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Unraveling the biogeochemical behaviour of arsenic and antimony in soils and bioavailability to agricultural plants

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DECLARATION

I, Lien Kim Ngo, declare that this thesis is submitted in partial fulfillment of the requirements for the conferral of the degree of Doctor of Philosophy in the School of Chemistry, the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Lien Kim Ngo

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ABSTRACT

The enrichment of soil arsenic (As) and antimony (Sb) is putting increasing pressure on the environment and human health. The biogeochemical behaviour of Sb and its uptake mechanisms by plants are poorly understood and generally assumed to be similar to that of As. Accurate assessment of the bioavailability of these toxic elements to agricultural crops grown in contaminated soils under different scenarios is important for the management of contaminated soils and minimising the risk of human exposure.

In this study, the diffusive gradients in thin films (DGT) technique and sequential extraction procedure (SEP) were applied to assess the partitioning and lability of As and Sb under agricultural conditions in historically contaminated soils with various physicochemical properties. The performance of DGT in predicting As and Sb uptake by two cultivars of radish (*Raphanus sativus*), water spinach (*Ipomoea aquatica*) grown in these soils were investigated, compared to soil solution analysis and sequential extraction. The competitive interactions of As and Sb in single (As-only and Sb-only) and multi-contaminant (As+Sb) soils and the interactive effects of As and Sb on accumulation by *I. aquatica* were also examined. Furthermore, the effect of changing redox conditions on the behaviour and speciation of As and Sb in soils using multiple *in situ* DGT samplers and their accumulation and speciation in *I. aquatica* cultivated in such changing environments were investigated.

The results showed that As and Sb exhibited contrasting biogeochemical behaviours. Irrespective of the method, all labile fractions showed that both As and Sb were firmly bound to the solid phases in historically contaminated soils, and that Sb was less mobile than As, even though total soil Sb concentrations were higher than total soil As. When the contaminated soils were subjected to anaerobic conditions (flooding), DGT-labile As increased with As(III) being the dominant species, but DGT-labile Sb decreased with Sb(V) dominating. The mobilisation of As was coupled with the reductive dissolution of Fe(III) oxyhydroxides, but this did not occur with Sb. Under aerobic condition, the lability of As in As+Sb soils was lower than that in As-only soils. In contrast, during the flooding period, labile As in As+Sb soils decreased and was higher than that in As-only soils, which was likely due to the presence of Sb.

The bioassays demonstrated very low bioaccumulation of As and Sb in the two cultivars of radish (*R.sativus*). The white icicle radish exhibited the same uptake trend as the cherry belle radish, but was less able to translocate As and Sb from roots to shoots than

the cherry belle cultivar. In contrast, much greater bioaccumulation of As and Sb in water spinach (*Ipomoea aquatica*) were observed in which As concentration in edible shoots of *I. aquatica* far exceeded the permissible limit of As in food set by the Food Standard Agency Australia and New Zealand and FAO/WHO. The bioaccumulation factor for As in *I. aquatica* was >1, being much higher than that of Sb, while the translocation factor of Sb was higher than that of As, indicating differences in uptake patterns. Compared with single-element soils, the average bioaccumulation factor of As in *I. aquatica* cultivated in aerobic As+Sb soils slightly decreased, while that of Sb significantly increased. *I. aquatica* was grown in flooded and non-flooded conditions. Flooded *I. aquatica* grew healthily and had greater biomass than non-flooded *I. aquatica*. Concentrations of As and Sb in *I. aquatica* tissues of flooded plants were over 2 times higher than those in non-flooded plants. Inorganic As species were found in plant tissues with both As(III) and As(V) present in non-flooded *I. aquatica* and mainly As(V) present in flooded *I. aquatica*. The reason for this difference is unknown.

Arsenic and Sb in tissues of *R. sativus* and *I. aquatica* were strongly correlated with their labile concentrations measured by DGT, soil solution, and SEP. These techniques are useful measures for predicting bioavailable As and Sb in the historically contaminated soil to the test plants. The coupling of SEP and DGT was useful in understanding the biogeochemical behaviour of As and Sb in aerobic soils. The combined mercapto-silica-DGT, Metsorb-DGT, and diffusive equilibration in thin films (DET) for Fe successfully examined the speciation and lability of As and Sb in flooded soils and differentiated the mechanisms of the mobilisation of As and Sb in soils under changes in redox conditions. DGT measurements in planted soils captured changes in As mobility induced by *I. aquatica*, leading to the better reflection of As uptake by tissues of *I. aquatica*. Importantly, the relationships between As(III)/As(V) in soils measured by DGTs and As(III)/As(V) in plants allowed predicting the preferential As species uptake by flooded *I. aquatica* and explaining the proposed As speciation in flooded *I. aquatica*.

Overall, the biogeochemical behaviour of Sb was different to As. The *in situ* sampling capability, which can be readily utilized in different environmental conditions, and the selective measurement capability for As(III) and Sb(III) made DGT an effective technique for this study. Therefore, DGT is a very promising tool for measuring the bioavailability and speciation of As and Sb in soils, identifying differences in their biogeochemical behaviour, and predicting their uptake by plants under various conditions.

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1.1. Statement of context

Accumulation of As and Sb in soils is putting increasing pressure on the environment and becoming a matter of great concern. This is because they are non-biodegradable, persistent, and toxic substances, and consequently pose a severe threat to human and the ecosystem health via various pathways (direct intake or the food web, interactions of the contaminated environment-plant-animal-human). Currently there is very little known about the biogeochemical behaviour of Sb and its uptake mechanisms by plants, and it is generally assumed to be the same as that of As. In order to assess the risk to human health associated with contamination of As and Sb in soils, there is a global initiative to develop suitable techniques for assessing labile As and Sb, the distribution of various chemical forms of As and Sb in soils, the dynamics of As and Sb in soils under different conditions, and their uptake by agricultural crops. Current approaches typically utilise conventional chemical methods, including total metal digests and sequential extraction techniques, however these have not been reliable measures of metal(loid) bioavailability in soils and nor have they been successful predictors of metal(loid) bioaccumulation in many cases. Despite limitations of sequential extraction such as lack of selectivity, and potential reprecipitation and readsorption during extraction, it is still useful for providing information on the partitioning of metal(loids) in various soil binding sites. The diffusive gradients in thin films (DGT) technique is a relatively novel approach that relies on time-integrated fluxes from soils to the device, which reflects similar metal exposure scenarios between soils and plant roots. DGT has been successfully applied for measuring metal lability in soils and metal uptake by plants, however its applications for assessing labile As and Sb in soils and predicting As and Sb accumulation by edible crops have been limited. This study will investigate the biogeochemical behaviour of Sb compared with As and evaluate the effectiveness of DGT in predicting As and Sb accumulation by a variety of edible plants in different agronomic practices to better understand the relationship between DGT measurements and plant uptake. These results will also be correlated with conventional approaches to identify the most appropriate techniques in measuring bioavailable As and Sb in soils and predicting their uptake by edible plants on the long-term basic.

1.2. Chemistry

Arsenic (As) and antimony (Sb) are metalloids belonging to Group 15 of the periodic table (Kabata-Pendias & Mukherjee 2007). As and Sb display the same range of oxidation states in environmental systems (-3 to +5) because they have identical s^2p^3 outer orbital electron configuration. They commonly exist as oxyanions in +5 states (arsenate and antimonate) in the relatively aerobic environments or in +3 states (arsenite and antimonite) in anaerobic environments. As and Sb in the environment occur in both inorganic and organic forms, with the two common inorganic forms being arsenite and arsenate, antimonite and antimonite, respectively.

While these similarities in the chemistry of As and Sb exist, there are differences in the structure of their pentavalent oxyanions. Arsenate (AsO_4^{3-}) is tetrahedral, a similar structure to phosphate (PO_4^{3-}), thus arsenate and phosphate compete for the binding sites in soil and the accumulation of arsenate by plants occurs via phosphate transporters. In contrast, antimonate ($Sb(OH)_6^-$) is octahedral. However, antimonite ($Sb(OH)_3$) is structurally similar to arsenite ($As(OH)_3$) (Wilson et al. 2010).

1.3. As and Sb contamination in soils

1.3.1. Sources of As and Sb

Both As and Sb naturally occur as trace elements in soil mainly from the weathering of soil minerals. The majority of natural sources of As and Sb are rocks, volcanic ore deposits, and hydrothermal metal sulphide deposits either associated or co-precipitated with metal ores, or separate mineral ores. Common As minerals are arsenopyrite (FeAsS), orpiment (As_2S_3), enargite (Cu_3AsS_4), realgar (AsS), and As metal oxides (Arai 2010; Smith & Huyck 1999). Higher As concentrations are found in sedimentary rocks than igneous rocks, thus soils and parent materials formed from sedimentary rocks often have elevated As concentrations (Smith 1998). The concentrations of As in the earth's crust and uncontaminated soils are 1.8 – 2.5 and 6.8 mg/kg, respectively. In terrestrial environments, inorganic forms (arsenite and arsenate) are generally dominant and highly affected by redox conditions and pH. Inorganic arsenic species under oxidising conditions can be methylated by microorganisms, producing monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide (TMAsO) (Mandal & Suzuki 2002). Ultimately organic As species are converted

into inorganic As and CO_2 by oxidative degradation, or into volatile As compounds, arsine (e.g. AsH_3) gas by reduction (Arai 2010).

Naturally occurring Sb is commonly found to combine with S, As, and Pb to form over 100 minerals. Sb is usually found in stibnite (Sb_2S_3), kermisite (Sb_2S_2O), guettardite ($Pb(Sb,As)_2S_4$), cervantite ($Sb(III)Sb(V)O_4$), docrasite ($Sb(III)_2(Sb(III),As)_2$), and various oxides (valentinite Sb_2O_3) of stibnite oxidation. Of these, stibnite is the only mineral commercially mined, being a source of metallic antimony. Sb is generally present in trace amounts in Ag, Cu, and Pb ores. Similar to As, Sb can be methylated and volatilised as SbH_3 in the environment (Arai 2010). The concentrations of Sb in the earth's crust and uncontaminated soils are 0.2 – 0.9 and 0.2 – 2.3 mg/kg, respectively (Kabata-Pendias 2011).

Substantial amounts of arsenic enter the environment through anthropogenic inputs including industrial processes, agricultural use, smelting, and mining activities. Industrial processes include smelting of As-containing ores and by-products of fossil fuel combustion (e.g. fly coal ash). Approximately 60% of As emissions are related to Cu-smelting and coal combustion (Matschullat 2000). Industrial uses include timber treatment, leather tanning, photoelectric devices, electronic, paints, Pb-acid batteries, Cu-based alloys, glassware, cosmetics, and fireworks (Kabata-Pendias 2011; Mahimairaja et al. 2005; Matschullat 2000; Smith 1998). Agricultural applications of As include pesticides, herbicides, algaecides, antibacterial agents for livestock, cattle dips, seed treatment, fertilizers, and As-enriched irrigation (Adriano 2001; Mahimairaja et al. 2005; Smith 1998).

Most Sb pollution originates from mining and smelting activities, coal and fuel combustion, industrial and municipal waste, outfall of sewage, shooting ranges, fertilizers, and emission from vehicles where Sb is used as a fire-retardant in brake linings (Adriano 2001; Arai 2010; Bhattacharya et al. 2007; He et al. 2012; Tschan et al. 2009b). Industrial uses include Pb-acid batteries, cable sheathings and ammunition, semiconductors, flame-retardants such as textiles, papers, plastics, and adhesives, a paint pigment, ceramic opacifier, catalyst, mordant, and glass decolouriser. The wide uses of Sb have led to substantial inputs of Sb into the environment under dust emissions and incineration of industrial and municipal wastes.

In general, Sb is a co-contaminant in soil. In mine tailings Sb is associated with the primary resource element (usually As), in orchards Sb is found with As, in shooting ranges with Pb, in dust and incineration of waste with other elements such as As, Cd, Cr, Cu, Co, Hg, Mn, Ni, Pb, V, and Zn.

1.3.2. As and Sb concentrations in contaminated soils

The concentrations of As and Sb in uncontaminated soils are generally less than 10 mg/kg (Kabata-Pendias 2011). However, their concentrations in contaminated soils vary greatly and can reach 310000 mg As/kg and 80200 mg Sb/kg, depending upon the nature and contamination sources (Mahimairaja et al. 2005; Wilson et al. 2010). Arsenic and Sb concentrations in soils contaminated by anthropogenic inputs are presented in Table 1.1, a review of recent publications. For all soils in Table 1.1, the total soil As concentrations exceeded the maximum tolerable concentration of As in agricultural soils set by EU (20 mg/kg) (Álvarez-Ayuso et al. 2016; Bhattacharya et al. 2010b) and the total soil Sb concentrations were several orders of magnitude larger than concentrations in unpolluted soils in China (< 3 mg Sb/kg) (He 2007) and Europe and North America (< 10 mg Sb/kg) (Filella et al. 2002a; Okkenhaug et al. 2011).

1.4. Chemical behaviour of As and Sb in soils

1.4.1. Speciation, solubility, and mobility

pH and redox potential (E_h) greatly influence metalloid oxidation state and consequently their solubility and mobility in soils. In geochemical environments, inorganic As is primarily present in two oxidation states (+3 and +5). In the oxic environment, arsenate, As(V), is the main species. As(V) exists as H_3AsO_4 (pK_1 = 2.20, pK_2 = 6.97, pK_3 = 12.13) at pH below 2, in the pH range (2 – 11) As(V) species are charged due to the dissociation of H_3AsO_4 to $H_2AsO_4^-$ and $HAsO_4^{2-}$. At low E_h value (-200 to +300 mV), As is found in the +3 oxidation state as H_3AsO_3 (pK_1 = 9.22, pK_2 = 12.1, pK_3 = 13.4) which does not dissociate until pH 9. At higher pH values H_3AsO_3 exists as $H_2AsO_3^-$ and $HAsO_3^{2-}$. At E_h below -250 mV the formation of insoluble compounds such as AsS, As_2S_3 occurs in the presence of sulphide. Elemental arsenic and arsine can be formed under very strong anaerobic conditions (Arai 2010; Bissen & Frimmel 2003). In the normal range of soil pH (4 – 9), As(III) species are uncharged, while As(V) species are usually negatively charged. In addition to the pH, the mobility of As in soils is influenced by the available sorbent types (e.g. Fe, Al, Mn hydroxides). The surfaces of

Fe, Al, and Mn oxides/hydroxides and clay minerals are only positively charged below soil pH 8, 5, 3, and 4, respectively (Lombi & Holm 2010). The lack of charge on As(III) species compared to successive deprotonated forms of As(V) species leads to less potential for association with solid phases such as oxides/hydroxides and clay minerals in soils (Wilson et al. 2010). Thus, As(III) species are generally more mobile than As(V) species in environmental systems, hence more toxic (Van Herreweghe et al. 2003; Wilson et al. 2010).

In the soil/water environment As can form solubility products with aluminum, iron, calcium, and sulphur. Arsenate ions precipitate with Fe(III) and Al(III) to form amorphous scorodite and aluminum arsenate at acidic conditions, while calcium arsenate precipitates at alkaline conditions (Fergusson 1990). The solubility constants for iron and aluminum arsenates are low (10^{-11}) being smaller than that for calcium arsenate (10^{-5}), illustrating that Fe and Al play a role in controlling As availability in soils (Arai 2010; Smith 1998; Smith et al. 1999). In oxic systems, arsenate is the predominant species and readily binds to mineral compositions. In reducing conditions, adsorbed As can be released via the reductive dissolution of adsorbents, for instance, the reduction of ferric ion into ferrous ion. In addition, a direct conversion of As(V) to As(III) increases mobile As concentration because of the weak sorption of As(III) on soil constituents. However, the decrease in mobile As concentration in long-term flooded soils has been observed (Onken & Hossner 1995). This can be explained by the resorption of As on solids (Onken & Adriano 1997) and co-precipitation of $Mn_3(AsO_4)_2$ (Masscheleyn et al. 1991) under long-term and moderately anoxic conditions (0 – 100 mV).

Table 1.1 As and Sb concentrations in contaminated soils (dry weight).

Soil concentration range (mg/kg)		Contamination source	Country	Reference
As	Sb			
826 - 1606	2735 - 4517	Mine sites	Australia	Wilson et al. 2013
479	2412	Historic gold-antimony mine	Australia	Doherty et al. 2017
4400	11152	Former Sb processing plant	Australia	Doherty et al. 2017
191 – 38600	2.5 – 237	Former mining activities	Australia	Ashley & Lottermoser 1999
1.8 – 40	0.1 – 39.4	Antimony mine 300 km upstream	Australia	Tighe et al. 2005a
59 – 176	180 – 554	Antimony mine 2 km upstream	Australia	Telford et al. 2009
42 – 4530	60 – 230	Abandoned mining areas	Spain	Casado et al. 2007
246 – 758	14.1 – 324	Former mining activities	Spain	Álvarez-Ayuso et al. 2012
7.4 – 3115	0.2 – 35.7	Mining-affected soils	Spain	Pérez-Sirvent et al. 2012
146 – 540	525 – 4463	Old mining sites	Slovakia	Vaculík et al. 2013
9.16 – 447	4.86 – 2058	Forest soils near the smelter	Czech Republic	Ettler et al. 2010
4.33 – 154	3.12 – 131	Agricultural soils near the smelter	Czech Republic	Ettler et al. 2010
7.4 – 596	74.2 – 16389	Mine and its surrounding sites	China	Li et al. 2014
40.02 – 400.2	610 – 54221	Old antimony mine	China	Wei et al. 2011
50 – 17428	10 – 1187	Former mining areas	Scotland	Gál et al. 2006
16 – 691	1.63 – 11.44	Former mining areas	Italy	Gál et al. 2006
11.1 – 651.1	30.5 – 5986.4	Mining-impacted soils	Portugal	Pratas et al. 2005
2220	80200	Smelter site	New Zealand	Wilson et al. 2004
4.4 – 65.1	2.5 – 175	Urban soils impacted by Pb-Zn smelter	France	Douay et al. 2008
37 – 13040	7.4 – 13610	Abandoned mining area	Slovakia	Rapant et al. 2006
	35 – 17500	Shooting range	Switzerland	Johnson et al. 2005
	26 – 1150	Mining contaminated area	France	Denys et al. 2009
	27.7 – 15112.9	Previous mining area	Italy	Baroni et al. 2000
	11.89 – 709.84	Mining and smelting areas	England	Flynn et al. 2003
	100.6 – 5045	Areas affected by mining activities	China	He 2007
	527 – 11798	Areas affected by mining activities	China	Okkenhaug et al. 2011
	14 – 15100	Areas affected by mining activities	Spain	Murciego et al. 2007
	585 – 3184	Areas affected by mining activities	Spain	Álvarez-Ayuso et al. 2013
	1300 – 17500	Shooting range	Switzerland	Scheinost et al. 2006
	629 – 8230	Shooting range	Switzerland	Robinson et al. 2008
200 - 310000		Abandoned gold mining sites	Canada	Meunier et al. 2010
16000		Former mining areas	Thailand	Visoottiviseth et al. 2002
5.3 – 2035.3		Former mining areas	Italy	Baroni et al. 2004
129 – 4779		Cattle dip sites	Australia	Rahman et al. 2017
21 – 1406		Cattle dip sites	Australia	Niazi et al. 2011

The speciation of Sb is also influenced by pH and redox potential and greatly affects its solubility and mobility in the geochemical environments. This means that the speciation influences Sb retention in soils and soil processes. In the typical soil environment, pH ranging from 4 – 9 and E_h ranging from – 300 to + 900 mV (Husson 2013), Sb(III) and Sb(V) are the dominant species (Filella et al. 2009). Over the relevant environmentally pH range, the negatively charged ion ($Sb(OH)_6^-$) is the dominant Sb(V) species, while in reduced conditions the uncharged $Sb(OH)_3$, antimonous acid with pK_a = 11.9 is the most abundant Sb(III) species (Filella et al. 2002a; Wilson et al. 2010). Thus, like As, Sb(III) species may be more mobile and toxic than that of Sb(V) (Filella et al. 2002b; Wilson et al. 2010). Under reducing conditions, the solubility of Sb is limited by the solubility of Sb(III) sulphides (e.g. stibnite) and oxides (e.g. $Sb(OH)_3$, Sb_2O_3, Sb_2O_4) (Arai 2010). In addition, Sb(III) can readily complex with Cl^- to form $SbCl^{2+}$, $SbCl_2^+$, $SbCl_3$, and $SbCl_4^-$ in acidic aqueous solutions (Oelkers et al. 1998).

Under oxic soil environments, the oxidation of Sb(III) to Sb(V) is likely to occur and Sb(V) should be the dominant sorbed Sb species. In a batch experiment using a water extraction of 12 Japanese soils, Nakamaru et al. (2006) reported that anionic Sb(V) was the major Sb species in soil solution. Oorts et al. (2008) also stated that the majority of Sb(V) (70%) was found within 2 days of the addition of Sb as Sb_2O_3 into soil. Using XAFS analysis, Mitsunobu et al. (2006) showed that Sb in mining-impacted soil was present as Sb(V) over a wide range of redox (- 140 to + 360 mV, pH 8) and suggested that Sb(V) was very stable in the soil. Under anoxic conditions, the reductive dissolution of Fe, Al, and Mn oxides could lead to the release of adsorbed Sb.

1.4.2. As and Sb retention in soils

Both As and Sb are strongly retained in soils (Ettler et al. 2010; Flynn et al. 2003; McLaren et al. 1998; Wilson et al. 2010). The degree of retention determines their labile and bioavailable fractions, which in turn determine their persistence, reaction, transformation, and toxicity. The retention is influenced by many factors such as metalloid species present and soil properties. Understanding the As and Sb retention processes in soils is a foundation for understanding their biogeochemical behaviour and for accurate and adequate risk

assessment in various systems. Adsorption is the most important mechanism in controlling the metalloid bioavailability (Wilson et al. 2010).

1.4.2.1. Adsorption

The adsorption of As and Sb in soils involves two different mechanisms, namely non-specific and specific adsorption. Non-specific adsorption refers to the electrostatic attraction between positive charges of adsorbents and negative charges of As and Sb anions (Manful et al. 1989; Sadiq 1997). Conversely, specific adsorption refers to the predominant sorb via ligand exchange, the incorporation of As and Sb species as ligands in the coordinated shell of mineral compounds (Manful et al. 1989; Sadiq 1997). The adsorption of As and Sb in soils is controlled by soil characteristics and environmental factors such as type of oxides/hydroxides and clay minerals and their amount present, organic matter content, pH, As and Sb species, and competing ions (Frost & Griffin 1977; Grafe et al. 2001; Manning & Goldberg 1996a; Wilson et al. 2010).

Adsorption on oxides and hydroxides

According to Arai (2010), anion adsorption on soil components is a function of the net surface charge density of the sorbent and the chemical speciation of sorbate, which in turn depends upon the pH. Generally, the adsorption of anions on minerals increases with decreasing pH due to the negatively charged chemical species and the positively charged mineral surfaces when pH < PZC (point of zero charge). Thus, As and Sb are expected to strongly sorb onto surfaces of metal oxides through electrostatic interaction when pH – PZC is <0 and to dominantly sorb through ligand exchange when pH – PZC is >0.

Of all the mineral components, oxides/hydroxides of Fe, Mn, and Al are actively involved in adsorption processes (Deschamps et al. 2003; Smith et al. 1999). Soils with high amounts of metal oxide minerals can retain higher amounts of As (Smith et al. 1999). The surface charge of various minerals is pH dependent (Figure 1.1). Under acidic systems, these minerals have positive surface charges, thus strongly retaining oxyanions of As and maybe Sb (Sadiq 1997); however, the positive surface charges vary with metal oxide/hydroxide type. The surfaces of Mn, Al, and Fe oxide/hydroxide limit the retention of As and potentially Sb when soil pH is greater than 3, 5, and 8, respectively (Figure 1.1, Sadiq 1997). In the pH range of 7 – 9, carbonate minerals are positively charged, thus play

an important role in As retention (Mahimairaja et al. 2005; Sadiq 1997). The adsorption of As onto minerals also depends on the As species (As(III) or As(V)) present. At acidic and neutral conditions, As(V) exists as negatively charged $H_2AsO_4^-$ and $HAsO_4^{2-}$, and the negative charge increases as medium pH increases (Smith 1998; Zhang & Selim 2008). Conversely, As(III) is present as neutral species, H_3AsO_3, at pH <9.2.

A rise in pH decreases As(V) adsorption but increases As(III) adsorption in soils (Smith et al. 1999). This decline of As(V) adsorption may be related to two interaction factors: (i) increased negative surface potential on adsorbents and (ii) increased amount of As(V) anions in soil solution due to deprotonation of As(V) compounds (Manful et al. 1989; Smith et al. 1999). Both arsenate and arsenite display a maximum adsorption at a certain pH. This pH value is variable and adsorbent-dependent. For instance, the adsorption As(V) maxima was achieved at pH 5 and for As(III) was pH 7 on alumina (Xu et al. 1988, 1991). As(III) sorption maxima was observed on amorphous $Fe(OH)_3$ at about pH 7, As(V) at about pH 4 (Pierce & Moore 1982).

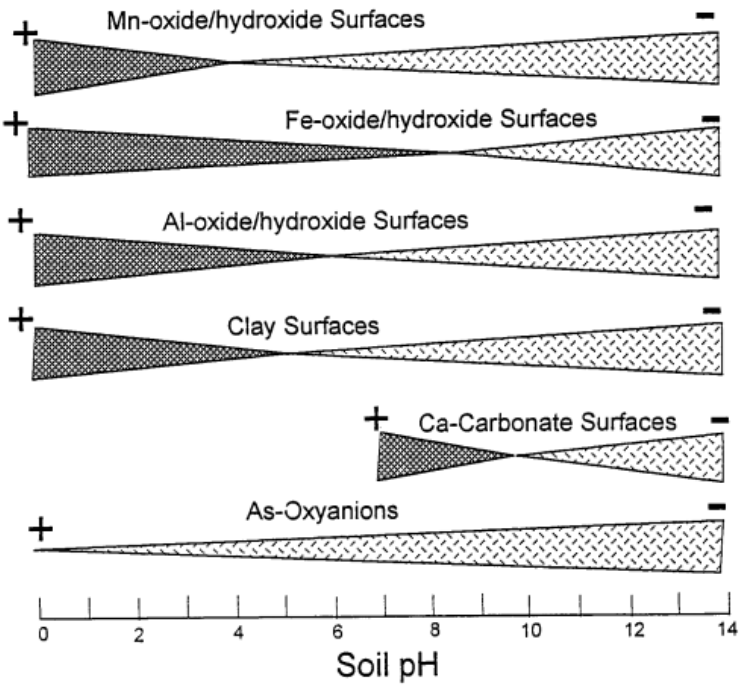


Figure 1.1 Generalised overview of charges distribution on soil colloids, showing the dependence of soil pH on charges formation of different soil compositions and minerals (adopted from Sadiq 1997).

Oxide/hydroxides are also known to be a sorbent for Sb in soil (Manaka 2006; Mitsunobu et al. 2006; Wilson et al. 2010). Sb(III) strongly sorbs to oxide/hydroxides and the sorption follows the order MnOOH > $Al(OH)_3$ > FeOOH, with a gradual decrease in the amount sorbed as pH rises above 6. Below pH 6, more than 80% of Sb(III) added is retained by these sorbents (Thanabalasingam & Pickering 1990). Adsorption of Sb(III) on goethite is stronger than As(III) given the same initial concentrations of anion and goethite (Leuz et al. 2006). In addition, goethite has a greater affinity for Sb(III) than Sb(V) (Xi & He 2013). Sb(V) adsorption to Fe hydroxide is more pH dependent than that of Sb(III) with an adsorption maxima below pH 7 (Leuz et al. 2006). According to Tighe and Lockwood (2007), 95% of Sb(V) was sorbed by crystalline Fe hydroxide across pH 2.5 – 7 with a maximum sorption at about pH 4. Sb(V) sorption on hematite is also strong and maximum at similar pH 4 (Pierce & Moore 1982). However, Sb(V) is not as strongly sorbed to goethite as As(V) over a wide pH range (Leuz et al. 2006), influencing on their mobility and bioavailability in specific systems. The primary sorption mechanism is inner-sphere surface complexes and binuclear bridging complexes (Fendorf et al. 1997; Leuz et al. 2006; Scheinost et al. 2006).

Adsorption on silicate clay minerals

Silicate clay minerals are also known to be important in As and Sb retention in soils (Frost & Griffin 1977; Gál et al. 2006; Manning & Goldberg 1996a). According to Wilson et al. (2010), adsorption of anions in clay minerals is associated with broken clay particle edges, commonly via surface ligand exchange mechanisms (e.g. –M–OH + $H_2AsO_4^-$ = –M–H_2AsO_4 + OH^-). More As is absorbed by illite and montmorillonite clay minerals than by kaolinite, possibly due to higher surface area of the former clay minerals (Frost & Griffin 1977; Manning & Goldberg 1997). The adsorption of two inorganic As species on silicate minerals also differs. Generally, clay minerals adsorb greater amounts of As(V) than As(III) (Lin & Puls 2000). As(V) adsorption on kaolinite and montmorillonite clay minerals increases at low pH, peaking at about pH 5, and thereafter decreasing (Frost & Griffin 1977; Goldberg & Glaubig 1988). Conversely, As(III) adsorption on kaolinite continuously increases between pH 4 – 9, but peaks near pH 7 on montmorillonite (Frost & Griffin 1977). Sb adsorption on clay minerals is less studied, yet the Sb adsorption on Al-

silicate minerals appears to be important, depending upon Sb origin (Gál et al. 2006). Like As(III), the adsorption of Sb(III) on kaolinite is strongly dependent upon pH and decreases with increasing pH (Xi et al. 2016). The presence of competitive anions have no obvious effect on the adsorption of Sb(III) on kaolinite.

Adsorption on organic matter

The role of organic matter in the adsorption of As is complex. Some studies have reported that organic molecules decrease As retention by competing with As for sorption sites on mineral surfaces (Grafe et al. 2001; Redman et al. 2002). The degree of adsorption is dependent on pH, As speciation, and type of competing organic molecules. According to Grafe et al. (2001), peat fulvic and humic acids diminished As(V) adsorption on goethite, whereas no effect was found with citric acid. The maximum reduction of As(V) adsorption on fulvic acid occurred at pH 3 – 8 and at pH 6 – 9 for humic acid. The decrease of As(III) adsorption on these organic acids followed the order of citric acid > fulvic acid ~ humic acid at a pH range of 3 – 8. Redman et al. (2002) reported that the adsorption of As(V) and As(III) on hematite was reduced by organic compounds. In contrast, the role of organic matter as an As adsorbent has been shown (Cao & Ma 2004; Cao et al. 2003; Saada et al. 2003), with the amine groups of humic acid being considered responsible for As retention on organic matter (Saada et al. 2003).

The association of Sb with soil organic matter has also been reported (Ceriotti & Amarasiriwardena 2009; Clemente et al. 2008). Up to 30% of total Sb(III) species readily bound to humic acid at environmentally relevant conditions (Buschmann & Sigg 2004; Filella et al. 2002b). The binding strength of Sb (III) is comparatively stronger than As(III). Sb(III) was found to constitute up to 34% of total Sb in soil organic acids (Ettler et al. 2007) and the interaction with humic acid significantly influenced mobility. In contaminated shooting range soils humic acid had a high capacity to complex and tightly bind the predominant Sb species, Sb(V), trapping most of it in the organic layer (Steely et al. 2007). Significant amounts of added Sb(V) (56%) sorbed on humic acid at pH 4 was also reported by Tighe et al. (2005b). Ligand exchange with Sb and formation of negatively charged Sb complexes with phenolic, carboxylic, and hydroxyl-carboxylic groups may be a mechanism of Sb association with organic matter. The association might be due to the

formation of bridging products between metalloid anions, organic matter, and Ca (Buschmann & Sigg 2004; Steely et al. 2007).

In general, the information of soil components and properties (e.g. % clay, % silt, % sand; Fe and Al oxide content; organic matter content, pH, redox potential) allows inferences of the formation of binding phases in soils and the availability of As and Sb in soils studied.

1.4.2.2. Competition

Phosphate ions are analogues of As(V) ions and can compete with As for adsorption sites on mineral compounds in soil, suppressing the adsorption of As (Manful et al. 1989; Roy et al. 1986). Phosphate is able to compete with arsenate for available adsorption sites, both non-specific and specific (Qafoku et al. 1999). According to Liu et al. (2001), phosphate and arsenate primarily compete for surface sites on goethite, but certain soil sites are uniquely specific for phosphate and arsenate adsorption. Other anions such as nitrate, chloride, sulphate, and molybdate also influence the adsorption of arsenate, but their effect on As adsorption is not profound (Livesey & Huang 1981; Manful et al. 1989; Manning & Goldberg 1996b). Competitive effects on Sb adsorption are relatively unknown. According to a recent study by Xi et al. (2016), the presence of competitive anions such as nitrate, sulphate, and phosphate have no obvious effect on the adsorption of Sb(III) on kaolinite. Addition of P as superphosphate enhanced the release of As and Sb in contaminated firing range soils (Kilgour et al. 2008). The addition of phosphate decreased the distribution coefficients of Sb in Japanese agricultural soils, with 20 – 40% of sorbed Sb (Nakamaru et al. 2006) and 0.2 – 1.3% of total soil Sb (Nakamaru & Sekine 2008) in phosphate exchangeable phase. It seems that the mobilisation of Sb by phosphate addition is dependent on the Sb species present and the extent of ligand exchangeable Sb.

1.4.3. Associations of As and Sb in soils

The mobility and bioavailability of As and Sb in soils are strongly influenced by their association with soil solid phases. The binding strength depends on the binding phase to which As and Sb are bound. Arsenic and Sb partitioning in soils can be operationally-defined by geochemical fractions including dissolved, soluble, labile, Fe and Al oxides (reducible), organic matter (oxidisable), and residual (Larios et al. 2013).

1.4.3.1. As and Sb in dissolved, mobile or labile fractions

The dissolved As and Sb in soils can be measured by extracting soils with deionised water at a certain ratio of soil and water. This phase can be isolated by centrifugation and ultrafiltration (Ettler et al. 2007). Casado et al. (2007) reported that the mobile As and Sb extracted from abandoned mining-impacted soils were 0.02 – 0.7% of total soil As and 0.02 – 0.27% of total soil Sb, reflecting that the plant-available fractions were extremely low compared to their concentrations in soils. Generally, the concentrations of dissolved or soluble As and Sb only account for a small proportion of their total soil concentrations (<2%) and greater than 99% of As and Sb in soil solution were arsenate and antimonate (Wilson et al. 2010). The readily available fraction, the non-specifically sorbed As and Sb, which are outer sphere complexes or freely exchangeable fraction, are obtained from the first extraction step with $(NH_4)_2SO_4$ of sequential extraction procedure (SEP) developed by Wenzel et al. (2001). This fraction contains the labile species comprised of free ions and soluble inorganic and organic complexes. It consists of the most labile and available chemical species for plant uptake (Anawar et al. 2008; Casado et al. 2007; Larios et al. 2012a). The freely exchangeable As is significantly positively correlated with As concentration in plant shoots (Niazi et al. 2011).

The adsorped fraction includes As and Sb that are specifically-sorbed to soil surfaces as inner-sphere complexes. Arsenic and Sb in this phase are released by anion exchange processes using ammonium phosphate, $NH_4H_2PO_4$ (Wenzel et al. 2001). Ammonium phosphate has been previously validated in SEPs for As in oxidized soils (Keon et al. 2001; Wenzel et al. 2001) and mine wastes (Drahota et al. 2014). It has been stated in section 1.4.2 that both As and Sb are strongly adsorbed onto mineral surfaces. Many studies have demonstrated that phosphate solutions are efficient in extracting adsorbed As in soils and mine wastes (Drahota et al. 2014; Keon et al. 2001; Larios et al. 2012b; Larios et al. 2013; Wenzel et al. 2001). These authors suggest that phosphate extracts the freely exchangeable As (outer-sphere complexes) as well as strongly adsorbed As (inner-sphere complexes), depending on the ionic strength and duration of extraction. The quantity of anions in these fractions is used to measure trace elements which are mobilised and readily released back to the environment. Arsenic and Sb in the non-specifically sorbed and specifically sorbed

phases are defined as the labile and bioavailable fractions (Wenzel et al. 2001). Thus, ammonium phosphate has been used as a single extraction to determine the labile fraction of As and Sb in soils (Ettler et al. 2007; Hamon et al. 2004). Labile As and Sb in long-term contaminated soils account for a small proportion of their total soil concentrations, less than 15% (Ettler et al. 2010; Kim et al. 2014; Ngo et al. 2016; Niazi et al. 2011; Wilson et al. 2010), and are highly correlated with As and Sb concentrations in plants (Gonzaga et al. 2008; Ngo et al. 2016; Niazi et al. 2011). In addition, in long-term contaminated soils labile Sb is much lower than labile As.

1.4.3.2. As and Sb incorporated into oxide/hydroxides

Mineral oxide/hydroxides are strong scavengers of As and Sb in soils (Drahota et al. 2014; Tighe et al. 2005a). Fe and Al oxides are typically the fraction binding the greatest proportion of As in the environment (Lock et al. 2016). The selected reagents used for extracting As and Sb associated with amorphous and crystalline Fe and Al are oxalate buffer and oxalate buffer plus ascorbic acid at 96 °C (Wenzel et al. 2001). The fractionation of As in 20 soils conducted by Wenzel et al. (2001) showed 12 – 73% of total As associated with amorphous Fe and Al oxides, but 13 – 39% associated with crystalline forms. Many later studies using or adapting the improved procedure of Wenzel et al. (2001) have reported the same trend (Álvarez-Ayuso et al. 2016; Gál et al. 2006; Kim et al. 2014; Müller et al. 2007; Niazi et al. 2011).

Similar to As, the proportion of Sb extracted from metal oxides is much greater than from the labile fraction. In a study on chemical fractionation of Sb in acid soil by Tighe and Lockwood (2007), 30 – 47% of total Sb was associated with non-crystalline Fe and Al hydroxides. In another study on speciation and availability of Sb in active mining impacted-soil by Okkenhaug et al. (2011), 22 – 66% of total Sb was reported to be associated with amorphous and crystalline Fe and Al oxides. The proportion of Sb associated with oxide fractions is dependent on Sb origin (He 2007).

1.4.3.3. As and Sb associated with organic matter and sulphide

As and Sb might be incorporated into different forms of organic and sulphide material known as the oxidisable fraction. The binding between As and Sb and organic matter is due to adsorption and complexation (as discussed in section 1.4.2.1). Oxidising reagents such as

hydrogen peroxide (H_2O_2) in acid medium (Jones et al. 1997; Lintschinger et al. 1998), $Na_4P_2O_4$ (Larios et al. 2013), and a mixture of $KClO_3$, HCl, and HNO_3 (Drahota et al. 2014) are usually applied to leach As and Sb bound to organic matter and sulphides. Applying a mixture of hydrogen peroxide and ammonium acetate to extract As associated with organic fraction, Jones et al. (1997) found about 10% of total As in this fraction but Lintschinger et al. (1998) found no significant amount of Sb in this fraction (<4.1%). In a comparative study on the partitioning of As and Sb in forest soils, (Ettler et al. 2010) used the same reagents and found that up to 32% of the total Sb bound in the organic and sulphide fraction, while 6 – 22% of the total As was in this fraction. In the same study with agricultural soils, Ettler et al. (2010) reported that Sb bound to organic matter and sulphide fraction was 2 – 12%, whereas As bound in the oxidisable fraction was 2 – 7%. The proportion of Sb in the organic and sulphide fraction appears to be higher than As. It can be generalised that the proportion of As and Sb in the oxidisable fraction depends on the contaminant, soil sources, and minerals. This fraction is negligible in soil surface horizons, but it may be important or even predominant in soil rich in sulphide or organic matter contents such as forest soils.

1.4.3.4. As and Sb in residual fraction

The residual fraction contains mainly silicates and other resistant minerals holding As and Sb in the crystalline lattice structure. These As and Sb are not soluble under natural environmental conditions, thus are not biologically available, and only become available as mineral weathering occurs over a long period of time. The destruction of the highly recalcitrant As and Sb fraction is obtained by strong acid digestion with a mixture of the following strong acids such as HF, $HClO_4$, HCl, and HNO_3 (He 2007; Tighe & Lockwood 2007). The residual fraction depends not only upon the contaminant source, but also factors controlling As and Sb mobility such as dissolution of primary phases, As and Sb adsorption by soil components, and interactions with other variables (e.g. pH, redox potential, competing ions) (Wilson et al. 2010). In general, the residual As and Sb is high in which the proportion of Sb in residual phase is higher than As.

1.5. Mechanism of metal(loid) uptake from soils by plants

This section will describe the processes by which (metal)loids are taken up by plant roots and how these (metal)loids are subsequently distributed within the plant tissues. This is relevant because it provides a foundation for the interpretation of soil processes around the root zone and As and Sb uptake by plants in this research.

1.5.1. Concentration and speciation of metal(loid)s in soil solution

The general paradigm in metal(loid) uptake from soils by plants is that plant roots absorb element ions via soil solution and the concentration and species of dissolved ions affects the uptake rate (McLaughlin et al. 2011). In soil solution, dissolved metal(loid)s exist as various forms categorised as free ions, inorganic complexes, and organic complexes (Hooda 2010; McLaughlin 2001). The total quantity of metal(loid) ions in soil solution affects the plant uptake of (metal)loid ions from the soil solution (Alloway 1995; Barber 1995). The metal(loid)s commonly enter the plant roots as ions via ion channels having capacity to concentrate elements from soil solution. According to Campbell (1995) and McLaughlin et al. (2011), if the metal(loid) uptake by plants is well predicted by free ion activity, the plant acquisition must be slow and the uptake does not induce the depletion of metal(loid) concentration in the rhizosphere.

The uptake rate generally increases with increasing ion concentrations in soil solution, which is called a concentration-dependent pattern or plant tissue concentrations increasing as soil concentrations increase. Such patterns are important in risk assessment in perspective of identifying soil concentrations at which plant tissue concentrations are below targeting values. This pattern is the basis of the application of bioaccumulation factor concept in risk assessment (McLaughlin et al. 2011).

1.5.2. Metal(loid) movements in soils to roots

The uptake of metal(loid)s from soil by plants depends on ion transport from the bulk soil to the root surface. There are three principal processes for metal(loid) transport in soils: 1) root interception, 2) mass flow (convection), 3) diffusion (Figure 1.2).

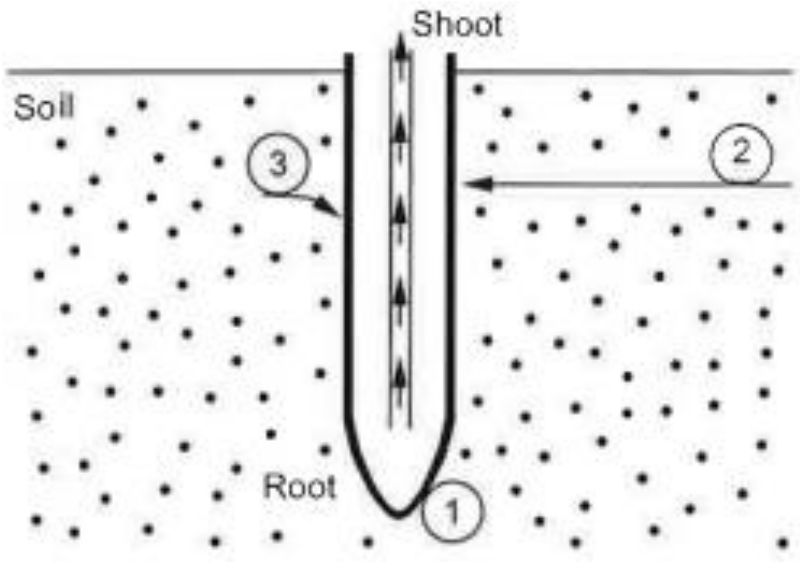


Figure 1.2 Movement of elements to the root surface (taken from Marschner and Rengel 2012); (1): root interception, (2): mass flow (convection), and (3): diffusion.

Root interception describes the movement of roots through the soil when they develop. As roots extend they replace spaces formerly occupied by soil, putting them in contact with elements sorbed to various solid phases. The proportion of elements intercepted by roots is based on a) the amount of available elements in the soil volume occupied by the roots, b) root volume as a percentage of total soil volume, and c) the percentage of the total soil volume occupied by pores (Marschner & Rengel 2012). In general, only a small proportion of available elements can be supplied by root interception.

Mass flow is the movement of dissolved ions through the soil to the root in the convective flow of water caused by plant water absorption. Transpiration from the plant shoots governs the convective transport; thus, the contribution of mass flow to the element supply depends on the portion of water transpired and the content of elements in the soil solution (Marschner & Rengel 2012). Comparison of the rate of supply with the rate of influx provides the significance of mass flow. Alternatively, mass flow can be calculated by multiplying water use per plant by the concentration in the soil solution. Differences in water use attributed to crop, climate, and moisture conditions result in variations in the significance of mass flow. Mass flow can have importance in the supply of water-soluble micro and macro-elements into plants (Barber 1995).

When a particular nutrient (micro- and macro- nutrient) is not sufficiently supplied to the root by mass flow and root interception, the concentration of available nutrients in the soil at the root surface is reduced because of continued uptake. This leads to a concentration gradient perpendicular to the root surface and subsequently nutrients diffuse along the gradient toward the root surface (Barber 1995). Equilibrium is not reached since roots absorb nutrients, causing the continuous diffusion to the root along the concentration gradient. In general, the diffusional supply of nutrients is the most important path for plant uptake. The distance in which the concentration gradient extends is dependent on the rate of diffusion. The distance for diffusive movement of nutrients through the soil to the root is usually in the range of 0.1 to 15 mm (Barber 1995; Marschner 1995). Thus, only the soil nutrients in this zone can contribute to the supply of diffusive nutrients to the root. Practically, if the depletion of ions occurs, ions are replenished from liquid or solid/complexed forms. This means that a fraction of complexed ions is also in the bioavailable form in soils, provided that the environmental condition favours the sufficient rapid desorption and dissociation. For this reason, bioavailability functionally includes both the activity in soil solution and the labile bound forms (McLaughlin et al. 2011). It is also reasonable that diffusive fluxes correlate well with the plant uptake, provided that the metal(loid) is depleted in the rhizosphere (Nolan et al. 2005).

Field-based data for some plants and soils show that the mass flow exceeded the root uptake of As, consequently As may have accumulated around the roots (Chen et al. 2009). This may be related to the As root absorption power and its mechanism regulating the uptake by plants. These will be discussed in sections 1.5.4 and 1.5.5.

1.5.3. Rhizosphere environment

The rhizoshpere is defined as a few millimetres (1 – 2 mm) of soil surrounding the plant roots and affected by plant activities (Alloway 1995). The rhizosphere conditions effectively control the supply of contaminants to plant roots. Factors affecting the rhizosphere include biological parameters (e.g. the root system including exudates, nutrient status, microorganisms), and physicochemical conditions (e.g. pH, redox potential, soluble organic matter, depletion and accumulation of ions) (Fitz & Wenzel 2002; Gobran et al. 2001). Plant roots have the ability to modify the solubility and availability of elements in

the soils, influencing the biogeochemical conditions in the root area (Hinsinger 2000; Marschner 1995; Mengel & Kirkby 2001). For instance, organic exudates are capable of mobilising nutrients, making anions (e.g. phosphates) and cations (Cu, Zn) more available. Moreover, plants are able to alter the pH of the rhizosphere thanks to the release of organic acids serving as soil solution buffer (Marschner 1995). In addition, the biogeochemical cycle in the rhizosphere is influenced by microorganisms. If bacterial activities in the rhizosphere are high, methylation and reduction is favoured (Renella et al. 2007).

Since phosphate and arsenate are chemically analogous, all processes for P mobilisation are likely to mobilise As. For example, organic acids having a low molecular weight (citric and malic acids) are capable of displacing arsenate from a position of retention in soils (Redman et al. 2002; Wenzel 2009). In addition, plant strategies include the attachment of Fe oxide/hydroxides to alter the mineral surfaces retaining the As, which can potentially solubilise As (Fitz & Wenzel 2002). Moreover, arsenate adsorbed by corn roots can be significantly reduced by corn mycorrhizae, association between fungi and plant roots (Yu et al. 2009).

1.5.4. Transfer of metal(loid)s from the root surface into roots

In general, there are two key mechanisms controlling the uptake of metal(loid)s by plant roots, passive and active (Alloway 1995; McLaughlin 2001). Passive (non-metabolic) uptake involves the diffusion of metal(loid) ions from soil solution into root endodermis without demanding energy and uptake of ions in response to the established concentration gradient. Active uptake takes place if ions have a net negative charge and acts against a concentration gradient. This process requires metabolic energy and thus can be inhibited by toxins. The barrier to plant uptake mechanisms which require energy is the plasma membrane inside the cell wall (Barber 1995). Two main ways for solutes to move from the soil solution to the xylem vessels in roots are apoplasmic or symplasmic flow (Figure 1.3). Apoplasmic pathways involve the movement of solutes through intercellular spaces with pores in cell walls of the cortex up to the Casparian strip. The Casparian strip around the endodermal cells is a barrier to the movement of solutes from the cell wall spaces into the stele. Symplasmic pathways involve the direct movement of solutes through the symplasm of cells in the cortex up to the Casparian strip (from cell to cell, selective transport crossing

membranes). In the Casparian strip, the solute ions in these two flows have to cross a plasma membrane in the cytoplasm to enter the xylem vessels. Then they can be transported to the shoots.

The adsorption of As by roots occurs by diffusion from soil solution in the root apoplast, followed by the symplast, penetrating to the interior of the plant cells (Mengel & Kirkby 2001). Ions pass through the plasma membrane via transport proteins specific for one or more elements with similar chemical characteristics. Both phosphate and aquaporins (water channel) are involved in the transmembrane transport and adsorption of As. Many studies have illustrated that arsenate uses a phosphate transporter (Esteban et al. 2003; Meharg & Macnair 1992; Zhao et al. 2009) and arsenite enters the membrane via aquaporin channel (Isayenkov & Maathuis 2008; Ma et al. 2008) since they share the same chemistry in terms of ion size and charge density.

