002). In the past, anthropogenic activities have been the major cause of As contamination in Australia, and still are responsible for widespread contamination in both urban and agricultural soils (Smith et al. 2003; Smith et al. 2006). For

example, the historical application of As^{III}-based pesticides and herbicides at disused cattle-dip sites and railway corridors across Australia has contaminated the surrounding soils with elevated As concentrations across the states of New South Wales (NSW), Queensland (QLD) and South Australia (SA). A brief overview of major anthropogenic causes of As contamination in soils is given as follows:

2.3.2.1 Mining and smelting activities

Arsenic is present as an impurity in Cu, Au, Pb and Zn ores and mining and smelting of these trace elements has created soil contamination problems to a large extent (Karczewska et al., 2007; McLaren et al., 2006; Smith et al., 1998). Erosion of the mining-waste rocks, tailings and slag potentially may contaminate nearby soils with As. The fine particles from this waste also migrated to surface waters as sediments and cause further site contamination. Secondary contamination occurs in the groundwater beneath or down the gradient on pits or ponds. Arsenic contamination arising from mining is reported in various places in the world, for example Thailand, England (Peterson et al. 1979) and Poland (Karczewska et al. 2007). In Australia, gold mining activities has resulted in much of the As contamination of soil. Most of the gold was mined from Victoria (2,500 tonnes), 40% from the primary lode depositions and the 60% from alluvial. The highest concentrations of As reported in mining waste disposal areas and mullock heaps ranged from 280–15,000 mg kg⁻¹ (Ellice et al. 2001; McLaren et al. 2006).

2.3.2.2 Industrialisation and urbanisation

Arsenic trioxide was the major As compound produced for many industrial uses including ceramic manufacturing, electronics, pigments and antifouling agents production, glass manufacturing, cosmetics production, fireworks preparation and use in the Cu-based alloys to increase resistance against corrosion. Arsenic trioxide was recovered from the smelting and

roasting of the non-ferrous metal ores or concentrates and as a result of these operations As was also released in the air. Use of arsenical compounds in agriculture was approximately 70% of the world's As production during the late 1970s to the early 1980s. At the same time when use of As decreased in the agriculture sector, As was added with chromium (Cr) and Cu to make CCA mixture, a wood preservative used in many countries including Australia and New Zealand to preserve the timber (Smith et al. 1998; Hingston et al. 2001; McLaren et al. 2006). Copper-chromium-arsenate timber contaminated the surrounding soils by way of spilling, leaking and run-off from the treated wood timber. Concentrations of As at these sites up to 500–10,000 mg kg⁻¹ soil were reported (McLaren et al. 2006). The contribution of commercial wastes was about 40%, coal ash 22% and approximately 13% due to the atmospheric fall out (Smith et al. 1998).

2.3.2.3 Irrigation

Irrigation with waste effluents is not only the source of As accumulation in soils; in some cases, water itself used for irrigating agricultural crops (e.g., rice) has led to As deposition in soils (McLaren et al. 2006; Dittmar et al. 2007; Roberts et al. 2007). The notable example of this occurrence is in Bangladesh where As-contaminated groundwater has resulted in the deposition of As in surface soils, with As concentrations in top-soil (0–10 cm) ranging between 11 and 35 mg kg⁻¹ (Dittmar et al. 2007; Roberts et al. 2007). The regular use of As-contaminated groundwater for irrigation is resulting in a gradual increase in the concentration of As in soils (Mahimairaja et al. 2005).

2.3.2.4 Agriculture activities

Agricultural use of As-based pesticides and herbicides has resulted in the elevated concentrations of As (up to > 1,000 mg kg⁻¹) in soils. For example, spraying PbAsO₄ on apple orchards (Queensland, Australia) has increased the As concentration up to 54.2 mg kg⁻¹ in the

top-soil (0–15 cm depth) and 20.9 mg kg⁻¹ in the subsurface soil depth (15–30 cm) (Smith et al. 2003). Arsenical pesticides (as sodium arsenite) were extensively used at (disused) cattle-dip sites to control cattle ticks in Australia and many other countries of the world including the USA, South Africa, New Zealand. This practice inadvertently led to the high and extremely variable concentrations of As in soils adjoining these sites across NSW in Australia with As concentrations ranging between 100 and 3000 mg kg⁻¹ (Smith et al. 1998; Kimber et al. 2002). The higher concentrations of As were found in the close vicinity of dip baths, draining pens and in scooping mounds (Table 2.3). Inorganic arsenical compounds were also used as selective soil sterilants and weed killers since the late 19th century. Application of monosodium methylarsinic (MSMA) at higher rates (1.1–11.2 kg ha⁻¹) on rice increased the sterility rate and enhanced the straight-head disease in rice grown in a Crowley silt loam soil (Smith et al. 1998).

Table 2.3 Arsenic concentrations in soil adjoining the cattle-dip sites in New South Wales (NSW), Australia (Smith et al. 2006).

Location around the dip	Arsenic concentration in soil (mg kg ⁻¹)	
	Mean	Range
Adjacent to dip bath	290	<0.5–1636
Draining pen	436	2–870
Disposal pit	467	<0.5–2600
Scooping mound	720	15–3000

Arsenite-based herbicides were extensively used along the railway corridors over 30 years ago in South Australia to suppress the grass growth. This resulted in the contamination of soils adjoining these corridors. Arsenic concentration in soils adjoining these corridors has been reported up to 1,400 mg kg⁻¹ (Smith et al. 2003).

Arsenic is also present as an impurity in phosphate (P) fertilisers. The concentration of As in commercial phosphorus fertilisers marketed in Iowa and phosphate rocks used in the manufacturing of P fertilisers was found to be higher than the other trace metals. Arsenic concentrations were in the range of 2.4–18.6 mg kg⁻¹ (tri-calcium phosphate, TCP), 8.1–17.8 mg kg⁻¹ (mono-ammonium phosphate, MAP), 6.8–12.4 mg kg⁻¹ (di-ammonium phosphate, DAP), and 3.2–32.1 mg kg⁻¹ in phosphate rocks (PRs) (Charter et al. 1995; Smith et al. 1998).

2.4 SPECIATION OF AS IN SOIL

Generally, there is no accepted definition of ‘speciation’. Akter and Naidu (2006) have defined the term speciation as follows:

- (i) Operationally; speciation in terms of characterising the molecule groups according to their similar behaviour during an analytical procedure such as extraction, for instance Fe oxides bound species;
- (ii) Oxidation states; chemical speciation which deals with determining redox form of an individual element in a system;
- (iii) Functionally; speciation delineates the function of species groups in biochemical pathways and their impact on organisms, for example phytoavailable species.

Hence the speciation of an element (e.g. As) in soil can be defined as the quantification and identification of various chemical (labile, sorbed, mineral bound), mineral and oxidation states of the contaminant in soil. Arsenic speciation in soil is determined using the sequential extraction procedures (SEP) and synchrotron-based X-ray absorption fine structure (XAFS) spectroscopy.

2.4.1 Sequential extraction procedure

Fractionation of As in soil using sequential extraction procedures have been previously used to delineate chemical forms of As in soils such as non-specifically and specifically sorbed As, As occluded in amorphous and crystalline Fe oxides, and recalcitrant As (McLaren et al. 1998; Lombi et al. 2000a; Wenzel et al. 2001; Cai et al. 2002; Novoa-Munoz et al. 2007). Such data can be used to evaluate the detailed fate of As in soils, which help in the risk assessment of contaminated sites. The sequential extraction procedures are considered to be more useful than the single extractions or total digestion procedures and provide information required for the risk associated with the various chemical forms of As (labile to least available) in soil.