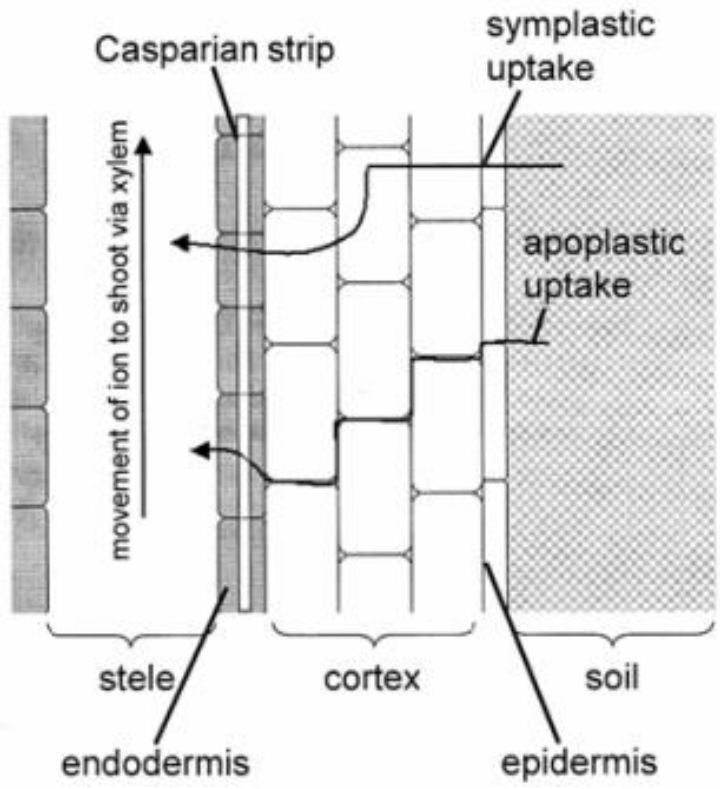


Figure 1.3 Simplified scheme of the root, displaying pathways of metal transport across the root to the stele (adopted from Luo 2008).

The mechanisms of Sb uptake by plants are relatively unknown. In the preliminary study by Tschan et al. (2008), antimonate uptake by plants was not affected by phosphate, suggesting that the same pathway as phosphate does not occur for antimonate. This difference is because the structure of antimonate is octahedral, while that of arsenate and phosphate is tetrahedral. Additionally, antimonate has a larger size and lower charge density, setting antimonate apart from the other oxyanions (Tschan et al. 2008). According to the review of Tschan et al. (2009b), antimonite may cross plasma membranes passively via aquaporins since $As(OH)_3$ and $Sb(OH)_3$ are similar in their structural conformation and charge distribution with glycerol. Such transport would agree with the observed proportionality between water-soluble Sb concentrations and Sb in plants. However, aquaporins are not open for antimonate. The uptake of antimonate by plants would require regulation by transporters.

1.5.5. Accumulation of metal(loid)s into root and transport to shoots

Once inside the cell, arsenate is rapidly reduced to arsenite, using glutathione as a reductant, with the catalysis of arsenate reductase ($AsO_4^{3-} + 2GSH \rightarrow AsO_3^{3-} + GSSG$) (Moreno-Jiménez et al. 2012; Verbruggen et al. 2009). Thus, it has been shown that the majority of As existing in plant tissues is arsenite (Tripathi et al. 2007), irrespective of which As species was in the plant growing medium. Arsenite is known to have a high affinity for –SH groups and to be complexed and stored in vacuoles, despite being able to be transported to shoots via the xylem. The complexation of As with glutathione (GSH) and phytochelatins (PCs), has been identified in various plants such as *Holcus lanatus, P.cretica, Brassica juncea, Rauvolfia serpentine,* and *Helianthus annuus* (Montes-Bayón et al. 2004; Pickering et al. 2000; Raab et al. 2004; Raab et al. 2005; Schmoger et al. 2000). The movement of ions via the xylem is regulated by both the flow of transpiration stream, and membrane transport proteins (Moreno-Jiménez et al. 2012). Two transporters (Lsi1 and Lsi2) have been recently described between the epidermis and endodermis and mediate arsenite entrance into xylem or its effux to the external medium (Moreno-Jiménez et al. 2012; Zhao et al. 2009). Accumulation of arsenite in vacuoles might explain the decrease in As transport into the xylem (Zhao et al. 2009). The transport of As in most plants is commonly not very effective, thus As tends to be accumulated in roots, with an exception

for some plant species unusually effective in accumulating As in shoots. The reduction process in plant roots may contribute to the physiological mechanism plants use to block the transport of As via the xylem and limit the flow of As into shoots, hence protecting them from the effect of As.

For the long-distance transport of As in plants, arsenate is considered to act similarly to phosphate, while no plant transporter has been identified for mediating transport of arsenite from root to shoot; furthermore, the role of chelators for this transport has not been described (Tripathi et al. 2007). Pickering et al. (2000) and Raab et al. (2005) identified unbound As(III) and As(V) in xylem sap of *Brassica juncea* and *Helianthus annuus*, in addition to As-PC complex. Furthermore, the majority of As was present as complexes in roots (Vazquez Reina et al. 2005), and the amount of As complexed by –SH groups was negatively correlated with the amount of As translocated to shoots (Huang et al. 2008). With these findings, many authors postulated that complexed As is not transported through the xylem and As(III) and As(V) are the main species transported from roots to shoots where similar reduction and sequestration occur (Farooq et al. 2016; Mendoza-Cózatl et al. 2011; Raab et al. 2005; Tripathi et al. 2007).

Generally, once the ions have been absorbed by the roots, the transport of solutes in plants may occur in the vascular system of xylem and phloem. Translocation is an important process in determining trace element concentrations in plant tissues. The translocation of solutes to the shoots depends on the element concerned, plant species and variety, plant age, external concentration of the element, and solution composition (Alloway 1995; Barber 1995). In the leaves, ions may be incorporated into proteins or translocated around the plant in the phloem with substances made by photosynthesis. Plants are grouped into different categories based on how much metal(loid) is accumulated in shoots (Baker 1981). A hyperaccumulator plant shows a high transfer factor, leading to extreme amounts of metal(loid)s in leaves (Baker 1981). It is reported that As hyperaccumulators can rapidly translocate a large proportion of metal(loid)s from roots to shoots, while non-accumulators are likely to sequester metal(loid)s in the roots (Baker 1981). The As concentration in non-accumulator shoots rarely exceeds 2 mg As/kg (Horswell & Speir 2006).

In contrast to As, the Sb accumulation in roots, the mechanisms of transport from roots to shoots, and Sb storage in leaves remains unknown (Feng et al. 2013). Based on the higher Sb(V) concentrations in rice shoots than in roots and pore water of growing media, Okkenhaug et al. (2012) postulated that rice may preferentially accumulate Sb(V) and oxidise Sb(III) to Sb(V) in shoots. Whether Sb is involved in redox reactions in plants is unclear. Additionally, whether Sb(V) is accumulated by roots and reduced to Sb(III), which is in turn complexed with phytochelatins is still unclear (Feng et al. 2013). In a recent review on cellular and molecular mechanisms of Sb transport, toxicity, and resistance there was evidence of intracellular reduction, however the mechanism of Sb(V) reduction to Sb(III) outside the cell is not understood (Tamás 2016). In the cytoplasm, Sb(V) may also be reduced to Sb(III) enzymatically or non-enzymatically and then sequestrated into vacuoles of yeast cells. However, the involvement of PCs in Sb(III) resistance may be limited due to trace amounts of $Sb(glutathione)_3$ detected. There is little information on the production and responses of PCs to Sb exposure in plants, further investigation is needed.

1.6. Bioaccumulation of As and Sb in edible vegetables

Metal(loid)s are accumulated by plants when they are exposed to soils or water contaminated with metalloids. Metal(loid) uptake by plants, especially edible crops, presents the risk to human health through the food chain (Wilson et al. 2014). The scenario of metal(loid)-enriched edible plants has led to many hydroponic and soil studies via greenhouse and field based experiments, conducted to understand their uptake potential by various vegetable species and subsequent risks that may occur to humans. The maximum permissible As concentration (MPC) of 0.1 mg/kg (wet weight, WW) recommended by FAO/WHO and Australian MPC of 1 mg/kg (WW) for foodstuffs, specific to cereals have usually been referred to when evaluating the risks to humans from the consumption of contaminated vegetables (Burló et al. 1999; Carbonell-Barrachina et al. 1999a; Rahman & Naidu 2009; Uddh-Söderberg et al. 2015; Wilson et al. 2014). Numerous studies reveal that As concentrations in edible tissues of crops grown in historically contaminated soils are lower than MPCs (Kabata-Pendias 2011; Kabata-Pendias & Mukherjee 2007; Warren et al. 2003). However, some vegetable species such as silverbeet, spinach, tomato, and turnip can accumulate As in their edible parts to concentrations exceeding the critical values (Burló et

al. 1999; Carbonell-Barrachina et al. 1999a; Carbonell-Barrachina et al. 1999b; Rahman & Naidu 2009).

Ample greenhouse and field based studies on As have been conducted to understand its mechanism of uptake by various plants. Generally, As accumulation in various parts of edible crops followed the order roots >> stems > leaves > fruits (Cobb et al. 2000; Kabata-Pendias 2011; Smith et al. 2009). Rice and wheat accumulated As from contaminated soils and waters in the order root >> stem > leaf > grain> husk (Kabata-Pendias 2011). The contaminated soil particles or As adsorbed on the iron layer on the root surfaces of edible root crops may contribute to high As bioaccumulation in roots. Where inadequate washing is exercised, this may result in direct intake of As by human, in spite of low As concentrations in plant tissues (Carbonell-Barrachina et al. 1999a; Carbonell-Barrachina et al. 1999b).

The soil-plant transfer model is commonly used to predict metal(loid) uptake by plants and estimate the potential risk present to humans via digestion of vegetables produced from contaminated soils. The simple and commonly-used parameters are bioaccumulation factor (BAF) determined by the ratio of metal(loid) concentration in plant to metal(loid) concentration in soil and translocation factor (TF) defined as the ratio of metal(loid) concentration in shoots to metal(loid) concentration in roots (Álvarez-Ayuso et al. 2012; Antunes et al. 2006; Murray et al. 2009; Sharifi et al. 2014; Vamerali et al. 2010). A higher BAF reflects the high availability of metal(loid)s in soil and thus greater plant efficiency in transferring metal(loid) from soil to the plant. A low BAF indicates the low availability of metal(loid)s or strong adsorption of metal(loid)s to soil constitutes (Douay et al. 2013). TF is an index to evaluate the ability of plants to translocate metal(loid)s from roots to shoots, used for classification of plants into different categories. Tolerant plants tend to limit soil-root and root-shoot transfers, thus have low bioaccumulation in tissues, while hyperaccumulator plants actively take up metal(loid)s from soil to roots and translocate metal(loid)s from roots to shoot tissues. Plants with high BAF (normally >1) are often classified as hyperaccumulators and can be used for phytoextraction. Plants with high BAF (>1) and low TF (<1) are suitable for phytostabilisation (Vamerali et al. 2010; Vithanage et

al. 2012). The existing literature on the As uptake by plants reveals that the typical bioaccumulation factor of As is about 0.2 (Wilson et al. 2013).

In terms of Sb research, a range of studies has been recently reported on Sb phytoavailability (He 2007; Wanat et al. 2014), toxicity to plants (Oorts et al. 2008; Sanderson et al. 2014; Shtangeeva et al. 2011; Tschan et al. 2009a; Vaculík et al. 2015), accumulation in plants (Álvarez-Ayuso et al. 2013; Conesa et al. 2011; Hajiani et al. 2017; Qi et al. 2011; Shtangeeva et al. 2014; Tschan et al. 2009a), and uptake mechanisms (Corrales et al. 2014; Mathews et al. 2011; Tisarum et al. 2015; Tisarum et al. 2014; Tschan et al. 2010; Tschan et al. 2008). In addition, a number of studies have focused on Sb uptake by rice with respect to the effect of the iron plaque and speciation on its uptake due to concerns with As uptake by rice (Cai et al. 2016; Cui et al. 2015; Huang et al. 2012; Okkenhaug et al. 2012; Ren et al. 2014). Most of these preliminary studies are field-based and hydroponic experiments. Only a few studies have investigated Sb accumulation in edible vegetables and food crops (Hammel et al. 2000; Ngo et al. 2016; Wilson et al. 2014). A recent review on Sb bioavailability with research perspectives for sustainable agricultures also reported Sb concentrations in a range of edible plants (Pierart et al. 2015). However, almost all edible plants in the cited studies were collected in the fields. This would lead to some limitations in terms of evaluating Sb bioaccumulation and understanding the mechanism of Sb uptake by edible vegetables. Some field studies showed that Sb concentrations in plant shoots were unusually high (exceeding 100 mg/kg) due to foliar uptake of Sb (Baroni et al. 2000; He 2007). According to McLaughlin et al. (2011), in air non-gaseous metal(loid) associated with small particulates can be deposited on plants.

Antimony accumulation varies widely among plant species (Pierart et al. 2015; Tschan et al. 2009b). In contrast to high Sb concentrations in plants collected in the fields, low Sb accumulation in plants grown in heavily contaminated soils was also reported (Tschan et al. 2009b). The typical bioaccumulation factor for Sb is about 0.02 in the reviewed literature (Wilson et al. 2013). Based on published data Tschan et al. (2009b) performed regression analysis and found that there was a significant correlation between extractable Sb concentrations in soils and Sb concentrations in plants. Interestingly, the slope of the

regression line was almost exactly to 1, indicating proportionality. Such proportionality is found to extend over five orders of magnitude of Sb concentrations. It was hypothesised that Sb uptake by plants would follow passive transport by convection with the stream of water transport into and through plants. Sb accumulated in edible plants may present a health risk to human via the food chain; however, there has been no maximum permissible concentration of Sb in foodstuffs, especially edible vegetables. The danger is when plants can accumulate high concentrations of Sb while still looking healthy.

1.7. Factors controlling bioavailability of As and Sb in soils and their uptake by plants

1.7.1. Arsenic and Sb speciation

Among As species, As(III) is more toxic than As(V) and inorganic species are more toxic than organic species with the order: As(III) > As(V) > MMA > DMA (Baig et al. 2010). Inorganic As species are dominant in plants (Abedin et al. 2002; Smith et al. 2008a) and can be transformed into organic species by methylation with microbial activities, especially in paddy soils (Takamatsu et al. 1982). Various As species have distinct solubilities and hence display different bioavailabilities to plants. The uptake of these four As species by common vegetable species showed a variable trend. For example, the As accumulated in tomatoes followed the trend: As(III) ~ As(V) >> MMA > DMA (Burló et al. 1999). Marin et al. (1992) reported that the As availability to rice followed the trend As(III) > MMA > As(V) > DMA. Similarly, Meharg and Hartley-Whitaker (2002) observed As(III) and MMA are more available to rice plants.

Inorganic species of Sb predominate over its organic species in environmental systems (Wilson et al. 2010). Sb(III) is generally more toxic than Sb(V) (Gebel 1997; He & Yang 1999). Sb speciation greatly influences Sb uptake by plants Feng et al. (2013). For example, rice was more efficient in accumulating Sb(III) than Sb(V), and Sb(V) was the main species in rice tissues (Okkenhaug et al. 2012; Ren et al. 2014). In addition, Shtangeeva et al. (2012) reported that wheat (*Triticum aestivum* L.) exposed to $SbCl_3$ accumulated more Sb in seeds and tissues than that exposed to $SbCl_5$. The opposite was true for rye (*Secale cereal* L.) under the same growing conditions. These reports indicate that the knowledge of As and Sb speciation in soils is important in assessing the As and Sb bioavailability and toxicity to plants.

1.7.2. Redox conditions and Fe, Al, and Mn oxide/hydroxides

Fe, Al, Mn oxides have been largely applied for immobilisation of As and Sb in contaminated soils (Bagherifam et al. 2014; Doherty et al. 2017; Okkenhaug et al. 2016; Warren et al. 2003), in which iron-based amendments were the most effective with up to 90% decrease of As and Sb in soil pore water (Doherty et al. 2017). In addition, Bagherifam et al. (2014) stated that natural metal oxides of Mn, Al, and Fe reduced As bioavailability (82%) and Sb (60%) and significantly decreased plant uptake and bioaccessibility of As and Sb in soils. In the field trials to assess As uptake by vegetables from contaminated soils and soils treated with iron oxides, Warren et al. (2003) reported that application of 0.5% of Fe oxides reduced As bioavailability and its uptake by beetroot, cauliflower, lettuce, potato, radish, and spinach by a mean of 32%. The pot experiment conducted on As uptake by lettuce cultivated in contaminated soils applied with ferric sulfate also observed reduction of As concentration in lettuce by 11% (Warren & Alloway 2003). For paddy soils, As and Sb mainly found in rice roots due to their adsorption by the iron plaque formed on the root surfaces, reducing As and Sb in rice plants (Cai et al. 2016; Okkenhaug et al. 2012). Many studies on paddy soils showed the significant correlations between As fractions and iron fractions, suggesting that iron redox cycling may directly influence As fractionation in soils, which may thereafter indirectly influence As bioavailability and uptake by rice plants (Fu et al. 2011; Liu et al. 2015). Additionally, significantly negative correlations between As in rice grain and As bound to amorphous Fe oxide were found, indicating that amorphous Fe oxide acts as a sink for As.

However, in reducing environments such as flooded paddy soils and waterlogged soil scenarios, arsenite and antimonite were predominant and the solubility of As and Sb increased sharply in soil solution (Hockmann et al. 2014a; Okkenhaug et al. 2012; Takahashi et al. 2004; Xu et al. 2008). The increase in As and Sb availability is due to the reductive dissolution of Fe (hydr)oxides and the reduction of As (V) and Sb(V) to As(III) and Sb(III), respectively, through microbial processes (Gorny et al. 2015; Hockmann et al. 2014a; Hockmann et al. 2014b; Islam et al. 2004; Masscheleyn et al. 1991; Nakamaru & Altansuvd 2014), resulting in more As and Sb available for plant uptake (Wan et al. 2013a; Xu et al. 2008). Flooding contaminated soils greatly enhanced Fe and Mn concentrations in

soil pore water, followed by an increase in As concentration in rice and reduction of rice growth (Hartley et al. 2010). According to Williams et al. (2007), the redox condition is driving force of the high bioaccumulation of As in paddy rice compared to other grain crops.

1.7.3. pH

Soil pH is one of the important parameters controlling the mobility and phytoavailability of As and Sb since it controls As and Sb speciation and retention in soils via adsorption. The specificity of As and Sb adsorption on the surfaces of soil components is influenced by pH, depending on the soil texture and types of mineral constitutes (Rosas-Castor et al. 2014). The difference in mobility of As and Sb is attributed to the effect of pH on surface charge of mineral constitutes and protonation or deprotonation of As and Sb species (as discussed in section 1.4.2.1). A few studies have described the effect of soil pH on As mobility in the presence of plants (Kim Anh et al. 2013; Tu & Ma 2003) who reported the enhanced As uptake by As-hyperaccumulating plants in an acidic environment. In a recent study, Ahmed et al. (2011) found a positive correlation between As in grain and soil pH. The As uptake can be enhanced at higher pH since the rise in soil pH increases negative surface charges on mineral components (as discussed in section 1.4.2.1), facilitating desorption of As from Fe oxides (Marin et al. 1992; Sahoo & Kim 2013), leading to the mobilisation of labile As in the rhizosphere, enhancing As accumulation in plants (Fitz & Wenzel 2002). The effect of soil pH on As and Sb mobility in the absence of plants (laboratory batch studies) could be different from studies where plant species are included since plants are capable of altering the rhizosphere pH by producing organic exudates (as discussed in section 1.5.3). There is very limited literature about the effect of pH on the uptake of metal(loid)s, especially Sb by vegetables.

1.7.4. Organic matter

The effect of organic matter (OM) on As and Sb mobility and their uptake by plants is inconsistent (Sahoo & Kim 2013). OM can control As and Sb mobility in soils since it forms soluble and insoluble complexes with As and Sb (Das et al. 2008; Wang & Mulligan 2006; Williams et al. 2011). An increase in OM can promote the desorption of As from solid phase (Buschmann et al. 2006; Redman et al. 2002; Sahoo & Kim 2013; Turpeinen et

al. 1999), increasing As mobility in soils. This is because it can enhance activities of soil microbes and decrease soil redox (Turpeinen et al. 1999), favouring the reductive dissolution of Fe oxyhydroxides (Harvey et al. 2002). The release of As through the reductive dissolution of oxyhydroxides linked to OM was observed (Selim Reza et al. 2010). In addition, dissolved organic matter (DOM) can increase As mobility since its negative charges have high potential to compete with As for adsorption sites in soils (Lin et al. 2008; Sahoo & Kim 2013). Arsenic solubility in soils was found to be positively associated with OM (Harvey et al. 2002; Huang et al. 2006; Rowland et al. 2006; Turpeinen et al. 1999). This may be the result of the positive correlation between As concentration in rice and soil OM (Bhattacharya et al. 2010a). An increase in DOM can enhance As(III) and As(V) mobility in soils (Dobran & Zagury 2006).

In contrast, OM has a high affinity for As because of the formation of insoluble organo-As complexes and their adsorption onto organic colloids (Cao & Ma 2004; Cao et al. 2003; Das et al. 2008; Sahoo & Kim 2013). Thus, soils with high contents of OM can significantly reduce As mobility (Lund & Fobian 1991), resulting in less As available to plants and accumulation in plants. Rahaman et al. (2011) investigated the effect of OM on the transport of As in rice and observed that the addition of organic amendments considerably reduce As concentration in rice grains, which was also supported by Fu et al. (2011) reporting a negative relationship between rice grain As and soil OM. However, it has been recently claimed that organic amendments may have insignificant effects on the As accumulation in plant tissues compared to unamended soils (McBride et al. 2013). The first study on the effect of biochar amendments on the mobility and bioavailability of As and Sb simultaneously present in contaminated soils conducted by Lomaglio et al. (2016) showed that the addition of biochar increased the solubility of As and Sb together with the increase in soil pore water pH. In addition, the indicator plants, dwarf beans (*Phaseolus vulgaris* L.), accumulated greater As and Sb concentrations in various tissues. This may be explained by an increase in soil pH which induced the mobilisation of humic acids, displacing As and Sb from inorganic and organic binding sites and made them available. Additionally, the alkaline conditions generated by the biochar addition resulted in the desorption of As and Sb from soil particles.

1.7.5. Soil texture

Soil texture also affects metal(loid) bioavailability to crops (Adriano 2001). In general, finer textured soils (clay and silt) have more surface active area and more Fe oxides present than coarse (sand) soils, thus clay soils have a greater metal(loid) adsorption potential compared with sandy soils due to greater sorption sites in clay (Fitz & Wenzel 2002; Heikens et al. 2007; Mahimairaja et al. 2005; McLaughlin et al. 2011). As a result, clay soils have less bioavailable metal(loid)s and less phytotoxicity of metal(loid)s than sandy or loamy soils (McLaughlin et al. 2011; Warren et al. 2003). O'Neill (1995) reported that As concentration in plants is lower in silty clay loam than loamy sand. A field study on potential health risks of As and Sb by Sharifi et al. (2014) showed that higher As and Sb uptake by plants at the site linked to coarse-grained texture (dominant by sand) compared to sites with finer texture (dominant by clay) because of higher bioavailable As and Sb in sandy soils.

1.7.6. Phosphate

Phosphate is also an important factor influencing As solubility in soils and its uptake by plants (Fitz & Wenzel 2002) as previously discussed (section 1.4.2.2). Since phosphate and arsenate are chemical analogues, they compete for the same adsorption sites in soils via ligand exchange mechanisms, resulting in the desorption of As and the increase in As solubility and bioavailability in soils and rhizosphere (Bogdan & Schenk 2009; Smith et al. 2002). Many studies reported that increasing phosphate concentration enhances As mobility in soils (Cao et al. 2003; Signes-Pastor et al. 2007; Smith et al. 2002). Hence, application of phosphate into soils can promote As accumulation in plants, supported by numerous studies using greenhouse experiments (Bogdan & Schenk 2009; Creger & Peryea 1994; Geng et al. 2005; Hartley et al. 2009; Hossain et al. 2009). Nevertheless, there is a contrasting effect in some cases since phosphate has an inhibitory effect competing with arsenate for the same transport channel when crossing the plasma membrane (Meng et al. 2011; Tripathi et al. 2007; Zhao et al. 2009). Some studies have reported that the addition of phosphate in solution decreases As accumulation in plants (Pigna et al. 2010; Rahman & Hasegawa 2011; Tu & Ma 2003; Wang et al. 2002; Wang & Duan 2009). The overall effect of

phosphate on arsenate accumulated into root cells may depend upon the balance between these two mechanisms.

The effect of phosphate on arsenite uptake by plants is negligible (Wang et al. 2002). This insignificant effect may be due to differences in their uptake mechanisms in which arsenite is transported through aquaporins, whereas phosphate and arsenate share the same transporter (Abedin et al. 2002; Meharg & Jardine 2003; Meharg & Macnair 1990). For Sb, the effect of phosphate on Sb mobility in soils and its uptake by plants is relatively unknown. In a study on Sb mobility in Japanese soils, Nakamaru et al. (2006) reported that the distribution coefficients of Sb decreases with increasing phosphate concentration used as an extractant, suggesting that Sb sorption and desorption was influenced by phosphate. However, the specific adsorption mechanism could not be explained since only 20 – 40% of Sb sorbed on soils was extracted by phosphate. In terms of plant uptake, Tschan et al. (2008) reported that the addition of phosphate did not affect the accumulation of antimonate in maize (*Zea mays*) and sunflower (*Helianthus annuus*) and deducted that plants did not use the phosphate uptake route to accumulate antimonate. Thus, further studies are needed to understand these mechanisms.

Apparently, these factors (e.g. pH, redox conditions, Fe and Al oxides, organic matter, phosphate) affecting the adsorption/desorption, precipitation/dissolution or complexation/decomplexation of As and Sb in soils determine the solubility, biovailability, and speciation of As and Sb in soils, which in turn influences their uptake by plants. This provides the background for the explanation of As and Sb biogeochemical behaviour in various soils and plants in this research.

1.7.7. Plant factors

1.7.7.1. Crop species and cultivars

The ability of plants to accumulate As in their tissues varies with crop species and varieties. For example, in a hydroponic study with similar growth conditions, tomatoes accumulated higher As concentrations than bean plants (Carbonell-Barrachina et al. 1997). Moreover, among various rice cultivars, IR 50, Red Minikit, and White Minikit accumulated higher As concentrations in grain (0.24 – 0.31 mg/kg) than Jaya, Nayanmani, Ratna, and Lal Sanna (0.14 – 0.20 mg/kg) (Bhattacharya et al. 2010c). A similar scenario was found for

Sb, demonstrated by a study of Cai et al. (2016) reporting that the japonica rice (cultivars N and Z) exhibited a higher accumulation and stronger translocation tendency of Sb from the root to stem than indica hybrid rice (cultivars F and G). The variation is likely due to genetic and morphological differences (Zhang & Duan 2008), although it may be influenced by other factors such as differences in As concentrations in soils and environmental conditions (Sahoo & Kim 2013). The genotype differences in As accumulation is probably due to differences in root structure, controlling root aeration and the formation of iron plaque on root surfaces (Chaturvedi 2006; Liu et al. 2004) or dissimilarities in As tolerance genes (Dasgupta et al. 2004). The variation of arsenic accumulation by rice amongst genotypes is affected by root structure (Dwivedi et al. 2010; Wu et al. 2011a). Thus, the selection of appropriate cultivars is crucial to limit As concentrations in edible parts of plants for food safety.

1.7.7.2. Duration of crop cultivation

The As accumulation by plants also depends upon the course of crop cultivation. Jedynak et al. (2010) reported that 6-week old mustard plants accumulated about 3 times higher As concentrations in tissues than 3-week old plants. This tendency indicates that As uptake by plants occurs continuously during their lifetime.

The accumulation of As and Sb by various edible vegetable species and different cultivars of the same edible vegetable cultivated in various contaminated soils will be performed in this research to evaluate their behaviour with crop species and plant cultivars. This is important for the appropriate selection of vegetable species and varieties in terms of food safety.

1.8. Conventional methods for estimating bioavailability of As and Sb in soils

As and Sb exist in various soil binding sites in which some forms are highly soluble, labile, and available for plant uptake, affecting the food chain, while others are inert and rarely affect the amount of metal(loid) in the soil solution. Thus, determination of As and Sb bioavailability in soil plays an essential role in the prediction of plant uptake and the assessment of environmetal toxicity problems (Wilson et al. 2010). A number of methods have been applied for assessing of bioavailability of soil As and Sb.

1.8.1. Soil solution

Plants take up nutrients and contaminants mainly from the soil solution (Barber 1995). The measurement of total dissolved elements in soils is considered as a mean of assessing the bioavailability and toxicity of trace elements in soils. Trace elements in the soil solution exist in a variety of chemical forms such as free hydrated ions, complexed with a wide range of naturally occuring organic and inorganic ligands and bound to colloidal material (Hooda 2010). The simple procedures to obtain soil solution are centrifugation (Thibault & Sheppard 1992) and a Rhizon soil solution sampler (Menzie & Guppy 2000; Tiensing et al. 2001). Centrifugation tends to extract a higher amount of solutes in the soil solution when compared to Rhizon samplers (Lorenz et al. 1994; Tiensing et al. 2001). The solution extracted by the Rhizon sampler is from larger pores and the physical structure of soil sample remains intact and is not disturbed. Hence, the Rhizon sampler is considered to better represent the soil pore water extracted by plants than centrifugation (Nolan et al. 2003; Tiensing et al. 2001). The use of soil solutions to predict bioavailability of metals to plants has given mixed results.

1.8.2. Chemical extraction

A variety of chemical extractions have been used as surrogate measurements for predicting bioavailable As and Sb in soils. The underpinning idea is that a chemical extraction procedure is capable of isolating species or forms of elements that represent their mobile pool in soil defined as exchangeable, mobile and plant-available fraction. Ideally, a test should extract only the labile fraction and should not alter the partitioning between the phases. Many attempts have been made to find a water-soluble extractant able to isolate the labile elements in soil to relate this faction to phytoavailability.

Single extraction procedures have been widely used and are still applied due to their simplicity from laboratory-operational aspects. The common extractants can be divided into three categories: organic chelators, mildly acidic solutions, and neutral salts. Chemical extractions have returned varying results in measuring bioavailable As and Sb in soils and predicting their uptake by plants. For Sb, there have been varying degrees of effectiveness in extracting Sb from soil found in decreasing order NH_4NO_3 > EDTA > CH_3COOH > CH_3COONH_4 (He 2007). For As, chemical solutions of $NaHCO_3$, NH_4Cl, NH_4F, NaOH,

HCl, and H_2SO_4 poorly predicted bioavailability of As to edible rape. The efficiency of various single extractants in predicting As bioavailability to plants is less successful when a range of soil types is included (Song et al. 2006). Neither ammonium sulphate nor ammonium phosphate extraction could explain phytotoxicity of arsenate added to 16 soils with various properties (Song et al. 2006). However, in other studies, phosphate solution has been proven to be a good predictor of As and Sb bioavailability in soils (Ettler et al. 2007; Huang et al. 2006).

Sequential extraction procedures (SEP) involve processing a sample of soils with a series of extractants in order to determine the concentrations of trace elements in different fractions Wenzel et al. (2001). Sequential extraction can provide information about geochemical partitioning of trace elements in soils, which can supply the distribution of trace elements in various phases and evaluate their lability, bioavailability, and thus toxicity. There are a number of sequential extraction procedures proposed by many authors, however the methods proposed by Wenzel et al. (2001) have been widely applied for As and Sb fractionation in soils. The associations of As and Sb with various binding phases in soils were described in section 1.4.3.

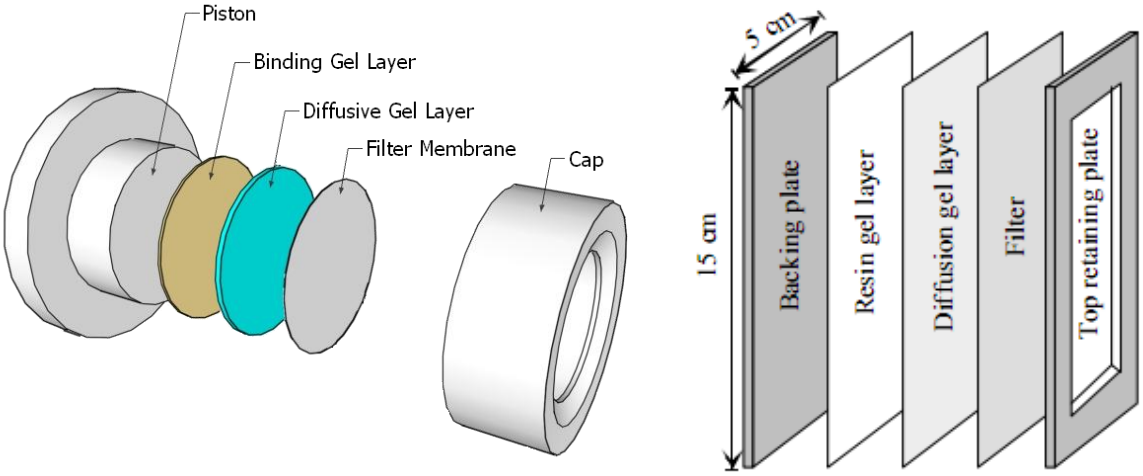
Chemical extraction approaches are commonly used in environmental research; however, most extractants are less specific than desired. The problems of these methods are non-selectivity of extractants and redistribution of elements during extractions (Young et al. 2005). However, the information about the partitioning of metal(loid)s in various soil binding phases can provide useful information on the potential mobility and availability of metal(loid)s under specific environmental conditions (Wilson et al. 2010). In addition, fractionation by sequential extraction can be useful in understanding the chemical reactions of metal(loid)s or their transformation in soils. Information obtained from validated sequential extraction procedures can be useful in making long-term risk assessment and providing supplementary information for remediation decisions (Hooda 2010).

In this research, SEP can provide valuable information on the partitioning of As and Sb in several solid pools, their potential mobility and labile pools, compared to the performance of the *in situ* technique, diffusive gradients in thin films (DGT), in measuring labile and bioavailable As and Sb in soils.

1.9. Diffusive Gradients in Thin Films (DGT) technique

DGT was initially developed for measuring trace metal species in aqueous media (Davison & Zhang 1994; Zhang & Davison 1995), then extended to soil and sediment deployments (Davison et al. 2000; Zhang et al. 1995). The DGT technique is capable of measuring various ionic species (e.g. cationic metals, anionic metalloids, phosphorus, sulphides) depending on the binding agent used. Some typical examples include Chelex 100 resin for trace metals (Cusnir et al. 2014; Dahlqvist et al. 2002; Garmo et al. 2003; Zhang & Davison 1995); Ferrihydrite (FeOOH), Metsorb (TiO_2), and ZrO_2 for As, Sb, Se, V, Mo, P, W (Bennett et al. 2010; Ding et al. 2010; Guan et al. 2015; Luo et al. 2010; Österlund et al. 2010; Panther et al. 2013; Panther et al. 2008; Panther et al. 2010; Price et al. 2013; Sun et al. 2014; Zhang et al. 1998a); silver iodide for sulphides (Teasdale et al. 1999); 3-mercaptopropyl-functionalised silica gel for As(III), Sb(III), and methyl-Hg (Bennett et al. 2016b; Bennett et al. 2011; Clarisse & Hintelmann 2006; Gao et al. 2014); and mixed binding agents (e.g. Chelex-Metsorb, Chelex-Ferrihydrite, Chelex-ZrO_2) for both cations and anions (Huynh et al. 2012; Mason et al. 2005; Panther et al. 2014; Sun et al. 2015; Wang et al. 2017). The DGT technique has been tested in various sample matrices including waters, soils, and sediments. For metal(loid)s, examples of some important applications of DGT are investigation of metal speciation in water (Davison & Zhang 1994; Zhang & Davison 2000), sediment biogeochemical processes (Bennett et al. 2012a; Gao et al. 2009; Stockdale et al. 2009; Wu et al. 2011b), bioavailability in sediments (Amato et al. 2014; Simpson et al. 2012), and bioavailability in soils (Huynh et al. 2010; Nolan et al. 2005; Wang et al. 2014; Zhang et al. 2001).

The DGT assembly consists of a plastic base in the form of a piston and a cylindrical cap that accommodates a resin layer overlaid with a layer of diffusive gel which is covered by a filter membrane to protect it from particles. The cap has a window of known surface area allowing the target elements to contact the filter membrane (Figure 1.4). A layer of hydrogel impregnated with binding resins or powders for target analytes is often used as the binding gel layer and a polyacrylamide gel is usually used as the diffusive gel layer (Zhang & Davison 1995). The principles of DGT application for measurements of solutes in solutions, soils, and sediments are described below.



(a) (b)

Figure 1.4 Cross section of DGT assembly: water and soil sampling piston (a) (taken from Bennett et al. 2010), sediment probe (b) (taken from Harper et al. 1998).

1.9.1. Principles of DGT in waters

The DGT technique is based on a simple device that is capable of accumulating labile chemical species on a binding agent after diffusion across a filter membrane and a hydrogel which serves as a diffusion layer (Figure 1.5). A binding agent, selective to the elements of interest or species of element in solution, is embedded in a thin layer of hydrogel (binding-gel). It is separated from the bulk solution by an ion-permeable gel layer (diffusive gel) and a filter membrane with thickness of Δg (Davison & Zhang 1994; Zhang & Davison 1995).

The diffusive gel layer is separated from the bulk solution by a diffusive boundary layer (DBL) with thickness of δ, where transport of target ions is limited to molecular diffusion. After the device is immersed in solution, a steady state linear concentration gradient is established between the bulk solution and the resin gel. Based on employing this simple steady state condition the DGT technique can be used to determine concentrations of chemical species in situ.

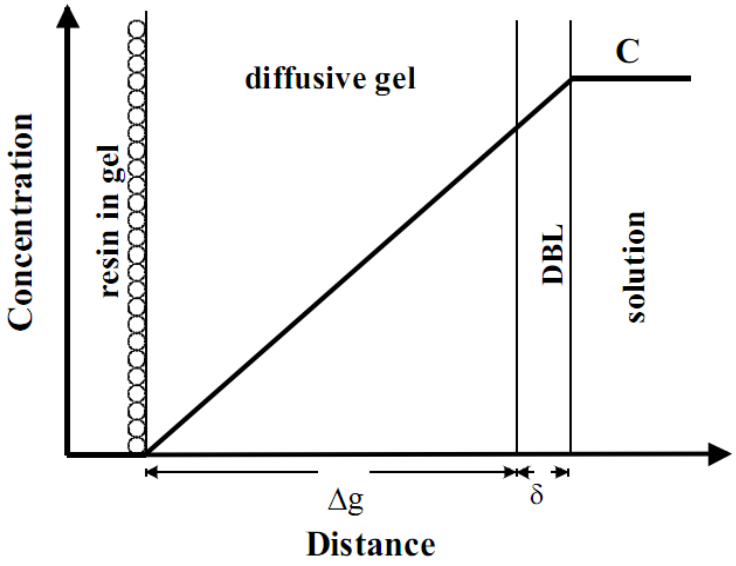


Figure 1.5 Schematic representation of a steady state concentration gradient of an analyte through a DGT assembly, where C: the concentration of analyte; DBL: diffusive boundary layer; Δg: thickness of diffusive gel layer; δ: thickness of diffusive boundary layer (taken from Davison et al. 2000).

Ion diffusion through the gel layer is driven by Fick's first law of diffusion, as shown in Equation 1.1.

$$J = \frac{D(C - C')}{\Delta g + \delta} \qquad \text{Equation 1.1}$$

where J (mol cm^{-2} s^{-1}) is the flux of metal(loid) ions diffusing through the diffusive gel layer to the resin gel; D (cm^{-2} s^{-1}) is the diffusion coefficient of metal(loid) ions in the gel; C is the concentration of a metal(loid) ion in the bulk solution; C' is the concentration of a metal(loid) ion at the interface between the binding gel and the diffusive gel. When the diffused metal(loid) ions are efficiently bound by resin gel, C' is effectively zero, providing that the binding resin is not saturated. In well-stirred solutions, Davison and Zhang (2012) have shown that the boundary layer thickness (δ) can be neglected. Therefore, Equation (1.1) can be simplified to Equation 1.2.

$$J = \frac{DC}{\Delta g} \qquad \text{Equation 1.2}$$

Following deployment, the resin layer is retrieved and the mass of the accumulated ions in this layer is measured. The mass can be measured directly in the binding-gel layer with techniques that are capable of analysing solids such as X-ray Fluorescence (XRF) or Proton-induced X-ray Emission (PIXE). More commonly, metal(loid) ions in the resin layer can be eluted with a known volume of extraction solution (e.g. 1 M HNO_3, 1 M NaOH, 1 M NaOH/1 M H_2O_2) (Bennett et al. 2016a). The extract can then be analysed instrumentally upon appropriate dilution, such as Atomic Absorption Spectrophotometry (AAS), Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) to determine the mass of accumulated metal(loid)s using Equation 1.3.

$$M = \frac{C_e(V_g + V_e)}{f_e} \qquad \text{Equation 1.3}$$

where C_e is measured concentration in the eluent; V_g is the volume of gel in the resin gel layer; V_e is the volume of added extraction solution; and f_e is extraction efficiency of extraction solution for the metal(loid). In practice, V_g is often neglected (Zhang & Davison 1995). The measured mass M can be used to calculate the flux through the known area of the exposed diffusive layer, A (cm^2) after a given deployment time.

$$J = \frac{M}{At} \qquad \text{Equation 1.4}$$

where A is a known exposure area; t is deployment time. Combining and rearranging Equation 1.2 and 1.4 give Equation 1.5.

$$C_{DGT} = \frac{M\Delta g}{DtA} \qquad \text{Equation 1.5}$$

The bulk concentration of metal(loid)s in the original sample (C_{DGT}) can be quantified from the measured mass of metal(loid)s in the resin layer, the thickness of the diffusive layer, ion diffusion coefficient, the time of immersion and exposure area. This feature of DGT makes it ideal for determination of labile chemical species in solution in situ.

1.9.2. Principles of DGT in soils and sediments

The principles for application of DGT in sediments and soils are different from those in solution. The interpretation of DGT measurements is not as easy in sediments and soils as in solutions. The DGT measurements of metal ions in solutions can be interpreted as concentrations because of the well-mixed conditions in solutions, while pore waters of sediments and soils are not well mixed, and so the concentrations of metal ions in the sediments and soil in the vicinity of the DGT assembly may be diminished (Figure 1.6).

Solute ions can be replenished from the solid phase to the solution in the layers of soils near to the device (Zhang et al. 1998b; Zhang et al. 1995; Zhang et al. 2001). If the mechanism of transport for solutes in pore water of sediments and soils is diffusion only, the depletion of metal ions in the pore water adjacent to the device becomes continuously larger with time. Therefore, the flux of solute ions to the DGT device, which is induced by the concentration gradients through the gel layer, continuously decreases with time. Harper et al. (1998) reported that in the case of no resupply of solutes to the pore water, after the 24-hour deployment the interfacial pore water concentration between the soils or sediments and DGT device (C_a) is about 0.06 C_{pw}, where C_{pw} is the labile pore water concentration in the bulk solution. In practice, C_a is often much greater than this value (Zhang et al. 1998b), meaning that there is a significant resupply of solutes. This is because the depletion of solute concentration in pore waters can mobilise solutes from the solid phase.

If the resupply from the solid phase is rapid and unchanged during the deployment time, the principles developed for solutions can be applied for deployments in sediments and soils, using C_a, the interfacial pore water concentration between the DGT device and sediments or soils, instead of the bulk solute concentration. Once the steady state is achieved throughout the deployment, C_a can be calculated from the mass obtained from DGT. Therefore, the concentration measured by DGT, C_{DGT}, is corresponding to the pore water concentration adjacent to the DGT device, C_a, as shown in Equation 1.6.

$$C_{DGT} = C_a = \frac{M\Delta g}{DtA} \qquad \text{Equation 1.6}$$

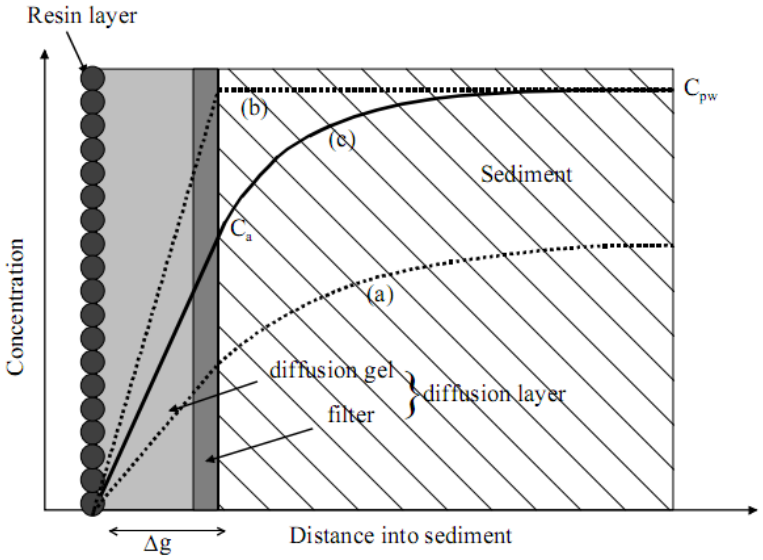


Figure 1.6 Schematic representation of a cross section through a DGT device in contact with sediments or soils. Pseudo steady-state concentration gradients are demonstrated for three cases: (a) unsustained, (b) sustained and (c) partially sustained case. Ca is the interfacial pore water concentration between the soils or sediments and DGT device (taken from Harper et al., 1999).

However, in practice the steady state is not constant during the deployment. C_a will change with the deployment time, and C_{DGT} is a time-averaged value of C_a. If the flux of solute ions to the resin layer at any given deployment time is governed by C_a, C_{DGT} can be rewritten:

$$C_{DGT} = \frac{1}{t}\int_0^t C_a(t_i)dt \qquad \text{Equation 1.7}$$

where $C_a(t_i)$ is C_a defined as a function of deployment time and t is the deployment time. In practice, C_{DGT} will rise rapidly at the beginning time of deployment thanks to the establishment of the steady state and then decrease gradually after several hours due to the depletion of resupply of solutes from the solid phase. The initial increase in C_{DGT} was observed to be negligible after 1-hour deployment (Zhang et al. 1995).

Solid phase resupply

Generally, the DGT depletes pore water concentrations adjacent to the device. Therefore, the mean interfacial concentration will be less than the concentration of labile species in the bulk pore water, C_{pw}. The extent of this underestimation can be interpreted as the ratio, R,

of DGT determined concentration to the pore water concentration measured by other techniques (Harper et al. 1999; Harper et al. 1998).

$$R = \frac{C_{DGT}}{C_{pw}} \qquad \text{Equation 1.8}$$

R varies between 0 and 1. R could reach the highest value if there was an infinite and rapid resupply of solutes from the solid phase. In practice, the rate of resupply is limited by the rate of the release of solutes from the solid phase, leading to an R value < 1. In sediments and soils, the higher the value of R, the faster the rate of the release of solutes, and the larger the capacity for remobilisation of solutes from the solid phase (Harper et al. 1999). Harper et al. (1999; 2000) stated that the response of DGT to deployment in sediments and soils will belong to one of three scenarios described below and demonstrated in Figure 1.6.

(a) Unsustained case ($R = R_{diff}$), where R_{diff} is the ratio obtained when solutes reach the resin layer through diffusion only. There are no resupply of labile species to the pore water and no steady state. This means that there are no metal ions in the exchangeable fraction of the sediments and soils, or the rate of desorption of metal ions from the solid phase is slow. Therefore, the DGT device is replenished only by the diffusion of dissolved species in the pore waters, resulting in continuous depletion. Characteristics of sediments and soils, the design of the DGT device, and the deployment time decide the value of R_{diff}. For a typical sampler deployed in high porosity sediments for 24 hours, $R_{diff} = 0.1$ ($C_{DGT} = 0.1C$) (Harper et al. 1998).

(b) Sustained case ($0.95 < R < 1$). Labile species removed from the solution by the DGT sink are rapidly resupplied from the solid phase. This is due to the fast rates of solute mixing in the sediments and soils, or the large capacity of the release of solutes from the solid phase, and that the rate of resupply from the solid phase is faster than the rate of removal by the DGT assembly. Well-mixed pore water only happens in surficial estuarine and sediments. The concentration measured by DGT can be considered as the concentration of labile metal species in the pore waters.

(c) Partially sustained case ($R_{diff} < R < 0.95$). There is a certain level of resupply of the solutes from the solid phase to the solution because of $R > R_{diff}$. However, it is insufficient

to maintain the initial bulk concentrations of pore water and to prevent some depletion of concentrations of pore water between DGT device and sediments or soils.

Kinetic of metal exchange and release

The response of DGT devices and thus the ratio, R, depends on a variety of factors including sampler design, diffusion coefficients, and the kinetics and capacity of solid phase resupply. If methods and sampler designs are standardised in use, R can be considered as a measurement of the capacity of the solid phase to resupply solutes to the pore water and the kinetics of this process.

Some studies were conducted to interpret the R value. Zhang et al. (1998b) described

$$R = K_d.K_1 \qquad \text{Equation 1.9}$$

where K_d is a distribution coefficient characterising the partitioning of labile metal species between solid phase and pore water and K_1 is the rate constant of the resupply of solutes from solid phase to pore water. Based on the Equation 1.9, the concentration of labile species in solution is fully sustained when the dissociation rate constant (K_1) and K_d are large (proportion of them in solution being high).

Harper et al. (1998; 2000) developed the DIFS (DGT Induced Fluxes in Sediments or soils) model (Figure 1.7), based on a model of the exchange of solutes between the solid phase and pore water to interpret the value of R. The exchange between a dissolved phase (pore water) and a solid phase (sorbed one) is assumed to be a first order reversible process, described by Equation 1.10.

$$C_d \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_s \qquad \text{Equation 1.10}$$

where C_d and C_s are the concentrations of solutes in pore water and in sorbed phase, respectively; k_1 and k_{-1} are the rate constants for sorption and desorption, respectively. At equilibrium, the ratio of sorbed to dissolved concentration is defined as the distribution coefficient, K_d. The relationship between k_1, k_{-1}, and K_d is shown by Equation 1.11, where P_c is the particle concentration.

$$K_d = \frac{C_s}{C_d} = \frac{1}{P_c}\frac{k_1}{k_{-1}} \qquad \text{Equation 1.11}$$

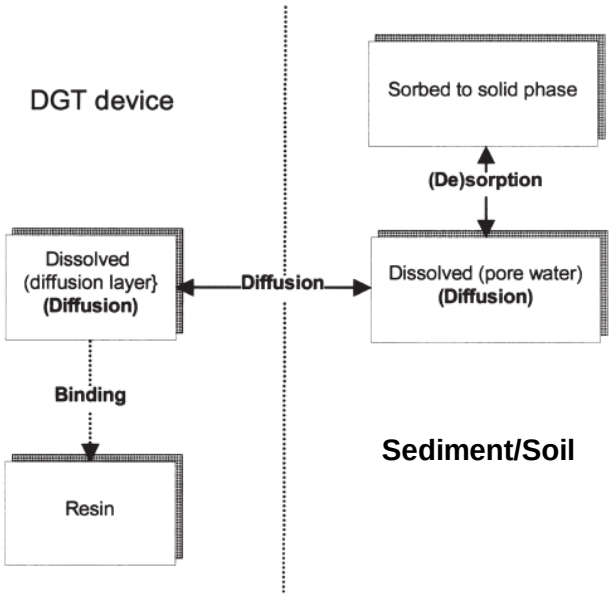


Figure 1.7 Basic concepts of solute transport and reaction in DIFS model (modified from Harper et al. 2000).