For instance, McLaren et al. (1998) used a sequential extraction method based on the phosphate fractionation, and Wenzel et al. (2001) developed a sequential extraction procedure based on a combination of the reagents commonly used for sequential extraction of metals and phosphate (McLaren et al. 2006). The two sequential extraction procedures were different in terms of using the extracting reagents, however, in both cases significant proportion of As was found to be associated with 'hydrous oxides' in soils. The first fraction (non-specifically bound or exchangeable As) in these two sequential extraction procedures contain the most labile form of As which is considered to be a useful indicator of instant As mobility and (phyto)availability in soil. Arsenic in the residual phase may be considered as immobile or inert under all conditions. The sequential extraction procedures are reported to have some limitations which include the partial dissolution of a required fraction (La Force and Fendorf 2000), dissolution of the nontarget phase and a partial recovery of the desired phase due to readsorption or reprecipitation reactions (Ostergren et al. 1999; Wenzel et al. 2001; Scheinost

et al. 2002). The redox forms of As in soils are also not detected using a sequential extraction procedure (Wenzel et al. 2001; Scheinost et al. 2002).

2.4.2 X-ray absorption fine structure (XAFS) spectroscopy

XAFS spectroscopy is a direct method of As speciation which includes X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS). Since the mid 1990s, synchrotron-radiation based XAFS spectroscopy has become one of the most popular way of *in situ* investigation of molecular properties of the elements (e.g., As, Zn, Fe) at the mineral-water interface and in soil and sediment systems (Grafe and Sparks 2006). XANES spectroscopy is useful to determine the oxidation state of As in soils and solid materials and EXAFS spectroscopy delineates the bonding environment of an element in the materials. Bulk- or micro-XAFS (beam size; e.g. 2×10 mm and 5×10 μ m, respectively) have been used to scan the element of interest in the soil and/or solid material over a specified energy range, which encompasses the energy of the target element (e.g. As K-edge = 11868 eV).

XAFS spectroscopy has been used to determine the oxidation state and chemically bound forms of As in contaminated soils and sediments (Cances et al. 2005; Arai et al. 2006; Cances et al. 2008; Meunier et al. 2010). For example, Cances et al. (2005) and Arai et al. (2006) employed XAFS spectroscopy for the solid-state speciation of As in soil near to a former arsenical pesticides manufacturing and processing plant. The authors observed that As predominantly occurred as As^V (71–80% of total As) mainly in association with the amorphous Fe oxides in top-soil (0–10 cm) and in the subsurface (20–90 cm) soils. In another study, Meunier et al. (2010) determined mineralogical composition of As in As-enriched tailings and soils from abandoned gold mine sites using XANES spectroscopy. The authors

reported that As was present in several mineral forms such as arsenopyrite, scorodite, kankite, orpiment and as Fe^{III}-As^V minerals.

2.4.3 Combination of SEP and XAFS spectroscopy

However, previous research has not used combination of SEP and XAFS spectroscopy, which could be very powerful in delineating various chemical (labile, sorbed, mineral bound), mineral and oxidation states of As in contaminated soils. XAFS spectroscopy alone may not be able to estimate the highly mobile and labile forms of As in soil. The combination of SEP and XAFS spectroscopy can provide a detailed knowledge on the chemical forms (labile, sorbed, mineral bound), minerals (e.g. scorodite, arsenopyrite) and oxidation state (As^V/As^{III}) of As in As-contaminated soils, particularly where As was historically used as As^{III}; for example, As-contaminated cattle-dip sites in Australia (Smith et al. 2006). To obtain such detailed knowledge on the quantification and identification of various chemical forms of As in soil is vital for the risk assessment purpose and developing suitable strategies for remediation and rehabilitation of As-contaminated soils.

2.5 AVAILABILITY OF AS IN SOIL

To determine the availability and solubility of As in soil is vital, which is thought to be related to different pools in the soil system. Wenzel et al. (2001) showed that the concentration of As in the soil is the sum of the As fractions within the following five pools:

- (i) Weakly adsorbed or exchangeable – As present as free ions or in soluble form (outer-sphere complexed);
- (ii) As specifically sorbed to the mineral surfaces, such as Al/Fe (oxy)hydroxides. This phase is also termed to as the phosphate extractable As, as As is desorbed due to phosphate ions (inner-sphere complexed);
- (iii) As bound to hydrous oxides of, Fe and Al (inner-sphere complexed);
- (iv) As associated with crystalline Fe/Al oxides; and
- (v) Residual As.

Arsenic availability is largely determined by the equilibrium between As in soil solution and the solid phase. Generally, the equilibrium is affected by various reactions including adsorption, ion-exchange, complexation with inorganic (and organic) ligands, redox reactions and precipitation-dissolution (Morel 1997). These reactions can potentially affect the free ion concentration of As at the soil-water interface, thereby affecting the solubility of As.

The plant available fraction is not the same as the total concentration in the soil; As is mainly bound to the solid phase. The phytoavailable form of As is either in soil solution (weakly adsorbed to the solid phase) or specifically sorbed to the solid phase but able to transfer in solution during plant growth. Therefore, of these five pools, the first three are considered bioavailable in terms of As availability for plant uptake. The unavailable or the least available

fractions (those rendered immobile or least mobile) are strongly bound within the mineral-matrix (McLaren et al. 1998; Wenzel et al. 2001). It is important to understand that high concentrations of As may not necessarily indicate its release in soil and/or availability for plant uptake (Novoa-Munoz et al. 2007; Devesa-Rey et al. 2008; Ko et al. 2008). For example, Devesa-Rey et al. (2008) revealed large differences in the amount of As extracted in (immobile) residual and exchangeable phases. The exchangeable and specifically sorbed fractions accounted for < 8% of the total As in soil (mean = 3.4% and 2.7% and range = 0.7–7.8% and 1.0–4.2%, respectively). Conversely, the mean As in the residual fraction was accounted 58.2% of the total As (46.0–74.7%). Similarly, Nova-Munoz et al. (2007) fractionated As in nine vineyard soils from a wine-producing area in Galicia (NW Spain) containing maximum As total content of 200 mg kg⁻¹. Their study revealed that As in the soluble and exchangeable fractions was < 4% of the total As. While As in the crystalline Fe and Al hydrous oxides bound (immobile or least available As) fraction showed, on average, higher than 80% of the total As. A schematic representation of As availability is presented in Figure 2.2.

The availability of As in soil is controlled not only by total As concentration, but also by physical, chemical and biological processes within the soil environment. Both physical and chemical aspects provide the framework in which biological factors can modify As availability. Physical processes are largely dependent on soil type and include physical resistance restricting root penetration, soil structure and low water storage capacity.

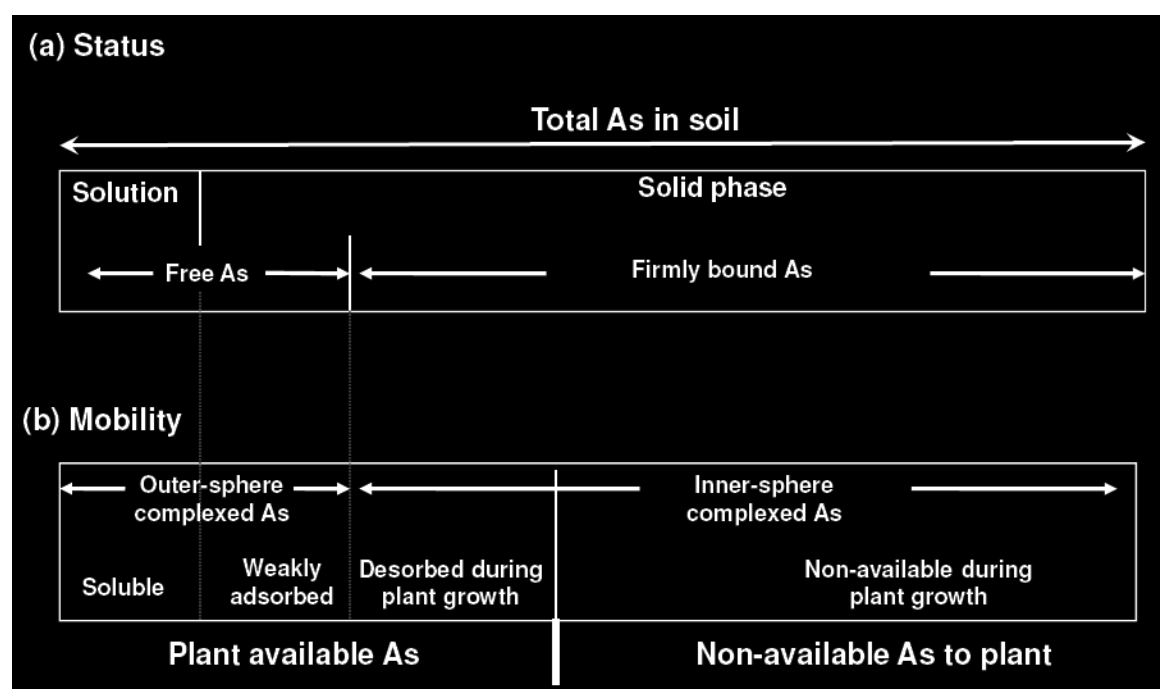


Figure 2.2 A model for determining available As concentration in soil (modified from Morel 1997).

Physical properties such as soil texture may also influence the distribution of As among soil fractions (Sheppard 1992; Jiang and Singh 1994; Smith and Naidu 2009). Jiang and Singh (1994) investigated the effect of As^{V} and As^{III} on the crop yield and plant As uptake of barley and rye grass in two different type of soils, sand and loam soil. The authors observed that both yield reduction and the increase in As concentration in crop tissue were lower in the loam soil than in the sand, indicating reduced phytoavailability of As in the loam soil.

Chemical properties such as soil acidity, E_h and speciation may also influence the lability and plant uptake of As (Smith et al. 1998; Mahimairaja et al. 2005). The role of soil pH is well documented in determining As mobilisation and availability in soil. Previous studies have indicated that a decline in soil pH increases the adsorption of As (As^{V}) in soil and decreases its availability for plant uptake (Smith et al. 1998, 1999; Xu et al. 2010). Similar to soil pH, soil E_h is also a well known soil parameter that can control the fate and speciation of As in

soils (Carbonell-Barrachina et al. 2000; Mandal and Suzuki 2002; Al-Abed et al. 2007). Generally, under reducing conditions, As availability is considered high (Masscheleyn et al. 1991; Carbonell-Barrachina et al. 2000). For example, Masscheleyn et al. (1991) studied the effect of pH and E_h on As mobility in a contaminated soil. The authors found that As solubility was low at higher soil redox levels (500–200 mV), and the major part (65–98%) of As in solution was present as As^V . The soluble and mobile As concentration increased 13 times upon reduction of E_h to -200 mV, as compared to 500 mV. This indicates that chemical speciation of As may affect its availability for plant uptake.

Arsenic availability in soil is associated with the adsorption strength with the solid particles. The factors influence the adsorption-desorption of As in soil have influence on the availability and plant availability of As in soil. The binding of molecules or ions (adsorptive) from the solution to the solid surface (adsorbent) involves both physical and chemical forces. Physical forces include van der Waals forces and electrostatic outer-sphere complexes, and chemical forces result in the formation of inner-sphere complexes, covalent bonding and hydrogen bonding (Stumm 1992; Sparks 2003). Arsenic has a high affinity of Fe/Al oxides surfaces (mainly Fe oxides) and reactivity of these oxides varies depending on the pH, charge density, time, E_h and soil solution composition (Smith et al. 1998; Grafe and Sparks 2006).

Effect of Fe oxides on As availability. Iron oxides are widely distributed in soil and sediments and exist as coatings on the surface of primary and secondary clay minerals (Smith et al. 1998, 1999; Grafe and Sparks 2006). They form various types of solid phases under different pH, temperature and pressure conditions. The processes of sorption/desorption, precipitation and coprecipitation are responsible for the As retention on Fe oxides surfaces which account for As availability in soil. Binding of As^V to the Fe oxides occurs through ligand exchange mechanism. As a result of this exchange monodentate mononuclear

complexes, at low loading levels and higher pH, bidentate binuclear complexes at intermediate and high loading levels and low pH or in special cases, tridentate polynuclear species at very high As/Fe ratios are formed (Waychunas et al. 1993; Sun and Doner 1996; Fendorf et al. 1997; Sun and Doner 1998; O'Reilly et al. 2001; Violante et al. 2007). Recently, it has been investigated using EXAFS spectroscopy that As^V only forms monodentate complexes at the surface of goethite and no bidentate complexes were determined (Loring et al. 2009). Arsenite has been reported to form outer-sphere surface complexes with the surface of Fe oxides, at high (1 mM) initial As^{III} concentration and increasing ionic strength, indicating that As^{III} adsorption decreased (Arai et al. 2001; Goldberg and Johnston 2001; Arai and Sparks 2002; Lin and Puls 2003). Adsorption of As^V on Al oxides has also been documented; however, their role to retain As in soil is lower than Fe oxides. Arsenate and As^{III} adsorption on γ -Al₂O₃ was examined by EXAFS and XANES spectroscopy analysis. Arsenate formed inner sphere bidentate binuclear complexes with the Al-As bond length of 3.11 and 3.14 Å at pH 4.5 and 7.8, respectively but As^{III} was found to be adsorbed both as inner and outer sphere complexes (Grafe and Sparks 2006).

Biological factors including soil bacterial and fungal rhizosphere associations and higher plants may significantly modify the chemical and physical conditions which determine As phytoavailability (Smith et al. 1998; Agely et al. 2005). For example, Agely et al. (2005) reported that arbuscular mycorrhizal (AM) fungi could increase aboveground biomass, As accumulation, translocation, and bioconcentration in *Pteris vittata*. Plant uptake of As continuously alters the concentration and speciation in contaminated soils through release of root exudates and rhizosphere acidification.

2.6 ARSENIC ACCUMULATION IN PLANTS

In plants, accumulation of As is attributed to the uptake capacity, intracellular binding sites, and is complicated by tissue and cell specific differences and intercellular transport mechanisms (Smith et al. 1998; Fitz and Wenzel 2006). Arsenic is detected in low concentrations in plants grown in the uncontaminated soils, with As concentration $< 1.5 \mu\text{g kg}^{-1}$ (McLaren et al. 2006). However, in contaminated soils As can be accumulated by plants and cause phytotoxicity which may eventually lead to plant death. Jiang and Singh (1994) showed that As concentrations as low as $2.5\text{--}8.7 \text{ mg kg}^{-1}$ in the barley and rye grass grown in As-contaminated soils reduced the dry matter yield using a glasshouse experiment. Following entry into plant tissues, As can affect various physiological and biochemical processes resulting in a reduction of plant growth, inhibition of photosynthesis and respiration, and degeneration of main cell organelles (Jiang and Singh 1994; Smith et al. 1998; Mandal and Suzuki 2002). For example, As^{V} uptake in plants inhibit the phosphate uptake pathway leading to insufficient levels of phosphorylated compounds and retarded plant growth. Arsenic in As^{III} form is known to have twice as much phytotoxicity as As^{V} , since it rapidly combines with the dithiol functional groups and destroys the functioning of sulfhydryl enzymes; thereby causing membrane degradation and immediate cell death (Jiang and Singh 1994; Mahimairaja et al. 2005; McLaren et al. 2006).