When DGT devices are deployed into soils and sediments, pore water concentrations adjacent to the devices become gradually depleted due to the accumulation of solutes onto the binding resin. In response to this perturbation, solutes desorb from the solid phase to replenish the pore water concentrations to re-establish the equilibrium. The response time T_c required to resupply solutes pore water concentrations from the solid phase is referred to the reciprocal of the sum of k_1 and k_{-1} as shown in Equation 1.12.

$$T_c = \frac{1}{k_1 + k_{-1}} \qquad \text{Equation 1.12}$$

The ability of the solid phase to resupply solutes to solution depends on the kinetics of sorption and desorption processes and the available reservoir of solutes sorbed to the solid phase. The DIFS model predicts a relationship between R, K_d, and the response time T_c. The R value is quantitatively related to K_d and the response time T_c of the (de)sorption process. If R and K_d are known, T_c can be obtained from equation 1.13, which is derived from plotting R against T_c for different values of K_d (c and d are parameters of the fitted sigmoidal curve).

$$T_c = c\left(\frac{1-R}{R-d}\right)^2 \qquad \text{Equation 1.13}$$

After the 24-hour deployment the one dimensional model (the domain comprising the horizontal transport) yields the ratio of DGT estimated to initial pore water concentration of 0.06 while the two dimensional approach (the domain comprising the vertical and horizontal diffusion) obtains a ratio of 0.1. If there is a rapid resupply from the solid phase and the depletion of pore water concentrations within 2 mm of the filter, the results from two models are not different (Harper et al. 1998).

1.9.3. DGT-determined effective concentration (C_E) as a predictor of metal bioavailability to plants

When DGT is deployed into a soil or sediment, it removes metals from solution phase by accumulating them on the binding resin. In response to the removal, the concentration of metals in soil solution immediately adjacent to the DGT device is lowered. Local depletion of metals in soil solution induces the release and resupply of labile metals from complexes in soil solution and from the exchangeable metal fraction of the solid phase. Like DGT, plants accumulate metals by removing them from soil, which locally lowers metal concentration in the soil solution, inducing the resupply of metals from labile species in soil solution and the labile metal pool in the solid phase (Zhang & Davison 2006; Zhang et al. 2001). The similarities between metal uptake by DGT and plant uptake under diffusion-controlled conditions have been discussed and modelled (Degryse et al. 2009; Lehto et al. 2006a, 2006b). The soil perturbation is similar if the rate of metal removal by DGT and the plant is similar, enhancing the ability of DGT to mimic plant uptake of metals. Models of metal uptake by DGT and plants showed that fluxes to DGT and plants are generally similar for typical values of diffusive layer thickness used in DGT devices (Lehto et al. 2006a, 2006b). Several studies have shown good correlations between C_{DGT} and concentrations of metals in plant tissues (Degryse et al. 2009; Zhang & Davison 2015). For DGT and plant uptake limited by diffusion, there is diffusional supply of metals from soil solution and additional resupply of labile metals released from the solid phase. This is because C_{DGT}, the time-averaged concentration at the soil and DGT interface, is less than

C_{sol}, dissolved metal concentration in soil solution, which can be confusing as the C_{DGT} has the additional resupply of metals from the solid phase (Lehto 2016). To overcome this conceptual difficulty and to allow further interpretation on DGT measurements, the DGT-measured concentration can be converted to an effective concentration (C_E) which was first introduced by Zhang et al. (2001) to predict the bioavailability of metals to plants. C_E is defined as the ratio of C_{DGT} to R_{diff}, as shown in Equation 1.14.

$$C_E = \frac{C_{DGT}}{R_{diff}} \qquad \text{Equation 1.14}$$

C_E represents the metal concentration which is available and exchangeable from both soil solution and solid phase. R_{diff} is the ratio of the mean interfacial concentration for the diffusion-only case (no resupply from the solid phase) to the concentration in soil solution. R_{diff} may be calculated using the numerical DIFS model (Harper et al. 2000; Harper et al. 1998; Sochaczewski et al. 2007). DIFS is used to simulate the deployment in a diffusion only scenario by accurately specifying the diffusion characteristics of soil, but assuming unsustained case of soil (e.g. very high response time T_c and very low available labile reservoir K_d). Therefore, the effective concentration of C_E can be obtained directly from the DGT measurements.

Input parameters of particle concentration (P_c, g/ml), soil porosity (φ) and diffusion coefficient in soil (D_s) were calculated with Equations 1.15, 1.16, 1.17 (Boudreau 1996).

$$P_c = m/V \qquad \text{Equation 1.15}$$

$$\varphi = d_p/(P_c+d_p) \qquad \text{Equation 1.16}$$

$$D_s = D_o/(1 - \ln(\varphi^2)) \qquad \text{Equation 1.17}$$

Where m is the total mass of soil particles, V is the soil solution volume, d_p is the density of soil particles, and D_o is the diffusion coefficient of each metals in water.

C_E is described as hypothetical constant dissolved concentration at the DGT interface needed to maintain the uptake by DGT device during the deployment, without the supply from the solid phase (Zhang & Davison 2015). The ratio between C_{DGT} and C_E (R_{diff}) depends on soil density, porosity, diffusion coefficient, and deployment time, and is usually

about 0.1 for a 24-hour deployment. C_E is usually around 10 times higher than C_{DGT}. Hence, C_{DGT} is generally very strongly correlated with C_E. In some datasets covering soils with various porosities, there may be less strong correlation between C_{DGT} and C_E due to variation in R_{diff} values. Some studies assessing the correlation between plant response with C_{DGT} and C_E did not observe any advantage in using C_E (Luo et al. 2014; Mason et al. 2010). Since the interpretation of C_{DGT} is easier and does not require additional calculation of R_{diff} by DIFS model simulation, C_{DGT} has still been used in predicting metal uptake by plants (Degryse & Smolders 2016).

1.9.4. Experimental evidence of DGT applications in measuring As and Sb bioavailability in soils to plants

With regard to assessment of the lability and bioavailability of elements, DGT is useful in estimating the labile fraction of elements in soil, and has been successfully employed to measure bioavailable metals to plants (Degryse et al. 2009). The dynamical DGT approach integrates a variety of soil properties influencing metal adsorption/release (Williams et al. 2011; Zhang et al. 2001), and can mimic metal uptake by plants where depletion of metal concentrations adjacent to plant roots induces the release and resupply of metals from the solid phase (Zhang & Davison 2006; Zhang et al. 2001). There is extensive literature relating soil and sediment DGT measurements to metal bioavailability (Zhang & Davison 2015). Most studies have focused on the bioavailability of divalent cationic metals (e.g. Cu, Zn, Cd, Pb, Ni) in soils to plants using standard Chelex-DGT devices, but there are a few studies that have compared DGT measurements with biouptake of As and none was for Sb. The first DGT use for sampling As in soil was conducted by Fitz et al. (2003) who successfully applied Ferrihydrite-DGT to investigate the resupply of labile As pools after phytoextraction and to monitor the efficiency of As phytoremoval. Cattani et al. (2009) assessed the availability of As to roots of As hyperaccumulators and illustrated the sensitivity of Ferrihydrite-DGT in monitoring root-induced changes of As in soils. The DGT technique (Ferrihydrite being the binding resin) was also considered as a sensitive tool for evaluating As mobility changes in rhizophere of two plants with different abilities in accumulating As (Senila et al. 2013) and changes in As availability during aging (Liang et al. 2014). Mojsilovic et al. (2011) applied Ferrihydrite-DGT measurements of As and P

to model wheat (*T. aestivum*) arsenic toxicity using a dataset of soils contaminated with As and reported that the DGT-derived As/P ratio was demonstrated as a promising predictor of As phytotoxicity. DGT was also used to predict As uptake by rice (*Oryza sativa*) collected in the fields (Williams et al. 2011) and edible rape grown in 43 soils (Wang et al. 2014). However, DGT has not previously been used to measure labile Sb in soils and predicting As and Sb uptake by edible vegetables.

Another advanced application of DGT in soil is evaluation of the distribution and dynamics of labile metal(loids) in the rhizophere of plants by high-resolution chemical imaging using localised DGT sampling combined with spatially resolved analysis by laser ablation - inductively coupled plasma - mass spectrometry (LA-ICP-MS), which is extensively reviewed by Santner et al. (2015). Santner et al. (2012) applied for the first time DGT coupled with LA-ICP-MS for chemical imaging of labile phosphorus in the rhizophere of two *Brassica napus* cultivars, advancing the understanding of P dynamics around the plant roots and plant P acquisition. In a recent study applying DGT-planar optode sandwich sensor, Williams et al. (2014) observed that elevated levels of As, Fe(II), and Pb exist at the boundary of the aerobic rhizophere region during the formation of iron plaque on the flooded rice roots. The release of As and Fe(II) was expected to co-occur since As was adsorbed on negatively charged surfaces through Fe(II)-bridges. pH decreases and Fe(II) mobilisation was co-localised, which can be because of slowed Fe(II) oxidation and/or Fe(II) desorption from freshly from iron oxide. These findings provide a new perspective in the mobilisation and accumulation of As in submerged rice.

1.10. Aim and objectives

The aim of this study is to investigate the biogeochemical behaviour of Sb compared to that of As in contaminated soils in relation to their uptake by importantly agricultural plants identified by the diffusive gradients in thin films (DGT) technique in comparison to selective extractions. The specific objectives of this research are:

- To investigate the partitioning of As and Sb in various soil binding phases and the performance of DGT in measuring labile As and Sb in different types of contaminated soils. DGT performance will be compared with conventional extraction techniques including soil solution and sequential extraction.

- To evaluate the performance of the DGT technique in measuring labile As and Sb in historically co-contaminated soils to root vegetables, two cultivars of radish (*Raphanus sativus*) in pot experiments and comparisons between predictions of As and Sb accumulation by radishes based on DGT measurements and current techniques (soil solution, sequential extraction) will be provided.
- To assess the performance of the DGT technique in measuring the lability of As and Sb in historically co-contaminated soils with varying physico-chemical soil properties and recently amended soils and predicting their accumulation by leafy vegetables, water spinach (*Ipomoea aquatica*) in pot experiments, in comparison to selective extractions. Differences in lability and bioaccumulation of As and Sb among soils will be evaluated in relation to the incorporation of bioavailable measurements into soil risk assessments.
- To investigate the fractionation and lability of As and Sb in single and multi-contaminant amended soils and their competitive interactions when they co-exist in soils in relation to their uptake by water spinach (*Ipomoea aquatica*) in pot experiments, identified by sequential extraction and DGT.
- To investigate the mobilisation and speciation of As and Sb in soils induced by redox changes using multiple binding resins in DGT. Changes in As and Sb biouptake by water spinach (*Ipomoea aquatica*) cultivated in contaminated soils subjected to different redox environmental conditions will be evaluated and linked to changes in As and Sb mobility in soils predicted by DGTs. The speciation transformation of As in *I. aquatica* determined by high pressure liquid chromatography - inductively coupled plasma - mass spectrometry (HPLC-ICP-MS) will be investigated and linked to As speciation in soils measured by DGTs.

Chapter 2. General methods

2.1. General washing methods

Deionised water (18.2 MΩ/cm; Milli-Q Academic Water System; Millipore) was used to prepare all solutions. All chemicals were analytical reagent grade or higher. All plasticware and glassware were washed by soaking in 10% (v/v) HNO_3 (BDH, AnalaR) for at least 24 h, followed by multiple rinses with deionised water.

2.2. Test soils

2.2.1. Historically contaminated soils

A soil highly contaminated with As and Sb was collected from a decommissioned antimony processing facility in Urunga, New South Wales (NSW), Australia. A second soil contaminated with only As was collected adjacent to a cattle dip site in Queensland (QLD), Australia. Uncontaminated soils were collected in Wollongong, NSW. All soils were air-dried and then crushed to pass through a 2-mm sieve. To establish an As and Sb concentration gradient of test soils, varying ratios of contaminated and uncontaminated soils were thoroughly mixed with a cement mixer to ensure homogeneity.

2.2.2. Recently contaminated soils

Clean soils were spiked to replicate recently contaminated soils. Each portion of a clean soil was amended with solutions of various concentrations of $Na_2HAsO_4.7H_2O$, $KSb(OH)_6$, and their mixture to achieve three series of soils contaminated with As only, Sb only, and both As and Sb, respectively. Following the addition of As/Sb solutions into soils, the spiked soils were homogeneously mixed and turned over vigorously several times. Moisture content of soils was retained at 60% of maximum water holding capacity (MWHC) after spiking. The artificially contaminated soils were left to equilibrate for eight weeks, during which time it was regularly stirred. After the equilibration period, all soils were air-dried, crushed using a mortar and pestle, and mixed homogeneously for the bioassays. A small proportion of soil samples was further crushed to pass a 2-mm sieve and stored in clean plastic bags for further analyses.

2.3. Test plants

This research investigated a range of plant species to evaluate the response of a variety of edible plant species and cultivars exposed to As and Sb in various contaminated soils under different conditions and the performance of different techniques in predicting As and Sb uptake by plants with different root structures and physiology, which is essential for appropriate soil risk assessments and ensures food safety.

Radish (*Raphanus sativus* L.) is widely grown in Europe, Asia, Africa, South America, and the United States. Radish is the easiest, quickest, and most commercial crop to grow. It grows best at the temperature at ~15 – 20 °C. Radish is cultivated on all types of soils from mud to sandy. Light-textured, fertile sandy loams produce the highest yield and best quality of roots. There are many radish cultivars, in which the two most popular ones with different root structures such as cherry belle and icicle were chosen for our studies. The cheery belle is a globe-shaped and bright red-coloured variety, while the icicle is a long, cylindrical-shaped and clear white-coloured variety. Both of them have short shoots and are mature in ~4 – 5 weeks after emergence (Hartmann et al. 1981).

Water spinach (*Ipomoea aquatica* L.) is a vascular plant and widely grown and distributed in Asia. It is tropical and subtropical plant and grow well at the temperature of >24 °C. *I. aquatica* can be cultivated in land or in semiaquatic systems. *I. aquatica* is easily cultivated with little care and develops fast. It has fibrous roots, hollow stems, and ~15 cm long and 2 cm wide arrowhead shaped leaves. The soft stems and leafy portions are edible and rich in important vitamins (A, C), iron, and nutrients, which is popular for daily food (Göthberg et al. 2002).

The chosen plants have important nutritional values; thus, the knowledge of the plant responses to As and Sb in contaminated soils and useful techniques for predicting As and Sb accumulation by these plants is important for the risk management action to ensure human health and the development of sustainable agriculture.

2.4. Pot experiments

2.4.1. Bioassays with radishes (*Raphanus sativus* L.)

Appropriate amounts of deionized water were added into test soils to have moisture content at ~60% of the water holding capacity (WHC). Nutrients were also added at the rate of 0.1 g N (as urea), 0.2 g P (as KH_2PO_4), and 0.3 g K (as K_2SO_4) kg^{-1} soil to ensure that plant growth was not affected by nutrient deficiencies. Soils were allowed to settle and equilibrate in a greenhouse for two weeks prior to the commencement of bioassays.

Following soil equilibration, prepared soils were added into plastic pots which were lined with high density polyethylene (HDPE) bags. 1.5 and 2.0 kg of soils were added into plastic pots (10 × 15 cm) and poly(vinyl chloride) (PVC) cylindrical pots (25 × 10 cm) for the cultivation of cherry belle radish (chapter 3) and white icicle radish (chapter 4), respectively. Four replicates were performed for each treatment. Seeds were directly sown into soil pots (Day 0), and after emergence, the seedlings were thinned to three per pot. The pots were randomly arranged in the growth chambers set at 14:10 h day:night cycle, 20:16 °C day:night temperatures, 75% relative humidity, and a minimum photon flux of 500 µmol m^{-2} s^{-1}. During the bioassay, pots were weighed daily and deionised water was used to maintain the soil moisture at ~60% WHC. After 35 days, plants were harvested, rinsed thoroughly in deionised water to remove adhering soil. Plants were sectioned into shoots, peel (including tap root), and flesh using a stainless steel blade and vegetable peeler. Wet tissue mass (g) was recoreded.

2.4.2. Bioassays with water spinach (*Ipomoea aquatica* L.)

Appropriate amounts of deionized water were added into test soils (historically contaminated and freshly contaminated soils) to have moisture content at ~60% of the water holding capacity (WHC). Nutrients were also added at the rate of 0.15 g N (as urea), 0.1 g P (as $NH_4H_2PO_4$), and 0.04 g K (as K_2SO_4) kg^{-1} soil to ensure that plant growth was not affected by nutrient deficiencies. Soils were allowed to settle and equilibrate in a greenhouse for two weeks prior to the commencement of bioassays.

Following soil equilibration, 1.5 kg of prepared soils was added into plastic pots (10 × 15 cm) which were lined with high density polyethylene (HDPE) bags. Three replicates were

performed for each treatment. Seeds were directly sown into soil pots (Day 0), and after emergence, the seedlings were thinned to six per pot. The pots were randomly arranged in the growth chambers set at 12:12 h day:night cycle, 30:25 °C day:night temperatures, 75% relative humidity, and a minimum photon flux of 500 µmol m^{-2} s^{-1}. During the bioassay, pots were weighed daily and deionised water was used to maintain the soil moisture at ~60% WHC. After 35 days, plants were harvested, rinsed thoroughly in deionised water to remove adhering soil, and sectioned into roots and shoots using a stainless steel blade. Wet tissue mass (g) was recorded.

2.5. Soil characteristic measurements

2.5.1. Maximum water holding capacity (MWHC)

Maximum water holding capacity was determined by placing 20 – 30 g of air-dried 2-mm-sieved soil in a funnel having a Whatman filter paper. The funnel was held in a container of water. Water was added to saturate the soil and then allowed to stand for 2 h. After that, the funnel was removed from the container and allowed to drain overnight. A blank measurement with only the filter paper was also performed simultaneously using the same procedure to get the weight of the wet filter paper. The maximum water holding capacity of soils was calculated using Equation 2.1.

$$MWHC\ (\%) = (W_{wet\ soil\ +\ wet\ filter\ paper} - W_{wet\ filter\ paper} - W_{dry\ soil}) \times 100\%/W_{dry\ soil}$$

Equation 2.1

where $W_{wet\ soil}$ is the mass of the wet soil and filter paper after drainage, $W_{wet\ filter\ paper}$ is the mass of the wet filter paper, and $W_{dry\ soil}$ is the mass of dry soil.

2.5.2. Moisture content

The measurement of the moisture content of soil was made by the gravimetric method based on quantification of the mass of water lost after drying at 105 °C (Buurman et al. 1996). The method involves weighing a proportion of homogenous sample of wet soil into a known weight crucible, placing the crucible in a drying oven at 105 °C overnight, then allowing it to cool to room temperature in a desiccator and reweighing. The difference between soil wet weight (WW) and soil dry weight (DW) was used to calculate the

percentage moisture content ([WW-DW] × 100/WW) which was then applied to transform values of chemical analyses on wet soil into dry weight.

2.5.3. Soil particle size analysis

Soil particle size distribution was determined by using a laser diffraction particle size analyser (Mastersizer 2000; Malvern Instruments Ltd, Worcestershire, United Kingdom). Soil was added into water and diluted to give an interference of 10 – 20% of signal detection. The percentage of particles was calculated by scattering of 4 mW, 632.8 nm red laser; and 0.3 mW, 470 nm blue laser in a convergent beam setting.

2.5.4. Soil pH

Measurement of soil pH involves weighing 5 g of air-dried 2-mm-sieved soil into a 50 ml centrifuge tube, adding 25 ml of deionised water, shaking for 2 h, and then allowing the tube stand for 1 h. When the suspension settled on the bottom, a pH meter was used to measure pH of the soil in the supernatant (Buurman et al. 1996). Caution was taken to avoid the pH electrode coming in to contact with the settled particles, leading to errors of pH readings. The pH electrode was calibrated using standard buffers of pH 4 and pH 7 before measuring. The electrode was rinsed well with deionised water between measurements and stored in the preservation solution, 3 M KCl, when it was not used.

2.5.5. Organic matter (OM)

Organic matter of soils was determined by 'loss on ignition', the weight loss between 105 °C for drying and 550 °C for ashing samples (Heiri et al. 2001). The procedure involves weighing a proportion of solid sample (approximately 1 g) into an ashing vessel, placing the vessel containing sample in the drying oven at 105 °C overnight. The vessel was then removed from the drying oven, allowed to cool at room temperature, and weighed accurately. The vessel was progressively ashed at 550 °C for 24 h in the muffle furnace, followed by cooling in the dry atmosphere and recording the accurate weight. The percentage of organic matter in soil was calculated by the formula:

$$\%OM = [(W_{105} - W_{550}) \times 100]/W_{105} \qquad \text{Equation 2.2}$$

where W_{105} is mass of soil at 105 °C and W_{550} is mass of soil at 550 °C. The sensitivity of this method for determining organic matter content approximates 0.2-0.5% OM.

2.5.6. Total Kjeldahl nitrogen

Total Kjeldahl nitrogen was determined by the digestion of ~1 g dried soil in 12 mL of concentrated H_2SO_4 with a catalyst mixture of $CuSO_4$, Na_2SO_4, and Se, followed by the distillation of ammonia by addition of an excess of NaOH solution. The ammonia was trapped in a boric acid solution. The formed ammonium was then titrated with sulphuric acid standardised with sodium carbonate solution (Rayment & Lyons 2011).

2.5.7. Extractable phosphorous

Extractable phosphorous (P-Olsen) was determined by the extraction of ~1 g dried soil with 20 mL of 0.5 M sodium bicarbonate solution at a pH of 8.5 for 30 min. The supernatant solution was filtered through a Whatman filter paper. The extracted phosphate was then analysed colourimetrically with ammonium molybdate (Rayment & Lyons 2011).

2.5.8. Amorphous Fe and Al

Amorphous Fe and Al in soils were determined by the extraction of 1 g soil with 50 mL of 0.2 M ammonium oxalate-oxalic acid solution at pH of 3.00 ± 0.05 for 4-h shaking in the dark at room temperature. The supernatant was centrifuged at 2200 × g for 15 minutes, filtered, and acidified to a final concentration of 2% HNO_3 for analyses of Fe and Al using inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Buurman et al. 1996; Rayment & Lyons 2011).

2.5.9. Total free Fe and Al

Total free Fe and Al in soils were determined by the extraction of 1 g soil with 1 g sodium dithionite and 50 mL of sodium citrate solution (22%) for 16-h shaking. The extract was then diluted 2 times with deionised water and 5 drops of superfloc solution (0.2%) was added and shaken vigorously. The supernatant was centrifuged at 2200 × g for 15 minutes, filtered, and acidified to a final concentration of 2% HNO_3 for analyses of Fe and Al using inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Buurman et al. 1996; Rayment & Lyons 2011).

2.6. Soil As and Sb concentrations

2.6.1. Sequential extraction procedure

A five-step sequential extraction procedure developed by Wenzel et al. (2001) was used for determination of As and Sb in various soil binding phases (Table 2.1). After each extraction step, the samples were centrifuged at 2200 × g for 15 minutes, filtered, and acidified to a final concentration of 2% HNO_3 for analyses of As and Sb in different fractions using ICP-MS (section 2.9).

2.6.2. Dissolved As and Sb concentrations in soil solution

Soil solution was extracted from a soil paste after DGT deployment by centrifuging at 2200 × g for 15 min at room temperature. Details of soil to water were as per section 2.7.5. The resulting soil solutions were taken up by syringe, filtered through a 13-mm diameter, 0.45-µm polysulphone filter and then acidified to achieve a final concentration of 2% HNO_3. The concentrations of As and Sb in the filtered soil solution were measured by ICP-MS (section 2.9).

2.6.3. Total soil As and Sb concentrations

Total As and Sb in soil samples were analysed using a microwave assisted aqua regia extraction procedure, as per US EPA method 3051A. Finely ground dried soil (<0.5 g) was combined with 12 mL of a mixture of HNO_3:HCl of 3:1 (v/v) (65% Suprapur®, Merck) in a TFM closed digestion vessel, left overnight, vented to allow gas to escape, then microwave heated (MARS Xpress, CEM). After cooling to room temperature, digests were filtered (<0.45 µm) and diluted to 2% acid with deionised water for analyses by ICP-MS (section 2.9).

Table 2.1 Sequential extraction procedure applied for determination of the partitioning of As and Sb in soils.

Fraction	Target phase	Extractant	Extraction conditions	Soil solution ratio	Wash step
1	Non-specifically sorbed	0.05 M $(NH_4)_2SO_4$	4 h shaking	1:25	
2	Specifically sorbed	0.05 M $(NH_4)H_2PO_4$	16 h shaking	1:25	
3	Bound to amorphous Fe oxides	0.2 M $(NH_4)_2C_2O_4/H_2C_2O_4$, pH 3.25	4 h shaking in the dark	1:25	0.2 M NH_4-oxalate buffer; pH 3.25, (1:12.5), 10 min shaking in the dark
4	Bound to crystalline Fe oxides	0.2 M $(NH_4)_2C_2O_4/H_2C_2O_4$ + 0.1 M $C_6H_8O_6$, pH 3.25	30 minutes in water basin at 96 ± 3 °C in the light	1:25	0.2 M NH_4-oxalate buffer; pH 3.25, (1:12.5), 10 min shaking in the dark
5	Residual	Differences in sum of four fractions and the total concentrations			

2.7. DGT measurements

2.7.1. DGT devices and assembly

DGT piston devices (an exposure window of 3.14 cm^2), sediment probes (24 cm × 4 cm × 0.5 cm, an open window of 1.8 cm × 15 cm), and agarose-based cross-linker were purchased from DGT Research Ltd (UK) (http://www.dgtresearch.com). Ammonium persulphate and tetramethylethylenediamine (TEMED) was purchased from Sigma-Aldrich. All equipment for DGT gel synthesis and handling (e.g. glass plates, plastic spacers, plastic containers, tweezers, chopping board, DGT pistons) was detergent-washed, rinsed with deionised water, and soaked in 10% HNO_3 for 24 h. An additional acid-wash in a second 10% HNO_3 for 24 h was applied to ensure the complete cleaning procedure. For washing of the filter membrane, 5% HNO_3 soaking for 8 h was applied. Gel synthesis and handling, DGT device manipulations were performed in an AURA SD4 Laminar Flow Cabinet

following standard procedures of DGT Research. For assembling DGT pistons (3.14 cm^2 sampling window), a titanium dioxide binding gel disc (0.4 mm thickness) was placed on base, followed by a polyacrylamide diffusive gel disc (0.8 mm thickness), and topped by a 0.45 µm cellulose nitrate filter membrane (~0.1 mm thickness), then clamped with a window (Zhang & Davison 1995; Zhang et al. 1995). For assembling DGT sediment probes, a titanium dioxide/mercapto-silica binding gel trip was placed onto the base plate followed by a diffusive gel trip and polysulphone membrane (0.45 µm pore-size), then covered by the window plate. Before deployment, DGT pistons/probes were conditioned in 0.01 M NaCl and degassed by purging nitrogen gas overnight. A sheet of titanium dioxide/mercapto-silica gel was added to the conditioning solution in order to minimise contamination.

2.7.2. Gel solution

Standard gel stock solution used in the synthesis of binding and diffusive gels was prepared as described by Zhang & Davison (1995), Zhang et al. (1998a) by combining 47.5 mL of deionised with 37.5 mL of 40% acrylamide solution (BDH, Poole). The solution was mixed thoroughly before adding 15 g of an agarose derived cross-linker (2%, DGT Research Ltd, Lancaster, UK). The gel solution was further mixed and stored at 4 ^oC in the refrigerator for 3 months.

2.7.3. Diffusive gel

To make diffusive gels, 10 ml of gel stock solution was transferred to an acid washed tube. A further 70 µL of fresh ammonium persulphate solution (10%) was added and well mixed, followed by an addition of 25 µL of TEMED (*N,N,N'N'*-Tetramethylethylenediamine, 99%). The solution was vigorously stirred for 3 – 4 min. The solution was cast between two acid-washed glass plates separated by a plastic spacer round three edges having an approximate thickness (0.5 mm) and placed in an oven at 42 – 45 °C for 1 h. After the removal of gels from the plates, the gels were hydrated in deionised water and changed water several times for 24 h to achieve a stable thickness. After hydration, the pH of the solution containing the gels should be around 6.5 – 7. Otherwise, more deionised water change was applied to flush out any impurities remained and reduce the pH. The thickness of the gels was ~0.8 mm when the gels were fully hydrated. The diffusive gels were stored

in deionised water until used (Davison et al. 2000; Davison & Zhang 1994; Zhang & Davison 1995).

2.7.4. Binding gels

Titanium dioxide powder (Metsorb) was used as binding agent for As and Sb measurements. 1 g of dry titanium dioxide was added to 10 mL of gel stock solution. The solution was then sonicated for 5 min, and mixed well for 1 min. A further 60 μL of fresh ammonium persulphate solution (10%) and 20 μL of TEMED (99%) was added to this solution and stirred well. Thereafter the gel solution was immediately cast between two acid-washed glass plates separated by a plastic spacer of appropriate thickness (0.25 mm). The gels were set at 45 °C in an oven for 30 min, then removed from the glass plates, hydrated in deionised water with 3-4 times changing water during 24 h. The hydrated gels were then stored in deionised water at 4 ^{o}C in the refrigerator until used (Bennett et al. 2010).

3-mercaptopropyl-functionalised silica gel was used as binding agent for As(III) and Sb(III) measurements. To prepare mercapto-silica binding layers, bisacrylamide-cross-linked polyacrylamide was replaced the standard agarose-cross-linked polyacrylamide since it leaded to homogeneous distribution of mercapto-silica. In brief, 1 g of dry mercapto-silica was added to 10 mL of bisacrylamide-cross-linked polyacrylamide gel stock solution. 200 μL of ammonium persulphate solution (10%) and 8 μL of TEMED (99%) was added. The gel mixture was well stirred and cast between two acid-washed Perspex plates using the 0.5 mm thickness spacer. The binding gels have the thickness of 0.4 mm because the gels shrink slightly after hydration (Bennett et al. 2011).

2.7.5. Procedure for deploying DGT in soils

Blank DGT pistons/probes were treated in the same way as the sample devices.

Preparation of soil samples

Subsamples of air-dried and 2-mm sieved soils (~80 g) were wetted to 100% maximum water holding capacity by adding the appropriate amount of deionised water. The soil and water were well mixed to have a smooth paste using an acid-washed plastic spatula. The paste was incubated in HDPE pots for 24 h at 20 ± 1 ºC. During the equilibration and

deployment, lids were loosely placed on pots to avoid evaporation and to allow air-flow into pots.

Deployment of DGT pistons in soils

DGT devices were carefully placed on the soil paste with gentle pressure to ensure complete contact between the filter membrane of the device and the soil surface. Each soil sample was performed in three replicates. DGT assemblies were deployed for 24 h at 20 ± 1 ºC. On retrieval, DGT devices were washed with deionised water to remove soil particles and then disassembled. The resin gel discs were removed from the DGT devices and placed in the clean vials. For DGT sediment probes, binding gel strips were sliced to the desired resolution using Teflon®-coated razor blades.

To elute elements, titanium dioxide and mercapto-silica gel disks/slices were immersed in 1 mL of solution with a final concentration of 1 M NaOH : 1 M H_2O_2 for 24 h (Bennett et al. 2016b; Panther et al. 2013). The concentration of elements in the eluent was measured by ICP-MS (section 2.9).

2.7.6. Calculation of DGT measured concentrations

The mass of elements in the resin gel (M) can be calculated using Equation 2.3.

$$M = C(V_{gel} + V_{eluent})/f_e \qquad \text{Equation 2.3}$$

where C is the concentration of elements in eluent, measured by ICP-MS, in (μg/L), V_{eluent} is the volume of eluents used for elution of elements accumulated on gels, V_{gel} is the volume of resin gel, and f_e is the elution factor for each analyte. The time averaged concentration of elements at the interface of the soil and the DGT device can be obtained using Equation 2.4.

$$C_{DGT} = M\Delta g / (DAt) \qquad \text{Equation 2.4}$$

where Δg is the thickness of the diffusive gel (0.8 mm) plus the thickness of filter membrane (0.1 mm), D is the diffusion coefficient of elements in the diffusive gel (4.97×10^{-6} cm^2s^{-1} for As and 5.42×10^{-6} cm^2s^{-1} for Sb at 20 °C), t is the deployment time (s), and A is the exposed area of the gel disc (A = 3.14 cm^2).

2.8. Plant analysis

2.8.1. Pre-digestion

After harvesting, plants were rinsed with tap water and then rinsed with deionised water thoroughly to remove soil particles, and excess of water was eliminated with tissue paper. Plant characteristics such as shoot length, root length, fresh weights were recorded. Plants were divided into shoots and roots with a stainless steel blade and dried in an oven at 70 ºC for 48 h (Agbenin & Welp 2012; Álvarez-Ayuso et al. 2012; Niazi et al. 2011; Soriano-Disla et al. 2010; Wang et al. 2014). Weights of dried shoots and roots were recorded. The oven-dried plant tissues were ground with a mortar and pestle and stored in clean plastic zip-bags for analyses of total As and Sb concentrations in plants. For analysis of concentrations of As and Sb species in plants, the washed plants were freeze dried for 48 h at –50 °C and subsequently ground to fine powder under liquid nitrogen.

2.8.2. Plant digestion

Finely ground plant sample (<0.5 g) was combined with 12 mL of a mixture of HNO_3:H_2O_2 1:2 (v/v) (65 and 30%, respectively, Suprapur®, Merck) in a TFM closed digestion vessel, left overnight, vented to allow gas to escape, and then microwave heated (MARS Xpress, CEM). After cooling to room temperature, digests were filtered (<0.45 µm) and diluted to 2% acid with deionised water for analyses by ICP-MS (section 2.9).

2.9. Analytical method

Total As and Sb analyses were performed using an Octopole Reaction Cell Inductively Coupled Plasma-Mass Spectrometer (ORC ICP-MS; Agilent 7500ce) by measuring the signal at m/z 75 for As and m/z 121 for Sb. Rh and In (final concentrations of 10 µg/L) were used as internal standards to account for instrument drift. Quality control standards prepared at 25 µg/L were analysed every 15 samples.

For quality control purposes, reagent blanks, duplicate analyses, certified reference materials, and matrix spike recovery were used. A soil certified reference material (Montana II soil, SRM 2711a; NIST) returned recoveries of 80-118% for the studied analytes. A plant certified reference material (Tomato leaves, SRM 1573a; NIST) had typical recoveries of 80-120% of the studied analytes.

2.10. Data analysis

The statistical testing and analysis and the correlations were carried out using SPSS 21 (IBM Corp.). All statistical tests were performed at the $\alpha = 0.05$ level, unless otherwise stated. Statistical significant differences in As and Sb labile fractions, the kinetic resupply of As and Sb in soils, As and Sb concentrations in plant tissues between treatments were identified using analysis of variance (ANOVA) with post hoc Tukey HSD comparisons where needed. Independent-sample T-test was used to evaluate any significant differences between As/Sb concentrations in roots and shoots, As/Sb bioaccumulation in two plant cultivars, As/Sb availability in individual and co-contaminated soils. Correlation analysis was performed to investigate the relationships between As and Sb labile fractions measured by different techniques and their concentrations in different plant tissues.

Chapter 3. Assessing the uptake of arsenic and antimony from contaminated soil by radish (*Raphanus sativus*) using DGT and selective extractions

This chapter has been published.

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3.1. Introduction

Arsenic and antimony are highly toxic elements, producing mutagenic and carcinogenic effects in humans (Mandal & Suzuki 2002; Winship 1987), and are considered priority pollutants by the United States Environmental Protection Agency (USEPA 1979). A major route of exposure is consumption of crops grown in contaminated soils. While background concentrations of arsenic and antimony in soils are low (<10 mg/kg) (Wilson et al. 2010), contamination can result from activities such as mining, waste disposal, incineration, fuel combustion, smelting, and shooting activities (He et al. 2012; Hockmann et al. 2015; Telford et al. 2009; Wilson et al. 2010), with mining operations making the greatest contribution (Takahashi et al. 2010). These activities can increase environmental concentrations by orders of magnitude (Wilson et al. 2010), with mining-affected soils previously reported to contain up to 17,400 mg As/kg and 16,389 mg Sb/kg (Gál et al. 2007; Li et al. 2014). A major concern is the disposal of waste material from mine sites, which has resulted in contamination of agricultural soils (Hammel et al. 2000), especially when the environmental conditions favour mobilisation of these contaminants. The biogeochemical behaviour of As and Sb is strongly dependent on chemical speciation and parameters like soil pH and redox potential, which can affect important retention mechanisms such as adsorption and precipitation (Wilson et al. 2010; Wilson et al. 2014).

The biogeochemical behaviour of As has been investigated widely, whilst the understanding of Sb biogeochemistry and bioavailability is comparatively limited (Filella et al. 2009; Wilson et al. 2014). Because they are both Group 15 metalloids and exist as oxyanion species with predominant oxidation states of +3 and +5, researchers have anticipated that As and Sb will behave in a similar manner (Wilson et al. 2010). Various speciation studies on As have identified its soil mobility and bioavailability, utilising procedures that are classic (soil water extracts/soil solution), operational (sequential extractions) and functional (plant bioassay) (Bacon & Davidson 2008; Meharg & Hartley-Whitaker 2002; Vithanage et al. 2012; Wenzel et al. 2001). A sequential extraction procedure developed by Wenzel et al. (2001) has been particularly useful in identifying the mobile fraction of As in soils and predicting As phytoavailability in long-term contaminated soils (Niazi et al. 2011), but few such studies have been done for Sb

(Okkenhaug et al. 2011). Recent studies by Hockmann et al. (2014a; 2015) have focused on the mobility of Sb under differing redox conditions in the laboratory and field, however, there is a general lack of information on the assessment of Sb soil pools and phytoavailability in soils. For this reason the As fractionation scheme was applied for Sb (Ettler et al. 2010; Okkenhaug et al. 2011). In terms of Sb uptake by plants, most studies to date have determined Sb accumulation for phytostabilisation and phytoremediation in field collected plants (Álvarez-Ayuso et al. 2013; Casado et al. 2007; Murciego et al. 2007) and preliminarily assessed Sb uptake by edible plants grown in field or greenhouse trials (He 2007; Tschan et al. 2009a; Wilson et al. 2014).

The diffusive gradients in thin films (DGT) technique is an *in situ*, passive sampling method that has been successfully used in soils to measure phytoavailable metals (Degryse et al. 2009). The DGT approach incorporates a variety of soil properties influencing metal adsorption/desorption (Williams et al. 2011), and can mimic metal uptake by plant roots where lower metal concentrations in the vicinity of plant root zones leads to the release and resupply of metals from the solid phase (Zhang et al. 2001). The first DGT application in soil for As sampling was conducted by Fitz et al. (2003) who successfully used DGT to monitor the efficiency of As phytoremoval and the resupply of labile As pools after phytoextraction. The suitability of DGT to monitor root-induced changes in soils was successful for available soil-As to roots in hyperaccumulator plants (Cattani et al. 2009); changes in the root-soil interface of two plants with differing As accumulation abilities (Senila et al. 2013); changes in As bioavailability during aging (Liang et al. 2014); and to predict As uptake by edible rape grown in 43 soils (Wang et al. 2014). However, DGT has not previously been used to measure labile Sb in soil, perhaps as the method validation has only recently been described (Panther et al. 2013).

The aims of this study were to investigate the lability of As and Sb in a contaminated soil serially diluted to produce a contamination gradient. Lability was measured using both chemical extractions and DGT techniques, and these were compared with As and Sb bioaccumulation in radish (*Raphanus sativus*), a common edible vegetable species worldwide. Importantly, this study assessed the ability of chemical extractions and DGT measurements to predict the bioavailability of As and Sb to *R. sativus*, which is critical for

future research on the risk of As and Sb phytoaccumulation by plants grown in contaminated soils.

3.2. Methods

3.2.1. General experimental

General washing methods were done as per section 2.1.

3.2.2. Experimental design

3.2.2.1. Experimental soils

Soil highly contaminated with As (4800 mg/kg) and Sb (7900 mg/kg) was collected from a decommissioned antimony processing facility in Urunga NSW, Australia. This was a sandy soil and had low organic matter (0.95%) and pH (3.2). The soil also contained a range of other metals including Cd, Cu, Zn, Cr, Co, Ni, and V at 0.003, 18, 28, 28.4, 2.2, 3.2, and 29.1 mg/kg, respectively, which are all below the reported global background soil concentrations (Kabata-Pendias 2011). This indicates that these metals are not main contaminants and their effects on As and Sb behaviour were negligible. The uncontaminated diluent sandy soil with higher organic matter (8%) and pH (6.6) was collected in Wollongong, NSW. All soils were air-dried and then crushed to pass through a 2-mm sieve. The historically contaminated soil was thoroughly mixed with the uncontaminated soil at various ratios from 1:200 to 1:10 to establish an As and Sb concentration gradient of 12 test soils (S1 - S12). The As and Sb concentrations were estimated to be in the concentration ranges of previous studies on As and Sb accumulation by plants in pot experiments (Niazi et al. 2011; Tschan et al. 2009a).

3.2.2.2. Pot experiment

Pot experiments were conducted with the cherry belle radish (*Raphanus sativus*) in controlled growth chambers as per section 2.4.1. Harvested plant tissues were oven-dried at 70 °C for 48 h, and dry mass recorded (g). Dried tissues were ground with a mortar and pestle and stored in clean plastic bags prior to As and Sb analyses.

3.2.3. Soil characteristics

Soil particle size, pH, total Kjeldahl nitrogen, and available phosphorous was determined as per section 2.5.

3.2.4. Soil and plant analyses

Finely ground soil (~0.4 g) was digested with aqua regia in a closed microwave digestion system (MARS Xpress, CEM) at 175 °C (ramp time 15 min, 5 min holding time). Finely ground plant (~0.3 g) was microwave digested with a mixture of HNO_3:H_2O_2 (1:2, v/v) (65% and 30%, respectively) at 180 °C (ramp and holding time 15 min each). After cooling to room temperature, digests were filtered (<0.45 µm) and diluted to 2% acid with deionised water for analyses. Total As and Sb was analysed by inductively coupled plasma-mass spectrometry (ICP-MS; Agilent 7500ce) by measuring the signal at m/z 75 for As and m/z 121 for Sb as per section 2.9.

For each analytical batch, reagent blanks, standard reference materials, spike recovery, and duplicate analyses were used as quality control measures. Soil standard reference material (Montana II soil, SRM 2711a; NIST) returned recoveries of As and Sb within the ranges of 101-105% and 80-90%, respectively. Plant standard reference material (Tomato leaves, SRM 1573a; NIST) were within 110-120% and 80-86% of certified values for As and Sb, respectively.

3.2.5. Sequential extraction procedure

The chemical fractionation (soil binding phases) of both As and Sb in the test soils was determined as per Wenzel et al. (2001), due to the expected geochemical similarities of As and Sb (Tighe & Lockwood 2007). A soil:solution ratio of 1:25 was used for all extractions and prepared as per section 2.6.1.

3.2.6. DGT and soil solution measurements

DGT and soil solution measurements were conducted as per sections 2.6.2 and 2.7.

3.2.7. Data analysis

The basic statistical analysis and the correlations were carried out using SPSS Ver. 21 (IBM Corp., Armonk, New York, USA). Statistically significant differences between As

and Sb in tissue dry masses were analysed using analysis of variance (ANOVA) at a significance level of $p<0.05$. Correlation analysis investigated the relationship between As and Sb labile fractions measured by various techniques and their concentrations in different tissue compartments. All results are presented as mean ± standard error (SE) (dry mass), unless otherwise stated. To evaluate the bioavailability of As and Sb for *R. sativus*, the bioaccumulation factor (BAF) was calculated by the ratio of the total As/Sb concentrations in roots as mg/kg dry weight (DW) and total As/Sb in soils (mg/kg DW) (Sharifi et al. 2014; Vamerali et al. 2010). To compare the distribution of As and Sb in *R. sativus* roots and shoots, the translocation factor (TF) was calculated by the ratio of the total As/Sb in shoots (mg/kg DW) and total As/Sb in roots (mg/kg DW) (Vamerali et al. 2010).

3.3. Results and discussion

3.3.1. Soil characteristics

Soil properties affect the solid phase speciation and mobility of As and Sb (Wilson et al. 2010). In this study 12 test soils were prepared by mixing a soil contaminated with both As and Sb with an uncontaminated soil having similar texture, and the physical and chemical properties of soils were analysed prior to use in pot experiments. The test soils properties were very similar for each of the 12 dilutions, as demonstrated by the low standard deviation in the following measurements: silty sand (73.0 ± 0.4% sand, 25.0 ± 0.3% silt and 2.00 ± 0.04% clay); slightly acidic (pH 6.50 ± 0.03); 7.11 ± 0.08% organic matter content; and mean extractable phosphorous and total nitrogen of 74 ± 8 and 102 ± 3 mg/kg, respectively. Thus, it is likely that the differences in As and Sb behavior were due to the differences in their concentrations. The total As and Sb concentrations in 12 bioassay soils ranged from 13.3 - 400 mg As/kg and 11.8 - 720 mg Sb/kg (the actual concentrations of each test soil presented in Table 3.1). These values were above the average background total As and Sb soil concentrations (<10 mg/kg, Wilson et al. 2010) and in the wide range of total soil As and Sb found in different areas around the world: 7.4 – 17,400 mg As/kg and 3.12 – 16,389 mg Sb/kg in mining, smelting, and industrial regions as well as soils surrounding these areas of Spain, China, Scotland, and Czech Republic (Álvarez-Ayuso et al. 2013; Casado et al. 2007; Ettler et al. 2010; Gál et al. 2007; Li et al. 2014; Okkenhaug et al. 2011).

Table 3.1 Total and labile concentrations measured by SEP, DGT, soil solution in a concentration gradient obtained from historically contaminated soils, and R value of As and Sb (mean ± SE, n = 3).

Soil	As					Sb				
	$Total_{As}$ (mg/kg)	$C_{SEP\text{-}labile\ As}$ (mg/kg)	$C_{DGT\text{-}As}$ (µg/L)	$C_{sol\text{-}As}$ (µg/L)	Ratio R_{As} ($R = C_{DGT}/C_{sol}$)	$Total_{Sb}$ (mg/kg)	$C_{SEP\text{-}labile\ Sb}$ (mg/kg)	$C_{DGT\text{-}Sb}$ (µg/L)	$C_{sol\text{-}Sb}$ (µg/L)	Ratio R_{Sb} ($R = C_{DGT}/C_{sol}$)
S1	13.3 ± 0.3	0.92 ± 0.01	1.9 ± 0.4	6.6 ± 0.7	0.29 ± 0.07	11.8 ± 0.6	0.140 ± 0.002	1.9 ± 0.1	10.3 ± 0.2	0.19 ± 0.01
S2	25 ± 3	1.51 ± 0.04	2.6 ± 0.6	6.8 ± 0.5	0.4 ± 0.1	26 ± 2	0.295 ± 0.005	2.5 ± 0.3	19 ± 1	0.14 ± 0.02
S3	30 ± 1	1.86 ± 0.09	2.7 ± 0.4	7.7 ± 0.2	0.35 ± 0.05	39 ± 1	0.41 ± 0.02	4.0 ± 0.3	28 ± 3	0.14 ± 0.02
S4	40 ± 2	2.51 ± 0.06	3.9 ± 0.2	8.4 ± 0.6	0.47 ± 0.04	56 ± 5	0.591 ± 0.008	5.1 ± 0.2	35 ± 3	0.15 ± 0.01
S5	49 ± 1	2.96 ± 0.06	4.4 ± 0.9	9.8 ± 0.4	0.4 ± 0.1	70 ± 2	0.727 ± 0.009	6.4 ± 0.3	43 ± 3	0.15 ± 0.01
S6	51 ± 1	3.31 ± 0.06	4.1 ± 0.3	10.9 ± 0.6	0.38 ± 0.04	80 ± 4	0.83 ± 0.01	6.9 ± 0.1	52 ± 5	0.13 ± 0.01
S7	77 ± 3	3.97 ± 0.01	5.7 ± 0.8	11.5 ± 0.6	0.49 ± 0.08	110 ± 10	0.853 ± 0.008	8.5 ± 0.4	57 ± 5	0.15 ± 0.01
S8	79 ± 3	4.23 ± 0.07	4.5 ± 0.5	14.1 ± 0.5	0.32 ± 0.03	127 ± 6	0.96 ± 0.03	8.7 ± 0.2	65 ± 4	0.13 ± 0.01
S9	90 ± 2	5.21 ± 0.09	4.40 ± 0.05	15.0 ± 0.6	0.29 ± 0.01	149 ± 3	1.34 ± 0.01	10.7 ± 0.1	84 ± 5	0.13 ± 0.01
S10	180 ± 10	8.07 ± 0.07	11 ± 2	22 ± 1	0.52 ± 0.07	280 ± 10	2.14 ± 0.04	17.7 ± 0.6	128 ± 3	0.138 ± 0.006
S11	210 ± 20	9.8 ± 0.1	13.8 ± 0.7	28.1 ± 0.7	0.49 ± 0.03	370 ± 30	2.60 ± 0.05	22.5 ± 0.4	167 ± 7	0.135 ± 0.006
S12	400 ± 20	18.5 ± 0.4	30 ± 4	67.1 ± 0.7	0.45 ± 0.06	720 ± 30	4.8 ± 0.1	46.7 ± 0.9	350 ± 1	0.133 ± 0.003

3.3.2. Labile As and Sb in soils assessed by sequential extraction

The As and Sb associated with the different soil binding phases are shown in Figures 3.1. The binding strength of As and Sb to soil increased with the strength of sequential extraction solutions, and for the test soils the extractable As and Sb in soils increased with the increasing total soil As and Sb concentrations (Table A.1). Although As and Sb have been assumed to have similarities in geochemical behaviour (Ettler et al. 2010), clear differences in association with soil binding phases were observed.

For As, the non-specifically sorbed (SO_4^{2-} extractable) As in the bioassay soils increased from 0.048 ± 0.004 (S1) to 1.49 ± 0.05 (S12) mg/kg, and only constituted 0.31 ± 0.01% of the total soil As. The specifically sorbed (PO_4^{3-}-extractable) As in soils varied from 0.87 ± 0.01 (S1) to 17.0 ± 0.4 (S12) mg/kg, which was 5.4 ± 0.2% of the total As. Arsenic was primarily associated with amorphous, crystalline Fe and Al oxides, and residual fractions, each represented 31-33% of the total soil As (Figure 3.1a).