In general, for essential elements such as Ca and Mg, there is a region of deficiency followed by a region of tolerance where concentrations of elements are enough to maintain plant physiological functions and growth. A breakdown in the metabolic control can occur if external (element) concentrations exceed toxicity threshold, which can lead to passive uptake and eventually plant death. However, there is no deficiency zone for non-essential elements

such as As, and concentrations in tissue generally increase until the external concentration is toxic and results in eventual death. These relationships are limited to plants that do not exhibit As-tolerance strategies. The response of plants to essential and non-essential elements (As in this thesis) is illustrated in Figure 2.3.

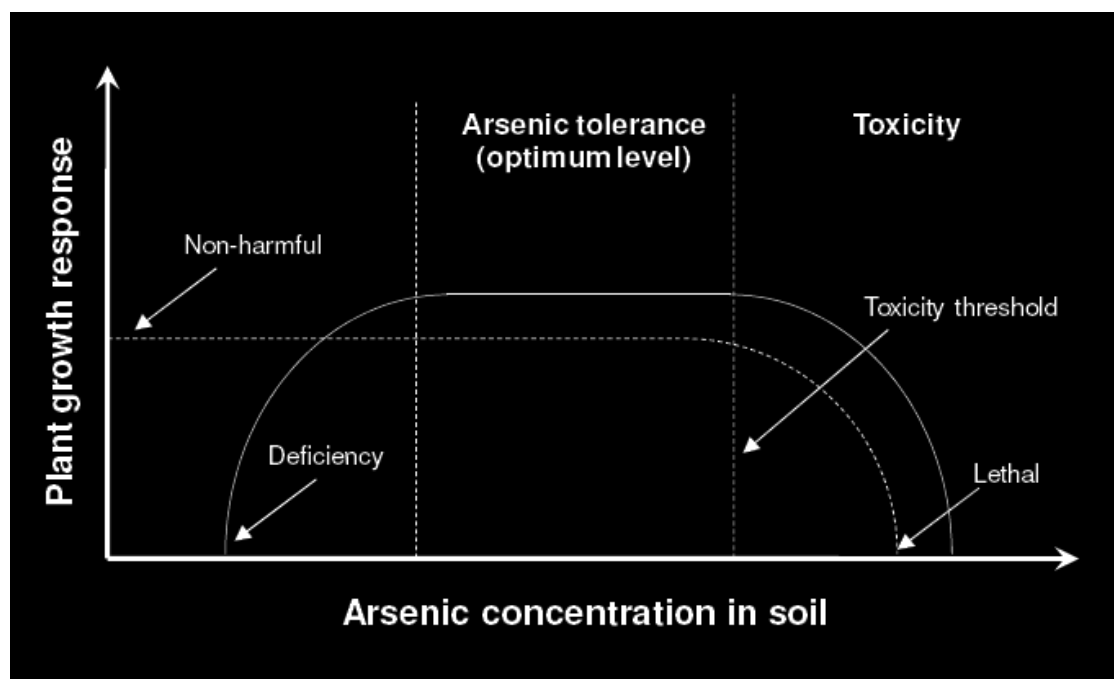


Figure 2.3 A dose response curve for the essential and non-essential (As in this thesis) elements in plants (modified after Morel 1997).

2.6.1 Tolerance strategies in plants

Terrestrial plants have evolved unique strategies to cope with the heavy metal(loid)s (e.g., Zn, Cu, Pb and As) stress. According to Baker (1981), plants have three distinct strategies of heavy metal(loid) tolerance. Based on his conceptual model of metal(loid) uptake in plants, the terrestrial plants can be characterised as excluders, indicators and accumulators (Figure 2.4).

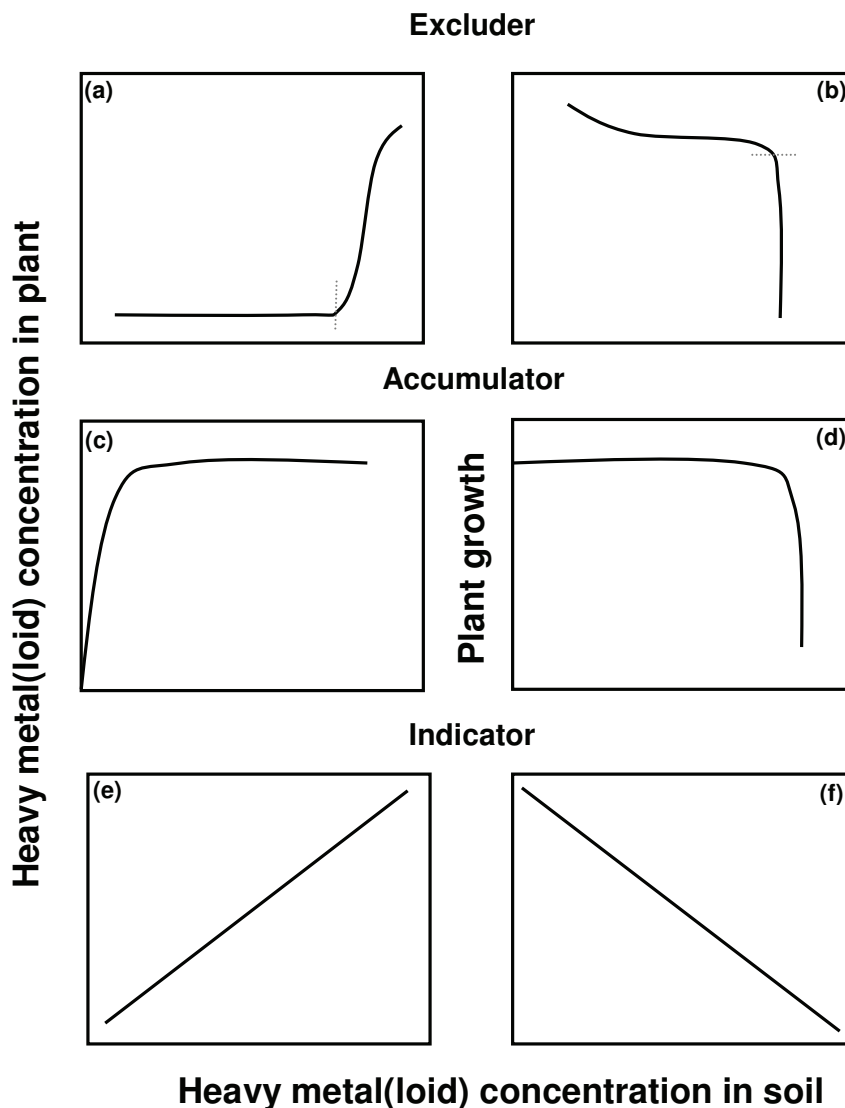


Figure 2.4 (a,c,e) Tolerance strategies in plants in relation to increasing heavy metal(loid) (As in this study) concentrations in soil; (modified after Baker 1981); (b,d,f) the plant growth in response to increasing heavy metal(loid) concentrations (in soil and plant), are also presented (Kachenko 2008).

When the roots act as a barrier to maintain the heavy metal(loid) concentration in the aboveground biomass at low levels, the strategy is termed as exclusion . These plants are referred to as excluders. According to Fitz and Wenzel (2006), the majority of non-As-hyperaccumulating plants can be termed as excluders. In the model proposed by these authors

(as as illustrated in Figure 2.5) the mean bioconcentration coefficient (As concentration in plant shoot to As concentration in soil) for all non-hyperaccumulators was 0.025, the highest was 0.28.