For Sb, the non-specifically sorbed fraction in the bioassay soils varied between 0.056 ± 0.001 (S1) and 1.75 ± 0.01 (S12) mg/kg, representing 0.33 ± 0.02% of the total soil Sb. The specifically sorbed Sb ranged from 0.084 ± 0.001 (S1) to 3.1 ± 0.1 (S12) mg/kg, constituting 0.60 ± 0.03% of the total Sb. The Sb associated with amorphous and crystalline Fe and Al oxides was 11.3 ± 0.8 and 15 ± 1%, respectively. Antimony was mainly present in the residual soil fractions accounting for 73 ± 2% of the total Sb (Figure 3.1b). This fraction is strongly associated with the soil minerals and is generally geochemically immobile (Filella et al. 2009; Okkenhaug et al. 2011). Antimony in this fraction is likely to exist as the antimony sulphide mineral, stibnite (Álvarez-Ayuso et al. 2013).

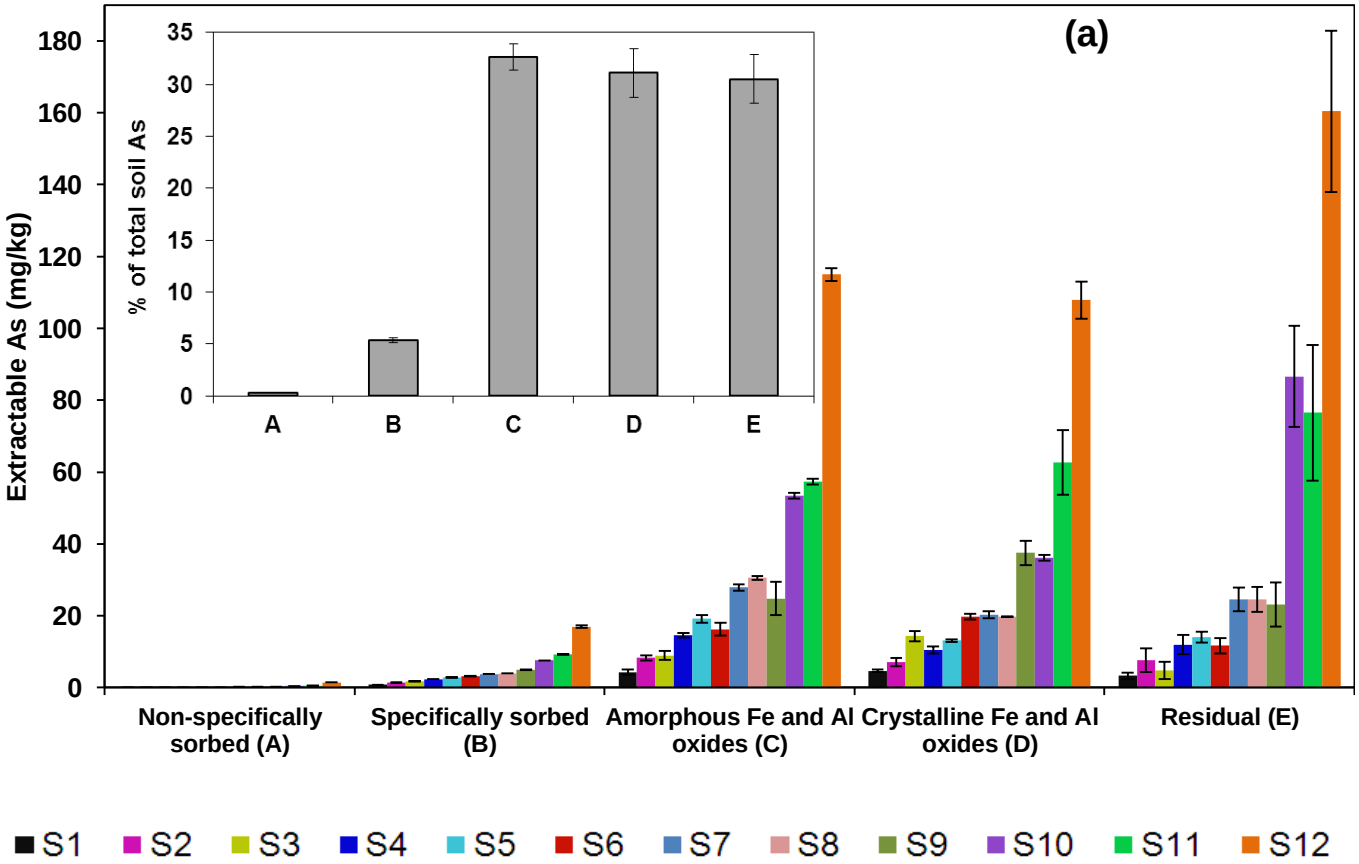

Figure 3.1 (a) As associated with different binding fractions for bioassay soils (mg/kg, dry mass, mean ± SE, n = 3). Soils are plotted from left to right as S1 to S12 for each binding phase. Inset graphs depict the As and Sb associated with each fraction as a percentage of the total soil concentration (mean ± SE, n = 36).

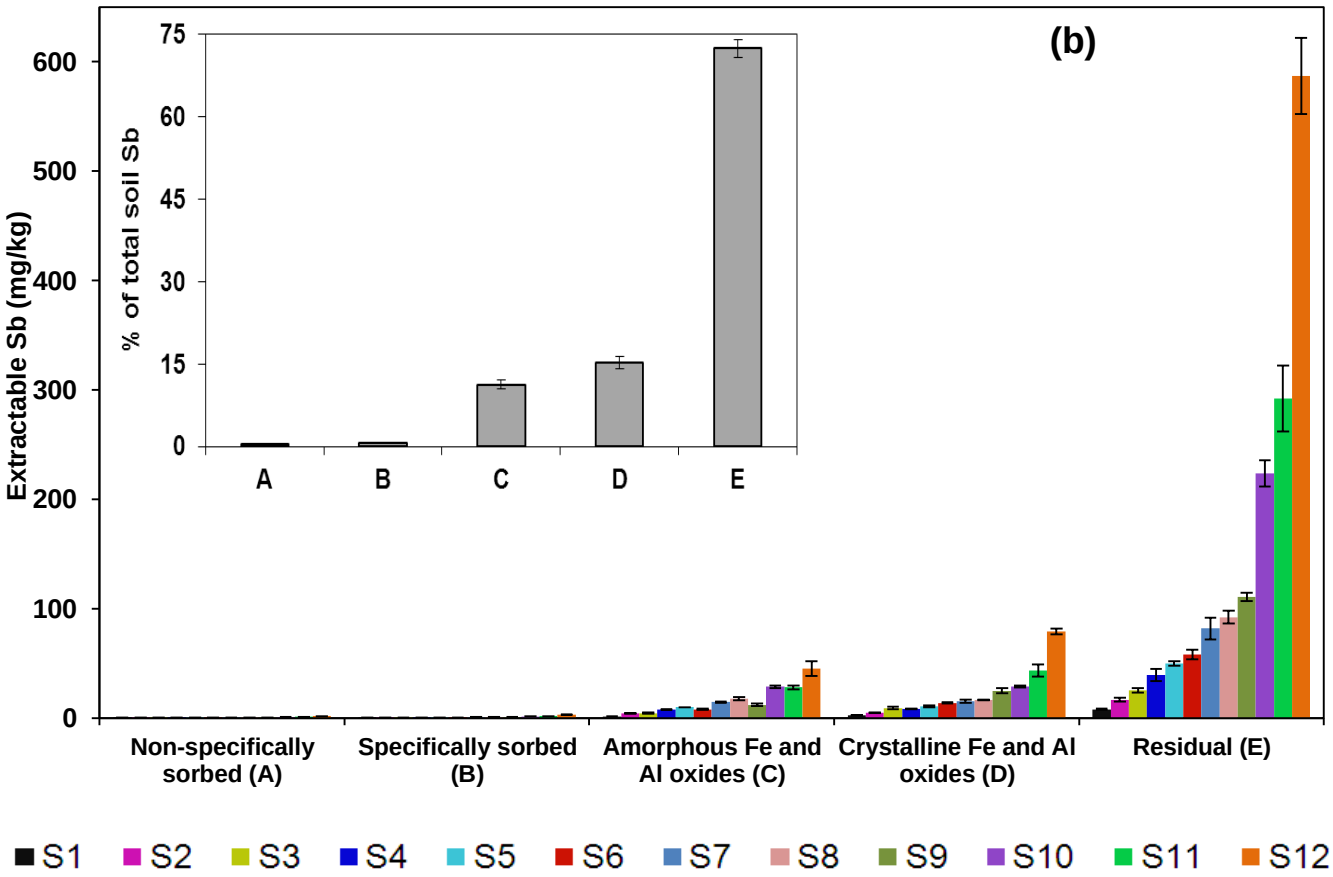

Figure 3.1 (b) Sb associated with different binding fractions for bioassay soils (mg/kg, dry mass, mean ± SE, n = 3). Soils are plotted from left to right as S1 to S12 for each binding phase. Inset graphs depict the As and Sb associated with each fraction as a percentage of the total soil concentration (mean ± SE, n = 36).

The soil binding phases that represent the bioavailable fractions are defined as the combination of the non-specifically sorbed and specifically sorbed fractions (Wenzel et al. 2001). Based on this, Sb was much less bioavailable (at 0.93 ± 0.04%) than As (5.7 ± 0.2%). The limited research comparing the As and Sb mobility in contaminated soils has also shown generally higher extractable/available As compared to Sb. For example, Ettler et al. (2007) showed that the extractability of Sb was systematically lower than As (0.1 M Na_2HPO_4 yielded 9% and up to 34% of Sb and As, respectively), and Müller et al. (2007) reported 10% and up to 15% of Sb and As, respectively, in the mobile fraction (0.05 M Na_2HPO_4). However, in soils from an historical mining site in Scotland, Gál et al. (2007) found higher Sb mobility (0.01-8.8% of the total Sb) than As (0.01-0.6% of the total As) in the exchangeable binding phase (with 1 M NH_4NO_3).

Binding to Fe and Al oxides was significantly lower for Sb (11-15%) than for As (31-33%), a trend which is supported by other studies (Ettler et al. 2010; Müller et al. 2007). This may be because arsenic has also been shown to outcompete Sb for soil sorption sites since As has a higher affinity for the Fe oxides than Sb (Casiot et al. 2007). The literature about the effect of redox state on the interaction of As and Sb with iron oxides also support the result. Under oxic conditions both As and Sb are present as As(V) and Sb(V) (Wilson et al. 2010), and As(V) interacts more strongly with Fe and Mn oxides than As(III), while the opposite is true for Sb (Fendorf et al. 2007; Leuz et al. 2006). In addition, As(V) has a higher affinity for iron oxides than Sb(V) (Wilson et al. 2010). These lead to higher proportions of As found in oxide phases compared with Sb. A study of As fractionation in 20 contaminated soils by Wenzel et al. (2001) also showed that As was predominantly associated with the amorphous Fe and Al oxides fraction, irrespective of the sources of arsenic contamination, making the Fe and Al oxides the most dominant sink for As in soils.

In the current study most of the Sb was strongly bound to the residual fraction of mining soils (73 ± 2 %), which was more than double the percentage of residual-As. Interestingly, this was unaffected by the diluent control soils or the interaction of the plants. Previous research has similarly found that Sb is predominantly partitioned in the residual fraction (Álvarez-Ayuso et al. 2013; Ettler et al. 2010). A higher proportion of Sb in the residual phase is most likely to be due to Sb present as stibnite, which is the mineral typically mined

(Álvarez-Ayuso et al. 2013) and known to be associated with the Urunga site. The residual fraction may also include Sb associated with organic matter; while As and Sb can both adsorb to humic acids, antimonite tends to accumulate more strongly in organic horizons of soils (Ceriotti & Amarasiriwardena 2009). Dousova et al. (2015) confirmed these trends by demonstrating that arsenate was predominantly bound to mineral soils, while antimonate was strongly adsorbed in organic soils. This suggests that Sb-lability may increase if concentrations of soil organic matter decrease (e.g. from microbial degradation). The amount of Sb or As associated with the organic fraction of soil is not identified in the SEP used here (Wenzel et al. 2001), and it is likely to be in measured as part of the residual fraction.

The differences in As and Sb chemical fractionation and lability may be related to the differences in their mineral solubilities, redox properties, and their pentavalent oxyanions structures, where arsenate is tetrahedral antimonate is octahedral (Okkenhaug et al. 2012; Wilson et al. 2010).

Based on the results, it is likely that As and Sb were both not very labile under the oxic conditions, and Sb was more strongly retained in the solid phase than As. However, these contaminants may be remobilised into soil pore water under anoxic conditions (Hockmann et al. 2015; Xu et al. 2008), and As is at more risk than Sb due to the slow rate of the release of Sb from mineral phases like stibnite. This warrants further investigation.

3.3.3. Labile As and Sb in soils assessed by DGT technique

DGT-labile concentrations, C_{DGT}, and total As and Sb concentrations in soil solution, C_{sol}, are reported in Table 3.1. The capacity of the solid phase to resupply As and Sb in soil solutions was calculated from these values as R ($R = C_{DGT}/C_{sol}$, $0 < R < 1$). An R value of 1 indicates that desorption/dissolution processes within the soils can sustain analyte soil solution concentrations in response to the flux into the DGT sampler (Harper et al. 1999; Harper et al. 1998), whereas a very low R indicates very limited or no resupply of labile species from the solid phase and only diffusional resupply.

In this study the soil solution concentrations (C_{sol}) of As and Sb and the DGT-labile (C_{DGT}) As and Sb generally increased with total concentrations. The values of C_{sol} and C_{DGT} of As and Sb were low ranging from 0.005 to 0.05% (1.9 – 67 µg As L^{-1}) and from 0.006 to

0.09% (1.9 - 350 µg Sb L^{-1}) of total soil As and Sb, respectively. The low lability could be due to the extent of aging with the contaminated soil and the stability of As associations (Liang et al. 2014). The average K_d value (sorbed/dissolved concentrations, mL/g) for As was 290 ± 20, which was much higher than that for Sb (15.4 ± 0.3), indicating more Sb in soil water. C_{DGT} of As and Sb were lower than C_{sol} in all soils, however the C_{DGT} increased linearly with soil solution concentrations (Figure 3.2). The average R, regression slope, for As was 0.41 ± 0.03 indicating partially sustained resupply from the solid phase and suggests that solid phase As was likely to be mobile for the plant bioassays. On the other hand, the average R of 0.14 ± 0.07 for Sb suggests that there was minimal resupply from the solid phase. The higher regression slope for As (0.41) compared to Sb (0.14) could be explained by the fact that DGT accumulated As more quickly than it was resupplied or more of the Sb being in the soil solution was in an inert form, perhaps as dissolved organic complexes, which were not DGT-labile.

It is also noticeable that despite total Sb concentrations in soils being nearly double those of As, the DGT-derived concentrations of Sb were only 26 to 34% higher than the DGT-As. The R ratios of As and Sb remained relatively constant throughout the soil concentration series, indicating that the behaviour of As and Sb in the bioassay soils were unaffected by the soil dilution. Thus the control soil (diluent) had no marked effect on the resupply rates of As and Sb from the solid to the dissolved phase.

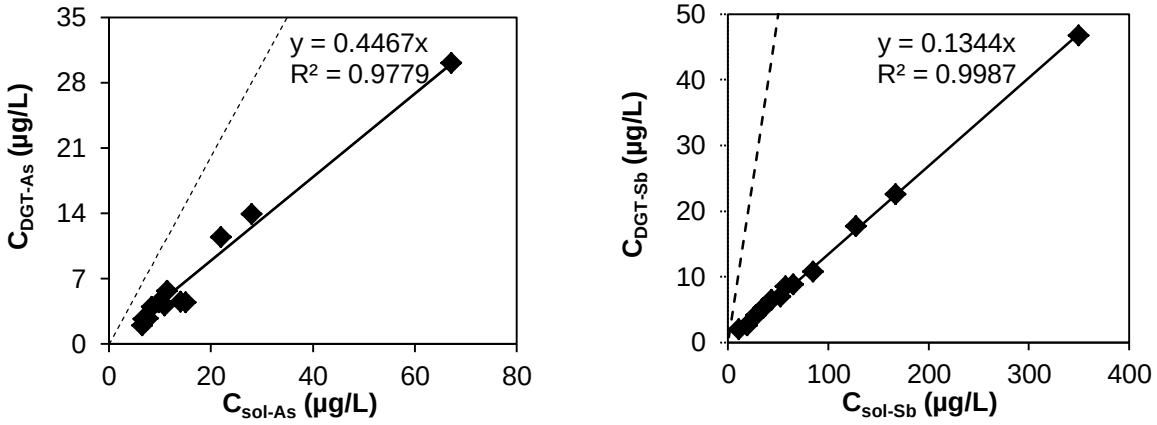

Figure 3.2 The relationship between DGT-labile (C_{DGT}) As and Sb after 24-h deployment and concentrations in extracted soil solutions (mean ± SE, n = 3). Dashed line represents a 1:1 gradient, regression set through zero. Note that total Sb soil concentrations were much higher than total As concentrations.

3.3.4. Uptake of As and Sb by radish (*Raphanus sativus*)

3.3.4.1. Plant yield

R. sativus had an average wet tissue mass of 23.4 ± 0.6 g (n = 48) which was not affected ($p>0.05$) by the contaminated soils following the 35-day exposure bioassay, as there was also no significant difference in tissue moisture (87.2 ± 0.3%, n = 48) between plants exposed to different concentrations. *R. sativus* responses in this study were not consistent with the study by Smith et al. (2008b), who observed the reduction of wet weight mass of *R. sativus* grown hydroponically (0.8 – 50 mg As/L) and in mine waste soil treatments (660 and 1100 mg As/kg). However, the As-lability in our test soils reached 0.067 mg As/L ($C_{sol\text{-}As}$) and had a total soil As of 400 mg/kg, which was much lower than that in Smith et al. (2008b).

3.3.4.2. Accumulation of As and Sb in *R. sativus* tissues

The As and Sb concentrations in different *R. sativus* tissues increased with increasing soil exposure concentrations (Figure 3.3). Tissue concentrations were low in comparison to the total soil concentrations, which is consistent with the low lability of As and Sb in soils as determined by both SEP, C_{sol} and DGT measurements. Both As and Sb concentrations in the *R. sativus* tissues followed the trend shoots > peel > root flesh. The flesh concentrations remained particularly low irrespective of soil As and Sb concentrations, and it accumulated <3 times the As and <9 times the Sb found in the shoots. For all total soil concentrations <70 mg As/kg (equivalent to DGT-labile As of <4.5 µg/L), the *R. sativus* peel and flesh concentrations were not significantly different ($p>0.05$), however, above this concentration the peel accumulated more As than the flesh. Antimony followed a similar trend, however the difference in Sb accumulation between the peel and flesh was less than for As. Overall As was accumulated to a greater concentration than Sb in all plant compartments even though total soil and soil water Sb concentrations were higher. The uptake pattern in this study is supported by Bhatti et al. (2013) who reported that the highest amount of As in *R. sativus* shoots, followed by peel and peeled roots when the plants were irrigated with arsenic-contaminated water every 10 days until harvest. This indicates that the distribution of As in various tissues of this *R. sativus* species is not affected by contamination exposure mode.

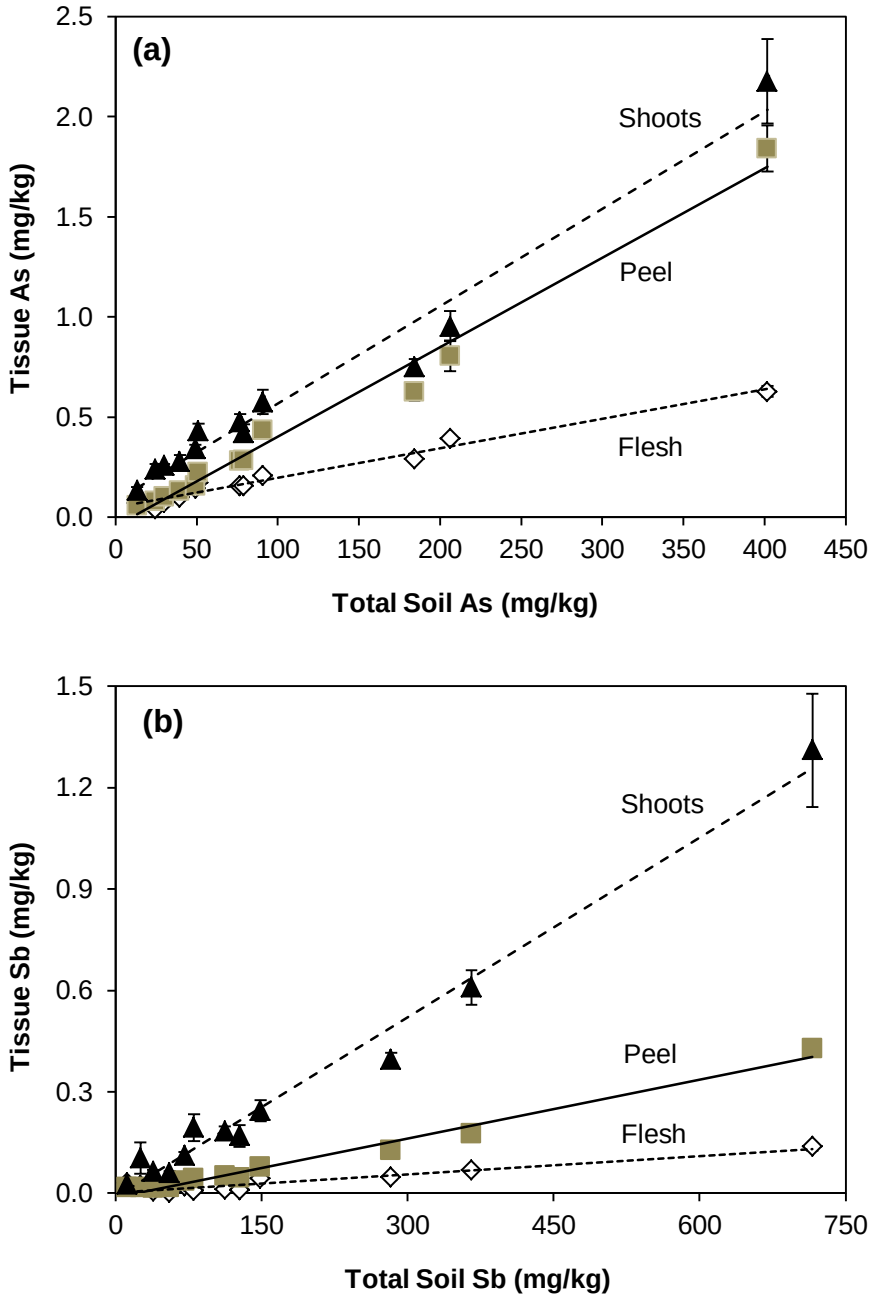

Figure 3.3 The relationship between total soil concentrations and the accumulation of As (a) and Sb (b) in *R. sativus* tissues (mg/kg dry mass, mean ± SE, n = 4) following cultivation in contaminated soils for 35 days. Shoots (▲), Peel (□), and Flesh (◇). All data have error bars; where they are not seen they are smaller than the symbols.

The actual As and Sb concentrations accumulated in different compartments ranged from 0.1 – 2.5 and 0.02 – 0.56 mg/kg in roots (flesh plus peel) and 0.1 – 2.2 and 0.03 – 1.31 mg/kg in shoots, respectively (Figure A.1). The distribution of As between *R. sativus* tissues was affected by the As soil concentrations. Below total soil concentrations of 90 mg As/kg slightly higher amounts of As were found in shoots, but As tended to be accumulated more in roots than in shoots when *R. sativus* were exposed to higher soil As concentrations. In comparison, there was significantly ($p<0.05$) higher Sb in shoots than roots in almost all studied soils, except for the lowest treatment (S1). This is consistent with previous studies on arsenic toxicity and accumulation in *R. sativus* exposed to different forms of As in media (Carbonell-Barrachina et al. 1999a; Tlustoš et al. 1998).

The soil-plant transfer model is one of approaches to predict contaminant uptake by plants, in which the translocation factor (TF) and the bioaccumulation factor (BAF) are computed to describe the scenario at the screening level of risk assessments (McLaughlin et al. 2011; Murray et al. 2009). Translocation factors evaluated the ability of plant to translocate As and Sb from roots to shoots and is based on the ratio of total concentrations in shoots compared to roots (mg/kg DW). In our study the TF were different for As (0.8 – 2) and Sb (3 – 4.1) (Table 3.2), with the translocation rate of Sb being approximately 2.5 times higher than that of As which means that the *R. sativus* had a significantly ($p<0.05$) stronger capacity to transport Sb from the roots to the shoots than As. The translocation factors of As and Sb tended to decrease with increasing soil As and Sb concentrations. The lower translocation of solutes could be from the reduction of xylem sap that occurs when plants were exposed to high concentrations of metals. A study of the impact of As on the metabolite profile and production of xylem sap in cucumbers (*Cucumis sativus* L.) grown hydroponically reported that 96% of xylem sap was reduced when cucumber was exposed to 1 mg As(V)/L (Uroic et al. 2012), possibly because the reduction of sap is a mechanism of plant to avoid As translocation to shoots to protect photosynthesis in shoots.

The bioaccumulation factor (BAF) (also known as the transfer factor, transfer coefficient, or uptake factor) is determined from the ratio of the total concentration in plant roots and the total soil concentration (mg/kg DW). The BAF is an index used to evaluate the transfer of a metal from soils to plants. A higher BAF reflects a greater efficiency of plants to accumulate a metal and a relatively more labile metal fraction (Douay et al. 2013). Plants

with a low BAF (<1) tend to have limited transfer between soil and the roots, so this plant is considered to be "tolerant". Plants with high BAF and TF (>1) have potential to be used for phytoextraction remediation purposes (Vamerali et al. 2010). However, if contaminants are highly accumulated in edible parts of plants or if the plants applied for phytotechnologies are accidentally consumed by animals, they pose a risk if consumed. Thus, the bioaccumulation factor is commonly determined to ensure food safety.

The BAF of As and Sb in *R. sativus* roots varied between 0.0049 – 0.008 and 0.00028 – 0.003, respectively (Table 3.2), showing that the uptake rate of As in roots was much higher (2.5 – 21 times) than that of Sb, however, the transfer of As from soils to *R. sativus* roots was more efficient than Sb. The BAF values for As and Sb were much lower than the typical BAF reported for As (about 0.2) and for Sb (about 0.01) reviewed from literature (Wilson et al. 2013), suggesting that the phytoavailable As and Sb in test soils were low and the plant acquisition of these metalloids from the historically contaminated oxic soils were small.

Table 3.2 Bioaccumulation factor and translocation factor for As and Sb in *R. sativus* (mean ± SE, n = 4).

Soil	Bioaccumulation factor (BAF)		Translocation factor (TF)	
	As	Sb	As	Sb
S1	0.008 ± 0.002	0.003 ± 0.001	1.4 ± 0.4	1.3 ± 0.5
S2	0.0049 ± 0.0004	0.0015 ± 0.0002	2.0 ± 0.3	3 ± 1
S3	0.0059 ± 0.0006	0.0005 ± 0.0001	1.5 ± 0.1	3.6 ± 0.5
S4	0.0058 ± 0.0004	0.00028 ± 0.00001	1.2 ± 0.1	3.9 ± 0.4
S5	0.0061 ± 0.0003	0.0008 ± 0.0001	1.15 ± 0.07	2.0 ± 0.2
S6	0.0078 ± 0.0007	0.0006 ± 0.0001	1.09 ± 0.05	4.1 ± 0.6
S7	0.0057 ± 0.0003	0.0004 ± 0.0001	1.1 ± 0.1	2.7 ± 0.1
S8	0.0056 ± 0.0002	0.00042 ± 0.00002	0.95 ± 0.07	3.1 ± 0.5
S9	0.0071 ± 0.0003	0.0008 ± 0.0001	0.89 ± 0.06	2.0 ± 0.1
S10	0.0050 ± 0.0003	0.0006 ± 0.0001	0.83 ± 0.06	2.4 ± 0.3
S11	0.0058 ± 0.0004	0.00066 ± 0.00004	0.81 ± 0.09	2.5 ± 0.3
S12	0.0061 ± 0.0004	0.00079 ± 0.00003	0.9 ± 0.1	2.3 ± 0.3

This is consistent with the low percentage of bioavailable As and Sb (sum of the non-specifically sorbed and specifically sorbed fractions obtained from the SEP), which was between 0.31 - 5.4% and 0.33 – 0.6% of the total As and Sb, respectively (Table 3.1). The low C_{DGT} of As and Sb also support this, with an average of 0.008% of total As and 0.009% of the total Sb. Our results support the well-established hypothesis that As phytoavailability is more closely related to available As than to total soil As (Cattani et al. 2009). These data suggest that the low lability of As and Sb in test soils resulted in low bioaccumulation of As and Sb in the *R. sativus*.

Our study was performed at much lower labile concentrations than previous publications, thus the As accumulated within our *R. sativus* tissues (Figure 3.3) was considerably lower than that reported by other studies: 36 ± 8 and 10 ± 3 mg As/kg (DW) in roots and shoots, respectively, of *R. sativus* grown hydroponically in 2 mg As/L nutrient solution (Smith et al. 2009), and 5 mg As/kg (DW) for both roots and shoots of *R. sativus* grown in soils with 1 N HCl-extractable As concentrations of 25 mg/kg (Gutierrez et al. 2010). In addition to our lower exposure concentrations, Australian soils are typically very high in Fe. Thus the most probable parameter limiting the As and Sb accumulation in our bioassay is adsorption by iron oxides and minerals within the soil particles, demonstrated by high percentages of As (64%) and Sb (27%) found in Fe and Al oxide phases and 31% of total As and 73% of total Sb found in residual phases (Figure 3.1). Arsenic is most prominently found in soils bound to Fe and Al-oxides (Kim et al. 2014; Niazi et al. 2011) and these minerals are dominant components of Australian soils (Smith et al. 2003).

Other plants have similarly accumulated more As in the roots than shoots (Cobb et al. 2000; Smith et al. 2009). The mechanism of metal and metalloid tolerance in plants may relate to plants limiting the upward movement of toxic elements by concentrating them in the plant roots, including via complexation in phytochelatins and sequestration in vacuoles (Schmoger et al. 2000; Zhao et al. 2010).

Greater As accumulation in comparison to Sb has also been reported in the literature (Wei et al. 2011; Wilson et al. 2014). The differences in bioaccumulation most likely arise from differences in analyte behaviour, due to their structural conformation of arsenate and antimonate, as the most common Sb species in soils is the octahedral oxyanion $Sb(OH)_6^-$, which differs from the tetrahedral oxyanions of As(V). The mechanisms of As uptake by

plants have been studied in depth, as arsenate and phosphate are chemical analogs, enabling arsenate to be taken up by plant roots through the phosphate pathway (Zhao et al. 2010). However, the uptake mechanisms of antimonate are still unclear (Feng et al. 2013), with research showing that antimonate was not accumulated via phosphate transporters by maize and sunflower in hydroponic experiments (Tschan et al. 2008). Further research on mechanisms of Sb uptake by plants is required to fully understand Sb bioavailability.

It is important to note that plants harvested from soils often contain a visible layer of iron plaque (e.g. precipitated iron oxyhydroxides) on the roots (Tripathi et al. 2014). If this layer is not removed prior to analysis, the tissue concentration of As and Sb could be erroneously high due to their sorption to this layer of iron plaque and thus the inclusion of a fraction that was not actually accumulated by the plant. The removal of root skin prior to cooking and eating is also a usual practice. In fact, as there was much higher As concentrations in the peel, it would be important to reduce contaminant exposure by peeling root vegetables before consumption.

3.3.5. The relationship between As and Sb bioaccumulation and soil exposure concentrations

The relationships between As and Sb concentrations in *R. sativus* tissues and the 'labile' soil exposure concentrations measured by SEP, DGT (as C_{DGT}), and soil solution (C_{sol}) are presented in Figure 3.4. The concentrations of As and Sb in plant tissues showed a highly significant positive correlation with all of the labile As and Sb fractions ($R^2 = 0.96 – 0.99$), indicating that As and Sb in these phases are readily available to plant uptake.

The 'bioavailable' fractions of SEP, and labile concentrations measured by DGT, and soil solution were better predictors for As and Sb in roots (greater correlation coefficients) than in plant shoots, with the exception of soil solution-As. Arsenic in shoots seems to be limited by that in roots, demonstrated by the low translocation factor. On the other hand, the Sb concentration in shoots is much higher than that in roots, consistent with the higher translocation factor. The better correlations between labile As and Sb in soils and their uptake by roots can be explained by the fact that roots are in direct contact with soils and may be better indicators for soil bioavailable elements than shoots, where bioaccumulation of elements is influenced by the translocation processes, normally governed by physiological characteristics of plants (Chaignon et al. 2003). This also confirmed the

findings that the bioaccumulation factor was one of the parameters reflecting the bioavailability of As and Sb in the test soils and their transfer from soils to roots, while the different translocation of As and Sb from roots to shoots solely depends upon the nature of As and Sb characteristic within this *R. sativus*. McLaughlin et al. (2011) also reported that the translocation factor is specific to the plant species, genotype, and a given element.

According to Degryse et al. (2009), metal uptake by plants can be governed by both plant and soil processes. If the plant acquisition of metals from soil is slower than the supply of metals by diffusion, the depletion of metals at the vicinity of root area is negligible. In this case, the metal accumulated by plants can be assumed to be controlled by the free ion in solution (Campbell 1995). If metal concentrations in soil solutions are proportional to free ion activities, good relationships between metals in plants and in soil solutions may be observed. If the metal accumulation by plants is rapid compared to the diffusional supply, depletion of free ion concentrations occurs within the rhizosphere, leading to the resupply of metals from the soil solution complexes and from the solid phase (Zhang et al. 2001). If the concentration of metals is depleted at the root zone, many processes affecting the metal supply to plants occur including the diffusional transport of metals to the roots and the kinetics of release of metals from solid phases to soil solutions. In these cases, free ion activities and soil solution measures are not successful in predicting metal uptake by plants (Lehto et al. 2006b). According to previous studies (Ernstberger et al. 2005; Luo et al. 2014), metal concentrations in soil solution are derived from labile and non-labile metal. The DGT measurement comprises the free ion in soil solution and the resupply of labile metal from complexes in solution and solid phases, but excludes inert metal bound to solid phases and solution complexes. In this study, the soil solution and DGT are equally effective in predicting As and Sb accumulation by *R. sativus*, suggesting that the plant accumulated the free ions and the resupply was not a governing factor, consistent with uptake being limited by the plant physiology.

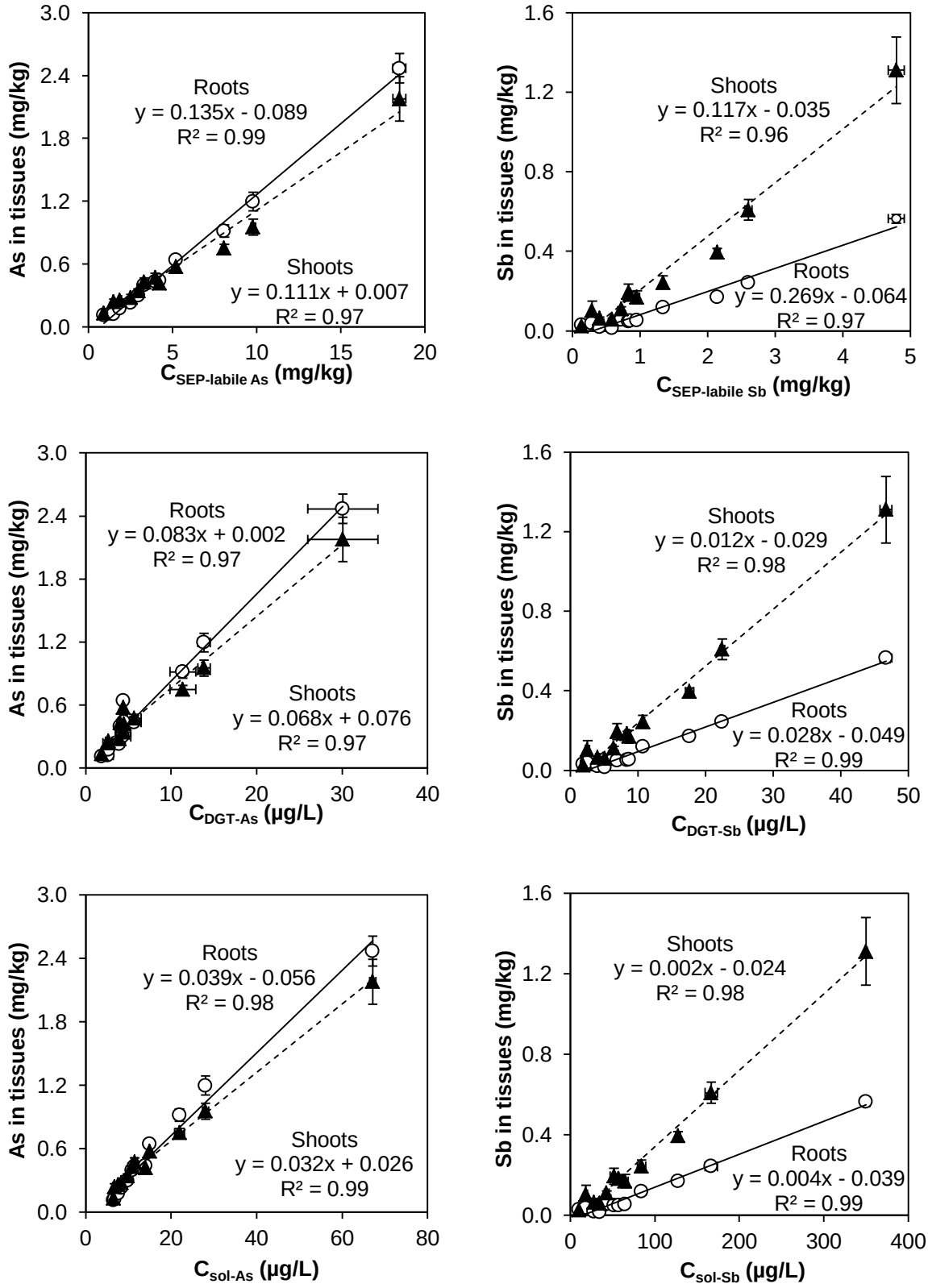

Figure 3.4 The relationship between tissue As and Sb (mg/kg, dry mass) and bioavailable fractions of As and Sb in soils, as measured by $C_{SEP\ labile}$, C_{DGT}, and C_{sol}. Shoot (▲), Root (o). All data have error bars; where they are not seen they are smaller than the symbols.

A previous study on the assessment of As bioavailability in 43 contaminated soils using DGT and conventional methods also demonstrated strong correlations between As in edible rape and As concentrations obtained from DGT and soil solution, while As contents in the plant were scattered with respect to As obtained from different single extraction (Wang et al. 2014). From these data, the authors concluded that DGT proved to be an effective tool for the assessment and prediction of As bioavailability in soils. However, the research presented in this chapter is the first study to report the application of DGT in predicting Sb accumulation by plants, and comparing this with other soil extraction procedures.

3.4. Conclusions

The soil historically contaminated with both As and Sb have different soil binding phases for these contaminants. As was chemically more labile and mainly bound to the Fe and Al oxides, yet Sb was much less labile and most likely predominantly associated with organic matter, silicates, or other resistant minerals like sulphides. The coupling of SEP, soil solution and DGT provided essential information on the soil As and Sb speciation, which may be useful in understanding the lability and phytoavailability of As and Sb in soils. The SEP procedure revealed that a high proportion of As was associated with Fe and Al oxides which is not mobile and not DGT-labile. Thus, it is not likely to be the bioavailable fractions in soils under oxic conditions.

Both As and Sb concentrations in *R. sativus* tissues displayed the trend of shoots > peel > flesh. Since there were higher amounts of As and Sb in the peel, peeling off radish skins before consumption would be important to reduce contaminant exposure. The *R. sativus* bioassay showed low bioaccumulation of As and Sb in comparison with total soil concentrations. Arsenic was accumulated more than Sb, however, Sb had a higher ability to translocate from root to shoot than As, to the extent that much higher Sb concentrations were observed in shoots, suggesting a quite different form of transport within the plant.

The DGT, soil solution, and SEP-labile fractions performed comparably well in predicting the accumulation of As and Sb in *R. sativus*. The ability of plants to accumulate As and Sb in their tissues is known to vary with varieties, thus, further studies are needed to identify if these observations are established with the other cultivar of radish and other soil types.

Chapter 4. DGT and selective extractions reveal differences in arsenic and antimony uptake by the white icicle radish (*Raphanus sativus*)

4.1. Introduction

In chapter 3, the DGT technique was used for simultaneous measurements of labile As and Sb in contaminated soils ranging from 13.3 to 400 mg As/kg and 11.8 to 720 mg Sb/kg and to predict uptake by the cherry belle radish (*Raphanus sativus*); the results indicated that these two elements behaved quite differently (chapter 3). In terms of their geochemical behaviour, Sb was mainly associated with residual phases and resistant minerals such as silicates or sulphides, while As was primarily bound to Fe and Al oxides. DGT measurements indicated that As was more labile and partially resupplied from the soil solid phase, while Sb was minimally resupplied and therefore most likely present in inert forms. With respect to their uptake by the cherry bell radish (*R. sativus*), arsenic accumulated in roots was 2.5 – 21 times higher that of Sb. However, Sb was translocated from roots to shoots at much greater extent than As by a factor of 2.5, illustrating different uptake trends between these two metalloids.

The ability of plants to accumulate As in their tissues varies with crop species and varieties. For example, the tomato variety Marmande accumulated much more As in roots than tomato cultivar Muchamiel, whereas the latter took up higher As concentrations in shoots and fruits than the former (Burló et al. 1999). Moreover, among various rice cultivars, the BR11 variety accumulated higher As concentrations in grain (0.104 – 1.835 mg/kg) than the others (0.058 – 0.117 mg/kg) (Meharg & Rahman 2003). Similar results were found for Sb, demonstrated by Cai et al. (2016) reporting that the Japonica rice (cultivars N and Z) exhibited a higher accumulation and stronger translocation tendency of Sb from root to stem than Indica hybrid rice (cultivars F and G). The selection of non-accumulating cultivars is an important risk management strategy to minimise human exposure to contaminants via vegetable consumption (McLaughlin et al. 2011). Thus, investigations at

the variety scale are also needed to determine if different cultivars of the same species behave similarly with respect to simultaneous uptake of As and Sb in a range of soil types.

In this study the DGT technique was combined with a sequential extraction approach to assess the lability of As and Sb in relation to their uptake by the *R. sativus* cultivar, the long white icicle radish. This cultivar has a very long taproot, which is different from the round shape of the root of the cherry belle radish examined in our previous study (chapter 3). The differences in uptake by these cultivars have not been reported previously. The aims of this study were to investigate (1) the biogeochemical behaviour of As and Sb in soils prepared from two historically contaminated soils; (2) the bioaccumulation of As and Sb in the white icicle radish grown in these test soils; and (3) the ability of sequential extraction and DGT measurements to predict As and Sb uptake by the plant. The results of this study on As and Sb bioaccumulation by the white icicle cultivar will be compared with recent data obtained for the cherry belle cultivar, thus providing an important comparison of bioaccumulation across two important vegetable cultivars of the same species.

4.2. Materials and methods

4.2.1. General methods

General washing methods and soil characterisation were done as per sections 2.1 and 2.5. Soil and plant digestion, and analysis of As and Sb in digested samples by ICP-MS were conducted as per sections 3.2.4 and 2.9. For quality assurance, reagent blanks, certified reference materials, spikes, and duplicates were analysed every 15 samples. A soil standard reference material (Montana II soil, SRM 2711a; NIST) returned recoveries of 101-105% and 80-90% for As and Sb, respectively. A plant standard reference material (Tomato leaves, SRM 1573a; NIST) yielded recoveries of 110-120% and 80-86% for As and Sb, respectively.

4.2.2. Soil preparation and plant bioassays

Soil highly contaminated with (4800 mg/kg) and Sb (7900 mg/kg) was collected from the area around a decommissioned antimony processing facility in Urunga, New South Wales (NSW), Australia (Warnken et al. 2017) (mining soil, “S”). Details about soil compositions and physical properties were described in section 3.2.2. A second contaminated soil was collected adjacent to a cattle dip site in Queensland (QLD), Australia (cattle dip soil, “Q”).

This was a silty-sand soil and contained 1360 mg As/kg and negligible concentrations of other contaminants. An uncontaminated soil was collected in Wollongong, NSW. All soils were air-dried and crushed to pass through a 2-mm sieve. In order to establish test soils, the uncontaminated and the contaminated soils were mixed in a cement mixer at various ratios from 1:200 to 1:10 to give a range of As and Sb concentrations for the mining soil (S1 – S12), and at ratios from 1:100 to 1:3 to give a series of As concentrations for the cattle dip soil (Q1 – Q12). Plant bioassays were conducted with the long white icicle radish (*Raphanus sativus*), an agriculturally important edible vegetable, as per section 2.4.1. Harvested plant tissues from the same pot were combined, weighed, and oven-dried at 70 °C for 48 h to a constant weight with the dry mass (g) recorded. Dried tissues were ground with a mortar and pestle and stored in clean plastic bags prior to As and Sb analyses.

4.2.3. Sequential extraction procedure

Sequential extraction was conducted as per section 2.6.1.

4.2.4. DGT and soil solution measurements

DGT and soil solution measurements were conducted as per section 2.6.2 and 2.7.

4.2.5. Data analysis

Statistical analysis and calculations of bioaccumulation factor (BAF) and translocation factor (TF) were performed as per section 3.2.7.

4.3. Results and discussion

4.3.1. Soil properties

The average physical and chemical properties of each series of test soils are summarised in Table 4.1. Soil characteristics are important parameters in controlling the mobility and distribution of As and Sb in solid phases (Wilson et al. 2010). The soils prepared from the cattle dip soil were contaminated with As only, while the mining soils were contaminated with both As and Sb. The physico-chemical properties of all test soils were characterised and analysed prior to performing pot experiments. The soils were a silty-sand (66 – 69% sand, 28 – 31% silt, and 3% clay), slightly acidic to slightly alkaline (pH 6.46 – 7.09), and had relatively similar amounts of nitrogen (83 – 90 mg/kg). The soils diluted from the cattle dip site had slightly higher amounts of organic matter and extractable phosphorus than

those prepared from the mining soil. The total As and Sb concentrations in bioassay soils varied between 13 – 456 and 11 – 680 mg/kg, respectively (Tables 4.2 and 4.3). These values are typical of the total As and Sb in various soils around the world.

4.3.2. Fractionation and mobility of As and Sb in soils

The differences in the percentage of As and Sb associated with various binding phases for the two contaminated soils are presented in Figure 4.1. Generally, the amounts of soil As and Sb extracted in each fraction increased with the increasing total soil As and Sb concentrations (Table B.1, B.2). In both soil types, relatively low amounts of As were released in the SO_4^{2-} extraction step targeting non-specifically sorbed (readily available) As and also in the PO_4^{3-} extraction step targeting specifically sorbed As. The non-specifically sorbed As in diluted cattle dip and mining soils only accounted for 1.70 ± 0.08% and 0.26 ± 0.01% of the total As, respectively. The specifically sorbed As in diluted cattle dip and mining soils only constituted 11.1 ± 0.2% and 5.1 ± 0.2% of the total As, respectively. The sum of the non-specifically sorbed and specifically sorbed As, known as the labile As (SEP-labile), in diluted cattle dip soils (~13%) was more than double that in diluted mining soils (~5%). Arsenic was predominantly found in the oxides and residual phases for both soils, relatively equally associated with amorphous and crystalline oxide fractions (33-35%) in the mine site soils, but was primarily bound to amorphous oxides (66 ± 1%) at the cattle dip site with a lower proportion bound to crystalline Fe and Al oxides (14 ± 1%). This is likely due to the higher concentrations of amorphous Fe in diluted cattle dip soils (Table 4.1).

Table 4.1 Physical and chemical properties of bioassay soils (dry mass, mean ± SE, n≥3).

Characteristics	Diluted cattle dip soils (Q1 – Q12)	Diluted mining soils (S1 – S12)
sand (%)	69 ± 1	66 ± 1
silt (%)	28 ± 1	31 ± 1
clay (%)	3.0 ± 0.3	3.0 ± 0.1
pH	7.09 ± 0.06	6.46 ± 0.03
organic matter (%)	8.0 ± 0.1	6.9 ± 0.1
extractable Phosphorous (mg/kg)	99 ± 7	70 ± 10
total Kjeldahl Nitrogen (mg/kg)	83 ± 7	90 ± 7
amorphous Fe (mg/kg)	8.3 ± 0.4	5.2 ± 0.1
crystalline Fe (mg/kg)	6.4 ± 0.5	9.4 ± 0.2

Table 4.2 Total and labile concentrations measured by SEP, DGT, soil solution in bioassay mining soils, including R-value ($R=C_{DGT}/C_{sol}$) for As and Sb (mean ± SE, n = 3).

Soil	As-Mining					Sb-Mining				
	Total (mg/kg)	$C_{SEP\text{-}labile}$ (mg/kg)	C_{DGT} (µg/L)	C_{sol} (µg/L)	Ratio R_{As} ($R = C_{DGT}/C_{sol}$)	Total (mg/kg)	$C_{SEP\text{-}labile}$ (mg/kg)	C_{DGT} (µg/L)	C_{sol} (µg/L)	Ratio R_{Sb} ($R = C_{DGT}/C_{sol}$)
S1	13.1 ± 0.3	0.86 ± 0.01	2.12 ± 0.02	13.2 ± 0.6	0.162 ± 0.007	11.0 ± 0.6	0.125 ± 0.002	1.3 ± 0.3	12.3 ± 0.5	0.11 ± 0.03
S2	18.3 ± 0.8	1.06 ± 0.07	2.83 ± 0.04	16.4 ± 0.5	0.173 ± 0.005	23.2 ± 0.6	0.255 ± 0.006	2.9 ± 0.2	22 ± 1	0.132 ± 0.006
S3	38 ± 3	2.22 ± 0.03	4.3 ± 0.2	20.6 ± 0.2	0.21 ± 0.01	42 ± 7	0.420 ± 0.06	4.1 ± 0.2	33 ± 3	0.13 ± 0.02
S4	44 ± 0.4	2.65 ± 0.06	5.93 ± 0.04	27.3 ± 0.4	0.218 ± 0.005	55 ± 7	0.59 ± 0.01	5.7 ± 0.1	45 ± 4	0.129 ± 0.008
S5	52 ± 6	2.93 ± 0.05	7.2 ± 0.5	31.6 ± 0.9	0.23 ± 0.02	69 ± 2	0.666 ± 0.007	8.0 ± 0.6	52 ± 4	0.154 ± 0.008
S6	60 ± 10	3.70 ± 0.09	7.6 ± 0.2	29.2 ± 0.7	0.259 ± 0.004	76 ± 2	0.738 ± 0.008	8.0 ± 0.4	55 ± 6	0.15 ± 0.02
S7	80 ± 9	3.85 ± 0.04	7.1 ± 0.9	35.8 ± 0.5	0.20 ± 0.02	120 ± 20	0.88 ± 0.01	11.6 ± 0.5	78 ± 2	0.15 ± 0.01
S8	82 ± 1	4.12 ± 0.05	9.6 ± 0.1	43 ± 2	0.22 ± 0.02	130 ± 20	0.92 ± 0.01	13.3 ± 0.6	102 ± 4	0.131 ± 0.008
S9	94 ± 4	5.08 ± 0.04	10.4 ± 0.4	47 ± 2	0.220 ± 0.004	190 ± 20	1.57 ± 0.04	14.9 ± 0.5	110 ± 4	0.136 ± 0.008
S10	142 ± 1	5.71 ± 0.07	12.2 ± 0.3	62 ± 2	0.199 ± 0.002	260 ± 4	1.80 ± 0.06	20.7 ± 0.6	167 ± 6	0.124 ± 0.007
S11	220 ± 30	9.81 ± 0.1	16 ± 2	73 ± 3	0.22 ± 0.01	400 ± 20	2.59 ± 0.04	28.1 ± 0.4	219 ± 3	0.128 ± 0.002
S12	420 ± 20	18.1 ± 0.2	34.9 ± 0.6	136 ± 4	0.256 ± 0.005	680 ± 50	4.1 ± 0.1	57.9 ± 0.9	484 ± 8	0.120 ± 0.003

Table 4.3 Total and labile concentrations measured by SEP, DGT, soil solution in bioassay cattle dip soils, including R-value ($R=C_{DGT}/C_{sol}$) for As (mean ± SE, n = 3).

Soil	As-Cattle dip				
	Total (mg/kg)	$C_{SEP\text{-}labile}$ (mg/kg)	C_{DGT} (µg/L)	C_{sol} (µg/L)	Ratio R_{As} ($R = C_{DGT}/C_{sol}$)
Q1	16 ± 1	1.98 ± 0.03	3 ± 1	14.5 ± 0.3	0.23 ± 0.07
Q2	27 ± 4	3.2 ± 0.1	5.2 ± 0.2	21.0 ± 0.2	0.25 ± 0.01
Q3	30 ± 1	4.9 ± 0.2	10.3 ± 0.5	39 ± 1	0.26 ± 0.01
Q4	47 ± 3	5.98 ± 0.09	15.3 ± 0.4	48.6 ± 0.7	0.32 ± 0.01
Q5	53 ± 4	7.97 ± 0.03	19 ± 1	59.3 ± 0.4	0.33 ± 0.02
Q6	62 ± 3	9.3 ± 0.2	23 ± 1	72.1 ± 0.3	0.31 ± 0.02
Q7	72 ± 2	10.95 ± 0.07	23.9 ± 0.8	81 ± 1	0.30 ± 0.01
Q8	86 ± 4	11.8 ± 0.07	27 ± 1	86 ± 1	0.31 ± 0.01
Q9	107 ± 6	15.0 ± 0.6	34 ± 3	104.3 ± 0.4	0.32 ± 0.03
Q10	153 ± 3	22.4 ± 0.2	46 ± 2	145 ± 2	0.32 ± 0.02
Q11	176 ± 8	30 ± 1	55 ± 2	169 ± 3	0.33 ± 0.01
Q12	460 ± 40	61.9 ± 0.6	120 ± 10	318 ± 3	0.39 ± 0.04

In general, the partitioning patterns of As in our test soils are consistent with those from other cattle dip (Niazi et al. 2011) and mining soils (Gonzaga et al. 2008), which also showed that the bioavailable As was only about 8% of the total As and that As was mostly (84.3%) associated with amorphous and crystalline Fe and Al oxides. The partitioning of As in these soil types indicates that Fe and Al oxides played a significant role in retention of As in contaminated soils, due to formation of inner-sphere, monodentate and mononuclear as well as bidentate binuclear complexes with soil Fe oxides (Wilson et al. 2010). The higher proportion of As in amorphous oxides and lower proportion of As in the residual fraction in the diluted cattle dip soils reflect the potentially mobile As in these soils, which could be released more easily than in the diluted mining soils, particularly under anoxic conditions. High fractions of As were found in mobile phases in the diluted cattle dip soils indicating that this soil could pose more environmental risk than the diluted mining soils.

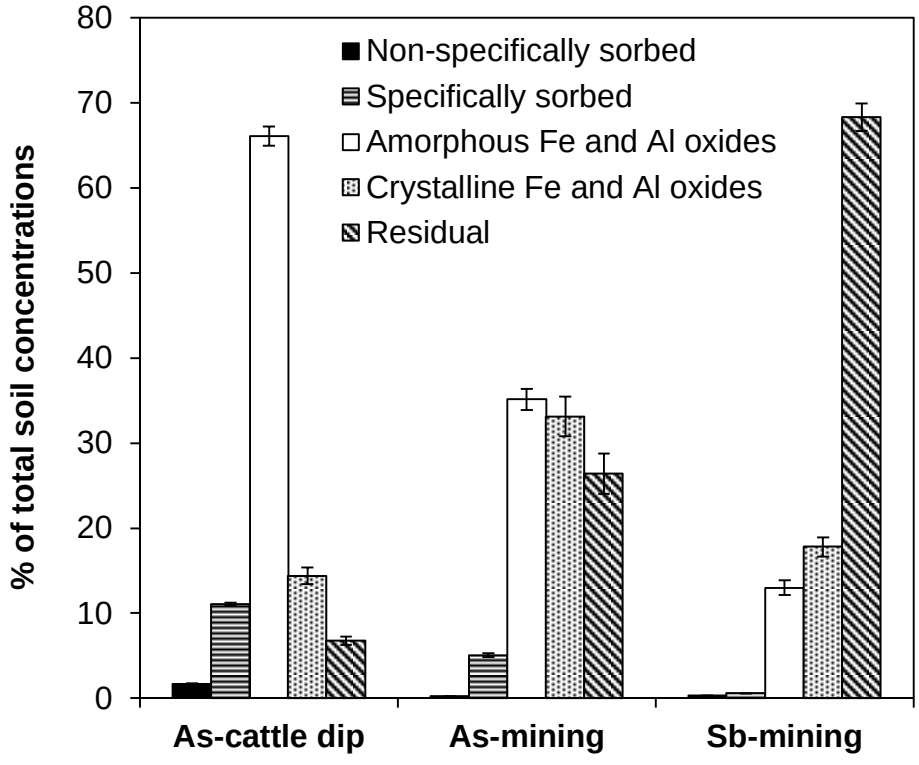

Figure 4.1 The percentage of As and Sb associated with different binding fractions for bioassay soils (mean of all concentrations for each soil, mean ± SE, n = 36).

Sb distribution between soil binding phases differed to that of As, confirming the results of our previous study (Ngo et al., 2016). The sum of the non-specifically sorbed and specifically sorbed Sb, known as the labile Sb (SEP-labile), was extremely low (0.86% of total soil Sb compared with 5.3% for As in the same soil). Sb in Fe and Al oxide phases (13-18%) were less than half that of As because As has a greater tendency to bind to metal oxides in comparison to Sb (Wilson et al. 2010). In contrast, Sb was primarily bound to the residual phase (68%), which is consistent with previous studies (Álvarez-Ayuso et al. 2012; Serafimovska et al. 2013). The lower proportions of Sb in the metal oxide phases and the higher percentage of Sb in the residual phase compared with As could be because of association with organic matter (Dousova et al. 2015; Tighe et al. 2005) or sulfides (Gál et al. 2007; Okkenhaug et al. 2011). These organic and sulfide phases are not specifically targeted by the SEP of Wenzel et al. (2001), thus in our study, the Sb in these phases was likely to be contained in the residual fraction.

Generally, the partitioning of Sb in the test soils is supported by previous studies on Sb mobility and distribution in soils impacted by smelting and mining activities. For example, in studies on the distribution and availability of Sb, generally low mobility was also observed in mining and smelting site soils (Macgregor et al. 2015; Pérez-Sirvent et al. 2011). In studies on soils contaminated with both As and Sb, Ettler et al. (2010) found that in a smelter-polluted soil, the labile concentrations of Sb ranged from 0.6 – 4% and the residual fractions accounted for 65 – 94% of total Sb, while labile As was 1.2 – 22%. The distribution of As and Sb in different fractions of mining soils showed lower proportions of Sb in oxide phases compared with As and a very high fraction of Sb in the residual phase, which suggests that As could be potentially mobilised more easily than Sb and that Sb is strongly bound to the soil matrix and less geochemically mobile in the environment.

4.3.3. Labile As and Sb in soils assessed by DGT

The DGT-labile concentrations (C_{DGT}) and total As and Sb concentrations in soil solutions (C_{sol}) are presented in Tables 4.2 and 4.3. Generally, the DGT-labile and soil solution concentrations of As and Sb in soils increased with increasing total concentrations. The C_{DGT} and C_{sol} of As in the diluted cattle dip soils accounted for ~0.03% (3 – 120 μg/L) and ~0.1% (14.5 – 318 μg/L) of the total soil As, respectively, while those of As in the diluted

mining soils constituted only ~0.01% (2.12 – 34.9 μg/L) and ~0.06% (13.2 – 136 μg/L) of the total As. The C_{DGT} and C_{sol} of Sb in the diluted mining soils were only ~0.01% (1.3 – 57.9 μg/L) and ~0.08% (12.3 – 484 μg/L) of the total soil Sb. The low lability and ratio of dissolved to total concentration is consistent with the SEP results, which highlights a low relative proportion of both As and Sb present in the SEP labile fraction.

The R ratio of C_{DGT} and C_{sol} ($0 < R < 1$) was calculated to estimate the capacity of the soil solid phase to resupply As and Sb into soil pore water as described in section 3.3.3. The R-values (Tables 4.2, 4.3, and slopes from Figure B.1) for both As and Sb exhibited a relatively constant trend with increasing total soil As and Sb concentrations. This is consistent with the concentration range being obtained by mixing two soils – there seems to be little redistribution during this process. The average R-values for As in diluted cattle dip and mining soils were 0.31 ± 0.01 and 0.214 ± 0.008, respectively, indicating partial resupply of As from the solid phases. A higher R-value for As from diluted cattle dip soils indicates a stronger resupply of As in this soil, consistent with the higher SEP-labile As in cattle dip soils compared with those in the mining soils. In contrast to As, the R-value of 0.132 ± 0.004 for Sb suggests virtually no resupply from the soil solid phase. The lower R-value, regression slope, for Sb (0.12) compared to As (0.24) could be because of the stronger partitioning of Sb to the solid phase and more Sb in inert forms or organic complexes in the soil solution that were poorly labile and thus not measured by DGT. This indicates that As was resupplied more strongly than Sb from the solid phase, which was supported by the partitioning of As and Sb in various SEP-derived binding phases stating that As was more chemically labile than Sb, and that Sb was primarily bound to the soil matrix (residual phase).

It is interesting to note that the resupply of As in cattle dip soil was higher than that in the mining soil, despite no significant differences in total concentrations of As between the two soils. The greater lability and bioavailability of As in diluted cattle dip soils could be due to higher concentration of phosphates because phosphates can enhance the release of As from solid phases via competition for sorption sites (Cao & Ma 2004). It was also interesting that the resupply rate of As was higher than that of Sb within the mining soil, although the total soil As was less than total soil Sb. The lower lability of As and Sb in diluted mining soils

may also be due to the presence of sulphide minerals reported to be responsible for the immobilisation of As and Sb in mine waste sites (Álvarez-Ayuso et al. 2013; Kim et al. 2014).

4.3.4. Uptake of As and Sb by radish (long white icicle, *Raphanus sativus*)

4.3.4.1. Plant yield

The long white icicle radishes grown in cattle dip and mining soils had an average dry biomass of 3.6 ± 0.1 g (n = 48), which was similar to controls and not affected ($p>0.05$) by the As and Sb exposure in the soils after the 35-day bioassay. These radish responses were consistent with the observation of cherry belle radishes (chapter 3).

4.3.4.2. Uptake trend

The accumulation of As and Sb in different white radish tissue compartments demonstrated a dose-dependent response and positive correlation with their total soil concentrations (Figure 4.2). Radish tissues (root flesh, peel, and shoot) accumulated As to greater concentrations than Sb even though total soil Sb concentrations were higher than total soil As, suggesting that As presents a greater risk than Sb in terms of food consumption/safety. However, bioaccumulation was low compared with the total As and Sb soil concentrations, which is in line with the low SEP and DGT labile As and Sb measurements. Tissue As displayed the trend of peel > shoot > flesh, while Sb in plant tissues followed a different trend of shoot > peel > flesh. The flesh As and Sb concentrations were consistently low, only accounting for 0.1 – 0.5% and 0.02 – 0.1% of their total soil concentrations, respectively, irrespective of soil concentrations and soil types. This trend was also observed by previous studies where the lowest As concentrations were in radish flesh (Bhatti et al. 2013) and peeled carrot (Codling et al. 2015; McBride 2013).

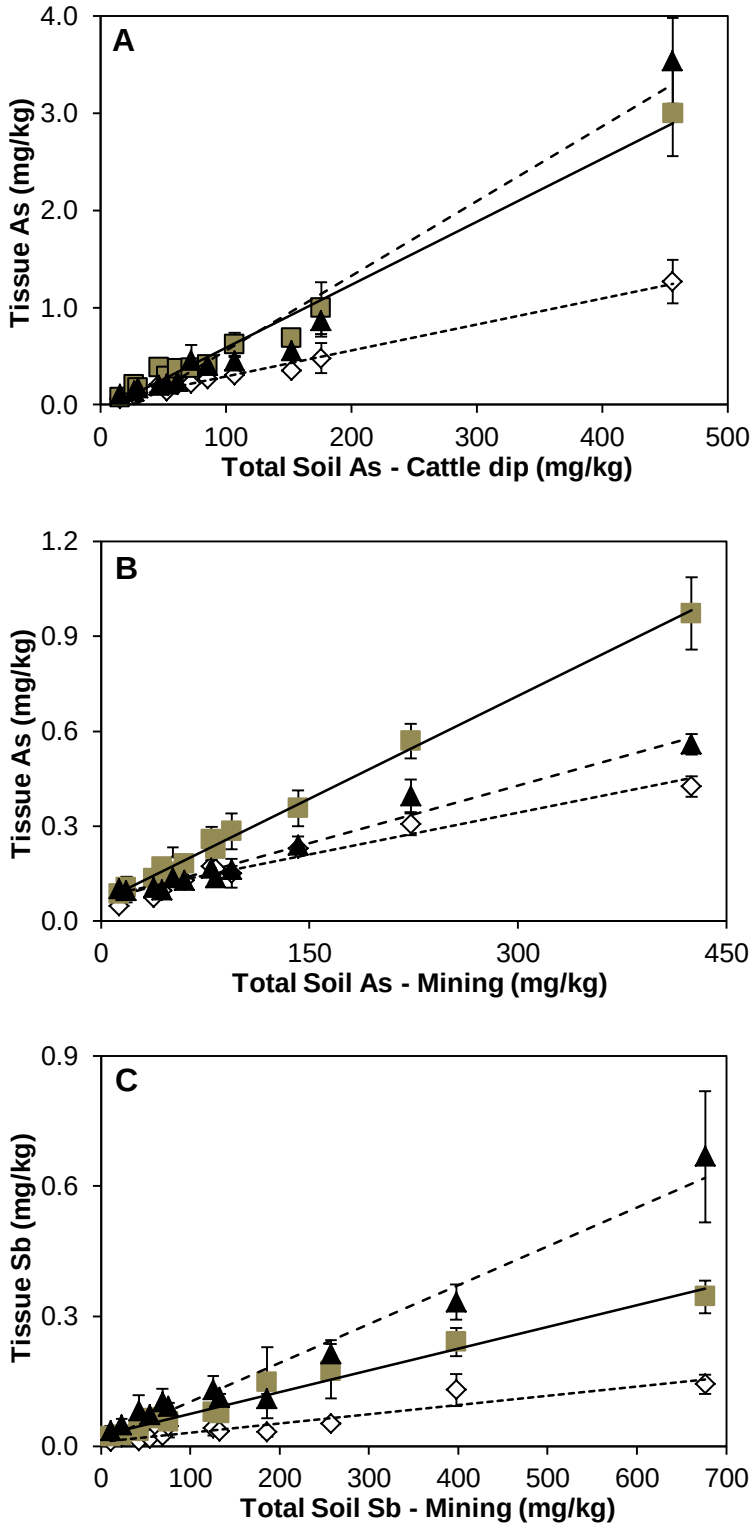

Figure 4.2 The relationship between total soil concentrations and the accumulation of As and Sb in white radish tissues (mg/kg dry mass, mean ± SE, n = 4) following cultivation in contaminated soils for 35 days. Shoots (▲), Peel (□), and Flesh (◇). Figures A, B, and C represent As in cattle dip soils, As in mining soils, and Sb in mining soils, respectively.

In the taproot of radishes, the peel accumulated more As than the flesh, the differences between peel and flesh concentrations being more pronounced with increasing soil concentrations, a trend that has been previously observed in radishes (Bhatti et al. 2013; Warren et al. 2003) and carrots (Bhatti et al. 2013; Codling et al. 2015). The higher peel concentrations may be due to the direct contact between roots and contaminated soils, resulting in the possible adsorption of As on the unwashed iron layer on the outside of roots. Iron is known to be an important sorption phase in soils (Wilson et al. 2010) and can act as a barrier for metalloid uptake by plant roots (Tripathi et al. 2014). Another possibility is that the cells of the outer layer might accumulate As and Sb to a greater extent than other radish cells. Peeling appeared to significantly reduce As in roots, especially in highly As contaminated radishes, which is also supported by previous studies where peeling is recommended for carrots (McBride 2013) and potatoes (Codling et al. 2016). Sb also exhibited a higher concentration in the peel compared to the flesh, although not to the same degree as As. If iron plaque is the dominant factor responsible for increased concentrations of contaminants in the peel, Sb may not bind as strongly to this sorption phase compared to As, as supported by previous studies (Wilson et al. 2010).

For Sb accumulation by radishes cultivated in diluted mining soils, the total Sb in roots and shoots varied between 0.03 – 0.49 and 0.04 – 0.67 mg/kg, respectively, which were lower by a factor of 2.4 – 4.5 and 1.1 – 2.9 relative to As in roots and shoots for the same soil. The As concentrations in radishes grown in cattle dip soils were higher than those from mining soils. Arsenic concentrations accumulated in different compartments of radishes grown in diluted cattle dip and mining soils ranged from 0.13 – 4.27 and 0.13 – 1.40 mg/kg in roots (flesh plus peel); 0.11 – 3.54 and 0.10 – 0.56 mg/kg in shoots, respectively (Figure B.2). For radishes grown in the lowest concentration soils (S1 and Q1), where the control soil was dominant, As concentrations in roots and shoots of radish from both soils were the same. For radishes cultivated in soils of above 20 mg As/kg, As in roots and shoots from diluted cattle dip soils were 1.3 to 3.1 and 1.5 to 6.4 times greater than those from diluted mining soils, even though there were no significant differences in total As concentrations. This finding indicates that the bioavailable As concentrations in cattle dip soils were higher than those in mining soils, probably due to the greater phosphate concentration in cattle dip soils and the As being sources from pesticides (not measured) rather than mineralogy,

which is in agreement with the SEP and DGT-measured labile concentrations described in the previous sections.

Comparing As and Sb uptake by radishes grown in mining soils, both roots and shoots accumulated more As than Sb, despite total soil Sb concentrations being higher. Higher concentrations of As in radish compartments compared to Sb can be explained by the greater amounts of labile As than labile Sb in soils. These results confirm that total soil concentrations are not sufficient to evaluate the bioaccumulation in plants and the potential risk of contaminated soils, and that bioaccumulation is both plant and chemical species specific.

4.3.4.3. Bioaccumulation and translocation factors

The bioaccumulation factor (BAF) defined as the ratio of total concentration in plant roots and the total soil concentration (mg/kg DW) was calculated to evaluate the transfer of As and Sb from soil to radish (Table B.3). The BAF of As in radish roots from diluted cattle dip and mining soils ranged from 0.007 – 0.013 and 0.003 – 0.011, respectively, showing that the average uptake rate of As in the cattle dip soils was 1.6 times higher than the mining soils (Figure 4.3). The BAFs of As were much higher (3.3 – 5.8 times) than that of Sb in radish roots with values ranging from 0.0007 – 0.003. Lower Sb bioaccumulation compared with As has also been reported in the literature (Wilson et al. 2013; Wilson et al. 2014). A recent study on the biogeochemical behaviour of As and Sb in water, soil, and tailings in China also showed that the bioaccumulation factors of Sb were ~10 times less than those of As in plant/tailing and vegetable/soil systems (Fu et al. 2016).

The BAF values suggest that As was transferred from soils to roots more efficiently than Sb. The BAF for As and Sb in this study were lower than the BAF typically reported for As (~0.2) and for Sb (~0.01) reviewed from literature (Wilson et al. 2013). This means that the bioavailability of As and Sb to radish in these test soils was low and the radish studied may have had exclusion mechanisms for As and Sb. The low accumulation of As and Sb in the white icicle radish is consistent with the very low bioavailability of As and Sb (Tables 4.2 and 4.3).

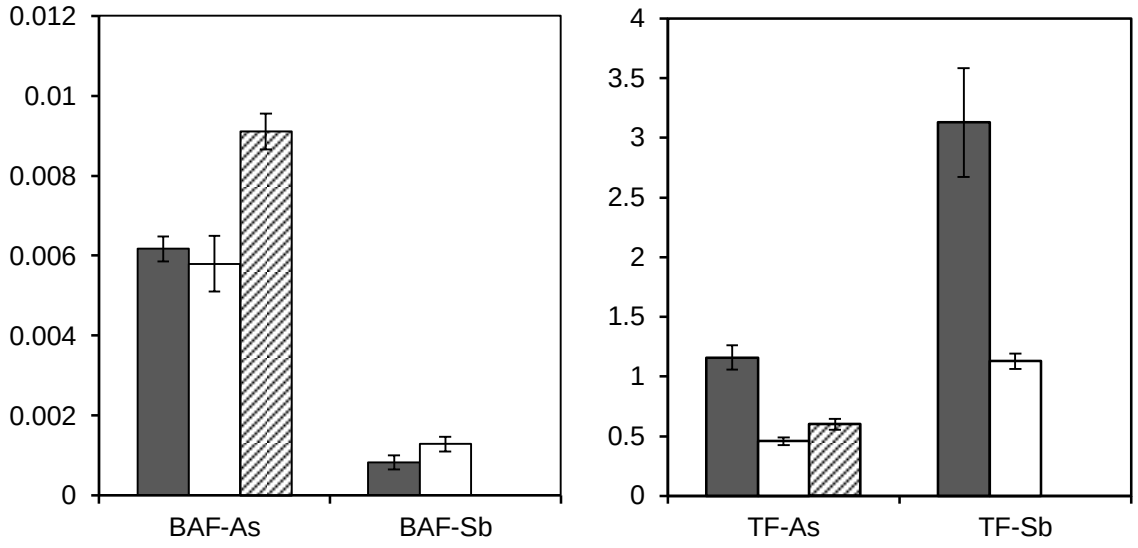

Figure 4.3 Bioaccumulation factors (BAF) and translocation factors (TF) for As and Sb of two cultivars of radish cultivated in various soils. Cherry bell radish – Mining (■) (chapter 3), White icicle radish – Mining (□), White icicle radish – Cattle dip (▨).

The translocation factor (TF) was obtained from the ratio of total concentrations of As and Sb in shoots compared to roots (mg/kg DW). The TF of As for radish from both diluted cattle dip and mining soils were < 1 with values varying between 0.37 – 0.9 and 0.35 – 0.77, respectively (Table B.3). The result illustrates that the translocation capacity is dependent on a given element and plant species, irrespective of soil types. The TF of Sb for radish grown in mining soils ranged from 0.85 – 1.69 being 1.3 – 3.3 times greater than for As. The limited translocation of As from roots to shoots for vegetables was also observed by Smith et al. (2009) and Bhatti et al. (2013). This may be a mechanism of As tolerance in plants, limiting the As movement to plant shoots by reducing root As(V) (the predominant As species in soils) to As(III) and sequestrating the reduced As in roots via phytochelatin (PC) complexation and stabilisation in vacuoles (Smith et al. 2008; Zhao et al. 2010).

The differences in As and Sb distribution in soil and radish compartments suggests different uptake mechanisms, possibly due to structural differences of the most common Sb and As species in soils, antimonate (Sb(V)) being octahedral and arsenate (As(V)) being tetrahedral (similar to phosphate) (Tschan et al., 2009). As a result, arsenate is taken up by roots via the phosphate transporter (Zhao et al., 2010). However, an analogous transport

path does not seem to occur for antimonate, with Tschan et al. (2008) showing that plant uptake was unaffected by phosphate. Nevertheless, the uptake mechanisms of Sb and the species accumulated by terrestrial plants and edible crops are still unknown (Feng et al., 2013; Tshan et al., 2009; Pierart et al., 2015). Thus, further studies on mechanisms of Sb uptake by a variety of plants are needed to fully understand Sb phytoavailability.

4.3.4.4. Comparison of As and Sb uptake by two radish cultivars

With respect to As and Sb uptake by two cultivars of radish grown in soils prepared from the same parent contaminated soil, differences in uptake mechanisms were observed (Figure 4.3). On average, the bioaccumulation factor of As was much higher than that of Sb (7.5 times for cherry belle radish and 4.5 times for white icicle radish). Arsenic was somewhat restricted in the roots, but Sb was translocated from roots to shoots, illustrated by the higher translocation factor of Sb compared to As (2.5 times). The sequestration of As in roots was also observed in the white icicle radish cultivated in cattle dip soils.

In terms of cultivar behaviour, the bioaccumulation factors for As in both radish cultivars were not significantly different. The bioaccumulation factor for Sb in the white radish was 1.5 times higher than that in cherry belle radish, but not statistically significant ($p>0.05$). According to Barber (1995), the uptake of elements by plants is affected by the adsorption area of roots; the larger the adsorption area the higher the effective uptake. The surface area of the taproot in contact with the soils is larger for the white icicle radish. However, significantly higher bioaccumulation factors for As and Sb for the white icicle radish were not evident, indicating the possibility that uptake is through the minor roots. The uptake of the elements by radishes would occur at the apical region, root tips rather than over the entire root surfaces.

Regarding translocation characteristics, the cherry belle radish had a higher capacity to translocate As and Sb from roots to shoots than the white icicle radish by a factor of 2.5 ± 0.2 and 2.8 ± 0.4, respectively. This is most likely due to the greater mean distance required to transport elements for the white icicle radish. The white icicle radish has a very long root, requiring more energy to move elements inside the xylem and plant organs compared to the small bulb of the cherry belle radish. Thus, the white icicle radish had lower efficiency to translocate As and Sb from roots to shoots.

These results confirm that the behaviour of As and Sb in relation to plant uptake was different and that the uptake and distribution trend are dependent on a given element and cultivar, irrespective of contamination sources. Taking the results of bioaccumulation and translocation of As into consideration, both the BAF and TF for As were low (<1) and therefore the white icicle radish can be considered to be an As tolerant plant (Vamerali et al. 2010).

4.3.5. Evaluating the effectiveness of geochemical measurements as predictors of biological uptake

The relationships between As and Sb concentrations in roots and shoots of the white icicle radish and their labile fractions in soils obtained from SEP, DGT, soil solution measurements are presented in Figures 4.4 and 4.5. The As and Sb concentrations in plant tissues were highly positively correlated with all labile As and Sb fractions (R^2 = 0.87 – 0.97 for As in cattle dip soils, R^2 = 0.93 – 0.99 for As in mining soils, and R^2 = 0.92 – 0.98 for Sb in mining soils), which indicates that tissue As and Sb were well predicted by these labile measures. The close relationships between SEP-labile As and Sb and tissue As and Sb indicates that the non-specifically sorbed and specifically sorbed As and Sb are the best predictors of the phytoavailability of As and Sb. The close relationships between plant As and SEP-labile As in this study are supported by the results of Gonzaga et al. (2008) stating that the total of the first two fractions from SEP was closely correlated with As uptake by the *P. vittata* (Chinese brake fern) and Niazi et al. (2011) reporting a positive correlation between exchangeable As from the first extraction and As in *B. juncea* (Indian mustard) shoots.

Arsenic and Sb uptake by the white icicle radish from the test soils had a stronger relationship with DGT-labile As and Sb than their soil solution concentrations, especially from cattle dip soils, which is demonstrated by the higher correlation coefficients for C_{DGT}. This suggests that there was some resupply of As and Sb from the solid phase or from the dissolved complexes of As and Sb in soil solution to sustain the depletion of As and Sb in the rhizophere. From the predictive perspective, DGT exhibited excellent performance in predicting the phytoavailability of As and Sb in the studied soils, compared to soil solution concentration method.

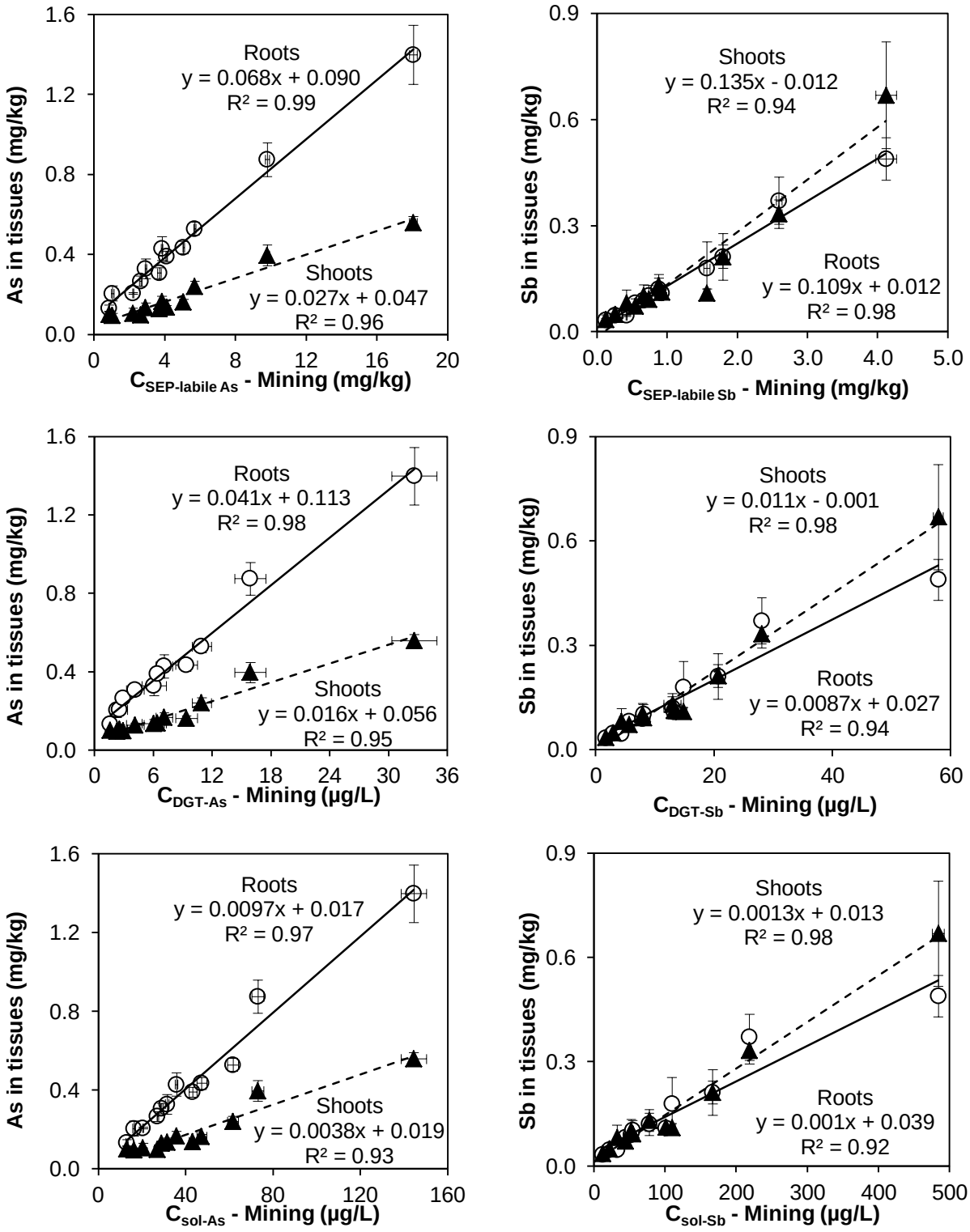

Figure 4.4 The relationship between tissue As and Sb (mg/kg, dry mass) and bioavailable fractions of As and Sb in bioassay mining soils, as measured by SEP, DGT, and soil solution. Shoots (▲), Roots (o). All data have error bars; where they are not seen they are smaller than the symbols.

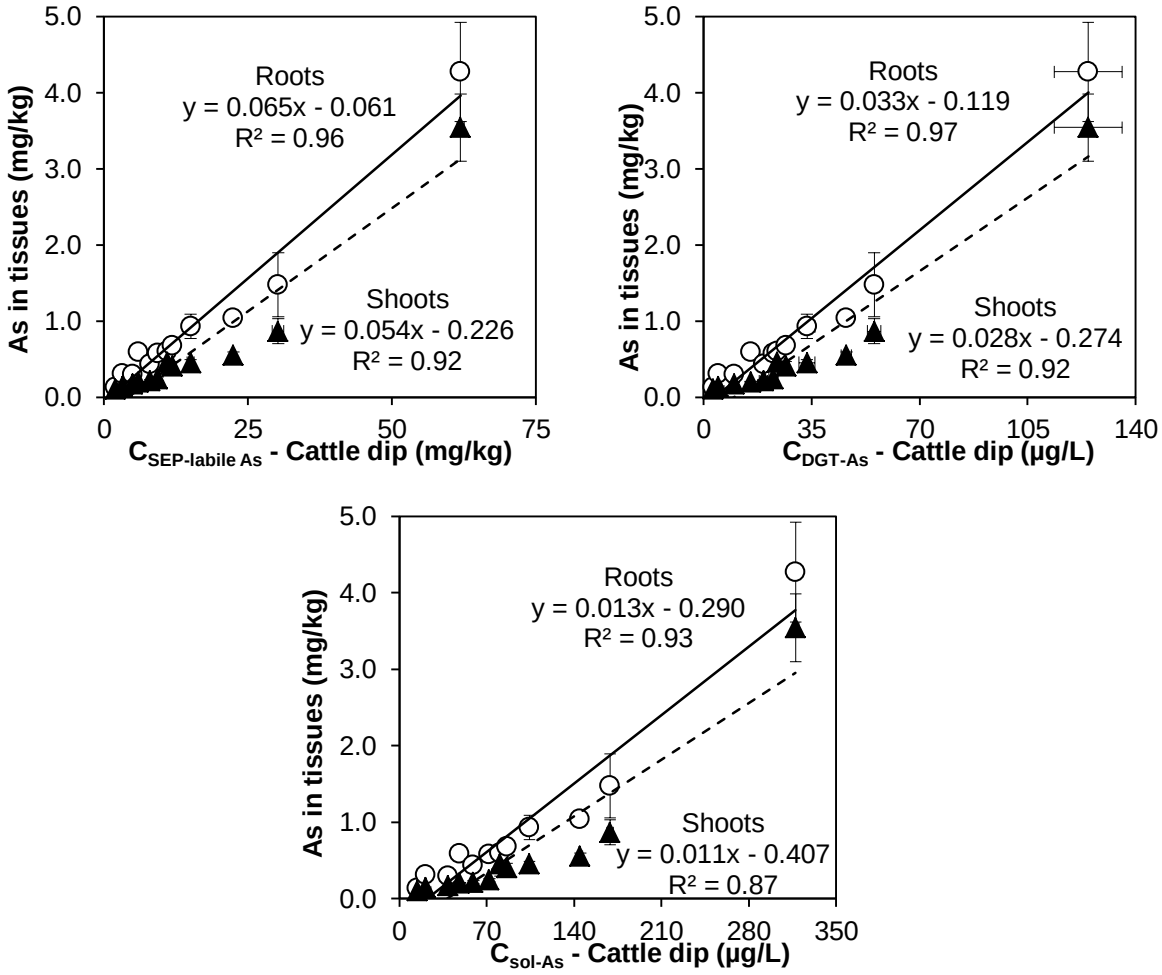

Figure 4.5 The relationship between tissue As (mg/kg, dry mass) and bioavailable fractions of As in bioassay cattle dip soils, as measured by SEP, DGT, and soil solution. Shoots (▲), Roots (o). All data have error bars; where they are not seen they are smaller than the symbols.

The good predictors of DGT are also supported by previous studies showing significantly strong correlations between As concentrations derived from DGT and As concentrations in edible rape from 43 contaminated soils (Wang et al. 2014) and As in rice grain from 39 contaminated soils (Williams et al. 2011). DGT-measured concentrations of Ni from low and high Ni treated soils were also strongly correlated with Ni concentrations in radish (*R. sativus*) grown in those soils (Luo et al. 2014). Recently, Dočekalová et al. (2015) found high correlations between flux to DGT and flux to radish (*R. sativus*) for Cu and Cd.

Better relationships were found for the prediction of As and Sb in roots than in shoots by all bioavailable fractions obtained from SEP, DGT, and soil solution, except the $C_{DGT\text{-}Sb}$ and

$C_{sol\text{-}Sb}$. This is expected because roots may be better indicators for bioavailable metals in soils due to the direct contact with soils, while the metal accumulation by shoots is determined by the translocation processes and governed by plant physiology. This pattern is supported by Nowack et al. (2004) who stated that shoots are not good indicators for bioavailable metals.

Considering As and Sb uptake by two cultivars of *R. sativus* grown in soils, labile measurements were better predictors for As and Sb uptake for cherry belle radish (chapter 3) (greater correlation coefficients) than white icicle radish, with the exception for root Sb predicted by SEP and root As predicted by DGT. This indicates that the capacity of these techniques to predict element uptake by plants is good, but depends on cultivars.

4.4. Conclusions

This study showed that As and Sb exhibited contrasting behaviour in soils and white icicle radish (*R. sativus*). Sb was most likely dominantly associated with resistant minerals, silicates, and organic matter, while As was mainly associated with Fe oxides, irrespective of contamination sources. Sb and As bound to these phases were poorly mobile and not DGT-labile. Thus, the bioavailable fractions of As and Sb in historically contaminated soils were low in which Sb was geochemically much less labile than As. The DGT measurements also identified that regardless of soil types, As was partially sustained and more labile than Sb which had virtually no resupply from the solid phase to soil solution. For plant uptake, As was accumulated in the plant tissues more than Sb, but translocated less to shoots than Sb. The white icicle radish exhibited the same uptake trend as the cherry belle radish in our previous study, but was less able to translocate As and Sb from roots to shoots than the cherry belle cultivar. These results suggest that the *R. sativus* uptake was clearly element and cultivar-specific.

This study demonstrated that SEP, DGT, and soil solution were good approaches in measuring the bioavailable As and Sb in historically contaminated soils and predicting their uptake by root vegetables, in which DGT had better performance than soil solution concentration method. Further studies are needed to identify if these observations are consistent with leafy vegetables and other soil types.

Chapter 5. Assessment of arsenic and antimony lability in historically co-contaminated soils and their uptake by water spinach (*Ipomoea aquatica*)

5.1. Introduction

The lability and mobility of As and Sb is governed by oxides of Fe, Al, Mn, clay, and organic matter (OM) via adsorption which is a pH-dependent process. These adsorbents are efficient in the immobilisation of As and Sb in mining soils, especially soils with lower pH and higher redox values (García-Sanchez et al. 2002; Juhasz et al. 2008; Mitsunobu et al. 2006). The availability of As can be reduced when oxide concentrations of Fe, Al, Mn, and OM increase (De Oliveira et al. 2015). The retention of antimonate by OM has also been observed in recent years (Clemente et al. 2008; Dousova et al. 2015), with humic acids having a high capacity to complex and bind antimonate trapped in the soil organic layer in a Sb-contaminated shooting range (Steely et al. 2007).

The lability of metal(loid)s is closely related to their accumulation in many plants (McLaughlin et al. 2011). To our knowledge, most of the research on metal(loid) uptake by plants has been conducted with freshly contaminated or spiked soils. However, this approach has some limitations since the system is unlikely to be in equilibrium. The distribution of metal(loid)s in spiked soils changes from more labile to less labile forms with time and metal(loid)s are more labile in spiked soils than in field contaminated soils (Liang et al. 2014), leading to an overestimate of their toxicity in spiked soils. Thus, the assessment of risks posed by historic contamination is important. In addition, these investigations have focused on responses to a single contaminant, despite very few environments containing only one contaminant. Thus, research conducted with a historically contaminated soil containing a mixture of contaminants is realistic, ecologically and environmentally relevant, and important for reliable risk assessment.

To study the lability of As and Sb and to determine their phytoavailable fractions in contaminated soils, sequential extraction and diffusive gradients in thin films (DGT) have been applied. Recently, the combination of sequential extraction and DGT was successful

in assessing labile As and Sb from contaminated soils to two cultivars of radish (*Raphanus sativus*) (chapters 3 and 4). The performance of DGT in predicting As and Sb uptake by leafy vegetables has not been conducted.

Water spinach (*Ipomoea aquatica*) is used as a popular edible leafy vegetable and herbal plant in some regions of the world, especially Asia. It has fibrous roots, hollow stems, and wide arrowhead shaped leaves. The soft stems and leafy portions are edible and rich in important vitamins, minerals, nutrients, and fiber, which is popular for daily food (Göthberg et al. 2002). *I. aquatica* has been shown to be a Cd, Cu, Pb, and Cr accumulator plant (Bedabati Chanu & Gupta 2016; Göthberg et al. 2002; Weerasinghe et al. 2008). The uptake of As and Sb by *I. aquatica* and the risks associated with consumption of *I. aquatica* grown on historically contaminated sites have not been evaluated adequately.

In this study a combination of selective DGT and sequential extraction measurements was applied to assess the lability of As and Sb in a historically co-contaminated soil, and compared to their uptake by water spinach (*Ipomoea aquatica*), a popular edible leafy vegetable in some regions of the world, especially Asia. The aims were to investigate (1) the biogeochemical behaviour of As and Sb in a soil series prepared by mixing a soil historically contaminated with both As and Sb and three uncontaminated soils with low iron (L), medium iron (M), and high iron (H); (2) the bioaccumulation of As and Sb in *I. aquatica* grown in these test soils; (3) the ability of sequential extraction and DGT technique to predict As and Sb uptake by *I. aquatica*; and (4) the risks to human health due to consumption of As-enriched edible shoots of *I. aquatica*. This study will provide a better understanding of the behaviour of As and Sb in historically co-contaminated soils and determine the extent of As and Sb accumulation by water spinach (*Ipomoea aquatica*).

5.2. Methods

5.2.1. General methods

General washing methods were done as per section 2.1.

5.2.2. Experimental design

Soil contaminated with both As and Sb was collected from a decommissioned antimony processing facility in Urunga NSW, Australia. Details about soil compositions and physical

properties were described previously (Ngo et al. 2016). Uncontaminated soils were obtained from three sites in Wollongong, NSW, with soil-L having the lowest iron and highest pH values, soil-M containing medium iron content, and soil-H having high iron content. All soils were air-dried and crushed to pass through a 2 mm sieve. The contaminated soil was thoroughly mixed with each uncontaminated soil (control soil) at different ratios from 1:150 to 1:0.5 to establish three treatment series of test soils with increasing As and Sb concentrations. Soil moisture was raised to and maintained at ~60% of the water holding capacity (WHC) with deionised water. Soils were allowed to settle and equilibrate in a greenhouse for two weeks prior to the commencement of bioassays.

The 35-day bioassays with *I. aquatica* were conducted in controlled growth chambers as per section 2.4.2. Harvested plant tissues from the same pot were combined, weighed, and oven-dried at 70 °C for 48 h to a constant weight with the dry mass (g) recorded. Dried tissues were ground with a mortar and pestle and stored in clean plastic bags prior to As and Sb analyses.

5.2.3. Soil characterisation

Soil properties including particle size, pH, organic matter, total nitrogen, available phosphorus, and amorphous and free Fe and Al were determined as described in section 2.5.

5.2.4. Soil and plant analyses

Finely ground soil (~0.2 – 0.3 g, dry mass) was digested with aqua regia in a closed microwave digestion system at 120 °C (10 min ramp, 15 min hold) and then 190 °C (5 min ramp, 15 min hold). Finely ground plant (~0.2 – 0.3 g, dry mass) was microwave digested with a mixture of HNO_3:H_2O_2 (1:2, v/v) (65% and 30%, respectively) at 110 °C (10 min ramp, 15 min hold) and then 180 °C (5 min ramp, 15 min hold). After cooling to room temperature, digests were filtered (<0.45 µm) and diluted to 2% acid with deionised water for analyses. Total As and Sb in soils and plants were analysed by ICP-MS as described in section 2.9.

For quality control, a combination of reagent blanks, duplicate analyses, certified reference materials, and matrix spike recovery were used. A soil certified reference material

(Montana II soil, SRM 2711a; NIST) returned recoveries of 90-115% for the studied analytes. A plant certified reference material (Tomato leaves, SRM 1573a; NIST) had typical recoveries of 95-110% for the studied analytes.

5.2.5. Sequential extraction procedure (SEP)

The procedure of Wenzel et al. (2001) was used to determine As and Sb associated with non-specifically sorbed, specifically sorbed, amorphous Fe and Al oxide, crystalline Fe and Al oxide, and residual fractions as described in section 2.6.1.

5.2.6. DGT and soil solution measurements

DGT and soil solution measurements were conducted as described in sections 2.6.2 and 2.7. The DIFS (DGT induced fluxes in soils) model was applied to predict (de)sorption kinetic parameters for soils (Ernstberger et al. 2002; Ernstberger et al. 2005; Harper et al. 2000; Zhang et al. 2004). The reponse time (T_c) of desorption process was obtained using the distribution coefficient (K_d) and R as input parameters. The rate constant of resupply from the solid phase (k_{-1}) was calculated from K_d, P_c (soil particle concentration), and T_c using Equation 5.1.

$$k_{-1} = 1/((1 + K_d P_c)T_c) \qquad \text{Equation 5.1}$$

5.2.7. Data analysis

Statistical analysis and calculations of bioaccumulation factor (BAF) and translocation factor (TF) were performed as per section 3.2.7. To assess the risk associated with the As and Sb calculated exposure, the average exposure was compared with the toxicological reference dose. Both non-carcinogenic and carcinogenic risks to humans were evaluated based on the formula obtained from Environmental Health Australia - enHealth (2012) and USEPA (2011). The risk of non-carcinogenic effect was assessed by the hazard quotient (HQ) (Equation 5.2) being the ratio of estimated daily intake (EDI) to the reference dose (RfD).

$$HQ = EDI/RfD \qquad \text{Equation 5.2}$$

where EDI and RfD represent the estimated daily intake of As/Sb (mg/kg/day) and the reference dose for As/Sb, respectively. The RfD for As and Sb are 0.0003 mg/kg/day and

0.0004 mg/kg/day suggested by USEPA (1991, 1998) and applied previously (Khan et al. 2014; Li et al. 2014; Rehman et al. 2016). An HQ < 1 defines no potential risk to the exposed humans (Environmental Health Australia - enHealth 2012; USEPA 1998, 2011). The EDI was calculated according to Equation 5.3.

$$EDI = (C \times IR \times EF \times ED)/(BW \times LE) \quad \text{Equation 5.3}$$

where C is the concentration of As in edible parts (shoots) of the plant (mg/kg wet weight, WW); IR is the ingestion rate of edible part (kg/person/day); EF is exposure frequency (days/year); ED is exposure duration (years, typically 70 years for an individual); BW is body weight (kg, 60 for adult and 24.5 for child); LE is life expectancy (days, 25550) (Rehman et al. 2016). The cancer risk (CR) was calculated by the multiplication of the estimated daily intake of As (mg/kg/day) to the cancer slope factor (CSF) according to Equation 5.4.

$$CR = EDI \times CSF \quad \text{Equation 5.4}$$

The CSF $(mg/kg/day)^{-1}$ for As for adult and child have been set to 1.5 and 4.5, respectively (USEPA 1998, 2011). The CR is defined as the probability of an individual suffering cancer risk over a lifetime. For instance, a CR of 1×10^{-4} indicates a probability of 1 in 10,000 individuals developing cancer. A range of 1 in 10,000 to 1 in 1,000,000 is considered acceptable As cancer risk (Environmental Health Australia - enHealth 2012; Uddh-Söderberg et al. 2015; USEPA 1998, 2011).

5.3. Results and discussion

5.3.1. Soil characteristics

The average physicochemical characteristics of each series of test soils are presented in Table 5.1. The soils were all sandy-silts that were slightly acidic, with the pH of soil-L being 1 unit higher than the other two soils. In terms of nutrients (N and P), soil-L was higher in P and lower in N compared to soil-M and soil-H. The capacity of soil to retain As and Sb is highly correlated with oxides of Fe and Al, and organic matter (OM). The soils were characterised by their mineral contents, with soil-L being low in Fe and Al, soil-M having medium Fe and Al, and soil-H having the highest amounts of Fe and Al. There was a similar trend in OM and dissolved organic carbon (DOC) with soil-L lower in OM and

DOC than soil-M and soil-H. Based on their characteristics, soil-L is expected to have the highest available As and Sb because of low Fe, Al, OM and high pH, followed by soil-M and soil-H.

The prepared soils covered a wide range of total contaminant concentrations, from low to very high (42 – 4180 mg As/kg and 52 – 6610 mg Sb/kg, Table 5.2), which were all above average concentrations in non-contaminated soils (<10 mg/kg), but within the range of total As and Sb found in mining and smelting-impacted soils around the world (Wilson et al. 2010). This allowed the availability of As and Sb and their uptake by plants under various conditions of soil properties and total metalloid concentrations to be assessed. The high soil concentrations of As and Sb prepared were based on our pilot study showing that *I. aquatica* grown in highly co-contaminated soils (>1000 mg/kg of As and Sb) did not exhibit growth reduction and toxicity symptoms. Thus, the largest concentrations of each soil type were estimated to be high enough for the plant growth reduction and toxicity to be detectable.

Table 5.1 Physical and chemical properties of bioassay soils (dry mass, mean ± SE, n≥3).