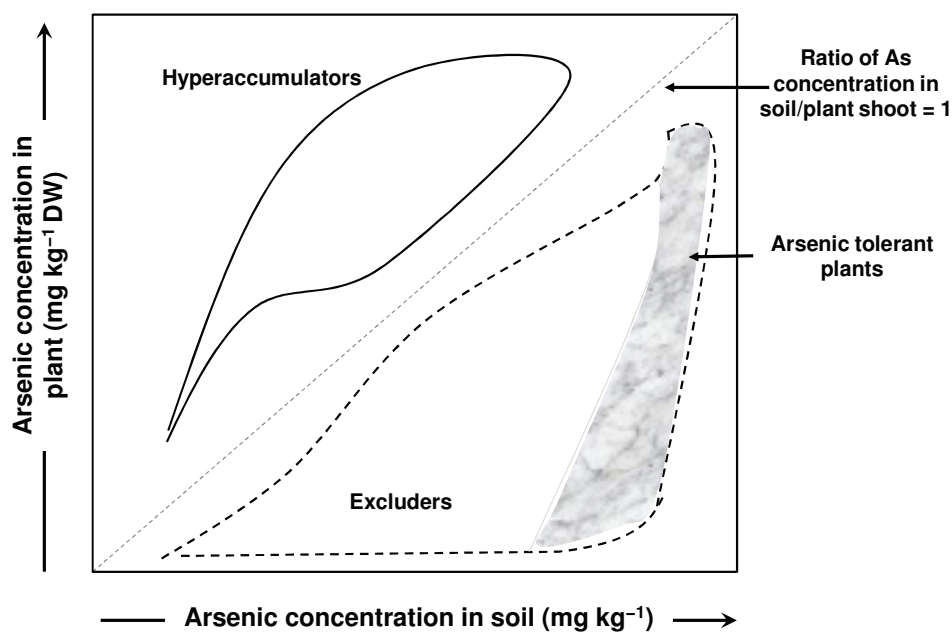


Figure 2.5 Generalised pattern of As uptake strategies by terrestrial plants (modified from Fitz and Wenzel 2006).

Accumulators can actively accumulate high levels of As in the aboveground biomass without adverse affects on plant growth until soil conditions become toxic and plant growth suppressed. These species are characterised by a leaf:root heavy metal(loid) concentration ratio of > 1 (see Figure 2.5) (Fitz and Wenzel 2006). Plants that fall into this category are termed As hyperaccumulators and all As-hyperaccumulators are ferns belonging to Pteridales from the genera *Pteris* and *Pityrogramma* (discussed in detail under Sections 2.7.1 and 2.8). Some As tolerant plant species are recognised to grow on extremely contaminated soils such as mine wastes and tailings. This may result in substantial As accumulation in shoots of non-

As-hyperaccumulating plants. For example, *Agrostis capillaries* has been reported to accumulate $> 3,000 \text{ mg kg}^{-1}$ As concentrations in shoot tissues from soil containing $26,500 \text{ mg As kg}^{-1}$. The calculated bioconcentration coefficient was 0.13; hence, *Agrostis capillaries* can be referred as a highly As-tolerant plant not a hyperaccumulator (Figure 2.5).

In addition to these tolerance strategies, there are some plants referred to as indicators, defined as plants showing a proportional relationship between soil and plant As concentrations. Fitz and Wenzel (2006) indicated that As-indicators may also exist in the plant kingdom, although their data was limited to differentiate between As indicators and excluders. Indicator species may be used as test plants to indicate the availability of As in contaminated soils and sediments (Ko et al. 2008).

2.6.2 Arsenic uptake mechanisms

Arsenate ion is chemically similar to phosphate. Both As^{V} and phosphate are in the same chemical group and have comparable dissociation constants and solubility products values for their acids and salts, respectively (Smith et al. 1998; Adriano 2001). Plant As uptake is influenced by As source and solubility (Smith et al. 1998; Anawar et al. 2008; Smith et al. 2009). Arsenite is thought to be taken up passively by aquaglyceroporins, or channels allowing movement of water and neutral solutes in the plant roots. The transport system for As^{V} is through the plasma membrane that is the same pathway used by phosphate in plants (Asher and Reay 1979; Meharg and Jardine 2003; Fitz and Wenzel 2006). Conversely, in *Deschampsia cespitosa*, *Agrostis capillaries* and *Holcus lanatus*, an altered phosphate transport pathway has been reported, which characterises these plants as As-tolerant ecotypes (see Figure 2.5) (Meharg and MacNair 1990a; Meharg and MacNair 1990b; Meharg and MacNair 1994; Mandal and Suzuki 2002).

Plant exposure to As and heavy metal enriched environments may result in the production of reactive-oxygen-species, including superoxide anions, hydrogen peroxide (H₂O₂) and hydroxyl radicals which can destroy the cell components (Hartley-Whitaker et al. 2001; Fitz and Wenzel 2006). The production of reactive-oxygen-species is thought to be attributed to the conversion of As^V to As^{III} upon exposure of plants to As (Hartley-Whitaker et al. 2001; Meharg and Hartley-Whitaker 2002a).

Plants can synthesize enzymatic and non-enzymatic antioxidants in response to the generation of reactive-oxygen-species. Hence, plants can cope with the detrimental effects of reactive-oxygen-species by using antioxidant molecules, such as L-ascorbic acid, reduced glutathione (GSH), α -tocopherols and carotenoids; particularly ascorbic acid (Adriano 2001; Kachenko 2008).

Under no As stress, *P. vittata* was found to have intrinsically higher concentrations of non-enzymatic antioxidants, ascorbate and glutathione (GSH), in its fronds compared to *Pteris ensiformis* (a non-As-hyperaccumulator). This suggests that the ascorbate-GTH pool may play a significant role in the ability of *P. vittata* to tolerate and hyperaccumulate As (Kertulis-Tartar 2005; Gonzaga et al. 2006). In previous studies, *P. vittata* has been reported to produce superoxide dismutase (SOD) and catalase (CAT) (antioxidants) when the ferns were exposed to As (Kertulis-Tartar 2005; Srivastava et al. 2005). Conversely, in the same conditions of the experiment *P. ensiformis* could not induce the generation of these antioxidants.

The terrestrial plants accumulate As and heavy metals from soil and reduce the toxic effects of the metal and metalloid using phytochelatins (Meharg and Hartley-Whitaker 2002b; Zhao et al. 2002). Phytochelatins containing thiol (-SH) functional groups are peptides and have the

ability to chelate heavy metals. Glutathione, the precursor for phytochelatins is a source of non-protein thiols. The phytochelatins are formed by the transpeptidase phytochelatin synthase enzyme using GSH (Adriano 2001; Zhao et al. 2002; Fitz and Wenzel 2006; Gonzaga et al. 2006). The synthesized phytochelatins can retain some metals and metalloids in the cytosol, thereby form phytochelatin-metal complex and then transported to vacuole in plant cell.

2.6.3 Factors affecting plant As uptake

The pH in the rhizosphere and bulk soil may vary significantly; and the differences in pH can be as high as up to two units depending on soil and plant factors (Fitz and Wenzel 2006). The factors that may influence the rhizosphere pH include the availability of plant nutrients (e.g. Fe and phosphorus deficiency), source of nitrogen supply (NO_3^- vs. NH_4^+ uptake), root exudates excretion from roots such as organic acids, CO_2 emission from roots, soil microbiota and buffering mechanism of soil (Zhao et al. 2002; Kachenko 2008). The effect of pH is considered to have great influence on the labile pool of As in soil, since As^{III} mobility increases with the decreasing pH and As^{V} is more mobile and available under the alkaline pH range (Smith et al. 1999).

The change in rhizosphere E_h can also affect the mobility and availability of As in soil. Arsenic availability is increased under reduced conditions either by transformation of As^{V} to As^{III} ; and/or release of As due to dissolution of Fe oxides which are considered to be the main sorbents of As in soil (Smith et al. 1998; Mandal and Suzuki 2002; Gonzaga et al. 2009). For example, As concentration in rice and wetland plants (e.g., *Aster tripolium*) has been found to increase under flooding and submerged conditions (Marin et al. 1993; Fitz and Wenzel 2006).