Characteristic	Soil-L (S1 – S5)	Soil-M (S6 – S10)	Soil-H (S11 – S15)
Sand (%)	30 ± 3	31 ± 1	33 ± 3
Silt (%)	63 ± 2	61.0 ± 0.5	60 ± 3
Clay (%)	7.0 ± 0.5	8.0 ± 0.4	7.0 ± 0.4
pH	6.5 ± 0.2	5.1 ± 0.2	5.3 ± 0.2
Organic matter (%)	6.75 ± 0.05	7.3 ± 0.3	7.6 ± 0.3
Extractable Phosphorous (mg/kg)	50 ± 20	34 ± 4	30 ± 9
Total Kjeldahl Nitrogen (mg/kg)	120 ± 30	280 ± 60	240 ± 20
Amorphous Fe (g/kg)	7.4 ± 0.3	7.4 ± 0.2	7.3 ± 0.4
Amorphous Al (g/kg)	0.55 ± 0.06	1.79 ± 0.09	2.2 ± 0.1
Free Fe (g/kg)	18.2 ± 0.6	25.0 ± 0.6	30 ± 1
Free Al (g/kg)	1.01 ± 0.08	2.0 ± 0.1	3.1 ± 0.2

Table 5.2 Total concentrations in a concentration gradient obtained from historically contaminated soils, R values, K_d (distribution coefficients, L/kg), and kinetic parameters including T_c (the response time of the desorption process, s), and k_{-1} (the desorption rate constant, s^{-1}) obtained from DIFS of As and Sb (mean ± SE, n = 3).

Soil	Treatment	As					Sb				
		$Total_{As}$ (mg/kg)	Ratio $R_{As}=C_{DGT}/C_{sol}$	K_d (L/kg)	T_c (s)	k_{-1} (10^{-6} s^{-1})	$Total_{Sb}$ (mg/kg)	Ratio $R_{Sb}=C_{DGT}/C_{sol}$	K_d (L/kg)	T_c (s)	k_{-1} (10^{-6} s^{-1})
L	S1	42.1 ± 0.2	0.65 ± 0.01	659	88	7.46	52 ± 6	0.1173 ± 0.0003	17	11170	2.76
	S2	160 ± 30	0.57 ± 0.06	707	182	3.48	200 ± 30	0.20 ± 0.02	24	2473	7.49
	S3	270 ± 9	0.60 ± 0.02	1010	149	2.87	330 ± 30	0.189 ± 0.001	28	3472	4.35
	S4	670 ± 100	0.682 ± 0.001	830	77	6.86	870 ± 170	0.21 ± 0.01	32	2138	6.37
	S5	1400 ± 50	0.609 ± 0.002	419	109	9.46	2277 ± 130	0.214 ± 0.001	35	2130	5.69
M	S6	65 ± 3	0.36 ± 0.04	486	820	1.12	90.7 ± 0.6	0.137 ± 0.006	20	7877	2.78
	S7	176 ± 2	0.46 ± 0.03	569	376	2.11	220 ± 30	0.166 ± 0.006	28	5125	3.10
	S8	330 ± 20	0.32 ± 0.01	327	1112	1.23	530 ± 180	0.187 ± 0.004	33	3827	3.53
	S9	950 ± 50	0.40 ± 0.03	295	577	2.65	1190 ± 60	0.189 ± 0.008	40	3872	2.92
	S10	2630 ± 90	-	-	-	-	3790 ± 180	-	-	-	-
H	S11	101 ± 8	0.39 ± 0.03	639	595	1.14	140 ± 8	0.143 ± 0.008	24	6975	2.55
	S12	200 ± 30	0.41 ± 0.03	498	512	1.64	230 ± 30	0.172 ± 0.007	26	4186	3.85
	S13	510 ± 30	0.38 ± 0.01	479	614	1.43	670 ± 70	0.183 ± 0.005	39	3817	2.79
	S14	1280 ± 40	0.48 ± 0.01	351	256	4.28	1600 ± 60	0.22 ± 0.01	41	1720	5.42
	S15	4180 ± 160	-	-	-	-	6610 ± 460	-	-	-	-

-: not measured due to analysis problems

5.3.2. Sequential extraction

The concentrations and proportions of As and Sb associated with different soil binding phases are presented in Table C.1 and Figure 5.1. Generally, the amounts of As and Sb extracted in each fraction increased with increasing soil concentrations. In all three soil types, As was mainly bound to the less labile fractions of the soil, amorphous Fe and Al oxides (38-45%), followed by crystalline Fe and Al oxides (33-40%). The residual As accounted for 10 – 24% of total As in all three soils. The available As (sum of non-specifically sorbed and specifically sorbed fractions) in soil-L (8.47% of total As) was significantly higher ($p<0.05$) than soil-M and soil-H (6.68 and 5.93%, respectively). Although the proportion of available As was quite low, it plays an important role in soil biogeochemical processes since they are the most mobile forms of As, especially in soils with high total As concentrations (Wenzel et al. 2001).

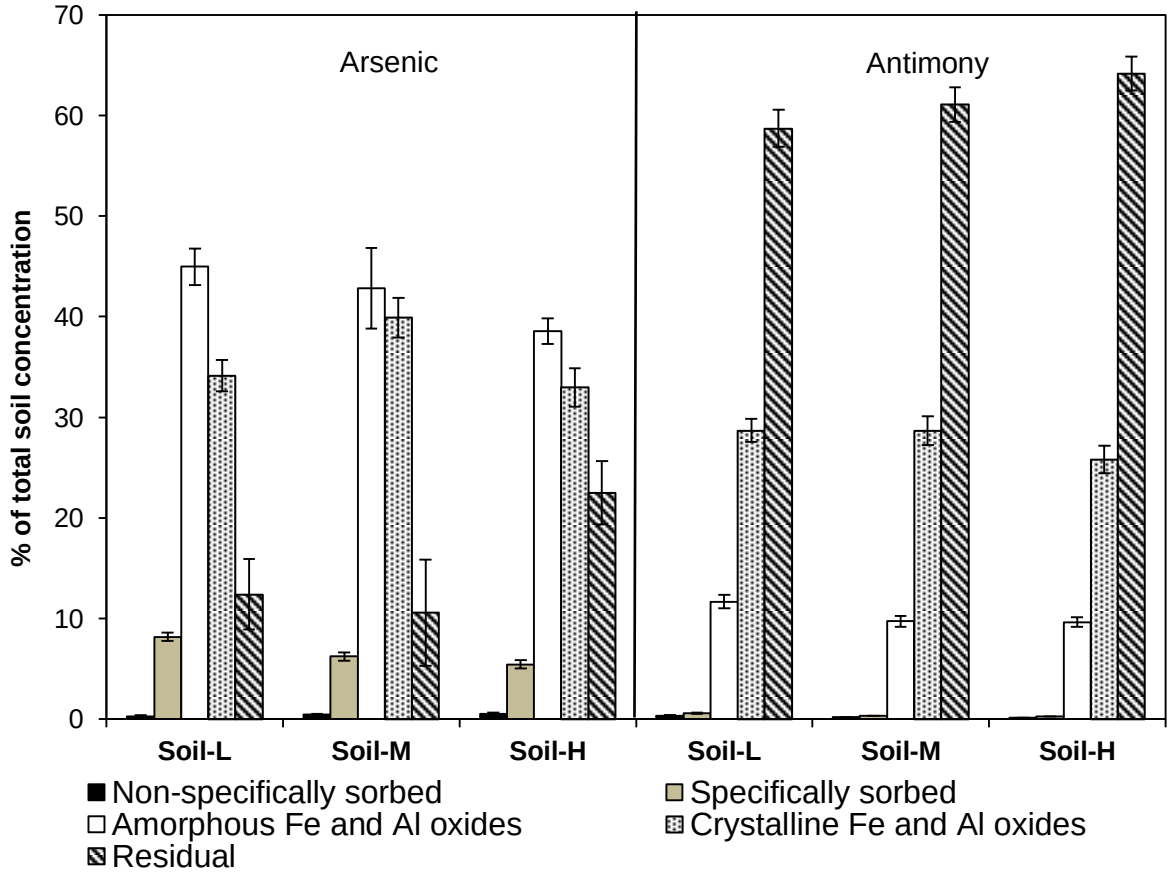

Figure 5.1 The percentage of As and Sb associated with different binding fractions for bioassay soils (mean of all concentrations for each soil, mean ± SE, n = 15).

Unlike As, Sb was primarily found in the residual fraction, representing 59-64% of total soil Sb, most likely as stibnite, which is the mineral typically mined (Álvarez-Ayuso et al. 2013). The Sb associated with crystalline (26-29% of total Sb) and amorphous (9.6-11.7%) Fe and Al oxides were the second and third most dominant phases. The high amount of Sb in the last three SEP fractions and the similar proportion within each fraction among all soil types (Figure 5.1) indicates that the partitioning of Sb in these fractions was not affected by soil Fe, Al, and organic matter from the different diluent control soils. The available Sb in soil-L (0.91%) was significantly higher ($p<0.05$) than soil-M (0.51%) and soil-H (0.41%). The very low proportion of Sb in available fraction (<1%) indicates that Sb was biogeochemically immobile and strongly bound to the soil matrix at all concentrations.

It is clear that both the available As and Sb in soil-L were significantly higher ($p<0.05$) than soil-M ~ soil-H, in which the As availability was much higher than that of Sb, even though total soil As was lower than total soil Sb. As expected, soil-L with the lowest concentrations of Fe, Al, and organic matter and the highest pH consequently had the highest available As and Sb. Soil-H with the highest amounts of Fe, Al, and organic matter and the lower pH had the lowest availability of As and Sb. This can be explained by the literature stating that As and Sb in soils were significantly sorbed by oxides of Fe and Al, organic matter under acidic conditions. This adsorption was reduced with increasing pH (Wilson et al. 2010). For example, in four Australian soils with pH ranging from 4.97 to 6.9, arsenate sorption was greater than arsenite and decreased as pH increased (Smith and Huyck 1999, cited in Wilson et al. 2010). The sorption of arsenate (Thanabalasingam & Pickering 1986) and antimonate (Tighe et al. 2005b) by humic acid was significant at around pH 5.5 and 4, respectively. Soils with a pH above 6 have less protonation of phenolic, carboxylic, and hydroxyl-carboxylic groups on particulate surfaces and a greater negative charge density on mineral surfaces, leading to the repulsion of As and Sb from binding sites and the release of As and Sb oxyanions (De Oliveira et al. 2015; Dousova et al. 2015). In addition, the greater phosphorous in soil-L may increase available As in soil solution due to competition of phosphate and arsenate binding on soil surfaces. The effect of phosphate on available Sb in soil is still unclear.

These observations are supported by previous studies on the bioavailability and fractionation of As in the whole soils. For example, Gonzaga et al. (2008) found that the amount of available As in a mining soil with 1626 mg Fe/kg was about six times lower than in a soil containing 509 mg Fe/kg. Juhasz et al. (2008) reported that soil with higher Fe and lower pH reduced As availability by 75% upon 12 months of aging. Wang et al. (2015) also stated that soil with pH 5.39 and total free Fe of 10.3 mg/kg had higher labile As fractions initially and one-year after spiking with 100 mgAs/kg than soil with pH 4.94 and total free Fe of 38.4 mg/kg.

The differences between As and Sb chemical fractionation and lability may be related to the differences in their pentavalent oxyanions structures, where arsenate is tetrahedral whereas antimonate is octahedral (Okkenhaug et al. 2012; Wilson et al. 2010). Arsenate has higher charge density and a smaller ionic radius (0.40), while antimonate has lower charge density and larger ionic radius (0.51), which results in a larger spatial structure for antimonate. As a result, As was more efficiently bound to active binding sites (e.g. metal oxides), explaining the higher proportions of As in Fe oxide fractions compared to Sb. The mineral solubility of As and Sb may also explain the differences in their lability. In mining soils, antimonate is primarily calcium antimonate (K_{sp} $10^{-12.55}$), while arsenate are mainly iron and aluminium arsenate (K_{sp} 10^{-11}) and calcium arsenate (K_{sp} 10^{-5}). Antimonite is possibly present as stibnite (K_{sp} 1.6×10^{-93}), whereas arsenite is possibly present as orpiment, As_2S_3 (K_{sp} 8.4×10^{-16}) (Arai 2010; Okkenhaug et al. 2011). The minerals of Sb have lower solubilities, which would contribute to the lower Sb availability. The fact that As and Sb existed together in the test soils may lead to an interaction and competition for binding sites which differed from previous studies based on single-contaminated soil. The mechanisms for As-Sb competition for soil binding sites are still unknown.

5.3.3. DGT measurements

The total soil concentrations, soil solution concentrations (C_{sol}), DGT-labile concentrations (C_{DGT}), and R values for As and Sb are presented in Tables C.2 and 5.2. C_{sol} and C_{DGT} of As and Sb increased with increasing total soil concentrations, but accounted for only a small proportion of total soil concentrations (0.004 – 0.02% for As, 0.002 – 0.04% for Sb). The soils had a low lability of As and Sb, possibly due to the aging of the parent

contaminated soil and the stability of complexes of As and Sb and solid phases of soils (Liang et al. 2014). As discussed in section 3.3.3, the R value ($R=C_{DGT}/C_{sol}$, $0< R <1$) reflects the ability of soils to resupply a solute from the solid phase to soil solution; the higher the R value, the greater the resupply rate. The average R values for As for three soil types ranged from 0.38 – 0.62, indicating that As was partially resupplied from soils but was not sufficient to sustain constant pore water concentration. The average R value of soil-L (0.62 ± 0.02) was significantly higher ($p<0.05$) than soil-M (0.38 ± 0.03) and soil-H (0.41 ± 0.02) (Table 5.2). The highest resupply of As in soil-L probably resulted from its lowest Fe, DOC, and highest pH because the desorption of As may be favoured under these conditions as discussed in section 5.3.2. According to Williams et al. (2011), DOC and DOC-Fe complexes can bind As and reduce the dissolved-As concentration. Thus, higher Fe and DOC in soil-M and soil-L (Table 5.1) possibly led to more As in complex forms with DOC or DOC-Fe complexes, which would be unavailable for DGT. The resupply of As followed the trend of soil-L > soil-M ~ soil-H, which is consistent with the trend of $C_{SEP\text{-}labile\ As}$.

For Sb, the average R values among soil types were relatively constant, ranging from 0.17-0.19. This means that the resupply of Sb from the solid phases to soil solution was lower than that of As, despite total soil Sb concentrations being higher than that of As. The relatively unchanged R values of Sb across soil types (varying soil properties) also support the conclusion that Sb was less labile and strongly bound to the solid phase.

DGT induced fluxes in soils (DIFS) was used to simulate the dynamic characteristics of As and Sb in soils using R and K_d (the distribution coefficients). The values of T_c (the response time of the resupply process) derived from DIFS model and K_d were used to calculate desorption rate constant (k_{-1}) as per Harper et al. (2000), Zhang et al. (2004), and Ernstberger et al. (2005). The calculated values of K_d, T_c, and k_{-1} are presented in Table 5.2. On average, for As, soil-L had significantly higher values of K_d, k_{-1} and lower values of T_c ($p<0.05$) than soil-M and soil-H. There were no significant differences in these average values between soil-M and soil-H. This indicates that soil-L had a higher labile pool of As in the solid phase, a higher rate constant of the As resupply process from the solid phase to soil solution, and shorter response time to the depletion of As than the other

two soils. Consequently, the release potential and kinetics of available As from the solid phase was highest in soil-L, followed by soil-M and soil-H. This is in agreement with the highest values of R_{As} and greatest $C_{SEP\text{-}labile\ As}$ in soil-M. For Sb, the average values of K_d, k_{-1}, and T_c in three soils were not significantly different ($p>0.05$), which is consistent with the trend of R values. This suggests that the kinetic release of Sb was not affected by soil types. Compared to As, Sb had significantly lower K_d and k_{-1} and higher T_c ($p<0.05$), suggesting the lower release and resupply of Sb from the solid phase to soil solution. This agrees with the lower R values for Sb and $C_{SEP\text{-}labile\ Sb.}$

The resupply of As and Sb were consistent with the previous studies on prediction of bioavailable of As and Sb in historically contaminated soils to plants (chapter 3 and 4) where As was partially resupplied, and the replenishment of Sb was minimal. Arsenic and Sb co-existed in test soils, thus competitive interactions for complex ligands in soil solutions and soil binding sites may occur, and this should be considered when comparing these results with single element studies.

5.3.4. Uptake of As and Sb by water spinach (*Ipomoea aquatica*)

5.3.4.1. Plant yield

The *I. aquatica* dried tissue masses are presented in Table 5.3. The root and shoot dry mass varied widely with increasing soil concentrations, irrespective of soil types, ranging from 0.28 – 0.9 g roots/pot and 0.4 – 4.5 g shoots/pot. The most significant decrease (size and area of leaves, numbers of leaves, root length and shoot height) occurred at the highest treatment of each soil type, but toxicity symptoms such as chlorosis and wilting were not observed during plant growth. *I. aquatica* responses in this study are supported by Shaibur et al. (2009), who observed the dramatic reduction of root and shoot length, and leaf area of *I. aquatica* grown hydroponically at 50 µM As treatment (equivalent to 3750 µg As/L).

Table 5.3 Tissue mass, As and Sb concentrations, bioaccumulation factor (BAF) and translocation factor (TF) for As and Sb in *I. aquatica* exposed to contaminated soils for 35 days (mean ± SE, n = 3).

Soil	Treatment	As				Sb				Root Dry weight (g)	Shoot Dry weight (g)
		Root As (mg/kg)	Shoot As (mg/kg)	BAF - As	TF - As	Root Sb (mg/kg)	Shoot Sb (mg/kg)	BAF - Sb	TF - Sb		
L	S1	40 ± 2	3.8 ± 0.4	0.96 ± 0.04	0.094 ± 0.007	0.84 ± 0.09	0.66 ± 0.03	0.016 ± 0.002	0.8 ± 0.1	0.6 ± 0.1	4.4 ± 0.9
	S2	107 ± 8	11.7 ± 0.6	0.66 ± 0.05	0.11 ± 0.01	3.0 ± 0.2	0.5 ± 0.1	0.015 ± 0.001	0.15 ± 0.05	0.55 ± 0.09	3.1 ± 0.5
	S3	360 ± 40	42 ± 4	1.4 ± 0.2	0.12 ± 0.03	4.6 ± 0.5	1.8 ± 0.6	0.014 ± 0.001	0.4 ± 0.1	0.9 ± 0.1	4.5 ± 0.3
	S4	700 ± 100	51 ± 4	1.0 ± 0.2	0.09 ± 0.02	15 ± 3	1.32 ± 0.04	0.018 ± 0.003	0.09 ± 0.01	0.6 ± 0.1	2.0 ± 0.3
	S5	2600 ± 200	126 ± 4	1.8 ± 0.1	0.049 ± 0.003	80 ± 20	10 ± 2	0.035 ± 0.008	0.14 ± 0.03	0.28 ± 0.08	1.41 ± 0.05
M	S6	41 ± 2	4.4 ± 0.2	0.63 ± 0.03	0.107 ± 0.005	1.5 ± 0.1	0.4 ± 0.1	0.017 ± 0.001	0.29 ± 0.06	0.83 ± 0.08	3.3 ± 0.4
	S7	170 ± 4	16 ± 3	0.96 ± 0.02	0.09 ± 0.02	4.0 ± 0.2	0.35 ± 0.02	0.018 ± 0.001	0.09 ± 0.01	0.9 ± 0.2	3.9 ± 0.8
	S8	610 ± 10	36 ± 6	1.84 ± 0.03	0.06 ± 0.01	11 ± 1	1.5 ± 0.2	0.021 ± 0.002	0.13 ± 0.01	0.57 ± 0.05	2.8 ± 0.2
	S9	1500 ± 100	73 ± 4	1.56 ± 0.08	0.050 ± 0.004	41 ± 5	2.3 ± 0.2	0.035 ± 0.004	0.06 ± 0.01	0.40 ± 0.05	1.3 ± 0.1
	S10	-	-	-	-	-	-	-	-	-	-
H	S11	102 ± 6	5.6 ± 0.8	1.01 ± 0.06	0.054 ± 0.005	3.43 ± 0.07	0.29 ± 0.02	0.0238 ± 0.0005	0.09 ± 0.01	0.90 ± 0.09	3.9 ± 0.7
	S12	240 ± 30	15 ± 2	1.2 ± 0.2	0.063 ± 0.005	5.7 ± 0.4	0.54 ± 0.04	0.025 ± 0.002	0.10 ± 0.01	0.621 ± 0.001	3.0 ± 0.5
	S13	580 ± 100	47 ± 2	1.1 ± 0.2	0.09 ± 0.02	11.6 ± 0.6	1.15 ± 0.04	0.017 ± 0.001	0.100 ± 0.002	0.7 ± 0.1	2.9 ± 0.6
	S14	1860 ± 200	89 ± 6	1.5 ± 0.1	0.048 ± 0.002	51 ± 4	1.9 ± 0.3	0.032 ± 0.002	0.04 ± 0.01	0.56 ± 0.08	1.4 ± 0.2
	S15	-	-	-	-	-	-	-	-	-	-

-: not measured for evaluating As/Sb uptake due to damaged roots and plant death

5.3.4.2. Accumulation of As and Sb in *I. aquatica* tissues

As and Sb concentrations in *I. aquatica* tissues increased with increasing soil concentrations, regardless of soil types (Table 5.3), and were significantly higher in roots than shoots. The As accumulated in *I. aquatica* roots ranged from 40 – 2600 mg/kg, which was 14 times higher than the shoot concentrations (3.8 – 126 mg/kg). A similar uptake trend occurred for Sb in which roots (0.84 – 80 mg/kg) concentrated 10 times more Sb than shoots (0.54 – 10 mg/kg). It is possible that root bioaccumulation is a detoxification mechanism for *I. aquatica* to respond to metalloid exposure. Shaibur et al. (2009) also found 10-15 times greater As in roots (than stems and leaves) of *I. aquatica* cultivated hydroponically. There is no known data in the literature for Sb uptake by *I. aquatica*. Arsenic concentrations in edible parts of plants in this study were much higher than that of plants grown in non-contaminated soils (<2 mg As/kg) which is also the reference value for non-contaminated plants in the literature (Kabata-Pendias 2011). In the present study, when *I. aquatica* were simultaneously exposed to soil concentrations of ≥160 mg As/kg (equivalent to $C_{sol\text{-}As}$ ≥18.5 µg/L and $C_{DGT\text{-}As}$ ≥10.4 µg/L) and ≥200 mg Sb/kg (equivalent to $C_{sol\text{-}Sb}$ ≥71 µg/L and $C_{DGT\text{-}Sb}$ ≥14 µg/L), As concentrations were ≥11.7 mg/kg dry weight (DW) in shoots, corresponding to 1.17 mg/kg wet weight (WW) in edible shoots (based on the measured average moisture content of 90%). This exceeds the permissible value of As in foodstuffs, specific to cereals being 1 mg As/kg WW set by the Food Standard Agency of Australia and New Zealand (FSANZ 2013). Although *I. aquatica* accumulated high concentrations of As in edible shoots, no visible toxicity symptoms in those treatments were observed. This phenomenon is a concern which poses a risk to people consuming *I. aquatica* grown in As and Sb contaminated soils. This indicates that the highly As-contaminated soil and underground water in some Asian regions pose significant risk and not suitable for cultivating water spinach (*I. aquatica*). There is no guideline value for Sb in foodstuffs.

The bioaccumulation factors (BAF) and translocation factors (TF) were also determined to evaluate As and Sb uptake by *I. aquatica*. The bioaccumulation factor (BAF) is based on the ratio of total root concentrations and total soil concentrations (mg/kg DW) and is an index used to assess the transfer of contaminants from soils to plant roots, and if >1 it is

defined as an accumulator (Vithanage et al. 2012). A higher BAF means a greater efficiency of plants to accumulate contaminants from soils. The BAF of As and Sb in roots of *I. aquatica* ranged from 0.63 – 1.84 and 0.014 – 0.035, respectively (Table 5.3). On average, the BAF of As was much higher (55.7 times) than Sb, indicating that As was more efficiently transferred from soils to *I. aquatica* roots than Sb. Greater As bioaccumulation in *I. aquatica* compared to Sb is consistent with the higher measured lability of As in soils. There was no significant difference ($p>0.05$) in the average BAF values for As (1.15, 1.27, and 1.19) and Sb (0.02, 0.023, 0.025) among soil-L, soil-M, and soil-H, respectively. *I. aquatica* with As BAFs > 1 can be defined as an As accumulator. The BAF values for As and Sb in *I. aquatica* roots were higher than the typical BAF for As (approximately 0.2) and Sb (approximately 0.01) reported in the literature (Kabata-Pendias 2011). This might suggest that *I. aquatica* was exposed to higher phytoavailable As and Sb or the *I. aquatica* acquisition of nutrients and metalloids was large compared to previous tested plants in the literature. Our study was performed at much higher As soil concentrations than other studies, thus the As accumulated in our *I. aquatica* tissues were considerably higher than those reported previously: 0.099 mg As/kg DW shoot of water spinach grown in soil of 33.1 mg As/kg (Yao et al. 2009); 0.195 and 0.362 mg As/kg shoot and root, respectively, for *I. aquatica* cultivated in soil amended with manure of 62.8 mg As/kg (Yao et al. 2010).

The translocation factor (TF) determined by the ratio of shoot to root concentrations (mg/kg DW) evaluates the ability of *I. aquatica* to translocate As and Sb from roots to shoots. Considering the TFs variation among soil types, the average TFs of As (0.09, 0.08, 0.06) and Sb (0.3, 0.14, 0.08) tended to decrease from soil-L, followed by soil-M and soil-H, but this was not statistically significant. On the whole, the translocation of both As and Sb were quite low, with all TF values <1, varying from 0.048 – 0.12 and 0.04 – 0.8, respectively (Table 5.3), in which the translocation factor of Sb was 2.2 times higher than As. This indicates that *I. aquatica* had a significantly ($p<0.05$) stronger capacity to translocate Sb from roots to shoots than As. This is supported by a previous study on As and Sb uptake by root vegetable (radish, *R. sativus*) showing that the translocation rate of Sb was 2.5 times higher than that of As (chapters 3 and 4). Thus, it appears that the movement of Sb from roots to shoots was more favoured than that of As, however the mechanism for this is still unknown. The limited translocation from roots to shoots (TFs <1) might be a mechanism of

plants to avoid toxicity, especially for As. Many researchers have shown that As detoxification occurs via reduction of arsenate to arsenite in roots, which is then complexed with thiols and sequestrated in root vacuoles to limit As transport from roots to shoots (Álvarez-Ayuso et al. 2016; Zhao et al. 2009). It is well known that the main transport pathway of arsenate uptake by plants is via the phosphate channel due to their chemical similarity, but this does not apply to Sb (Feng et al. 2013).

In general, As in roots was significantly more concentrated than Sb in *I. aquatica*, despite the total soil As being lower than Sb. The larger size of antimonate might prohibit its transport through the root cell wall compared to arsenate, thus restricting its accumulation. However, once accumulated in roots, Sb was translocated to shoots at the greater rate than As, illustrating the differences in uptake patterns of As and Sb by *I. aquatica*. Since As and Sb co-existed in the studied soils, the presence of As may affect Sb accumulation and translocation in *I. aquatica* and vice versa. The question of the occurrence of their competitive uptake by edible plants is still unknown. Greater accumulation of As compared to Sb has been observed for other plants grown in As and Sb co-contaminated soils (Wei et al. 2011; Wilson et al. 2014).

5.3.5. The relationship between As and Sb bioaccumulation and soil concentrations

The relationship between As and Sb concentrations in *I. aquatica* tissues and their labile concentrations in soils measured by SEP ($C_{SEP\text{-}labile}$), DGT (C_{DGT}), and soil solution (C_{sol}) are illustrated in Figure 5.2. $C_{SEP\text{-}labile}$, C_{DGT}, and C_{sol} predicted tissue concentrations for As better than Sb, demonstrated by greater correlation coefficients for As. Root As was better predicted than shoot As, which can be explained by the fact that roots directly contact soils, whereas bioaccumulation in shoots depends on the translocation from roots to shoots, usually governed by plant physiology (Nowack et al. 2004). This is in agreement with results from the soil-plant transfer model in which the bioaccumulation factor was a reflection of the bioavailable fraction, while translocation factor was dependent on the element and plant characteristics (McLaughlin et al. 2011). These significant positive correlations between As concentrations in *I. aquatica* tissues and As labile fractions are also supported by previous studies on As uptake by plants (Ngo et al. 2016; Wang et al.

2014). In contrast, Sb concentrations in shoots of *I. aquatica* were better predicted than root Sb concentrations.

Sb concentrations in *I. aquatica* tissues were not as well correlated with $C_{SEP\text{-}labile}$, C_{DGT}, and C_{sol} as As, illustrated by lower correlation coefficients (Figure 5.2). This indicates that As was well sustained and readily available to plant uptake than Sb, which is supported by the fact that the lability and release capacity of As from the solid phase and As bioaccumulation in all soils were much higher than those of Sb (sections 5.3.2, 5.3.3, and 5.3.4). This also suggests that the bioavailable fraction of Sb might not be the primary pool of Sb to plant uptake. When the soil Sb extracted from amorphous Fe and Al oxides were considered, a better correlation between the extractable Sb in soil and Sb in roots was observed (Figure 5.3), which is not the case for As. This indicates that Sb associated with amorphous Fe and Al oxides may contribute to the Sb uptake by *I. aquatica* or the plant induced the release and resupply of Sb from the solid phase, which was not captured by labile measures for Sb in soils, while As associated with amorphous Fe oxides may not be important for As uptake by the plant. In terms of the predictive ability, SEP performed well in predicting As and Sb concentrations in *I. aquatica* compared to DGT and soil solution analysis.

The release of As and Sb from amorphous Fe oxides occurred because Fe oxides bound As and Sb were less stable than those strongly bound to crystalline and residual fractions and could potentially be solubilised under favoured environmental conditions (Hockmann et al. 2015; Niazi et al. 2011). The release and solubilisation of As and Sb can be due to the effects of oxalic and citric acids excreted by *I. aquatica* roots and changes in surrounding conditions such as soil pH and redox potential. Another possible explanation is that the decrease in organic matter content due to microbial degradation over the test duration may result in the release of As and Sb associated with the organic fraction (Cattani et al. 2009; Lomaglio et al. 2016), providing more As and Sb for plant uptake. These processes were not included in the lability measurements. This is supported by Ptak and McBride (2015) reporting that organic acid exudates were able to readily mobilise Sb trapped by organic Fe complexes. According to Cattani et al. (2009), plant exudates such as oxalic and citric acids may complex elements such as Fe (and other metals complexed with As) or generate

environmental conditions for the development of soil microfauna and microorganisms. These processes would enhance the transformation and mobilisation of As in soils.

5.3.6. Risk assessment of As and Sb in *I. aquatica* to human health

The As concentrations in edible parts of water spinach (*I. aquatica*) were higher than the limit in food, specific to cereals (1 mg As/kg WW) set by the Food Standard Agency of Australia and New Zealand (FSANZ 2013); the permissible value for food (0.1 mg As/kg WW) recommended by joint Food and Agricultural Organisaion (FAO)/World Health Organisation (WHO) (JECFA 2011); and far exceeded the Chinese maximum permissible concentration of As in food (0.05 mg As/kg WW) (Bhatti et al. 2013). Thus, there is a potential risk of consuming this leafy vegetable cultivated in As-contaminated soils. The joint FAO/WHO Expert Committee on Food Additives (JECFA) has not reported guidelines relating to Sb safety. Nevertheless, the USEPA has recommended an oral reference dose (RfD) for Sb being 0.0004 mg/kg of body weight (BW)/day. The risk to individual health also depends on the vegetable intake rate, exposure time, lifetime, exposure duration, target population, and their body weight (Environmental Health Australia – enHealth 2012; USEPA 1998, 2011). The hazard quotient (HQ) and cancer risk (CR) calculations include these parameters and were used to further explore the potential risks to human health. Considering the dietary pattern of people in Asian countries where vegetables are consumed in three meals per day, the intake of 0.345 kg/day for adults and 0.232 kg/day for children (Khan et al. 2008; Khan et al. 2010; Wang et al. 2005), and the exposure frequency (EF) of 52 days in a year (Bhatti et al. 2013) were used for calculations. Both adults and children are considered in this assessment.

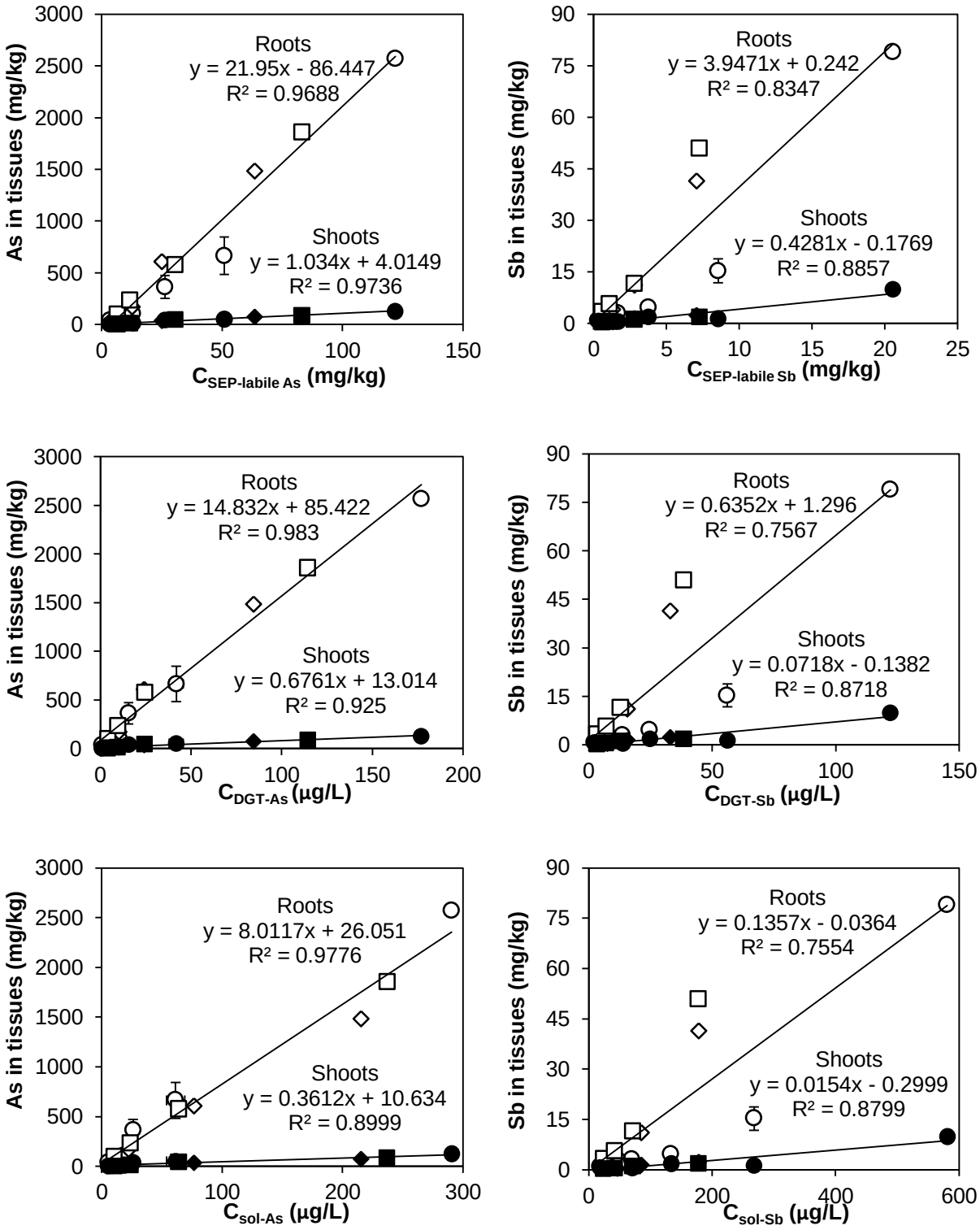

Figure 5.2 The relationship between tissue As and Sb (mg/kg, dry mass) and bioavailable fractions of As and Sb in soils, as measured by $C_{SEP\text{-}labile}$, C_{DGT}, and C_{sol}. Shoot (filled symbols), Root (unfilled symbols), Soil-L (o), Soil-M (◇), Soil-H (□). All data presented have error bars; many are smaller than symbols.

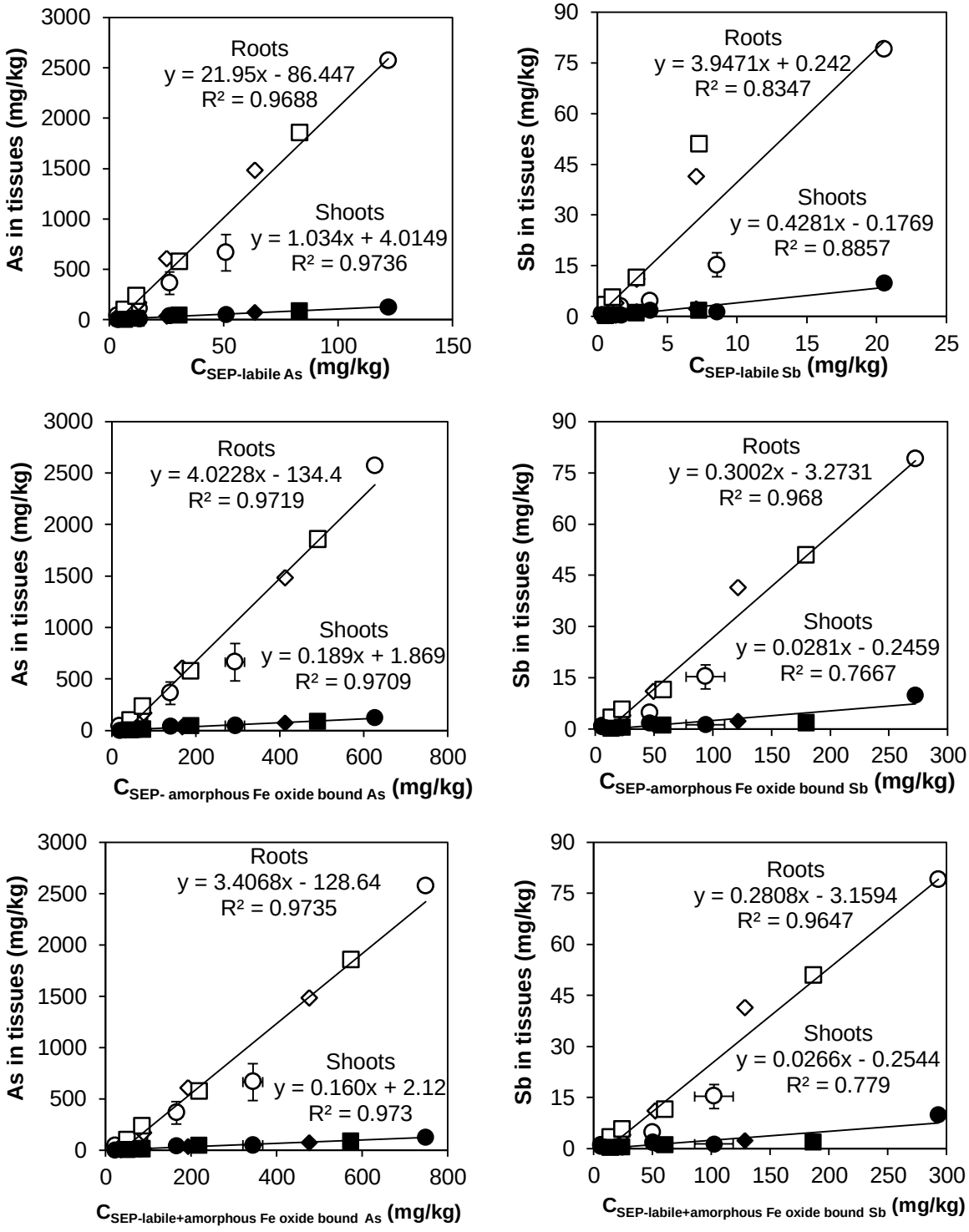

Figure 5.3 The relationship between tissue As and Sb (mg/kg, dry mass) and bioavailable fractions of As and Sb and As and Sb extracted from amorphous Fe oxide phase in soils. Shoot (filled symbols), Root (unfilled symbols), Soil-L (o), Soil-M (◇), Soil-H (□). All data have error bars; many are smaller than symbols.

The estimated daily intake (EDI), hazard quotient (HQ), and cancer risk (CR) of As and Sb via the consumption of *I. aquatica* shoots as a function of soil As and Sb are presented in Tables 5.4 and 5.5, respectively. Since there is no cancer slope factor for Sb, the CR value of Sb was not calculated. The EDI values of As for both adults and children were higher than the acceptable limit or the reference dose (RfD, 3×10^{-4} mg/kg/day) set by (USEPA 1998, 2011). The HQ values of As and Sb were calculated to assess the potential risk of non-cancer adverse health effect to humans through the As and Sb ingestion. The HQ values of As were > 1 for all cases, ranging from 1.04 – 34.5 for adults and 1.72 – 54.8 for children, indicating the substantial risk of non-carcinogenic effects for both adults and children consuming *I. aquatica* grown in contaminated soils with As ≥42 mg/kg corresponding to $C_{sol\text{-}As}$ ≥5.27 µg/L or $C_{DGT\text{-}As}$ ≥3.42 µg/L. The HQ value of Sb was >1 only in S5. The HQ values of As were much higher than the HQ values of Sb, suggesting that the non-carcinogenic risks of As in *I. aquatica* shoots is much higher than that of Sb. Cumulatively, the HI (hazard index) values being the sum of HQ values of As and Sb were >1 for all cases. Higher HQ for children compared to adults inferred greater potential health risks for this group. Previous studies also reported that teenagers and children were at higher risk associated with the consumption of As-enriched vegetables (Jiang et al. 2015).

The carcinogenic risk was calculated to assess the probability of the exposed population developing cancer risk through the ingestion of As-contaminated vegetables during their lifetime. The CR values of As increased with the increasing soil As and far exceeded the probability of 1 in 10,000, indicating an increase in probability of the cancer development via consumption of *I. aquatica* shoots cultivated in soils contaminated with ≥42 mg As/kg ($C_{sol\text{-}As}$ ≥5.27 µg/L or $C_{DGT\text{-}As}$ ≥3.42 µg/L). The CR values of As were higher for children than adults, indicating that children were at higher risk of cancer. Taking *I. aquatica* grown in the lowest treatment as an example, the cancer risk was 4.69 per 10,000 for adults, but was 23.2 per 10,000 for children. The average cancer risk development via consumption of *I. aquatica* shoots cultivated in the studied soils was 4.9 times higher for children than adults. Rehman et al. (2016) also reported that the development of cancer risk through ingestion of vegetables was lower in adults than children.

Table 5.4 Values of EDI (mg/kg/day), HQ, and CR of As for the ingestion of *I. aquatica* shoots grown in studied soils.

Soil	Treatment	Soil As (mg/kg DW)	Shoot As (mg/kg WW)	Estimated daily intake (EDI)		Hazard quotient (HQ)		Cancer risk (CR)	
				Adults	Children	Adults	Children	Adults	Children
L	S1	42	0.38	3.13×10^{-4}	5.15×10^{-4}	1.04	1.72	4.69×10^{-4}	23.2×10^{-4}
	S2	160	1.17	9.55×10^{-4}	15.7×10^{-4}	3.18	5.24	14.3×10^{-4}	70.7×10^{-4}
	S3	270	4.20	34.4×10^{-4}	56.6×10^{-4}	11.5	18.9	51.6×10^{-4}	255×10^{-4}
	S4	670	5.10	41.8×10^{-4}	68.8×10^{-4}	13.9	22.9	62.6×10^{-4}	309×10^{-4}
	S5	1400	12.63	103×10^{-4}	170×10^{-4}	34.5	54.8	155×10^{-4}	767×10^{-4}
M	S6	65	0.44	3.60×10^{-4}	5.93×10^{-4}	1.20	1.98	5.40×10^{-4}	26.7×10^{-4}
	S7	176	1.59	13.0×10^{-4}	21.5×10^{-4}	4.35	7.16	19.6×10^{-4}	96.6×10^{-4}
	S8	330	3.60	29.5×10^{-4}	48.6×10^{-4}	9.83	16.2	44.2×10^{-4}	218×10^{-4}
	S9	950	7.35	60.2×10^{-4}	99.1×10^{-4}	20.1	33.0	90.3×10^{-4}	446×10^{-4}
	S10	2630	-	-	-	-	-	-	-
H	S11	101	0.56	4.58×10^{-4}	7.54×10^{-4}	1.53	2.51	6.87×10^{-4}	34.0×10^{-4}
	S12	200	1.47	12.1×10^{-4}	19.9×10^{-4}	4.02	6.63	18.1×10^{-4}	89.5×10^{-4}
	S13	510	4.73	38.8×10^{-4}	63.9×10^{-4}	12.9	21.3	58.2×10^{-4}	287×10^{-4}
	S14	1280	8.87	72.7×10^{-4}	120×10^{-4}	24.2	39.9	109×10^{-4}	539×10^{-4}
	S15	4180	-	-	-	-	-	-	-

-: not measured for evaluating As/Sb uptake due to damaged roots and plant death

Table 5.5 Values of EDI (mg/kg/day) and HQ of Sb for the ingestion of *I. aquatica* shoots grown in studied soils.

Soil	Treatment	Soil Sb (mg/kg DW)	Shoot Sb (mg/kg WW)	Estimated daily intake (EDI)		Hazard quotient (HQ)	
				Adults	Children	Adults	Children
L	S1	52	0.066	0.54×10^{-4}	0.89×10^{-4}	0.14	0.22
	S2	200	0.045	0.37×10^{-4}	0.60×10^{-4}	0.09	0.15
	S3	330	0.182	1.49×10^{-4}	2.45×10^{-4}	0.37	0.61
	S4	870	0.132	1.08×10^{-4}	1.79×10^{-4}	0.27	0.45
	S5	2277	0.990	8.11×10^{-4}	13.4×10^{-4}	2.03	3.34
M	S6	91	0.044	0.36×10^{-4}	0.60×10^{-4}	0.09	0.15
	S7	220	0.035	0.29×10^{-4}	0.47×10^{-4}	0.07	0.12
	S8	530	0.145	1.19×10^{-4}	1.96×10^{-4}	0.30	0.49
	S9	1190	0.232	1.90×10^{-4}	3.13×10^{-4}	0.48	0.78
	S10	3790	-	-	-	-	-
H	S11	140	0.029	0.24×10^{-4}	0.40×10^{-4}	0.06	0.10
	S12	230	0.054	0.44×10^{-4}	0.73×10^{-4}	0.11	0.18
	S13	670	0.115	0.94×10^{-4}	1.55×10^{-4}	0.24	0.39
	S14	1600	0.188	1.54×10^{-4}	2.53×10^{-4}	0.39	0.63
	S15	6610	-	-	-	-	-

-: not measured for evaluating As/Sb uptake due to damaged roots and plant death

It is noticeable that *I. aquatica* accumulated As at high levels exceeded the permissible limit for food consumption, but physical toxicity symptoms were not evident. The cancer risk assessment model also showed unacceptable non-carcinogenic (all HQ > 1) and carcinogenic risks (all CR > 10^{-4}) to both adults and children ingesting edible parts of *I. aquatica* cultivated in the studied soils, even in the lowest contaminated soils.

5.4. Conclusions

This study illustrated the contrasting behaviours of As and Sb in soils, their uptake by *I. aquatica*, and the importance of bioavailability in risk assessments. The SEP and DGT showed the lability of As in contaminated soils and its partial resupply from the solid phase varied with soil types, while for Sb, these were low and relatively constant across soil types. This confirms that irrespective of soil types, As was more geochemically labile than Sb, which was dominated by associations with recalcitrant minerals. Soil physicochemical properties especially Fe and Al oxides, DOC, and pH are important in controlling the lability and phytoavailability of As and Sb in contaminated soils. Thus, the incorporation of bioavailability measurements into soil risk assessments was essential for the management of As and Sb-contaminated soils.

I. aquatica had a high capacity to accumulate As and Sb with concentrations in roots being 10 and 14 times higher than shoots, respectively. A different transport pathway of As and Sb within *I. aquatica* was observed and *I. aquatica* was considered as an As accumulator plant. Arsenic accumulated in edible shoots at very high concentrations far exceeded the permissible limit of As in food set by the Food Standard Agency of China, Australia and New Zealand, and FAO/WHO, but no visible toxicity symptoms was observed in the plant, which is an alarming concern. Consumption of *I. aquatica* cultivated in studied soils, even at the lowest treatment (low contamination) posed the cancer risks to both adults and children. Thus, cultivation of *I. aquatica* in As-contaminated soils or water or irrigation of *I. aquatica* with As-enriched water should be restricted in terms of food safety and human health.

DGT, soil solution, and SEP-labile fractions were excellent in predicting metalloids uptake by *I. aquatica* cultivated in historically contaminated soils with the greater extent for As prediction. The analyses of relationships between As and Sb concentrations in *I. aquatica*

and their concentrations in various soil fractions support the conclusion that the activity of *I. aquatica* roots could induce the solubilisation and release of Sb from the solid phase. The combination of SEP and DGT was useful in determining the partitioning of As and Sb in soils and measuring their lability in different soil types. DGT was sensitive to differentiate the lability, capacity and kinetics of As and Sb release from the solid phase of various soils. The dynamic DGT technique integrated soil properties affecting soil processes and was less laborious than other measurements, thus it provides a promise for assessing bioavailable As and Sb in historically contaminated soils.

The question is does the presence of Sb influence the partitioning, lability and bioaccumulation of As by *I. aquatica* or vice versa. This is still unknown and warrants for further investigation. To do this a study on the fractionation and lability of As and Sb in its singly contaminated soil compared to that in co-contaminated soil and their uptake by *I. aquatica* grown in those soils needs to be performed.

Chapter 6. Biogeochemical behaviour of As and Sb in soils and their uptake by water spinach (*Ipomoea aquatica*): Implications for lability, distribution, and competition

6.1. Introduction

Arsenic is of concern due to its high concentrations in both anthropogenically contaminated sites and naturally in several areas such as India, West Bengal, and Bangladesh (Chowdhury et al. 2000; Fu et al. 2016). Intensive research has explored the biogeochemical behaviour and toxicity of As (Abernathy et al. 1999; Dixit & Hering 2003; Seyfferth et al. 2010), while studies on Sb have been limited. The behaviour and toxicity of Sb have been assumed to be comparable with that of As since they are both in group 15 of the periodic table. Due to insufficient data for Sb, the observed behaviour for As (uptake, detoxification mechanisms, methylation) has been commonly extended to Sb (Filella et al. 2007). However, Sb and As have different coordination geometry: arsenate is tetrahedrally coordinated to four oxygen atoms (e.g. similar to phosphate) whereas antimonate is octahedrally coordinated to six oxygen atoms and has a greater spatial structure than that of arsenate (radius ratios: Sb(V)/O = 0.51, As(V)/O = 0.4) (Qi & Pichler 2017). Recent evidence shows that these two metalloids behave differently under certain conditions, which is a challenge for interpreting Sb behaviour on the basis of As characteristics (Fu 2016). For example, previous chapters have shown that As was primarily associated with Fe oxides, while Sb was mainly found in residual phase, possibly sulphide or organic matter. Arsenic was present as As(III) and As(V), while Sb was dominantly present as Sb(V) in a soil-water system near an Sb mine (Mitsunobu et al. 2006), and bioaccumulation factors for Sb, determined by the ratio of plant and soil concentrations, were 10-fold less than those of As (Fu 2016; Ngo et al. 2016).

Arsenic and Sb are non-essential elements that are toxic to plants (Filella et al. 2002a; Gebel 1999); however, some plants can readily take up As and Sb from soils and accumulate them in edible tissues posing a risk to human health via the food chain. Ample

research has been previously conducted to investigate the accumulation of As by a variety of plants in relation to its bioavailability in soils (Álvarez-Ayuso et al. 2016; Bhatti et al. 2013; Niazi et al. 2011; Rosas-Castor et al. 2014; Smith et al. 2009) and some research specific to Sb has been recently performed (Okkenhaug et al. 2011; Shtangeeva et al. 2014; Tschan et al. 2009a). There are a few studies focused on the simultaneous accumulation of As and Sb by edible crops cultivated in soils contaminated with both As and Sb (Ngo et al. 2016; Wilson et al. 2014), but their interactive effects remains unclear. Recent work on the effect of As on the uptake and bioaccumulation of Sb by As-hyperaccumulators, *P. cretica* (Feng et al. 2011) and *P.vittata* (Müller et al. 2013), reported that the presence of As enhanced Sb uptake by plants; however, they did not consider the effect of Sb on the As uptake as well as their competitive interactions in edible vegetables.

It is well established that the bioavailability of As and Sb in soils is controlled by many factors such as pH, redox potential (Mitsunobu et al. 2006; Okkenhaug et al. 2012), organic matter (Dousova et al. 2015; Williams et al. 2011), clay (García-Sanchez et al. 2002), mineral oxides (Dixit & Hering 2003; Kim et al. 2014; Leuz et al. 2006), and aging time (Juhasz et al. 2008; Li et al. 2014; Tang et al. 2007). However, these studies have only focused on single-element contaminated soils. Some studies investigated the fractionation and bioavailability of As and Sb that were co-existing in long-term contaminated soils (Ettler et al. 2010; Li et al. 2014; Müller et al. 2007; Ngo et al. 2016). Only one study by Liang et al. (2014) examined the bioavailability of As in As-contaminated soils in comparison to As+Pb contaminated soils. However, little attention has been given to the effect of As and Sb on their respective bioavailability in co-contaminated soils. To our knowledge, no study has been conducted to investigate whether the presence of As influences the partitioning, lability, and solubility of the co-existing Sb in soils and vice versa, which is important for the prediction of their bioavailability and potential environmental risk. In addition, the competitive uptake of As on the uptake and bioaccumulation of Sb by edible plants and vice versa in co-contaminated soils has not been explored.

Chapter 5 investigated the biogeochemical behaviour of As and Sb in historically co-contamianted soils with various soil properties. However, the competitive interactions

between As and Sb in soils and competitive uptake by water spinach (*Ipomoea aquatica*) could not be interpreted due to the lack of soils having identical mineralogy and properties and contaminated with As only and Sb only, acting as references. To do this a study on the behaviour of As and Sb in soils spiked with As only and Sb only compared to those in soils spiked with As+Sb in the same mannner is needed. In addition, *I. aquatica* is a very popular edible vegetable in Asia and was known to accumulate high amounts of As, posing cancer risks to human health (section 5.3.6, chapter 5). The question that the presence of Sb enhanced As accumulation by *I. aquatica* has been unknown and should be further explored.

The aims of this study are (1) to investigate the biogeochemical behaviour of As and Sb in single and multi-contaminant amended soils, (2) to investigate the interactive effects of As and Sb on accumulation by water spinach (*Ipomoea aquatica*), (3) to assess the performance of sequential extraction and the DGT technique for predicting As and Sb uptake by *I. aquatica*. This study will provide a better understanding of interactive effects of As and Sb in individual and co-occurring soils and in the studied plant.

6.2. Methods

6.2.1. General methods

General washing methods were done as per section 2.1.

6.2.2. Experimental design

6.2.2.1. Soil preparation

An uncontaminated soil was collected in Wollongong, NSW, then air-dried and crushed to pass through a 2-mm mesh sieve. Each portion of soil was amended with either single or mixed solutions of $Na_2HAsO_4.7H_2O$ and $KSb(OH)_6$ at various concentrations to create three series of soils contaminated with As only, Sb only, and both As and Sb. Eight treatments of each contaminated soil series were prepared, denoted as As1 – As8, Sb1 – Sb8, and AsSb1 – AsSb8. The amounts of As and Sb added into soils were targeted in the wide range of 20 – 600 mg As/kg and 20 – 6000 mg Sb/kg. The chosen concentrations of As and Sb were based on published studies on As bioavailability in As-spiked soils compared with that in soils spiked with a mixture of As+Pb (Liang et al. 2014) and As and

Sb uptake by plants cultivated in singly-spiked soils (Niazi et al. 2011; Tschan et al. 2010). In addition, the previous study (chapter 5) showed that *I. aquatica* grown in highly co-contaminated soils did not exhibit toxicity symptoms. Thus, the largest concentrations were estimated to be high enough for the plant toxicity to be detectable. Spiking soils was done as per section 2.2.2. These spiked soils are considered as recently contaminated soils.

6.2.2.2. Pot experiment

Spiked soil moisture was then raised to and maintained at ~60% of the water holding capacity (WHC) with deionised water. Soils were allowed to settle and equilibrate in a greenhouse for two more weeks prior to the commencement of bioassays. The 35-day bioassays with water spinach (*Ipomoea aquatica*) using pots were conducted in controlled growth chambers as per section 2.4.2. Harvested plant tissues from the same pot were combined, weighed, and oven-dried at 70 °C for 48 h to a constant weight with the dry mass (g) recorded. Dried tissues were ground with a mortar and pestle and stored in clean plastic bags prior to As and Sb analyses.

6.2.3. Soil characterisation

Soil physicochemical properties including particle size, pH, organic matter, total nitrogen, available phosphorus, and amorphous and free Fe and Al were determined as described in section 2.5.

6.2.4. Soil and plant analyses

Total As and Sb in soils and plants were digested and analysed as described in sections 5.2.4 and 2.9. For quality control, a combination of reagent blanks, duplicate analyses, certified reference materials, and matrix spike recovery were used. A soil certified reference material (Montana II soil, SRM 2711a; NIST) returned recoveries of 90-115% for the studied analytes. A plant certified reference material (Tomato leaves, SRM 1573a; NIST) had typical recoveries of 95-110% of the studied analytes.

6.2.5. Sequential extraction procedure

The improved sequential extraction scheme developed by Wenzel et al. (2001) was applied to partition both As and Sb in the studied soils as per section 2.6.1. The five extracted

fractions were As and Sb associated with non-specifically sorbed, specifically sorbed, amorphous Fe and Al oxide, crystalline Fe and Al oxide, and residual fractions.

6.2.6. DGT and soil solution measurements

DGT and soil solution measurements were done as described in sections 2.6.2 and 2.7.

6.2.7. Data analysis

Statistical analysis and calculations of bioaccumulation factor (BAF) and translocation factor (TF) were performed as per section 3.2.7. To assess the non-carcinogenic and carcinogenic risks associated with As and Sb exposure through diet, the hazard quotient (HQ) and cancer risk (CR) were calculated as described in section 5.2.7.

6.3. Results and discussion

6.3.1. Soil characteristics

The test soils were a sandy-silt (29.5% sand, 62.4% silt, 8.1% clay), slightly acidic (pH ~6.0), and had a total organic matter of 8.1%. The total Kjeldahl nitrogen and extractable phosphorous in soils were 92 and 30 mg/kg, respectively. The amorphous and crystalline Fe concentrations in soils were 7.9 g/kg and 27.1 g/kg, respectively. The measured concentrations of As and Sb in amended As-only, Sb-only, and As+Sb soils were presented in Tables 6.1 and 6.2.

6.3.2. As and Sb fractionations in soils

The partitioning of As and Sb in different soil fractions are presented in Tables 6.1 and 6.2. In general, reagents with increasing dissolution strength were used to sequentially extract As and Sb bound to various soil constituents, meaning that As and Sb extracted in the later fraction was less labile and bioavailable than those in the earlier fractions (Kim et al. 2014). These extractions are not perfectly selective to the target phase, so there can be some interactions between them. On average, As in the As-only soils was partitioned in the following order: associated with amorphous Fe and Al oxides (54 ± 1%) > specifically sorbed (32 ± 2%) > associated with crystalline Fe and Al oxides (17 ± 1%) > non-specifically sorbed (3.2 ±0.7%) (Table 6.1). The pattern of Sb distribution in various soil binding phases differed from As. The partitioning of Sb in Sb-only soils followed the

sequence: associated with amorphous Fe and Al oxides > bound to crystalline Fe and Al oxides > specifically sorbed > non-specifically sorbed, except for high treatments (≥1560 mg Sb/kg) where the order was associated with amorphous Fe and Al oxides > non-specifically sorbed > bound to crystalline Fe and Al oxides > specifically sorbed (Table 6.2).

Considering changes of As and Sb in each fraction with their total loads in the soils, both As and Sb in non-specifically sorbed and specifically sorbed fractions increased in concentration and proportion. In contrast, As and Sb in amorphous Fe and Al oxides increased with each addition but proportions increased initially to ~60% (at total 84 mg As/kg) and ~70% (at total 78 mg Sb/kg), and then decreased to ~50% and ~55% at higher total concentrations of As and Sb, respectively. Arsenic in crystalline Fe and Al oxides increased but proportionally decreased from about 20 to 15% over the As-added series. This trend was also observed by Huang et al. (2016) and Tang et al. (2007).

When As and Sb were added together, the distribution pattern for As and Sb in As+Sb soils was similar to that in their singly amended soils but with some interesting differences. In As+Sb soils at low concentrations, the amounts of added As and Sb were comparable, but the Sb partitioning in various binding sites was different from that of As. The proportions of Sb in non-specifically sorbed, amorphous Fe and Al oxide, and crystalline Fe and Al oxide fractions were higher than that of As, while the proportions of Sb in specifically sorbed fraction was lower. There was relatively unchanged As proportions in the non-specifically sorbed and specifically sorbed fractions, but the higher tendency was apparent for Sb in co-amended soils. Compared to As and Sb fractionation in singly amended soils, the proportions of As and Sb in the non-specifically sorbed fraction were higher (e.g. the percentage of As partitioned to the non-specifically sorbed fraction was double that in As-only soils at two highest treatments) while those in the amorphous Fe and Al oxide fraction tended to be lower. This may be because the simultaneous addition of As and Sb into soils increased amounts of dissolved co-contaminants added, which decreased soil adsorption binding sites. As a result, the sorbed and bound As and Sb decreased and more As and Sb were found in non-specifically sorbed fraction. The As distribution pattern in this study is

supported by Niazi et al. (2011) who studied As fractionation in As spiked soils with various soil properties.

The difference in proportion of As and Sb in each fraction between soil treatments was most likely due to the soil surface adsorption sites being initially unsaturated in the native soil. Thus, when soluble As and Sb was added, As and Sb were first partitioned to the soil solution, followed by the rapid adsorption onto active binding sites. The adsorbed As and Sb then transformed into the inner-sphere complexes over time, with the excess As and Sb added in the high treatments being redistributed among other soil binding phases. When large amounts of As and Sb added (>400 mg As/kg, >1500 mg Sb/kg), the soil adsorption sites may be saturated. This explains the significant portions of As and Sb in non-specifically sorbed fraction in soils AsSb6 and AsSb7.

Overall, As in the non-specifically sorbed and specifically sorbed fractions was higher than Sb in all treatments, indicating that As was more labile than Sb. In our soils the competitive interaction of As and Sb for their soil binding sites appeared to affect the distribution of As and Sb in various soil binding phases and make them more available in the As+Sb co-amended soils, compared to singly amended soils. To our knowledge, this is the first study on comparative interactions of As and Sb in soils.

The partitioning of As and Sb in As+Sb soils was different from that in As and Sb historically co-contaminated soils (chapter 5) (Figure D.1) where As and Sb were low in labile fractions and high in Fe and Al oxides and residual fractions. This possibly occurred because of the variation in aging time and equilibration of soils. Arsenic and Sb in non-specifically sorbed and specifically sorbed fractions (the available fraction) accounted for ~30% and ~18% of total soil As and Sb, respectively, and it is not surprising that they are significantly higher than that of available As and Sb in historically contaminated soils in chapter 5 (Figure D.1). This may be attributed to the soluble form of added As and Sb, and their shorter equilibration time in amended soils compared to the long aging in historically contaminated soils (Tang et al. 2007).

Table 6.1 As concentrations in each fraction of soils amended with (i) As only and (ii) with a mixture of As and Sb (mean ± SE, n = 3). Numbers in brackets are % of total soil concentrations.

Soil	As concentrations in each fraction (mg/kg)				
	Total As	Non-specifically sorbed	Specifically sorbed	Amorphous Fe and Al oxide	Crystalline Fe and Al oxide
As only					
As1	46 ± 3	0.457 ± 0.006 (1.0)	10.37 ± 0.05 (22.7)	25.9 ± 0.4 (56.5)	9.2 ± 0.5 (20.1)
As2	84.2 ± 0.7	1.12 ± 0.007 (1.3)	22.2 ± 0.1 (26.4)	49.7 ± 0.2 (59.0)	17.9 ± 0.3 (21.2)
As3	165 ± 3	3.11 ± 0.08 (1.9)	49.2 ± 0.7 (29.8)	92.4 ± 0.7 (56.0)	30 ± 2 (18.0)
As4	196 ± 2	4.5 ± 0.2 (2.3)	64.1 ± 0.3 (32.6)	110 ± 1 (56.3)	39 ± 3 (19.9)
As5	300 ± 5	10.4 ± 0.2 (3.5)	103.8 ± 0.8 (34.8)	160 ± 4 (53.4)	44 ± 7 (14.7)
As6	410 ± 7	16.7 ± 0.7 (4.0)	142 ± 0.1 (34.3)	210 ± 3 (50.0)	58 ± 6 (14.0)
As7	510 ± 7	27 ± 1 (5.3)	180 ± 3 (35.8)	260 ± 6 (50.5)	77 ± 8 (15.2)
As8	600 ± 1	36.8 ± 0.5 (6.1)	220 ± 3 (37.1)	300 ± 3 (49.9)	86 ± 10 (14.3)
As + Sb					
AsSb1	60 ± 6	0.520 ± 0.005 (0.9)	11.62 ± 0.09 (19.4)	27.2 ± 0.5 (45.3)	10.4 ± 0.8 (17.3)
AsSb2	87 ± 2	1.19 ± 0.03 (1.4)	23.0 ± 0.6 (26.3)	47.1 ± 0.9 (53.8)	15.9 ± 0.5 (18.2)
AsSb3	175.3 ± 0.9	3.9 ± 0.1 (2.2)	54 ± 1 (30.7)	94 ± 2 (53.9)	35 ± 2 (19.9)
AsSb4	220 ± 4	5.24 ± 0.08 (2.4)	65.1 ± 0.3 (29.3)	110.9 ± 0.6 (50.0)	39 ± 1 (17.6)
AsSb5	310 ± 3	10.5 ± 0.2 (3.3)	100 ± 1 (31.9)	160 ± 2 (52.2)	49 ± 2 (15.7)
AsSb6	405 ± 2	43.7 ± 0.4 (10.8)	120 ± 6 (29.8)	200 ± 1 (50.2)	72 ± 3 (17.6)
AsSb7	510 ± 10	63.9 ± 0.9 (12.5)	150 ± 4 (29.3)	250 ± 2 (48.9)	90 ± 2 (17.5)
AsSb8	600 ± 3	-	-	-	-

-: not measured due to analysis problems

Table 6.2 Sb concentrations in each fraction of soils amended with (i) Sb only and (ii) with a mixture of As and Sb (mean ± SE, n = 3). Numbers in brackets are % of total soil concentrations.

Soil	Sb concentrations in each fraction (mg/kg)				
	Total Sb	Non-specifically sorbed	Specifically sorbed	Amorphous Fe and Al oxide	Crystalline Fe and Al oxide
Sb only					
Sb1	40 ± 1	2.54 ± 0.04 (6.4)	3.61 ± 0.06 (9.1)	25.0 ± 0.2 (62.7)	8.0 ± 0.1 (20.2)
Sb2	78 ± 2	5.58 ± 0.04 (7.2)	7.4 ± 0.1 (9.6)	54.7 ± 0.5 (70.5)	17.1 ± 0.8 (22.0)
Sb3	160 ± 9	10.69 ± 0.04 (6.8)	14.9 ± 0.3 (9.5)	107.7 ± 0.5 (68.9)	32.6 ± 0.3 (20.8)
Sb4	300 ± 10	21.2 ± 0.4 (7.1)	28.5 ± 0.2 (9.6)	200 ± 2 (66.8)	58 ± 2 (19.6)
Sb5	650 ± 20	48.5 ± 0.3 (7.5)	59.6 ± 0.4 (9.2)	380 ± 6 (58.9)	120 ± 4 (19.0)
Sb6	1560 ± 60	370 ± 4 (23.5)	200 ± 1 (13.0)	860 ± 80 (55.2)	330 ± 10 (21.2)
Sb7	3000 ± 60	-	-	-	-
Sb8	5300 ± 40	-	-	-	-
As + Sb					
AsSb1	45 ± 4	2.73 ± 0.02 (6.0)	3.71 ± 0.04 (8.2)	26.6 ± 0.6 (58.8)	9.0 ± 0.3 (20.0)
AsSb2	73 ± 7	5.58 ± 0.09 (7.7)	7.1 ± 0.1 (9.8)	51 ± 1 (70.8)	16.1 ± 0.4 (22.2)
AsSb3	170 ± 5	14.5 ± 0.3 (8.6)	16.6 ± 0.6 (9.8)	103 ± 1 (61.1)	33.6 ± 0.9 (19.9)
AsSb4	310 ± 4	28.2 ± 0.2 (9.1)	29.8 ± 0.2 (9.6)	168 ± 1 (54.2)	55.7 ± 0.5 (17.9)
AsSb5	320 ± 8	37.1 ± 0.4 (11.4)	36.6 ± 0.9 (11.3)	195.8 ± 1 (60.4)	62 ± 1 (19.2)
AsSb6	1620 ± 30	570 ± 5 (35.4)	230 ± 9 (14.2)	800 ± 10 (49.0)	240 ± 9 (14.9)
AsSb7	3400 ± 10	1310 ± 20 (38.3)	490 ± 10 (14.4)	1650 ± 20 (48.4)	460 ± 9 (13.6)
AsSb8	5800 ± 80	-	-	-	-

-: not measured due to analysis problems

6.3.3. Lability of As and Sb in soils

DGT-labile concentrations (C_{DGT}), soil solution concentrations (C_{sol}), K_d, and R values for As and Sb are presented in Tables 6.3 and 6.4. $C_{DGT\text{-}As}$ and $C_{sol\text{-}As}$ increased with As soil treatment concentrations (Table 6.3). $C_{DGT\text{-}As}$ and $C_{sol\text{-}As}$ in As-only soils ranged from 0.02 – 0.14% (9.7 – 853 µg/ As/L) and 0.04 – 0.64% (17.36 – 3860 µg/ As/L) of total soil As concentrations, respectively. Similarly, $C_{DGT\text{-}Sb}$ and $C_{sol\text{-}Sb}$ in Sb-only soils increased with soil Sb concentrations (Table 6.4), with 0.08 – 0.17% (31.6 – 5260 µg Sb/L) and 0.38 – 1.12% (150 – 33700 µg Sb/L) of total soil Sb concentrations, respectively.

The C_{DGT} of As and Sb were less than their C_{sol} in all cases, especially for Sb. This is because C_{DGT} represents labile As and Sb, while C_{sol} includes both free and complexed As and Sb, and colloids (Zhang & Davison 2015). Some complexes found in soil solutions may be too large to diffuse through the polyacrylamide diffusive gel in DGT, making them non-DGT labile. Decreases in the distribution coefficients (K_d) of soil solid phase to soil solution concentration of As and Sb occurred with the increasing additions of As and Sb due to an increase in As and Sb in soil solutions, especially at their high treatments. This is consistent with the trend of non-specifically sorbed As and Sb obtained from SEP, the increasing As and Sb load, and potential increasing saturation of soil binding sites.

The R value ($R=C_{DGT}/C_{sol}$, $0 < R < 1$) obtained from the ratio of DGT-labile and soil solution concentration reflects the capacity of soil to resupply solutes from the solid phase to the soil solution and the kinetics of this process (Harper et al. 1998; Zhang et al. 2006; Zhang et al. 2004). The R values for As and Sb are presented in Tables 6.3 and 6.4. The average R value for As in As-only soils was 0.38, indicating that As was partially resupplied from the solid phase, but insufficient to sustain pore water concentrations. The average R value for Sb in Sb-only soils was 0.2, suggesting that the resupply of Sb from the solid phase was limited and slow. This demonstrates that the sorption of As and Sb onto soils and their resupply to soil solution were controlled by different factors.

Changes in soils that were simultaneously co-contaminated with As and Sb were examined. Soil solution As (0.03 – 0.31% of total As) in As+Sb soils were slightly lower than the As-only soils (0.04 – 0.64%), whereas soil solution Sb (0.52 – 2.01% of total Sb) in As+Sb soils were about two times higher than the Sb-only soils (0.38 – 1.12%). This phenomenon

also occurred for $C_{DGT\text{-}As}$ and $C_{DGT\text{-}Sb}$, but to a lesser extent. The average percentage of $C_{DGT\text{-}As}$,$C_{sol\text{-}As}$, $C_{DGT\text{-}Sb}$, and $C_{sol\text{-}Sb}$ of their total soil concentrations is illustrated in Figure D.2. This implies that some As and Sb may not be sorbed to the same soil binding sites, possibly because As has a higher affinity for Fe oxides compared to Sb (Qi & Pichler 2017; Wilson et al. 2010), while Sb has stronger binding strength with organic matter (OM) than As. Liang et al. (2014) showed that soluble Fe and dissolved organic carbon (DOC) concentrations increased significantly, especially in high As-amended soils. Based on the Langmuir adsorption model, the binding stability of As/Sb in soil types was also presented in order of As(V) – Fe(III) >> Sb(V) – OM > As(V) – OM > Sb(V) – Fe(III) (Dousova et al. 2015). Thus, when As and Sb were added together, the energetically favourable binding sites such as Fe oxides gave preference to As, leading to more added As in solid phases and more Sb in soil solutions. This is clear from the $C_{sol\text{-}Sb}$ and reflected in the decrease of K_d for Sb to low values in the As+Sb soils.

Consequently, the resupply of As in As+Sb soils was slightly lower than the As-only soils, evident by the lower average R value (0.33 ± 0.02 compared to 0.38 ± 0.04, respectively). As the proportion of $C_{sol\text{-}Sb}$ increased and K_d decreased, it was expected that Sb in As+Sb soils would have the greater supply for counteracting the Sb depletion at the vicinity of DGT devices and a proportionally increased C_{DGT} compared to that in Sb-only soils. This expectation was based on maintaining the concentration of Sb needed to be resupplied from a non-DGT labile source as a result of the depletion. However, the resupply of Sb in As+Sb soils remained limited or was even lower than that in Sb-only soils, demonstrated by the average R values (0.115 ± 0.009 compared to 0.20 ± 0.01, respectively). This may be because (1) most Sb in soil solutions were inert or in large complexes, making the diffusional transport through polyacrylamide DGT gels restricted, which were unavailable for DGT measurements over short periods and (2) the release of Sb from the solid phase was minimal. Quantification of DOC in soil solutions would be needed for further explaining the reason for low Sb lability. It is possible that sorbed As had higher rates of desorption from the solid phase and little interaction with DOC compared with Sb, which determined the kinetic processes and differences between As and Sb behaviour in soils.

Table 6.3 Total and labile As concentrations measured by SEP, DGT, soil solution in contaminated soils, and R value of As (mean ± SE, n = 3).

Soil	As in As-only amended soils						Soil	As in As+Sb amended soils					
	$Total_{As}$ (mg/kg)	$C_{SEP\text{-}labile\ As}$ (mg/kg)	$C_{DGT\text{-}As}$ (µg/L)	$C_{sol\text{-}As}$ (µg/L)	Ratio R_{As} ($R=C_{DGT}/C_{sol}$)	K_d (L/kg)		$Total_{As}$ (mg/kg)	$C_{SEP\text{-}labile\ As}$ (mg/kg)	$C_{DGT\text{-}As}$ (µg/L)	$C_{sol\text{-}As}$ (µg/L)	Ratio R_{As} ($R=C_{DGT}/C_{sol}$)	K_d (L/kg)
As1	46 ± 3	10.83 ± 0.05	9.7 ± 0.5	17.36 ± 0.06	0.56 ± 0.03	624	AsSb1	60 ± 6	12.14 ± 0.09	8.2 ± 0.5	18.6 ± 0.1	0.44 ± 0.03	652
As2	84.2 ± 0.7	23.4 ± 0.1	22.2 ± 0.3	47.6 ± 0.5	0.47 ± 0.01	491	AsSb2	87 ± 2	24.2 ± 0.6	15.7 ± 0.2	50 ± 1	0.31 ± 0.01	485
As3	165 ± 3	52.3 ± 0.7	63 ± 1	157 ± 5	0.40 ± 0.02	333	AsSb3	175.3 ± 0.9	58 ± 1	50 ± 3	150 ± 4	0.34 ± 0.02	384
As4	196 ± 2	68.5 ± 0.4	88 ± 4	233 ± 4	0.38 ± 0.02	294	AsSb4	220 ± 4	70.3 ± 0.3	90 ± 5	260 ± 20	0.34 ± 0.01	268
As5	300 ± 5	114.2 ± 0.8	232 ± 5	650 ± 40	0.36 ± 0.03	175	AsSb5	310 ± 3	110 ± 2	143 ± 8	600 ± 10	0.24 ± 0.01	185
As6	410 ± 7	158.5 ± 0.7	330 ± 20	1231 ± 3	0.27 ± 0.02	129	AsSb6	405 ± 2	165 ± 6	367 ± 5	850 ± 20	0.31 ± 0.01	138
As7	510 ± 7	210 ± 3	582 ± 6	2560 ± 80	0.23 ± 0.01	82	AsSb7	510 ± 10	214 ± 4	503 ± 9	1580 ± 40	0.32 ± 0.01	136
As8	600 ± 1	260 ± 3	850 ± 20	3860 ± 200	0.22 ± 0.01	67	AsSb8	600 ± 3	-	-	-	-	-

-: not measured due to analysis problems

Table 6.4 Total and labile Sb concentrations measured by SEP, DGT, soil solution in contaminated soils, and R value of Sb (mean ± SE, n = 3).

Soil	Sb in Sb-only amended soils						Soil	Sb in As+Sb amended soils					
	$Total_{Sb}$ (mg/kg)	$C_{SEP\text{-}labile\ Sb}$ (mg/kg)	$C_{DGT\text{-}Sb}$ (µg/L)	$C_{sol\text{-}Sb}$ (µg/L)	Ratio R_{Sb} ($R = C_{DGT}/C_{sol}$)	K_d (L/kg)		$Total_{Sb}$ (mg/kg)	$C_{SEP\text{-}labile\ Sb}$ (mg/kg)	$C_{DGT\text{-}Sb}$ (µg/L)	$C_{sol\text{-}Sb}$ (µg/L)	Ratio R_{Sb} ($R = C_{DGT}/C_{sol}$)	K_d (L/kg)
Sb1	40 ± 1	6.15 ± 0.07	31.6 ± 0.3	150 ± 3	0.210 ± 0.005	41	AsSb1	45 ± 4	6.44 ± 0.04	38.2 ± 0.7	236 ± 1	0.161 ± 0.002	27
Sb2	78 ± 2	13.0 ± 0.1	74.8 ± 0.9	340 ± 3	0.222 ± 0.001	39	AsSb2	73 ± 7	12.7 ± 0.1	67 ± 2	670 ± 5	0.095 ± 0.002	18
Sb3	160 ± 9	25.6 ± 0.3	170 ± 1	740 ± 10	0.226 ± 0.002	34	AsSb3	170 ± 5	31.1 ± 0.7	167 ± 5	1767 ± 2	0.095 ± 0.003	18
Sb4	300 ± 10	49.7 ± 0.5	360 ± 10	1650 ± 20	0.219 ± 0.005	30	AsSb4	310 ± 4	57.9 ± 0.6	406 ± 6	3130 ± 60	0.130 ± 0.001	19
Sb5	650 ± 20	108.1 ± 0.5	850 ± 10	4250 ± 20	0.201 ± 0.002	25	AsSb5	320 ± 8	74 ± 1	450 ± 20	4550 ± 20	0.098 ± 0.004	16
Sb6	1560 ± 60	570 ± 4	2690 ± 20	16850 ± 100	0.159 ± 0.001	34	AsSb6	1620 ± 30	806 ± 10	3620 ± 70	32650 ± 400	0.111 ± 0.002	25
Sb7	3000 ± 60	-	5260 ± 100	33700 ± 200	0.156 ± 0.004	-	AsSb7	3400 ± 10	1800 ± 20	6410 ± 200	57080 ± 200	0.112 ± 0.003	32
Sb8	5300 ± 40	-	-	-	-	-	AsSb8	5800 ± 80	-	-	-	-	-

-: not measured due to analysis problems

Arsenic and Sb in As+Sb soils were much more labile than As and Sb in historically co-contaminated soils (chapter 5) on account of the greater proportion of their C_{DGT} and C_{sol} (Figure D.3), despite their lower soil concentrations. This could de due to the shorter equilibration time and soluble As and Sb added.

6.3.4. Uptake of As and Sb by water spinach (*Ipomoea aquatica*)

6.3.4.1. Plant growth yield

Water spinach cultivated in As-only soils showed a decrease in root and shoot biomass with increasing As concentrations (Figure 6.1A). Although the plants exhibited growth inhibition, toxicity symptoms such as wilting or chlorosis were not visible. The yield decreases were significant ($p<0.05$) when total soil As concentrations reached 300 mg/kg, corresponding to 653 µg As/L in soil pore water. At soil concentrations of 510 mg As/kg, the root and shoot yield were 0.09 and 1.63 g/pot, respectively, which were 15.2% and 7.7% of the corresponding yield in control soils. Plant death occurred at the highest treatment of 600 mg As/kg soil.

For Sb, compared to controls, there was a slight decrease in the yield of *I. aquatica* grown in Sb-only soils of <300 mg/kg, followed by a gradual increase in plant biomass cultivated in soils ≥300 mg Sb/kg, and a significant increase in plant tissues grown in soils >3000 mg Sb/kg (Figure 6.1B). This trend possibly resulted from the additional amounts of potassium accompanied with the use of $KSb(OH)_6$ as the Sb source, which stimulated the plant growth. For *I. aquatica* cultivated in As+Sb soils, the plant yield significantly decreased compared with the control yield, even when the plant was exposed to the lowest As+Sb treatment (Figure 6.1C). This indicates that the plants were sensitive to being exposed to a high amount of available As and Sb, leading to plant stress.

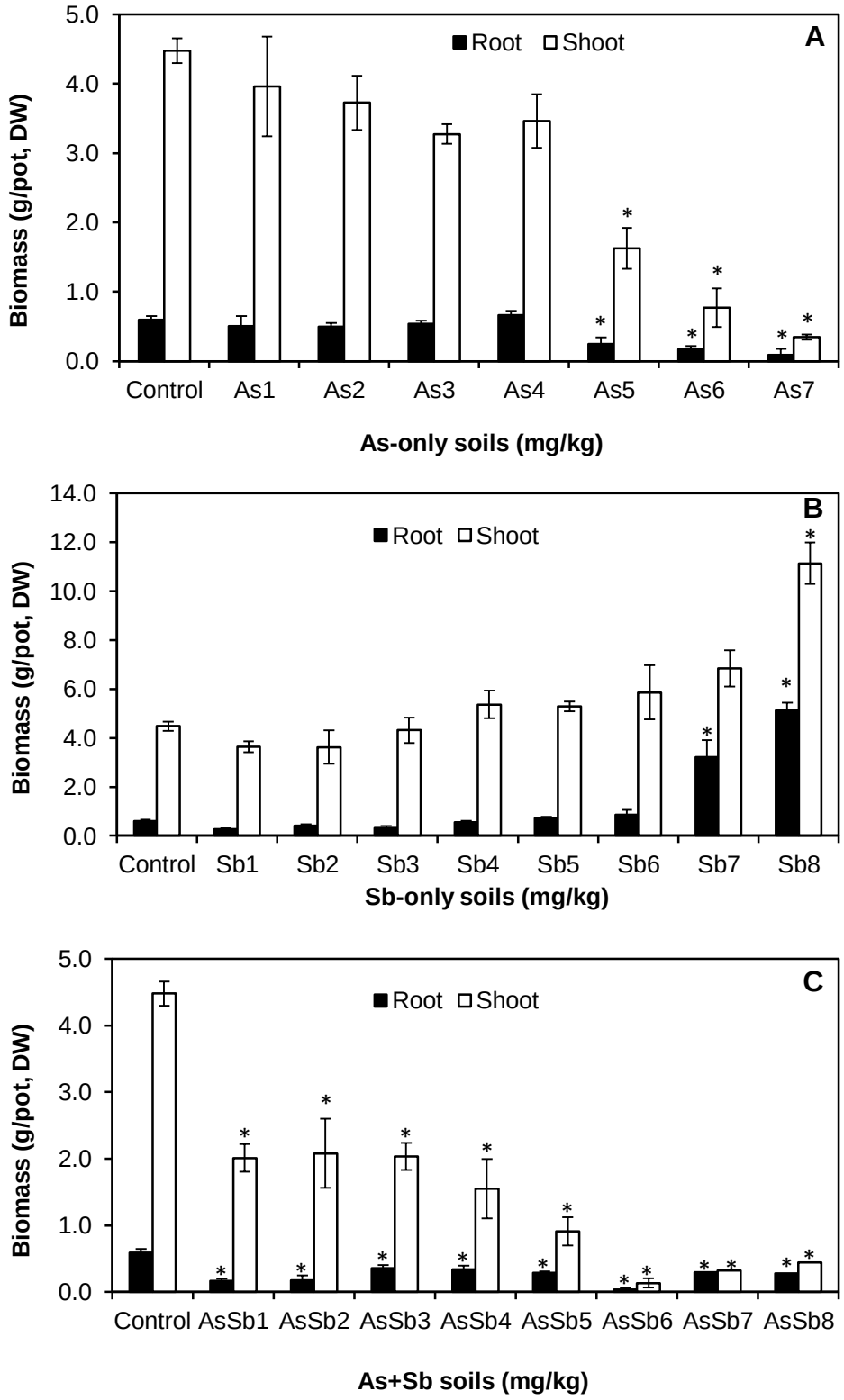

Figure 6.1 Biomass of *I. aquatica* cultivated in soils amended with different concentrations of As and Sb. Each value is mean ± SE (n = 3). The asterisks in the figure indicate significant differences ($p<0.05$) compared with the control plant tissues.

6.3.4.2. Accumulation of As and Sb in *I. aquatica* tissues

Arsenic and Sb concentrations in roots and shoots of *I. aquatica* grown in contaminated soils are presented in Figure 6.2. In As-only soils, As concentrations in roots linearly increased with increasing soil As concentrations, reaching at 1260 mg As/kg roots when *I. aquatica* was exposed to 410 mg As/kg. Above this concentration, there was a decrease of root As. The similar trend was observed for shoot As, but the bioaccumulation being highest at 82 mg As/kg shoots in a lower soil concentration (Figure 6.2A). The decrease in the translocation of As to shoots corresponded with the significant decrease in plant biomass. On average, As accumulation in *I. aquatica* roots (100 – 1260 mg As/kg) was 13 times higher than shoots (14.3 – 82 mg As/kg). The average bioaccumulation factor (BAF) based on the ratio of total root concentration and total soil concentration (mg/kg, DW) of As was 3.62. The average translocation factor (TF) determined by the ratio of total shoot concentration and total root concentration (mg/kg, DW) of As was 0.09.

For Sb-only soils, Sb concentrations in roots linearly increased with increasing soil Sb until 1560 mg Sb/kg, after which it decreased. The same pattern for Sb concentrations in shoots was observed, but it was highest at the higher soil concentration (3000 mg Sb/kg) (Figure 6.2C). This occurred at the same time as a significant increase in root biomass (Figure 6.1B) which may have stimulated the translocation of Sb to shoots. The decrease in Sb bioaccumulation occurred at much higher soil concentration compared to the As scenario, suggesting that Sb was accumulated via a different uptake mechanism to As, or Sb induced a lower degree of toxicity. Like As, Sb bioaccumulation in roots was 12.6 times higher than shoots. The average BAF and TF of Sb was 0.16 and 0.1, respectively.

Root and shoot As uptake by *I. aquatica* cultivated in As+Sb soils was similar to that in As-only soils. The root As concentration was highest in 310 mg As/kg soil and then declined as the soil As concentration increased, while the shoot As reached the accumulation saturation at 220 mg As/kg soil (Figure 6.2B). This may have occurred because the plant biomass significantly decreased. Roots also accumulated much more As than shoots, but to a lesser extent (a factor of 11) than that in As-only soils. The BAF of As (2.8) in As+Sb soils was lower than that in As-only soils, which can be explained by the slightly lower labile As

discussed in section 6.3.3. The competitive uptake of the co-occurring Sb might also contribute to the lower BAF.

In regards to the response of *I. aquatica* exposed to As+Sb soils, the saturation of Sb bioaccumulation in roots and shoots followed the same pattern as Sb-only soils, but to a greater extent (Figure 6.2D). The average BAF of Sb in As+Sb soils was 0.39, 2.4 times higher than in Sb-only soils, which might be due to the higher labile Sb discussed in sections 6.3.2 and 6.3.3. However, based on the DGT measurements the resupply of Sb in As+Sb soils was known to be lower than that in Sb-only soils. This indicates that the sustained Sb concentration in soil solution was likely related to *I. aquatica* roots activities. Plants produce root exudates such as organic compounds which may displace adsorbed Sb from the solid phase to increase Sb solution concentration, replenishing Sb in the rhizosphere soil. Another possibility for the increase of Sb bioaccumulation is that As enhanced the Sb uptake by plant. Although *I. aquatica* accumulated more Sb in both roots and shoots in As+Sb treatments, the average translocation factor of Sb was slightly lower in co-contaminated soils than that in Sb-only soils. This might be due to the reduction of plant biomass which inhibited the translocation of Sb.

The decrease in As bioaccumulation corresponded with the significant decrease in plant yield. The occurrence of the lowest root and shoot As concentrations in the highest As treatments is most likely because of the severe growth retardation, which may have limited the transfer of As from soil to roots. In addition, plant detoxification mechanisms may have restricted the transport of As to roots and subsequent translocation to shoots. This is supported by previous studies in spinach shoot, amaranth and silverbeet (Helgesen & Larsen 1998; Rahman & Naidu 2009). According to Rahman and Naidu (2009), the likely mechanism utilised by vegetable crops to tolerate As is avoidance via limiting the transport of As from roots to shoots, possibly due to diverting energy from biomass production and increasing As accumulated in roots. This mechanism seems to be appropriate to *I. aquatica* in this study, which explains the accumulation saturation of shoots occurring earlier than that of roots.

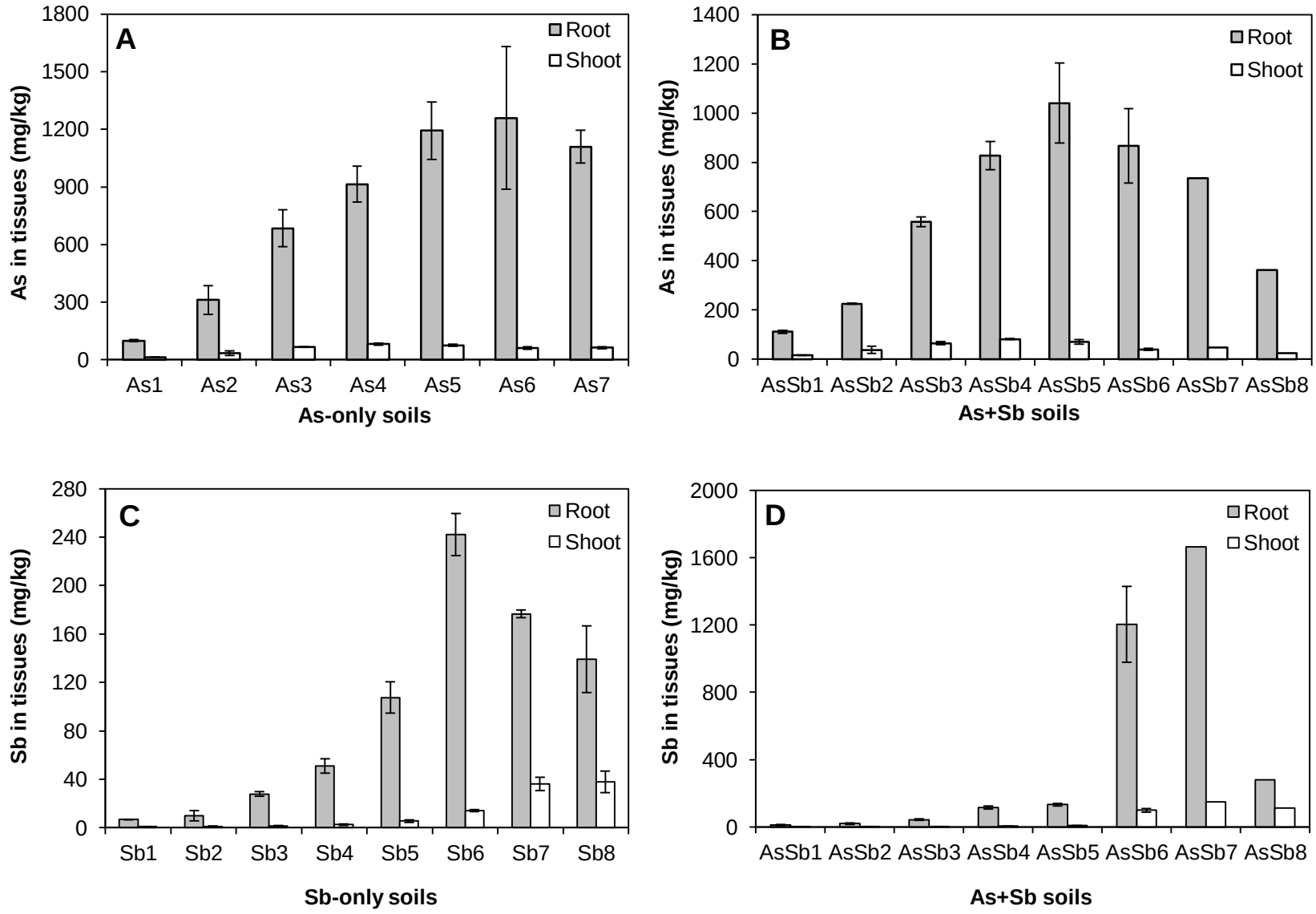

Figure 6.2 As and Sb concentrations in *I. aquatica* tissues (mean ± SE, n = 3) following cultivation in contaminated soils for 35 days.

The bioaccumulation factors of As and Sb varied among soils, depending upon their phytoavailabilities in soils. Compared to the historically As+Sb contaminated soils (chapter 5), the recently As+Sb amended soils exhibited substantial plant yield reduction and much greater bioaccumulation of As and Sb in *I. aquatica* cultivated in these soils, although the total soil As and Sb in these soils were much lower than historically contaminated soils (Figure 6.3). This is in agreement with the SEP and DGT data showing greater proportion of C_{SEP}, C_{DGT}, and C_{sol} of As and Sb. The results reconfirm that total soil concentration is not appropriate for risk assessments of contaminated soils.

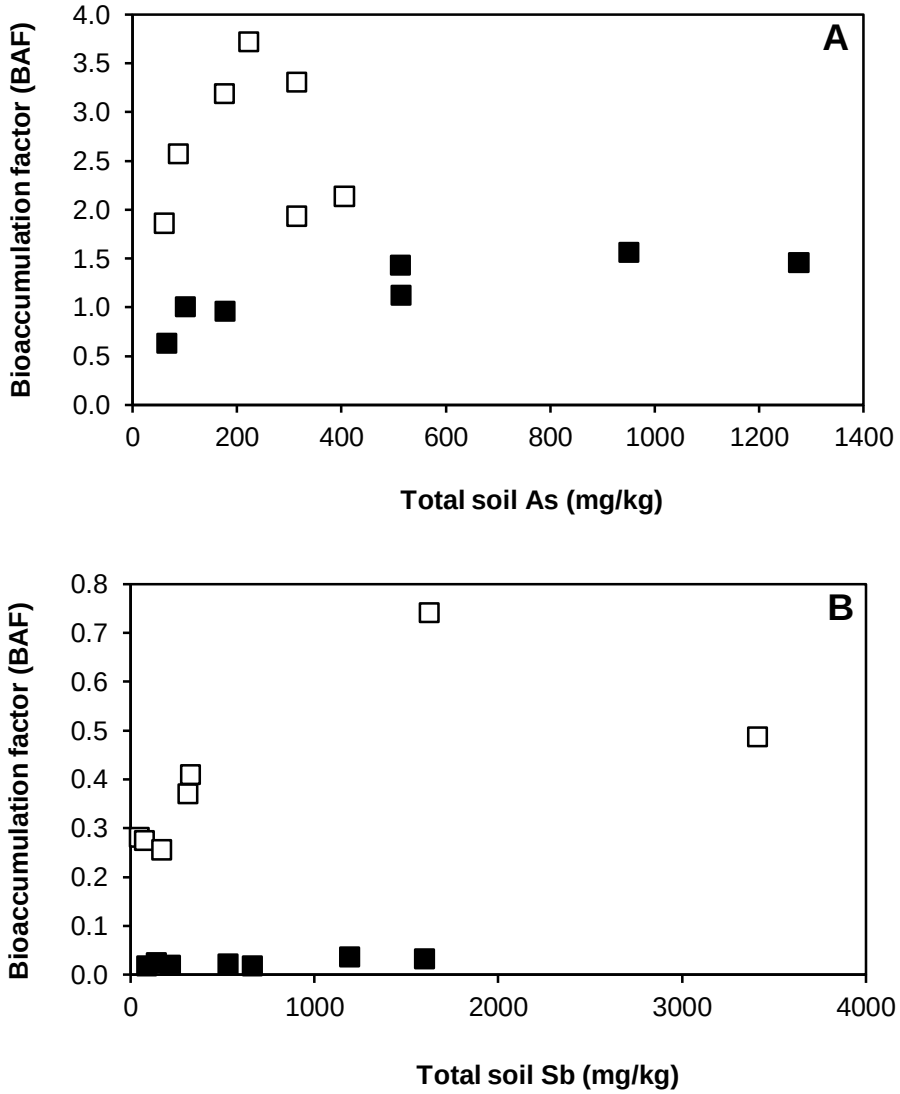

Figure 6.3 Comparison of bioaccumulation factors (BAF) of As and Sb in water spinach (*I. aquatica*) cultivated in recently As+Sb amended soils (□) and historically As+Sb contaminated soils (■) (chapter 5).

Overall, there is strong evidence of interaction between As and Sb with respect to *I. aquatica*. One hypothesis is competitive accumulation between As(III) and Sb(III) through aquaporins due to the similarities of glycerol, $As(OH)_3$, and $Sb(OH)_3$ in terms of structure, neutral charge, and molecular size (Bhattacharjee et al. 2008). As(III) is transported via Si transport pathways which belong to the aquaporin family (Ma et al. 2008). The aquaporins allow the uncharged molecules with similar size relative to Sb to pass through (Mathews et al. 2011). Meharg and Jardine (2003) observed an inhibition of As accumulation by the Sb for rice. However, the interaction between As(III) and Sb(III) was not expected to be dominant in the current study since the As(V) and Sb(V) are the dominant species in aerobic soils (Okkenhaug et al. 2011).

It has been established that As(V) is transported through phosphate transporters (Meharg & Hartley-Whitaker 2002), but such interactions were not observed for Sb(V) and phosphate in *Zea mays* Tschan et al. (2008). Competition between As(V) and Sb(V) for the same membrane channels has not been established (Tschan et al. 2009b). Thus, since the mechanism of Sb(V) uptake is still unknown, it can only be speculated that As(V) altered membrane integrity and hence the permeability of Sb(V). For the present study, it is possible that the addition of a more toxic element (As) into soils damaged the cell membrane and allowed Sb adsorption and transportation. The stimulation of Sb uptake due to As presence and the inhibition of As uptake due to Sb presence observed here are supported by hydroponic and quartz substrate studies by Feng et al. (2011) and Müller et al. (2013) reporting that the addition of As resulted in an increase of Sb uptake by As-hyperaccumulator *Pteris cretica* and *Pteris vitta*. Further analysis of subcellular distribution by Feng et al. (2011) showed that the increase of Sb concentrations associated with more transfer of Sb into the cytosol fractions, and less in the cell wall and cytoplasmic organelles. In contrast, the presence of Sb decreased As uptake by *Pteris cretica* when exposed to high concentrations of As and Sb, leading to the reduction of As in cytosol fractions in plant tissues, suggesting that cytosol may be an important reservoir. In the current study, water spinach (*I. aquatica)* can be classified as an As accumulator plant due to BAF of As being > 1 in both historically and recently contaminated soils. Thus it is not unreasonable to extrapolate these mechanisms of uptake and distribution in “accumulators”

of As and Sb by *I. aquatica*. The determination of As and Sb in subcellular fractions of *I. aquatica* tissues needs to be done to confirm those similarities.

6.3.5. The relationship between As and Sb bioaccumulation and soil concentrations

The relationship between As and Sb bioaccumulation and soil concentrations was only linear until a certain concentration after which bioaccumulation decreased corresponded with growth inhibition (Figure 6.2). Correlation analyses were performed on data where dose responses of plant roots gave linear relationships (expressed as correlation coefficients) between tissue As and Sb concentrations and their labile fractions measured by SEP, DGT, and soil solution concentrations (Table 6.5). Arsenic concentrations in roots were significantly correlated with all measures of labile fractions compared to shoot As concentration. This is probably because roots were in direct contact with soils, while As concentrations in shoots may be strongly controlled by plant physiology. This is evident by the fact that at the 300 mg As/kg soil treatment, the roots continued to accumulate As, while shoot As decreased. The shoot As concentrations were weakly correlated with soil As concentrations (low correlation coefficients, Table 6.5), which can be explained by the contrasting relationships between increased As concentrations in soils and decreased shoot As concentrations and plant yield.