The excretion of root exudates from plant roots such as citric and malic acids can also enhance the mobilisation of As in rhizosphere soils (Smith et al. 1998; Gonzaga et al. 2006; Gonzaga et al. 2009). The release of As in soil could be attributed to the dissolution of amorphous Fe oxides (Slowey et al. 2007; Mikutta et al. 2010). In a previous study, Gonzaga et al. (2009) showed that both *P. vittata* and *P. biaurita* increased the dissolved organic carbon and water soluble As concentrations in the rhizosphere soil than that in the bulk soil, where the two fern species were grown in rhizo-boxes for 8 weeks in As-contaminated soils. Their results indicate the possible role of organic acids in the displacement of As from soil, thereby increasing its mobility. In addition, a role of mycorrhizas has also been demonstrated to interfere with As uptake by plants (Gonzaga et al. 2006; Kachenko 2008). Gonzalez-Chavez et al. (2002) found in a pot experiment that a mixed population of tolerant arbuscular mycorrhiza fungi (AMF) conferred increased As^V resistance on tolerant plant species, *Holcus lanatus* by reducing As accumulation in both shoots and roots. Arsenic accumulation in plants is also dependent on the plant species. In most studies beans are classified as having low or no tolerance, while tomato and carrot are referred to as very tolerant species (Sheppard 1992; Fitz and Wenzel 2006). A considerable variation in plant sensitivities to As exists among plant species (Jian and Singh 1994). The vegetable crops grown in three soils (Lakeland loamy sand, Hagerstown clay loam, and Christiana clay loam) in a glasshouse pot experiment exhibited a range of sensitivities to As applied (as sodium arsenate) @ 0–500 mg As kg⁻¹ (Smith et al. 1998; Fitz and Wenzel 2006). The sensitivities of plants followed the order: green beans > lima beans = spinach > radish > tomato > cabbage.

Anions such as phosphate ($\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$), chloride (Cl^-), sulfate (SO_4^{2-}), have a greater influence (mainly phosphate) on the adsorption-desorption reactions and availability of As in

soil. The anions affect the mobility of As in soil depending on the As species present in soil (Smith et al. 1998; Goh and Lim 2005). Phosphate has been found to be the major anion displacing sorbed As from soils and increasing its phytoavailability in the soil solution. These reactions mainly occur at the surfaces of Fe/Al oxides and/or clay minerals (Smith et al. 1998; Meng et al. 2002; Violante and Pigna 2002; Frau et al. 2008; Stachowicz et al. 2008). Application of relatively high rates of phosphate fertilisers have been reported to increase the As^V concentration in soil solution in batch and column studies. The presence of phosphate in the equilibrating solution has been found to decrease the As adsorption, while the addition of other anions such as Cl⁻, NO₃⁻, SO₄²⁻ showed very minute effect on the adsorption reactions. Both phosphate and As^V occupy the same adsorption sites on the oxides and mineral surfaces and compete with each other. Studies show that As^V adsorption has been decreased by SO₄²⁻ on Al oxides surfaces but increasing concentration of the anion did not decrease the As^V adsorption. This showed that SO₄²⁻ did not occupy the same sites as As^V (Smith et al. 1998; Meng et al. 2002). The studies indicated that PO₄²⁻ is the only major anion which decreases the As^V adsorption in soil and increases its mobility and other anions have very little effect.

Organic matter can also have influence on the availability and mobility of As in soil. It has two major portions: fulvic acid (FA) and humic acid (HA) which affect the adsorption of As in soil and water systems (Smith et al. 1998; Grafe et al. 2001; Grafe et al. 2002). The presence of FA showed a great influence on the adsorption of As^V on alumina between pH 3 and 7.5. Fulvic acid may be adsorbed on alumina by columbic interaction or directly form complexes with As (Smith et al. 1998), which decrease the sorption of As complex. Several studies reveal that HA and FA compete with As on oxides and mineral surfaces and enhance its mobility and availability in soil solution (Gustafsson 2006; Wang and Mulligan 2006; Sisr

et al. 2007; Gadepalle et al. 2008; Lin et al. 2008). These studies suggest that organic matter addition in soil increases its availability by enhancing the desorption of As from soil.

Biotransformations of As create further complexity between the solid and solution phase association of As. These include oxidation, reduction and methylation reactions. However, toxicity of As is related to its oxidation state (Smith et al. 1998; McLaren et al. 2006). The oxidation of As by bacteria was first identified by Green in 1918 when a bacterium (*Bacillus arsenoxydans*) was isolated from the cattle dipping solution. Several other *Bacillus* or *Pseudomonas* spp. have been characterised to be involved in the oxidation of As^{III} to As^V. For example, the bacterium, *Alcaligenes faecalis* oxidizes As^{III} to As^V using As^{III} as a terminal electron acceptor (Frankenberger and Arshad 2002; McLaren et al. 2006). Oxidized forms of As is As^V and can be transformed to As^{III} under reduced soil conditions and finally to AsH₃ gas. Soil microbes are able to convert As^V and As^{III} into many volatile reduced forms such as methyl arsines. Methylphenyl arsenic acid and dimethylphenyl arsine oxide are reduced to dimethylphenylarsine by *Candida humicola*. Seven diverse species of Eubacteria and two species of Crenarchaea have been isolated for the reduction of As^V to As^{III} (Frankenberger Jr and Arshad 2002). In addition to the microbial reduction, chemical reduction also occurs in the soil environment (McLaren et al. 2006).

2.7 REMEDIATION OF AS-CONTAMINATED SOILS

Anthropogenic activities has led to the contamination of large expanses of land with As. Arsenic-contaminated soils, in many parts of the world (e.g. India, Bangladesh, Australia, the USA, New Zealand), are reported to have impact on the sustainability, productivity and health of soil environment, leaving large areas of land uninhabitable and unproductive. Increasing growth in the global population has put a demand for remediating the contaminated soils in order to create housing or land to cultivate. Therefore, it is crucial that efforts concentrate on economically and environmentally viable techniques to remediate these contaminated landscapes. Current physico-chemical methods employed to remediate As-contaminated soils are costly and are often restricted to small scale applications (Gonzaga et al. 2006). During the past 10 years, there has been increasing interest toward the growing of plants (i.e. As-hyperaccumulating ferns) to remove As from contaminated soils. The present remediation methods for As-contaminated soils include soil removal and washing, physical stabilisation, and/or the use of chemical amendments. All these approaches are thought to be expensive and disruptive, with an average cost of (US dollars) \$404,700 ha⁻¹ (Gonzaga et al. 2006).

Excavation. A commonly used *ex-situ* remediation method which involves the physical removal of the contaminated soil and disposed of it in landfill sites. Although excavation results in fast and quick remediation of the site, however, it is often costly because of the operation, transport, and special landfill requirements.

Capping. An *in-situ* method of remediation in which a hard cover is placed on the surface of the contaminated soil. Capping is also a quite simple method that masks the contaminant exposure. However, it does not remove contaminants from the soil.

Solidification and stabilisation. This is an *in-situ* method where the contaminated soil is mixed with stabilisers to decrease the mobility of As in soil.

Vitrification. *In-situ* method, where As is chemically bonded inside a glass matrix forming silico-arsenates.

Soil washing/acid extraction. *Ex-situ* method of treating the suspension or dissolution of As in a water-based wash solution to concentrate the contaminant.

Soil flushing. *In-situ* method that uses water, chemicals or organics to mobilise As and flush it from the soil.

2.7.1 Phytoremediation

Phytoremediation can be defined as a process in which green plants extract, sequester or stabilise As to render them harmless (Salt et al. 1998). This is an emerging remediation technology for the remediation of As-contaminated soils. The ongoing advancement in the field of phytoremediation has been largely driven by the spiralling costs associated with conventional soil remediation methods and the need to use a 'green', sustainable process. It has been indicated that in some cases the costs associated with phytoremediation were 15 times less expensive than that of conventional physicochemical remediation strategies (Glass 1999); the author suggested that phytoremediation was an economically feasible remediation technology. Moreover, the current physicochemical technologies are aimed mainly for rigorous *in situ* or *ex situ* remedy of highly contaminated sites, and thus are not suitable for immensely and extremely variable contaminated areas where contaminants exist at low concentrations and demonstrate high spatial variation (Kertulis-Tartar et al. 2006). A summary of the advantages and possible disadvantages of phytoremediation is provided as follows (Glass 1999; Kachenko 2008):

1. Advantages

(a) Cost

- (i) No requirement for the expensive equipment or highly skilled personnel.
- (ii) Metal and/or metalloid recycling provides further economic gain.

(b) Performance

- (i) The extent of soil disturbance is minimum compare to conventional methods.
- (ii) Adaptable to a range of inorganic and organic compounds.
- (iii) Application (*in situ/ex situ*) is possible in effluent or soil.
- (iv) *In situ* applications decrease spread of contaminant via air and water.
- (vi) Capable of remediating bioavailable fraction.

(c) Other

- (i) Publically acceptable; aesthetically pleasant.
- (ii) Compatible with risk-based remediation, brownfields.
- (iii) Can be employed during site investigation or after closure.
- (iv) In large scale applications the potential energy stored can be utilised to generate thermal energy.

2. Disadvantages

(a) Time

- (i) Many years may be required to remediate a contaminated site.
- (ii) Several hyperaccumulating plants are slow grower species.

(b) Performance

- (i) Remediation is restricted to shallow contamination within rooting zone of remediative plants.
- (ii) 100% reduction may not be achieved.
- (iii) Limited to sites containing low contaminant concentrations.

(iv) Consumption or utilisation of contaminated plant biomass is a cause of concern for secondary pollution.

(v) Harvested plant biomass from phytoextraction may be classified as a hazardous waste hence treatment/disposal should be proper.

(vi) Adaptation to the climatic conditions is a growth-limiting factor.

(c) Other

(i) Lack of recognised economic performance data.

(ii) Need to displace existing facilities (e.g. wastewater treatment).

(iii) Introduction of non-native species may affect biodiversity.

(iv) Regulators may not be familiar with the technology and its capabilities.

The term phytoremediation includes the following strategies (Gonzaga et al 2006):

Phytoextraction. The use of hyperaccumulating plants to extract the contaminant from soil and translocate it to the aboveground biomass. For example, hyperaccumulating ferns such as *Pteris vittata* to remove As from soil (Ma et al., 2001).

Phytostabilisation. The pollutant-tolerant plants are used for mechanical stabilisation of contaminated soil in order to prevent bulk erosion, decrease air-borne transport, and leaching of contaminants. It is used to provide a cover of vegetation for a moderately to heavily contaminated site, thus preventing wind and water erosion (Kramer 2005).

Phytoimmobilisation. It refers to the use of plants to reduce the mobility and bioavailability of contaminants in soil by formation of precipitates and insoluble compounds, as well as by sorption on roots.

Phytovolatilisation. The use of plants to volatilise contaminants has been demonstrated for Hg and Se. For Hg, such mechanism was developed by genetic manipulation of plants

whereas in the case of Se phytovolatilisation naturally occurs in plants (Gonzaga et al. 2006). Limited information available on the As-volatilisation in soil indicated that volatile compounds account only for little proportions of total As, in the absence of plant roots.

2.8 PHYTOEXTRACTION OF AS USING HYPERACCUMULATING FERN

SPECIES

Brooks et al. (1977) used the term *hyperaccumulator* for the first time, which they defined as a plant species that could accumulate substantial amounts of a given heavy metal and/or metalloid in aboveground tissue without deleterious effects to the plant. The authors developed this definition particularly for Ni hyperaccumulating plants, however, several hyperaccumulators for the heavy metal(loid)s As, Cd, Cu, and Zn have also been described. Hyperaccumulating plants are reported to contain $> 1,000 \text{ mg kg}^{-1}$, or 0.1%, of an element. Generally, hyperaccumulators are reported to have a high rate of accumulation, fast growing behaviour, and have a potential to yield larger amount of biomass (Brooks et al. 1977; Gonzaga et al. 2006). Also, bioconcentration factor (BF; ratio of contaminant concentration in plant aboveground biomass to soil) and translocation factor (TF; ratio of contaminant concentration in plant above ground biomass to plant root) of the hyperaccumulating plants are considered to be > 1 . However, the soil properties, such as (low) pH and (high) Fe oxides content can reduce the availability of As in soil and substantially decrease the accumulation rate of a contaminant (e.g. As) by the hyperaccumulating plants. This is particularly important to consider when these plants are grown under the field conditions.

The ladder brake fern, (*P. vittata*) is the first known example of a plant that extracts As from soil and can be referred as an As-hyperaccumulator (Ma et al. 2001). Ferns are lower plants,

unlike several of the other identified hyperaccumulating plants, which are dicots or monocots (e.g. plants in mustard family, such as *Thlaspi* spp. and *Brassica* spp.). *Pteris vittata* has long been associated with arsenical mine dumps (Wild 1974) and Cu/Co rich substrates (Brooks and Malaisse 1985). However, its ability to hyperaccumulate As was discovered by Ma et al. (2001). The authors observed that it can accumulate up to 22,630 mg As kg⁻¹ DW in the fronds. This fern was highly tolerant to As in soil containing up to 500 mg As kg⁻¹, and soils spiked with 50 mg As kg⁻¹ were best for fern growth resulting in biomass production of 3.9 g plant⁻¹ (Tu and Ma 2002). Similarly, the highest BF (63) and TF (25) were observed in soils spiked with 50 mg As kg⁻¹ (Tu and Ma 2002).

Since the discovery of *P. vittata*, several other fern species have been identified as potential As hyperaccumulating species (Table 2.4) such as the silver fern, *Pityrogramma calomelanos* (Francesconi et al. 2002). Zhao et al. (2002) assessed As accumulation in three different accessions of *P. vittata*, two cultivars of *Pteris cretica* and, *Pteris longifolia* and *Pteris umbrosa*. Arsenic concentrations among all accessions and species ranged from 6200–7600 mg kg⁻¹ DW and these authors indicated that As hyperaccumulation is a constitutive property of the *Pteris* genus. It has been shown, however, that *Pteris* species such as *P. straminea*, *P. tremula* (Meharg 2003) and *P. semipinnata* (Wang et al. 2006) do not hyperaccumulate As. Recently, Kachenko et al. (2007) identified gold dust fern (*Pityrogramma calomelanos* var. *austroamericana*) in Australia. The fern has shown a consistent As-hyperaccumulating pattern in the glasshouse conditions and can accumulate As up to 16,400 mg kg⁻¹ DW in fronds.

Table 2.4 Review of the confirmed As-hyperaccumulating species.*

Fern species	Family name	Maximum frond As concentration (mg kg⁻¹)	Reference
<i>Pteris vittata</i>	Pteridaceae	22,630	Ma et al. (2001)
<i>Pityrogramma calomelanos</i>	Pteridaceae	8,350	Francesconi et al. (2002)
<i>Pteris cretica</i> var. <i>albo-lineata</i>	Pteridaceae	7,600	Zhao et al. (2002)
<i>Pteris cretica</i> var. <i>alexandrae</i>	Pteridaceae	7,600	Zhao et al. (2002)
<i>Pteris longifolia</i>	Pteridaceae	7,600	Zhao et al. (2002)
<i>Pteris umbrosa</i>	Pteridaceae	7,600	Zhao et al. (2002)
<i>Pteris cretica</i> var. <i>nervosa</i>	Pteridaceae	2,594	Chen et al. (2003)
<i>Pteris cretica</i> var. <i>chilsii</i>	Pteridaceae	1,358	Meharg (2003)
<i>Pteris cretica</i> var. <i>crista</i>	Pteridaceae	1,506	Meharg (2003)
<i>Pteris cretica</i> var. <i>mayii</i>	Pteridaceae	1,239	Meharg (2003)
<i>Pteris cretica</i> var. <i>parkerii</i>	Pteridaceae	2,493	Meharg (2003)
<i>Pteris cretica</i> var. <i>rowerii</i>	Pteridaceae	1,425	Meharg (2003)
<i>Pityrogramma calomelanos</i> var. <i>austroamericana</i>	Pteridaceae	16,400	Kachenko et al. (2007)
<i>Asplenium australasicuma</i> [†]	Aspleniaceae	1,240	Sridochan et al. (2005)
<i>Asplenium bulbiferum</i> [†]	Aspleniaceae	2,630	Sridochan et al. (2005)
<i>Pteris multifida</i> Poir.	Pteridaceae	1,145	Du et al. (2005)
<i>Pteris oshimensis</i>	Pteridaceae	2,142	Wang et al. (2006)
<i>Pteris biaurita</i> L.	Pteridaceae	3,650	Srivastava et al. (2006)
<i>Pteris quadriaurita</i> Retz	Pteridaceae	3,650	Srivastava et al. (2006)
<i>Pteris ryuensis</i>	Pteridaceae	3,650	Srivastava et al. (2006)
<i>Pteris faurier</i>	Pteridaceae	1,362	Wang et al. (2007)
<i>Pteris aspericaulis</i>	Pteridaceae	2,410	Wang et al. (2007)

[†]The plants showed As toxicity symptoms when exposed to concentrations > 50 mg L⁻¹.

*modified from Kachenko (2008).

Xu et al. (2010) compared the phytoremediation potential of *P. calomelanos* var. *austroamericana* and *P. vittata* grown in As-contaminated soils with contrasting soil properties in a glasshouse experiment. They demonstrated that *P. vittata* possessed higher As accumulation and produced greater frond biomass than that of *P. calomelanos* var. *austroamericana*. However, no field study has been conducted to compare the phytoremediation efficiency of *P. calomelanos* var. *austroamericana* and *P. vittata*. Field evaluation of the phytoextraction potential *P. calomelanos* var. *austroamericana* is vital as this species is well adapted to the subtropical Australian conditions.

Detoxification and tolerance of As in ferns. Arsenic is a non-essential element for plants, however, in As-hyperaccumulators such as *P. vittata*, As is accumulated at high rates and at concentration proportional to As concentrations in soil or growth media (Ma et al. 2001; Gonzaga et al. 2006). *Pteris vittata* has been reported to survive in soil contaminated with 1,500 mg kg⁻¹ As and (bio)concentrate 2.3% of As in its aboveground biomass (fronds). This feature of *P. vittata* indicates that the hyperaccumulating ferns possess efficient mechanisms to detoxify As accumulated from soil. Such mechanisms may include chelation, compartmentalisation, biotransformation and cellular repair (Salt et al. 1998; Gonzaga et al. 2006). For instance, heavy metals are generally transported and deposited in a vacuole as metal-chelates. Once free metal ions in soil solution are taken up by plants into their tissues, they get reduced greatly when chelated by particular, high-affinity ligands (such as sulfur-donor, oxygen-donor, or nitrogen-donor ligands). Sulfur-donor ligands including, metallothioneins and phytochelatins have the capability to form highly stable complexes with heavy metals, since sulfur is a better electron donor than oxygen. Previous studies show a prominent role of PCs in the detoxification of As in plants (Hartley-Whitaker et al. 2001). Reina et al. (2005) demonstrated that both GSH and PCs were able to complex the majority of

As in shoots of lupin plant. However, the function of PCs appears to be negligible in As-hyperaccumulating fern species (Zhang et al. 2004; Gonzaga et al. 2006). In *P. vittata* and *P. calomelanos*, reduction of As^V to As^{III} occurs inside plant cells (Pickering et al. 2000; Ma et al. 2001). This reduction of As^V inside the plant cells is an intriguing process as As^{III} is more toxic than As^V. Additionally, *P. vittata* was shown to have only 4.5% of its As complexed with PCs, as a GSH-As^{III}-PCs complex (Gonzaga et al. 2006). In a study by Raab et al. (2004), *P. cretica* demonstrated only 1% of its As complexed with PCs. From these studies, the authors conclude that the PCs may act as a carrier to transport As in a non-toxic form through the cytoplasm and into the vacuoles. However, As complexation with PCs may not be the highly efficient detoxification mechanism in As-hyperaccumulating ferns (Gonzaga et al. 2006). Recently, Kachenko et al. (2010) investigated speciation of As in As-hyperaccumulating fern *P. calomelanos* var. *austroamericana* using X-ray absorption spectroscopy. The authors indicated that As^V absorbed by roots was partially reduced to As^{III} prior to transport into aboveground tissues and reported that As^{III}-S²⁻ compounds might be involved for the biochemical reduction of As^V to As^{III}.

2.8.1 Background on *P. calomelanos* var. *austroamericana*

Pityrogramma calomelanos (L.) Link var. *austroamericana* (Domin) Farw. (Pteridaceae) fern, is native to Southern America and is naturalised across the paleotropics including Australia (Chaffey 2002). This species is a terrestrial, rhizomatous fern and is characterised by a yellow waxy indumentums on the abaxial frond surface (Kachenko 2008). It is largely confined to the coastal regions of south-eastern Queensland and north-eastern NSW in Australia. The fern is often found thriving in disturbed areas such as road cuttings, mine overburden and tailings and has also been reported as a weed in banana and pineapple plantations (Ashley et al. 2003). In a survey of Mt Perry Cu mine, Australia, it was reported

as a possible As-hyperaccumulator with concentrations in fronds ranging from 249–3,330 mg As kg⁻¹ DW (Ashley et al. 2003).

It is evident from the above discussion that considerable effort has been devoted to investigate the phytoextraction potential of the As-hyperaccumulating fern species (e.g. *P. vittata*, *P. calomelanos*, *P. calomelanos* var. *austroamericana*) under glasshouse conditions. Few field studies have explored the performance of *P. vittata* for the phytoremediation of As-contaminated sites. However, no field study has been performed to evaluate the phytoremediation efficiency of *P. calomelanos* var. *austroamericana* (a lesser-known As-hyperaccumulating fern) against the well-recognised As-hyperaccumulator *P. vittata*. It is imperative to determine the phytoextraction potential *P. calomelanos* var. *austroamericana* in the field as this species is well-adapted to the (subtropical) Australian conditions.

In addition, the inherent spatial variability in soil As concentration around the cattle-dip sites (as discussed in Section 2.3.2.4) is imperative to define, employing geostatistical methods. So far, no research has been done to estimate the spatial variability of soil As in the vicinity of cattle-dip sites. Thus, the information on spatial variation of As in soil surrounding the cattle-dip sites can be useful for the management and remediation purposes of these sites.

Considering that As was applied in highly toxic and mobile form (sodium arsenite) at cattle-dip sites, further research is required to determine the speciation and phytoavailability of As in contaminated soils, using the combination of SEP-XAFS spectroscopy-plant As uptake (as discussed earlier in Section 2.4.3). The combination of SEP and XAFS spectroscopy can provide a detailed knowledge on the chemical forms (labile, sorbed, mineral bound), minerals (e.g. scorodite, orpiment, arsenopyrite) and oxidation states ($\text{As}^{\text{V}}/\text{As}^{\text{III}}$) of As in As-contaminated soils. To obtain such detailed information on As speciation in soils is not possible using either SEP or XAFS spectroscopy alone.

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