As discussed in section 6.3.4, shoot As concentrations increased with increasing soil As concentrations up to a certain exposure concentration, after which As accumulation decreased corresponding to the reduction in plant biomass. When the saturated bioaccumulation data point was excluded (from soil treatment >300 mgAs/kg soil), shoot As concentrations were significantly correlated with all measures, C_{SEP}, C_{DGT}, and C_{sol} (correlation coefficients for shoot As concentrations > 0.9 for all cases). This illustrated that all measures worked well in predicting As uptake by *I. aquatica* grown in the low range of labile As in soils ($C_{SEP\text{-}As}$ <70 mg/kg, $C_{DGT\text{-}As}$ <90 µg/L, $C_{sol\text{-}As}$ <260 µg/L). For *I. aquatica* grown in As-only soils, $C_{SEP\text{-}As}$ best predicted As concentrations in tissues. This implies that the readily available and exchangeable As phases in the soils were important and responsible for plant uptake. For *I. aquatica* cultivated in As+Sb soils, the performance of DGT in predicting As in plants was improved and $C_{SEP\text{-}As}$ and $C_{DGT\text{-}As}$ better predicted As in plant tissues than $C_{sol\text{-}As}$. These results suggest that there was a labile pool of As from the

solid phase and dissolved complexes that were consistent with the R-value results (section 6.3.3), which is important from the bioavailability perspective. It is interesting that the labile extraction (C_{SEP}) was more highly correlated than DGT, perhaps there was direct uptake by roots in contact with soils.

In contrast, all lability measures were similar in predicting Sb accumulation by *I. aquatica*. C_{SEP}, C_{DGT}, and C_{sol} and tissue concentrations correlated more strongly for Sb than for As, which suggests that the reduction of plant yield resulted more from As than from Sb exposure. The Sb concentrations in shoots obtained from As+Sb soils were slightly less correlated with $C_{SEP\text{-}Sb}$ and $C_{DGT\text{-}Sb}$ in the soils than from Sb-only soils. This might be the higher Sb bioaccumulation in As+Sb soils due to the presence of As in soils which contributed to the slightly weaker relationships between shoot Sb concentrations and these soil labile fractions. Similar to As, Sb concentrations in shoots of *I. aquatica* grown in As+Sb soils were not as well predicted by $C_{SEP\text{-}Sb}$ and $C_{DGT\text{-}Sb}$ in the soils as the root Sb concentrations. The explanation for As can be applied for the Sb scenario here, which is because root accumulation was affected by soil processes, while shoot uptake was regulated by plant physiology to control the translocation of Sb from roots to shoots. The lower biomass may inhibit the transfer of Sb from roots to shoots.

Table 6.5 Correlation coefficients (R^2) between As and Sb concentration in tissues of *I. aquatica* cultivated in different soils and C_{SEP}, C_{DGT}, and C_{sol} of As and Sb in soils.

Treatments	As		Treatments	Sb	
	$C_{Root\text{-}As}$	$C_{Shoot\text{-}As}$		$C_{Root\text{-}Sb}$	$C_{Shoot\text{-}Sb}$
As-only			Sb-only		
$C_{SEP\text{-}As}$	0.96^{**}	0.72	$C_{SEP\text{-}Sb}$	0.99^{**}	0.99^{**}
$C_{DGT\text{-}As}$	0.82^{*}	0.49	$C_{DGT\text{-}Sb}$	0.99^{**}	0.99^{**}
$C_{sol\text{-}As}$	0.80^{*}	0.47	$C_{sol\text{-}Sb}$	0.99^{**}	0.99^{**}
As+Sb			As+Sb		
$C_{SEP\text{-}As}$	0.97^{**}	0.69	$C_{SEP\text{-}Sb}$	0.98^{**}	0.91^{*}
$C_{DGT\text{-}As}$	0.96^{**}	0.63	$C_{DGT\text{-}Sb}$	0.99^{**}	0.85^{*}
$C_{sol\text{-}As}$	0.86^{*}	0.46	$C_{sol\text{-}Sb}$	0.96^{**}	0.95^{**}

**: statistically significant ($p<0.01$)

*: statistically significant ($p<0.05$)

6.3.6. Risk assessment of As and Sb in *I. aquatica* to human health

Similar to water spinach (*I. aquatica*) grown in historically contaminated soils (chapter 5), the As concentrations in edible shoots of *I. aquatica* cultivated in amended soils (mg/kg WW, Table 6.6) were higher than the maximum permissible value in food, specific to cereals (1 mg As/kg WW) set by the Food Standard Agency of Australia and New Zealand (FSANZ 2013) and permissible value for food (0.1 mg As/kg, WW) recommended by joint Food and Agricultural Organisaion (FAO)/World Health Organisation (WHO) (JECFA 2011). Thus, there is high risk of consuming this leafy vegetable cultivated in the studied As-contaminated soils. The joint FAO/WHO Expert Committee on Food Additives (JECFA) has not reported guidelines relating to Sb safety. Nevertheless, the USEPA has recommended an oral reference dose (RfD) for Sb being 0.0004 mg/kg of body weight (BW)/day. The exposure parameters used for the calculation of estimated daily intake (EDI), hazard quotient (HQ), and cancer risk (CR) in sections 5.2.7 and 5.3.6 of chapter 5 were applied in this chapter.

The values of EDI, HQ, and CR of As and Sb through the consumption of *I. aquatica* shoots are presented in Tables 6.6 and 6.7, respectively. The EDI values of As for both adults and children were higher than the acceptable limit or the oral reference dose of As (RfD, 3×10^{-4} mg/kg/day) set by USEPA (2011). The HQ values were calculated to assess the potential risk of non-cancer adverse health effect to human through As and Sb ingestion. The HQ values of As were > 1 for all cases, ranging from 3.91 – 22.3 for adults and 6.43 – 36.7 for children, indicating the substantial risk of non-carcinogenic effects for both adults and children consuming *I. aquatica* cultivated in contaminated soils with As ≥46 mg/kg corresponding to $C_{DGT\text{-}As}$ ≥9.7 µg/L and $C_{sol\text{-}As}$ ≥17.36 µg/L. The EDI values for Sb for both adults and children consuming *I. aquatica* from Sb-only soil (≥650 mg Sb/kg corresponding to $C_{DGT\text{-}Sb}$ ≥850 µg/L and $C_{sol\text{-}Sb}$ ≥4250 µg/L) and from As+Sb soil (≥310 mg Sb/kg corresponding to $C_{DGT\text{-}Sb}$ ≥406 µg/L and $C_{sol\text{-}Sb}$ ≥3130 µg/L) were higher than the oral reference dose of Sb (RfD, 4×10^{-4} mg/kg/day) set by USEPA (1991). The HQ values of Sb were > 1 for Sb-only soil (≥650 mg Sb/kg) and As+Sb soil (≥310 mg Sb/kg), ranging from 1.13 – 30.5 for adults and 1.87 – 50.2 for children, indicating the significant risk of

non-carcinogenic effects for both adults and children consuming *I. aquatica* cultivated in Sb-contaminated soils in those concentration ranges.

The higher potential risk of *I. aquatica* from As+Sb soil compared to Sb-only soil is due to its higher lability of Sb in these As+Sb soils resulting in higher Sb bioaccumulation. It is noticeable that As presented higher non-carcinogenic risks than Sb, demonstrated by the much higher HQ values of As derived from the consumption of *I. aquatica* grown in soils with comparable concentrations of As and Sb. In addition, HQ values of As were much higher than 1 when *I. aquatica* grown in the lowest soil treatments was consumed, while HQ values of Sb were only higher than 1 when *I. aquatica* exposed to Sb-only soil (≥650 mg Sb/kg) and As+Sb soil (≥310 mg Sb/kg) was ingested.

The carcinogenic risk (CR) was calculated to assess the probability of the exposed population developing cancer risk through ingestion of As-contaminated vegetables during their lifetime. Due to the lack of the cancer slope for Sb, only CR derived from the consumption of As-enriched *I. aquatica* was calculated. The CR values increased with the increasing soil As and far exceeded the probability of 1 in 10,000, indicating an increase in probability of cancer development via consumption of *I. aquatica* shoots cultivated in soils contaminated with As ≥46 mg/kg corresponding to $C_{DGT\text{-}As}$ ≥9.7 µg/L and $C_{sol\text{-}As}$ ≥17.36 µg/L.

In general, the potential health risks and cancer risks were greater for children than adults. The consumption of *I. aquatica* cultivated in amended soils with such concentrations poses much higher risks (both non-carcinogenic and carcinogenic effects) to humans than that in historically contaminated soils (section 5.3.6, chapter 5). This is due to the higher edible shoot bioaccumulation resulting from the higher lability of As and Sb in freshly amended soils compared to historically contaminated soils.

Table 6.6 Values of shoot As (mg/kg, WW), EDI (mg/kg/day), non-carcinogenic risk (HQ), and cancer risk (CR) for the ingestion of water spinach (*I. aquatica*) shoots grown in As-only and As+Sb soils.

Soil	Treatment	Soil As (mg/kg DW)	Shoot As* (mg/kg WW)	Estimated daily intake (EDI)		Hazard quotient (HQ)		Cancer risk (CR)	
				Adults	Children	Adults	Children	Adults	Children
As-only	As1	46	1.43	11.7×10^{-4}	19.3×10^{-4}	3.91	6.43	17.6×10^{-4}	86.9×10^{-4}
	As2	84	3.49	28.6×10^{-4}	47.0×10^{-4}	9.52	15.7	42.8×10^{-4}	212×10^{-4}
	As3	165	6.63	54.3×10^{-4}	89.5×10^{-4}	18.1	29.8	81.5×10^{-4}	403×10^{-4}
	As4	196	8.16	66.8×10^{-4}	110×10^{-4}	22.3	36.7	100.2×10^{-4}	495×10^{-4}
As+Sb	AsSb1	60	1.65	13.5×10^{-4}	22.3×10^{-4}	4.51	7.42	20.3×10^{-4}	100×10^{-4}
	AsSb2	87	3.75	30.7×10^{-4}	50.5×10^{-4}	10.2	16.8	46.0×10^{-4}	227×10^{-4}
	AsSb3	175	6.45	52.9×10^{-4}	87.1×10^{-4}	17.6	29.0	79.3×10^{-4}	392×10^{-4}
	AsSb4	220	8.08	66.2×10^{-4}	109×10^{-4}	22.1	36.3	99.3×10^{-4}	490×10^{-4}

* Concentration of As in shoots of *I. aquatica*; parameters are presented where a dose response was observed.

Table 6.7 Values of shoot Sb (mg/kg, WW), EDI (mg/kg/day), non-carcinogenic risk (HQ), and cancer risk (CR) for the ingestion of water spinach (*I. aquatica*) shoots grown in Sb-only and As+Sb soils.

Soil	Treatment	Soil Sb (mg/kg DW)	Shoot Sb* (mg/kg WW)	Estimated daily intake (EDI)		Hazard quotient (HQ)	
				Adults	Children	Adults	Children
Sb-only	Sb1	40	0.08	0.68×10^{-4}	1.11×10^{-4}	0.17	0.28
	Sb2	78	0.11	0.91×10^{-4}	1.50×10^{-4}	0.23	0.37
	Sb3	160	0.15	1.26×10^{-4}	2.07×10^{-4}	0.31	0.52
	Sb4	300	0.24	2.01×10^{-4}	3.30×10^{-4}	0.50	0.83
	Sb5	650	0.55	4.54×10^{-4}	7.47×10^{-4}	1.13	1.87
	Sb6	1560	1.41	11.6×10^{-4}	19.0×10^{-4}	2.89	4.76
	Sb7	3000	3.63	29.7×10^{-4}	48.9×10^{-4}	7.43	12.2
As+Sb	AsSb1	45	0.06	0.51×10^{-4}	0.84×10^{-4}	0.13	0.21
	AsSb2	73	0.10	0.78×10^{-4}	1.29×10^{-4}	0.20	0.32
	AsSb3	170	0.19	1.59×10^{-4}	2.63×10^{-4}	0.40	0.66
	AsSb4	310	0.46	3.75×10^{-4}	6.18×10^{-4}	0.94	1.54
	AsSb5	320	0.89	7.27×10^{-4}	12.0×10^{-4}	1.82	2.99
	AsSb6	1620	9.96	81.6×10^{-4}	134×10^{-4}	20.4	33.6
	AsSb7	3400	14.89	122×10^{-4}	201×10^{-4}	30.5	50.2

* Concentration of Sb in shoots of *I. aquatica*; parameters are presented where a dose response was observed.

It is important to note that the consumption of *I. aquatica* cultivated in Sb-only soils (≥650 mg Sb/kg) poses unacceptable non-carcinogenic risk to humans, but *I. aquatica* grew healthily, had no physical toxicity symptoms, and greater biomass of edible shoots. As discussed in section 6.3.4.1, the tissue biomass of *I. aquatica* grown in Sb-only soils displayed the increasing trend with the addition of soluble Sb into soils. Consequently, the Sb-enriched edible shoots of *I. aquatica* cultivated in Sb-only soils cannot be recognised and distinguished compared to As-enriched shoots of *I. aquatica* cultivated in As-contaminated soils where shoot biomass decreased when the plant exposed to high soil concentrations. This implies that growing *I. aquatica* in Sb-contaminated soils should be in caution because the plant presents hidden risks and may pose non-carcinogenic risks to human health via the food chain.

6.4. Conclusions

The SEP data showed that amorphous and crystalline iron oxides were the most two dominant fractions for Sb, whereas As was mainly found in amorphous iron oxide and specifically sorbed fractions. High loads of both As and Sb into soils increased the proportions of As and Sb in non-specifically sorbed fraction. The presence of As in As+Sb soils significantly increased Sb concentrations in soil solutions. However, the resupply of Sb in As+Sb soils was lower than that in Sb-only soils and the resupply of Sb was slower than that of As.

The *I. aquatica* response varied according to concentration ranges of As and Sb in soils, demonstrated by bioaccumulation of As and Sb saturated at a certain soil concentration, then decreasing with the significant decline in plant yield. Root As and Sb concentrations were >10 times higher than their shoot concentrations. The interesting finding was the clear enhancement of Sb uptake by *I. aquatica* cultivated in As+Sb soils compared with Sb-only soils. On average, the increase of Sb accumulation was not accompanied by an increased translocation of Sb from roots to shoots.

The strong positive correlations between labile fractions of As and Sb in soils and their concentrations in *I. aquatica* tissues were only observed in non-toxic ranges, confirming that labile measures do not work well in predicting the bioaccumulation of elements when plants suffer from toxic effects. C_{SEP}, C_{DGT}, and C_{sol} more strongly correlated with tissue concentrations of Sb than As and root concentrations than shoots. SEP, DGT, and soil solution concentrations were relatively similar in predicting As and Sb

accumulation by *I. aquatica*. DGT performed comparatively well with other measures, but DGT measurements are probably simpler than some of the other measurements which required single or multiple extractions of soil samples and soil characterisation. The combination of these techniques was useful in understanding the biogeochemical behaviour of As and Sb in individual and co-occurring soils as well as interactive effects of As and Sb on accumulation by *I. aquatica*.

The As concentrations in shoots of *I. aquatica* far exceeded the maximum permissible values set by the Food Standard Agency of Australia and New Zealand (1 mg As/kg, WW) and FAO/WHO (0.1 mg As/kg, WW) and As posed higher non-carcinogenic and carcinogenic risks than Sb. This is the first study illustrating the competitive interactions of As and Sb in aerobic soils and in an important edible vegetable, thus is of value for risk assessments of As and Sb contaminated soils and managements of their concentrations in edible vegetables for food safety.

Chapter 7. Mobilisation of As and Sb in soils induced by redox changes identified by DGT and their uptake by water spinach (*Ipomoea aquatica*)

7.1. Introduction

Arsenic (As) and antimony (Sb) frequently co-occur in extremely high concentrations on mining and smelting sites and surrounding soils (Anawar et al. 2011; Ashley et al. 2007; Okkenhaug et al. 2012; Pratas et al. 2005). The retention and mobilisation of As and Sb in soils depends on the soil properties (e.g. Fe oxides, organic matter), environmental conditions (e.g. redox potential, pH), and their speciation (Wilson et al. 2010). Iron (hydr)oxides are important sorbents for the immobilisation of As and Sb in soils via adsorption, precipitation, and co-precipitation (Mitsunobu et al. 2006; Wilson et al. 2010), thus the lability and solubility of As and Sb in oxic soils were low (Álvarez-Ayuso et al. 2012; Ettler et al. 2010; Kim et al. 2014; Liu et al. 2015; Okkenhaug et al. 2012).

When As contaminated soils were subjected to anoxic conditions (e.g. waterlogging), the As was rapidly mobilised, leading to increased soluble As in soil pore water, dominated by As(III) (Garnier et al. 2010; Takahashi et al. 2004; Xu et al. 2008). Xu et al. (2008) reported that arsenic concentrations in soil pore water were 7-16 and 4-13 times higher under the waterlogged than under the non-waterlogged conditions in soil without As addition and in soils amended with 10 mg As/kg, respectively. In contrast to As, studies on Sb behaviour in anoxic soils are inconsistent and mechanisms controlling Sb speciation and concentrations have been unclear. Mitsunobu et al. (2006) reported only 6% of Sb present as Sb(III) in the solid phase and soil solution under reducing conditions. Later studies reported the effect of redox condition on Sb speciation with Sb(III) constituting 5 – 19% of total Sb in mining-impacted soil pore water (Okkenhaug et al. 2012) and 60% of total Sb in anaerobic shooting range soil pore water (Wan et al. 2013a). It has been reported that reducing conditions generally decreased Sb mobility (Mitsunobu et al. 2006; Wan et al. 2013b), whereas an incubation study by (Hockmann et al. 2014a) stated that reducing conditions actually increased Sb mobility. The varied results of Sb speciation in reduced soil pore water are likely due to changes in Sb

species during sampling and analysis. According to Oorts et al. (2008), Sb(III) is very unstable and is oxidized to Sb(V) within 2 days under oxic conditions. It is difficult to preserve and analyse Sb species in environmental samples (Hansen & Pergantis 2008). Thus, collection and analysis of Sb species in soil pore water for accurate assessment of Sb behaviour in contaminated soils and its risks still remains a challenge.

As discussed in previous chapters, diffusive gradients in thin films (DGT) and diffusive equilibration in thin films (DET) techniques are *in situ* samplers allowing the *in-situ* study of distribution and concentration profiles of solute in sediment pore water at higher spatial resolution than conventional techniques (Davison et al. 2000; Zhang et al. 1995; Zhang et al. 2002). The Metsorb DGT coupled with DET for Fe was applied to investigate the sediment biogeochemistry of As and Fe in spatial resolution and demonstrated the mechanism of As mobilisation in sediments (Bennett et al. 2012b). The As and Sb species in sediments have not been investigated using DGT because the mercapto-silica DGT for As(III) and Sb(III) measurements has recently been developed and initially applied to measure As(III) in water (Bennett et al. 2011) and Sb(III) in water (Bennett et al. 2016b). With the newly developed techniques, we are able to measure *in-situ* total inorganic As and Sb, together with individual As and Sb species in water, sediment, and soil pore water by simultaneous use of the combination of Metsorb DGT and mercapto-silica DGT, avoiding artifacts of species changes during sample collection and analysis. The use of Metsorb DGT and mercapto-silica DGT, coupled with Fe-DET, will allow the mechanisms of As and Sb mobilisation in soils to be investigated.

In terms of the effect of waterlogging on As and Sb uptake by plants, waterlogging As-contaminated soils increased As accumulation in plants cultivated in those soils (Punshon et al. 2017; Wu et al. 2017; Xu et al. 2008). Xu et al. (2016) stated that growing rice in As-contaminated soils subjected to flooding dramatically enhanced As concentrations in rice grains by a factor of 10-15 fold. Arsenic concentrations in rice roots were markedly higher in anoxic treatments (147 - 243 mg/kg) compared to oxic treatments (88.8 - 218 mg/kg) (Wu et al. 2017). A preliminary study on Sb uptake by plants grown in waterlogged contaminated soils showed a contrasting result. Waterlogging Sb-contaminated soils increased Sb concentrations in shoots of *Lolium perenne* by 10 times, but decreased uptake by *Holcus lanatus* by 80% (Wan et al. 2013a). Most studies on the effect of waterlogging on As and Sb uptake by plants used

singly contaminated soils as test soils and rice and grass as test plants. There has been no published study on the effects of flooding on simultaneous uptake of As and Sb by edible crops cultivated in co-contaminated soils and their competitive uptake by vegetables exposed to flooded contaminated soils.

Previous studies (chapters 3, 4, 5) showed that As and Sb in historically contaminated soils were mainly present in the Fe oxides and residual phases and their lability in these aerobic soils were low. It is expected that when the historically contaminated soils are subject to waterlogging, As and Sb will be more labile and mobilised, which leads to an increase in their accumulation in the cultivated edible plants. In addition, the results of chapter 5 showed that water spinach (*Ipomoea aquatica*) grown in historically contaminated soils accumulated As in edible shoots at high concentrations exceeding the maximum permissible value for food (1 mg As/kg, WW) set by the Food Standard Agency of Australia and New Zealand (FSANZ 2013), although the plant did not exhibit toxic symptoms such as wilting and chlorosis. Furthermore, *I. aquatica* is a common edible vegetable in Asia and can grow in both terrestrial and semiaquatic soil systems. All these matters become alarming concerns. Thus, it is essential to examine the behaviour of As and Sb in contaminated soils and *I. aquatica* exposed under different scenarios and determine As and Sb bioaccumulation and speciation in *I. aquatica* cultivated under various soil conditions to further accurately assess risks to humans and ensure food safety.

This study aims to investigate (i) changes in solubility of As and Sb and their mobilisation mechanisms in historically co-contaminated soils, freshly co-contaminated soils, and soils freshly contaminated with As only and Sb only in response to waterlogging using several DGT/DET techniques; (ii) the effect of using semiaquatic cultivation on the solubility and speciation of As and Sb in soils; (iii) the uptake of As and Sb by water spinach (*I. aquatica*) grown in terrestrial and flooded contaminated soils; and (iv) the speciation transformation of As in the *I. aquatica* by HPLC-ICP-MS. To our best knowledge, this is the first study to measure the dynamics and chemistry of As and Sb species in flooded contaminated soils using the DGT and DET and to determine for the first time As speciation in flooded *I. aquatica* compared to that in non-flooded *I. aquatica*. In addition, the study on As-only amended soil, Sb-only amended soil, and As+Sb amended soil allows us to understand if there is the competitive effect of As on the solubility of Sb in anoxic soils and vice versa.

7.2. Methods

7.2.1. General method

General washing methods were done as per section 2.1.

Materials, reagents, and standards for HPLC-ICP-MS

Sub-boiled double distilled nitric acid (Aristar, BDH) and deionised water were used in the extraction of As species from plant samples. Ammonium dihydrogen orthophosphate (Suprapur, Merck) and pyridine (Extra Pure, Merck) were used for the mobile phases. Ammonia solution (>99.9%, Aldrich) and formic acid (Extra Pure, Fluka) were used for pH adjustment of mobile phases for HPLC. Arsenous acid (As(III)), arsenic acid (As(V)), methylarsonic acid (MA), and dimethylarsinic acid (DMA) were prepared by dissolving sodium arsenite, sodium arsenate heptahydrate (AJAX Laboratory Chemicals), disodium methylarsenate (Alltech-Specialists), and sodium dimethylarsinic (Alltech-Specialists), respectively, in deionised water immediately before use.

7.2.2. Experimental design

7.2.2.1. Soil preparation

A historically As and Sb-contaminated soil was collected from a decommissioned antimony processing facility in Urunga NSW, Australia. Details about soil physicochemical properties were described previously (section 3.2.2.1). An uncontaminated soil (control soil) was collected in Wollongong, NSW. The soils were air-dried and crushed to pass a 2-mm sieve. The historically contaminated soil was thoroughly mixed with the uncontaminated soil to establish one low treatment (Aged-L) and one high treatment (Aged-H) of As and Sb. In addition, separate portions of uncontaminated soil was amended with either solutions of sodium arsenate, potassium antimonate, or their mixture to establish one treatment of each spiked soil, namely As-only soil (As50), Sb-only soil (Sb300), and As+Sb soil (As50Sb300). Following the addition of As/Sb solutions into soils, the spiked soils were homogenised by mixing and turning over vigorously. Moisture content of soils was retained at 60% of maximum water holding capacity (MWHC) after spiking. The artificially contaminated soils were left to equilibrate for 12 weeks. After the equilibration period, all spiked soils were air-dried, crushed using a mortar and pestle, and mixed homogeneously for the bioassays.

All test soils were applied nutrients and allowed to equilibrate as described in section 2.4.2. Soils As50, Sb300, and As50Sb300 are considered recently contaminated soils.

7.2.2.2. Experimental treatment and management

Pots were filled with 4 kg of each processed soil. Soil pots without water spinach (*Ipomoea aquatica*) and pots with *I. aquatica* were prepared in triplicate. Two water regimes were applied: (i) non-flooded, in which soils were maintained at 60% of MWHC and (ii) flooded, in which soils were thoroughly mixed with water and flooded to about 4-5 cm above the surface which is typical of semiaquatic agronomy practices. All soil pots were transferred to controlled growth chambers, as described in section 2.4.2. After one week of flooding, a pair of Metsorb-DGT and mercapto-silica-DGT probes was gently inserted into the centre of each flooded soil treatment pot for 24 h to measure labile As and Sb (see DGT procedures below for details). *I. aquatica* were then transplanted into all soil pots (both flooded and non-flooded treatments) the following day. Water was added daily to maintain flooding of soils and pots were randomly rearranged every week.

After 5 weeks of growth in soils, the second DGT deployment was conducted in the same manner as the first deployment. *I. aquatica* were harvested the following day and washed vigorously with deionised water to remove adhering soil before being separated into shoots and roots with a stainless steel blade. After removal of the iron layer from the roots (see procedure below) and washing with deionised water, roots and shoots were freeze dried for 48 h at –50 °C. All dried samples were ground to a fine powder under liquid nitrogen with a ceramic mortar and pestle and stored in clean plastic bags for further analysis.

Extraction of iron plaque

Iron plaque on fresh roots was extracted using dithionite-citrate-bicarbonate (DCB) method as described previously (Liu et al. 2004). The whole fresh root system was soaked in 50 mL of 0.03 M sodium citrate ($Na_3C_6H_5O_7.2H_2O$) and 0.125 M sodium bicarbonate ($NaHCO_3$) for 10 min at room temperature (20 – 25 °C). Then 0.6 g of sodium dithionite ($Na_2S_2O_4$) was added and each root was incubated for another 60 min. Subsequently, roots were taken out and rinsed three times with deionised water. All rinsed water was collected and combined with its corresponding DCB extract. The volume of final solutions was made up to 100 mL with deionised water.

7.2.3. Soil and plant analyses

7.2.3.1. Total As and Sb analysis

The digestion and analysis of soil and plant samples were done as per sections 5.2.4 and 2.9. For quality control, reagent blanks, duplicate analyses, certified reference materials, and matrix spike recovery were used. A soil certified reference material (Montana II soil, SRM 2711a; NIST) returned recoveries of 86-118% for the studied analytes. A plant certified reference material (Tomato leaves, SRM 1573a; NIST) had typical recoveries of 88-116% of the studied analytes.

7.2.3.2. HPLC-ICP-MS inorganic As analysis

The extraction of inorganic As species was performed as per Foster et al. (2007) and Maher et al. (2013) with minor modifications. Briefly, an accurately known mass (approximately 0.2 g) of water spinach sample (*I. aquatica*) was weighed into 55 mL polytetrafluoroacetate (PTFE) digestion vessels (CEM) and combined with 5 mL of 2% v/v HNO_3. Samples were heated in a Mars Xpress microwave oven at 110 °C for 10 min, cooled to room temperature and combined with 5 mL of deionised water. The supernatants were removed by centrifuging at 5000 rpm for 10 min and filtering. The extract was further diluted 1:1 (v/v) with deionised water prior to chromatography to get adequate peak separation between arsenite and DMA.

The HPLC system consisted of a Perkin-Elmer series 200 mobile phase delivery and autosampler system (Perkin-Elmer). Column conditions used for the separation of anionic As species are described by Kirby et al. (2004). A PEEK PRP-X100 anion exchange column (250 mm × 4.6 mm, 10 μm) (Phenomenex) using 20 mM ammonium phosphate buffer mobile phase at pH 4.5, flow rate 1.5 mL min^{-1}, column temperature of 40 °C, and injection volume of 40 μL was used for the identification and quantification of As(III), As(V), DMA, and MA. The eluant from HPLC columns was directed by PEEK (polyether-ether-ketone) (i.d. 0.02 mm) (Supelco) capillary tubing into a Ryton cross-flow nebulizer of a Perkin-Elmer Elan-6000 ICP-MS, which was used to monitor the signal intensity of As at m/z 75. The potential interference to As (m/z 75) from chloride ($^{40}Ar^{35}Cl$) was determined by monitoring chloride at m/z 35, $^{40}Ar^{37}Cl$ at m/z 77. Selenium was monitored at m/z 82 as a cross check on $^{40}Ar^{37}Cl$ interference.

7.2.4. DGT procedures

7.2.4.1. DGT probe preparation

Metsorb DGT probes for measuring total labile inorganic As and Sb and mercapto-silica DGT probes for measuring labile As(III) and Sb(III) were prepared as per section 2.7.

7.2.4.2. DGT deployment and retrieval

All DGT probes were deoxygenated by immersion in a clean 0.01 M NaCl solution and bubbled with nitrogen for 24 h prior to deployment. A pair of deoxygenated DGT probes (one Metsorb-DGT and one mercapto-silica-DGT) arranged back to back, was gently inserted into the flooded soil pots. DGT deployment was performed one day before transplanting plants and one day before harvesting plants at 25 ± 1 °C for 24 h. On retrieval, the soil water interface (SWI) was marked, the probes were rinsed well with deionised water to remove sediment particles, immediately put in cleaned plastic zip lock bags, and kept in a refrigerator until disassembly. The diffusive gels from mercapto-silica DGT probes were used as diffusive equilibration in thin films (DET) for measuring Fe, thus mercapto-silica DGT probes were disassembled immediately. On disassembly, binding and diffusive gels for Fe were sliced at 10 mm intervals with two slices above the SWI and eight slices below the SWI using a Teflon coated razor blade. Each DET slice was eluted in 3 mL of 2% HNO_3 for 24 h (Gao et al. 2007; Garnier et al. 2015; Gregusova & Docekal 2013). Each DGT resin slice was eluted in 1 mL of solution with a final concentration of 1 M NaOH : 1 M H_2O_2 for 24 h (Bennett et al. 2016b; Panther et al. 2013). The DGT extracts were diluted 20 fold prior to analyis by ICP-MS.

Blank DGT probes were treated in the same way as the sample probes. DGT-measured concentrations, C_{DGT}, of As and Sb were calculated using Equation 7.1 (Zhang & Davison 1995).

$$C_{DGT} = M\Delta g / (DAt) \qquad \text{Equation 7.1}$$

Where M is the mass of elements on the binding gel, Δg is the thickness of the diffusive gel (0.8 mm) plus the thickness of filter membrane (0.1 mm), D is the diffusion coefficient of elements in the diffusive gel (5.69×10^{-6} cm^2 s^{-1} for As(V), 9.04×10^{-6} cm^2 s^{-1} for As(III) and 6.21×10^{-6} cm^2 s^{-1} for Sb(V), 9.42×10^{-6} cm^2 s^{-1} for Sb(III) at 25 °C), A is the exposed area of the gel slice (cm^2), and t is the deployment time (s).

7.2.5. Data analysis

Statistical analysis and calculations of bioaccumulation factor (BAF) and translocation factor (TF) were performed as per section 3.2.7.

7.3. Results and discussion

7.3.1. DGT measurements in flooded historically contaminated soils

7.3.1.1. Dynamics of lability and speciation of As and Sb in flooded historically contaminated soils

The C_{DGT} profiles of As, As(III), As(V), Sb, Sb(III), and Sb(V) in soil Aged-L were determined one and five weeks after flooding with and without plants, and are presented in Figure 7.1. After 1 week of flooding (before transplanting), $C_{DGT\text{-}Sb}$ was higher than $C_{DGT\text{-}As}$ in both the overlying water (OLW) and soil layer. $C_{DGT\text{-}As}$ and $C_{DGT\text{-}Sb}$ slightly increased at mid-profiles and then decreased (Figure 7.1A). The higher $C_{DGT\text{-}As}$ and $C_{DGT\text{-}Sb}$ in the OLW indicates that there were fluxes of As and Sb into the OLW. For speciation, both $C_{DGT\text{-}As(III)}$ and $C_{DGT\text{-}Sb(III)}$ were constantly low until a depth of 10-mm, after which $C_{DGT\text{-}As(III)}$ exhibited a sharp increase from 20 to 40-mm depth, followed by a slight increase, while $C_{DGT\text{-}Sb(III)}$ displayed a sharp increase to from 20 to 50-mm depth, followed by a decrease. The average proportion of $C_{DGT\text{-}As(III)}$ and $C_{DGT\text{-}Sb(III)}$ in deep soil layer (from 20-mm depth downwards) accounted for 62% and 11% of total labile As and Sb species, respectively (Figure 7.1B). The presence of trivalent species indicates that the anaerobic condition developed and the mobilisation of As and Sb occurred at the initial stage of plant bioassay. In contrast, both $C_{DGT\text{-}As(V)}$ and $C_{DGT\text{-}Sb(V)}$ were high in the OLW and displayed a decreasing trend from the OLW until the bottom of the probe (Figure 7.1C). $C_{DGT\text{-}Sb(V)}$ showed virtually the same patterns to $C_{DGT\text{-}Sb}$ because Sb(V) was the dominant inorganic Sb species.

After 5-week flooding (at the end of the plant bioassay), the profiles of $C_{DGT\text{-}As}$, $C_{DGT\text{-}Sb}$, and their species in flooded soils varied greatly compared to the initial stage. In unplanted soils, interestingly, $C_{DGT\text{-}As}$ increased considerably from 10 to 40-mm depth, followed by a gradual increase and was higher than that in 1-week flooded soil. In contrast, $C_{DGT\text{-}Sb}$ was only higher in the OLW, but significantly lower in deep soil (below 10-mm depth) compared to that in 1-week flooded soil (Figure 7.1D). $C_{DGT\text{-}Sb}$ profile also had a peak at 40-mm soil depth. $C_{DGT\text{-}Sb}$ was higher than $C_{DGT\text{-}As}$ in the OLW and 10-mm soil depth, but was much lower than $C_{DGT\text{-}As}$ below 10-mm depth. For

speciation, the profile of $C_{DGT\text{-}As(III)}$ exhibited the same trend as $C_{DGT\text{-}As}$, with the average proportion of $C_{DGT\text{-}As(III)}$ in deep soil (below 10-mm depth) constituting 91% of $C_{DGT\text{-}As}$ (Figure 7.1E). This suggests that As(III) played an important role in the changes in As profile and mobilisation. $C_{DGT\text{-}Sb(III)}$ significantly increased from 10 to 30-mm depth, followed by fluctuation with sub-peaks at 40-mm and 60-mm depth, which is similar to the trend of $C_{DGT\text{-}As(III)}$. The average proportion of $C_{DGT\text{-}Sb(III)}$ in deep soil (below 10-mm depth) constituted 43% of $C_{DGT\text{-}Sb}$ (Figure 7.1E). The increase in proportion of As(III) and Sb(III) indicates that the anaerobic condition increasingly developed with depth and over time in the soil system. In contrast to $C_{DGT\text{-}As(III)}$ and $C_{DGT\text{-}Sb(III)}$, $C_{DGT\text{-}As(V)}$ and $C_{DGT\text{-}Sb(V)}$ were high in the OLW and to 10-mm soil depth and tended to be depleted in the deep layers with sub-peaks at 40-mm depth for both $C_{DGT\text{-}As(V)}$ and $C_{DGT\text{-}Sb(V)}$ (Figure 7.1F). Overall, in deep soils (below 10-mm depth) flooded for 5 weeks, the average values of $C_{DGT\text{-}As(III)}$ and $C_{DGT\text{-}As(V)}$ significantly increased, whereas $C_{DGT\text{-}Sb(III)}$ and $C_{DGT\text{-}Sb(V)}$ considerably decreased. This is consistent with the substantial increase in $C_{DGT\text{-}As}$ and significant decrease in $C_{DGT\text{-}Sb}$ and also led to the much lower $C_{DGT\text{-}Sb}$ compared to $C_{DGT\text{-}As}$.

The increase in As(III) with flooding time and soil depth is supported by numerous studies which showed that As is mobilised and released into the soil solution in response to flooding (Stroud et al. 2011; Takahashi et al. 2004; Xu et al. 2008; Yamaguchi et al. 2011). According to Stroud et al. (2011), As(III) was dominant in pore waters of soils contaminated with As to different degrees and from different sources under flooded conditions (66 – 100%, average 93%, on day 21). The flooded incubation of soils also led to a decrease in redox potential and a rise in As(III) fraction in the solid phase up to 80% of the total soil As (Yamaguchi et al. 2011). A study on As speciation in soil water collected under various soil depths showed that As was dissolved as As(V) (94%) at 10-mm depth, while As(III) was the primary species (99%) at 90-mm depth.

In contrast to As, previous studies on Sb speciation in soil solution under different redox conditions showed inconsistent results. The current study shows that the average Sb(III) proportion in anoxic zone (below 20 mm depth) was 43% for soils, while Mitsunobu et al. (2006) reported that Sb(V) was a stable form and Sb(III) accounted for only 6% of total Sb in soil solution collected at a depth of 90 mm. According to Okkenhaug et al. (2012), Sb(III) concentration in flooded soil pore water accounted for 5 – 19% of total Sb. The lower proportions of Sb(III) for Mitsunobu et al. (2006) and Okkenhaug et al.

(2012) may be due to oxidation of Sb(III) to Sb(V) during the sampling soil at different depths, transportation, and soil solution extraction, and storage or the less reducing conditions. Another study on the release of Sb from contaminated soil under various redox conditions by Hockmann et al. (2014a) mentioned that 85% of soluble Sb was present as Sb(III) on day 64 of incubation in lactate-amended microcosms.

The As concentration profiles closely match with its own Fe concentration profiles, illustrated by the fact that both As and Fe concentrations significantly increase from 10-mm to 40-mm depth, but the contrasting trend was observed for the Sb and Fe concentration relationship (Figure E.2). This indicates that the increased mobilisation of As in flooded soils was strongly associated with the increased reductive dissolution of Fe oxyhydroxides, releasing Fe(II) and previously adsorbed or co-precipitated As into pore water. This mechanism of As mobilisation has been observed previously and known as the primary mechanism of flooded soil and sediment As mobilisation (Bennett et al. 2012b; Masscheleyn et al. 1991; Takahashi et al. 2004). Furthermore, the reduction of As(V) to As(III) led to the As mobilisation. It has been debated if As(V) was released into the soil solution along with the dissolution of Fe oxyhydroxides and subsequently reduced to As(III) in the soil solution or if adsorbed As(V) was reduced to As(III) on the soil solid phase and subsequently partitioned into the soil solution. Yamaguchi et al. (2011) and Takahashi et al. (2004) reported that the As desorption followed by As(V) reduction on the solid phase contributed half of the increase in As concentration in soil pore water under reducing conditions.

The current results are supported by previous studies on the mobilisation of As in sediment by DGT reporting that the mobility of As increased with sediment depth and closely related with the dissolution of Fe oxyhydroxides (Bennett et al. 2012a; Gao et al. 2017). Hossain et al. (2012) studied As dynamics in mangrove sediments and reported that As concentrations down depth profiles were more associated with elevated Fe and Mn concentrations than with organic matter. Conversely, the negative or no relationships for Sb indicates that the mobilisation of Sb was not directly associated with the chemistry of Fe in this study, which is supported by a recent study on the remobilisation of Sb in sediment cores collected in the field (Gao et al. 2016).

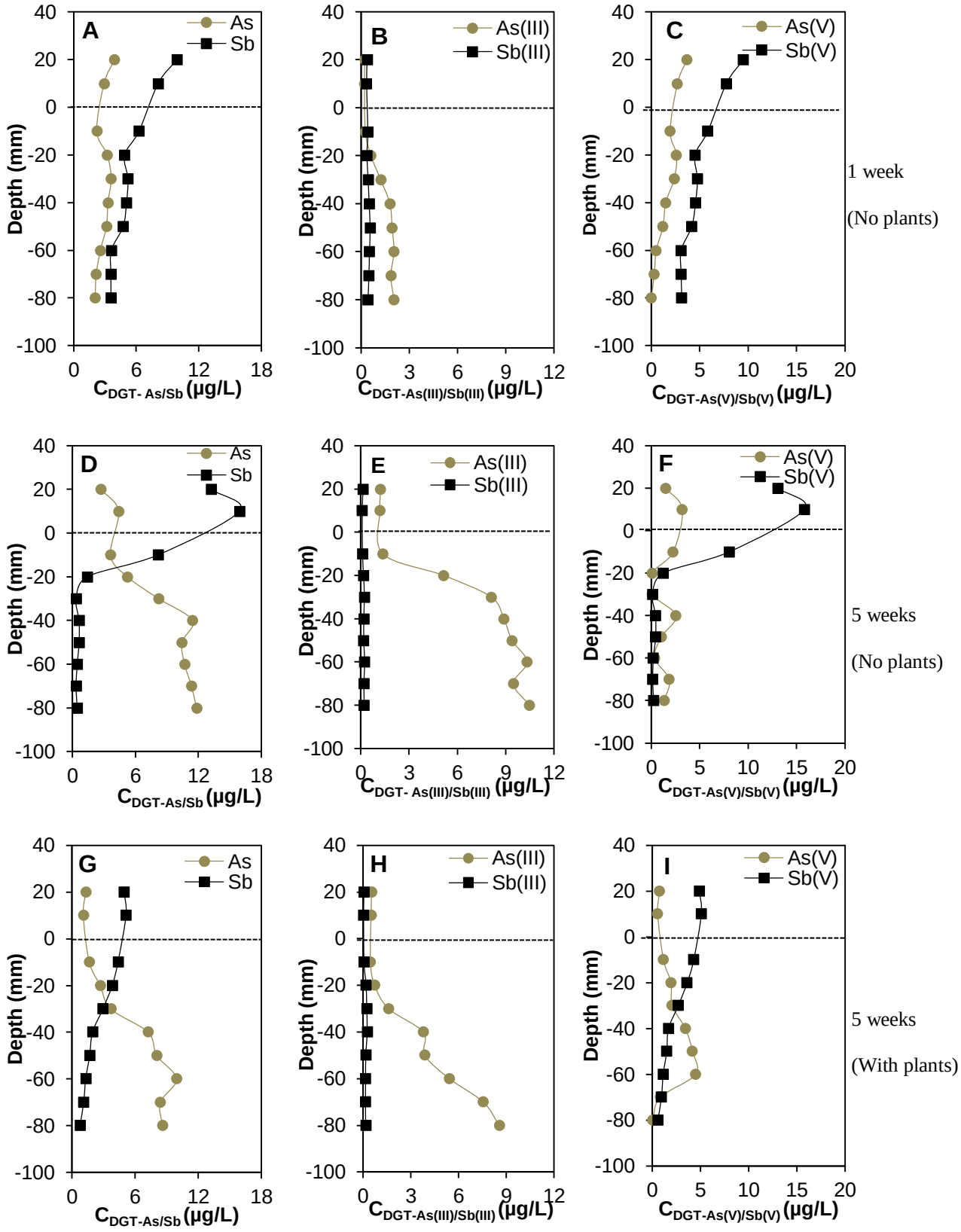

Figure 7.1 Depth profiles of As and Sb species in soil Aged-L after 1-week flooding without plants (A, B, C) and 5-week flooding without plants (D, E, F) and with plants (G, H, I). The dotted line represents the soil water interface.

The concentrations of Sb, Sb(III), and Sb(V) in deep soil after 5 weeks of flooding was lower than that after 1 week of flooding (reflected by lower $C_{DGT\text{-}Sb}$, $C_{DGT\text{-}Sb(III)}$, $C_{DGT\text{-}Sb(V)}$), which is in contrast with a study by Okkenhaug et al. (2012) who observed the increased dissolved Sb concentrations in flooded soil solution over time. However, the current result is in agreement with Wan et al. (2013b) who studied on the solubility and speciation of Sb in waterlogged soil in a tank experiment. The decrease in Sb lability after 5-weeks of flooding in the current study may be due to (1) Sb(V) being adsorbed to re-precipitated less crystalline Fe oxyhydroxides to a larger extent, a process suggested by Mitsunobu et al. (2006), (2) Sb(V) being reduced to Sb(III) which was then immobilised by adsorption to Fe oxyhydroxides due to its higher affinity to Fe oxyhydroxides (Hockmann et al. 2014a; Leuz et al. 2006; Mitsunobu et al. 2010; Wan et al. 2013a), (3) Sb(III) precipitated with sulphide resulted from the microbial reduction of sulphate (Hockmann et al. 2014b), and (4) more Sb was in inert forms or in complexes having large molecules, which are non-DGT labile. The third and fourth explanations seem to be more reasonable since (1) the water table was maintained during the flooding period and the re-precipitation of Fe oxyhydroxides was not observed and (2) the reduction of Fe oxyhydroxides occurred, which would release re-adsorbed Sb into soil pore water together with As, however this was not the driving mechanism as a decrease in labile Sb was observed. Thus, the coupled Sb release and Fe mineral reduction was not the case for our results. The DGT probe for sulphide measurement and Sb K-edge XANES analysis of solid phase speciation in flooded soil should be applied for further explanation of the Sb mobilisation mechanism.

In comparison of $C_{DGT\text{-}As}$ and $C_{DGT\text{-}Sb}$ in the flooded soil system, As and Sb show contrasting trends with concentrations increasing for As and decreasing for Sb with depth. $C_{DGT\text{-}Sb}$ was much lower than $C_{DGT\text{-}As}$ below 10-mm depth, even though total soil Sb was much higher than total soil As. These results might be due to (1) more Sb being in particulate colloids, (2) Sb being released and resupplied to soil solution very slowly in response to DGT depletion over the 24-h deployment, leading to less Sb measured, and (3) more Sb being present in inert forms or in large organic complexes which were not measured by DGT. However, the first possibility can be ruled out since $C_{DGT\text{-}Sb}$ was much higher than $C_{DGT\text{-}As}$ in the OLW and surface soil.

7.3.1.2. Effect of plants on the speciation and solubility of As and Sb in flooded historically contaminated soils

For planted soil, the C_{DGT} profiles of As and its species generally displayed the same patterns as those in unplanted soil. $C_{DGT\text{-}Sb}$ and $C_{DGT\text{-}Sb(V)}$ shared the same pattern which was a gradually decrease from the OLW to the bottom of probe, but was less pronounced than their decreasing trend in unplanted soil. Both As and Sb were present at lower concentrations of trivalent species and higher concentrations of pentavalent species. Compared to unplanted soils, the proportion of $C_{DGT\text{-}As(III)}$ and $C_{DGT\text{-}Sb(III)}$ decreased to ~59 and ~12% of $C_{DGT\text{-}As}$ and $C_{DGT\text{-}Sb}$, respectively. The presence of these lower trivalent species is likely due to diffusion of oxygen from plant root aerenchyma into the rhizosphere creating a narrow zone of oxidising environments around plant roots, enhancing the oxidation of trivalent species to pentavalent species (María-Cervantes et al. 2010; Nishiuchi et al. 2012). The average $C_{DGT\text{-}As(V)}$ and $C_{DGT\text{-}Sb(V)}$ (below 10-mm depth) in the planted soil was 9.8 and 6.7 times higher than that in the unplanted soil, respectively. In addition, due to the likely higher oxidising environments around the root zones, the reduction of Fe oxyhydroxides releasing adsorbed As and Fe(II) and the reduction of As(V) to As(III) occurred to a lesser extent in soils with plants, leading to the decrease in trivalent species and increase in pentavalent species.

Overall, $C_{DGT\text{-}As}$ decreased, but $C_{DGT\text{-}Sb}$ increased in the planted soil system (below 10-mm depth). The decrease in $C_{DGT\text{-}As}$ is most likely due to As accumulated by plants, and Fe plaque coating plant roots adsorbing and co-precipitating As, counteracting the increase of As mobility. The overall increase in $C_{DGT\text{-}Sb}$ in the flooded soil system might be because Sb(V), the dominant Sb species, was adsorbed less effectively than As(V) on the Fe plaque (Qi & Pichler 2017), enhancing more soluble Sb in the solution phase. Another possibility is that Sb was accumulated less by plants than As, leading to an increase in Sb in the soil solution. The current results reconfirm the effect of plant roots on the mobility of surrounding contaminants reported by previous studies (María-Cervantes et al. 2010; Wan et al. 2013b). It is noticeable that C_{DGT} of As, Sb, and their species in the OLW in planted soil system were lower than those in unplanted soil system. As observed, plant roots developed from the plant node below the water table. This suggests that the absorption of As and Sb in the OLW in planted soil system by newly developed roots possibly occur, which may contribute to their overall uptake by plants.

The C_{DGT} profiles of As, As(III), As(V), Sb, Sb(III), and Sb(V) in soil Aged-H after one-week flooding and five-week flooding with and without plants are presented in Figure E.3. In general, all profile shapes closely resemble those in soil Aged-L, illustrated by the increasing trends, decreasing trends, minima, and maxima occurring at the same locations, but to a greater extent due to soils contaminated with higher concentrations of As and Sb. This indicates that the performance of DGT in assessing labile As and Sb in flooded soils is reproducible and these profiles accurately reflect the dynamic of labile As and Sb species. This is an important observation as no replicate measurements were performed due to the volume of data collected in this study. Thus, the interpretation of As and Sb behaviour in soil Aged-L can be applied for soil Aged-H.

In summary, historically contaminated soils subjected to waterlogging resulted in the significant increase of total labile As in soils with As(III) being the dominant species. Despite labile Sb decreased after 5 weeks of flooding, Sb(III) was present >10% of total labile Sb in the flooded soil system. As(III) and Sb(III) are more toxic than As(V) and Sb(V); hence, waterlogging contaminated soils poses higher risks of As and Sb toxicity to the environment and human health.

7.3.2. DGT measurements in flooded recently contaminated soils

7.3.2.1. Dynamics of lability and speciation of As in flooded recently contaminated soils and the effect of Sb on As lability

The C_{DGT} profiles of As, As(III), and As(V) in As-only and As+Sb soils flooded for 1 and 5 weeks, with and without plants, are presented in Figure 7.2. After 1 week of flooding, C_{DGT} in As-only soil generally fluctuated with small peaks along the depth profile, whereas C_{DGT} in As+Sb soil gradually decreased with depth. For speciation, $C_{DGT\text{-}As(III)}$ in As-only significantly increased in deep soil with a maxima from 40 to 70-mm depth, while $C_{DGT\text{-}As(III)}$ in As+Sb soil was relatively unchanged with depth. The average proportion of $C_{DGT\text{-}As(III)}$ in deep soil (below 10-mm depth) was <20%. As(V) was a dominant species, thus the profiles of $C_{DGT\text{-}As(V)}$ displayed the same patterns as $C_{DGT\text{-}As}$ in both soils. Overall, after 1 week of flooding C_{DGT} of As and its species were higher in deep As-only soil than As+Sb soil.

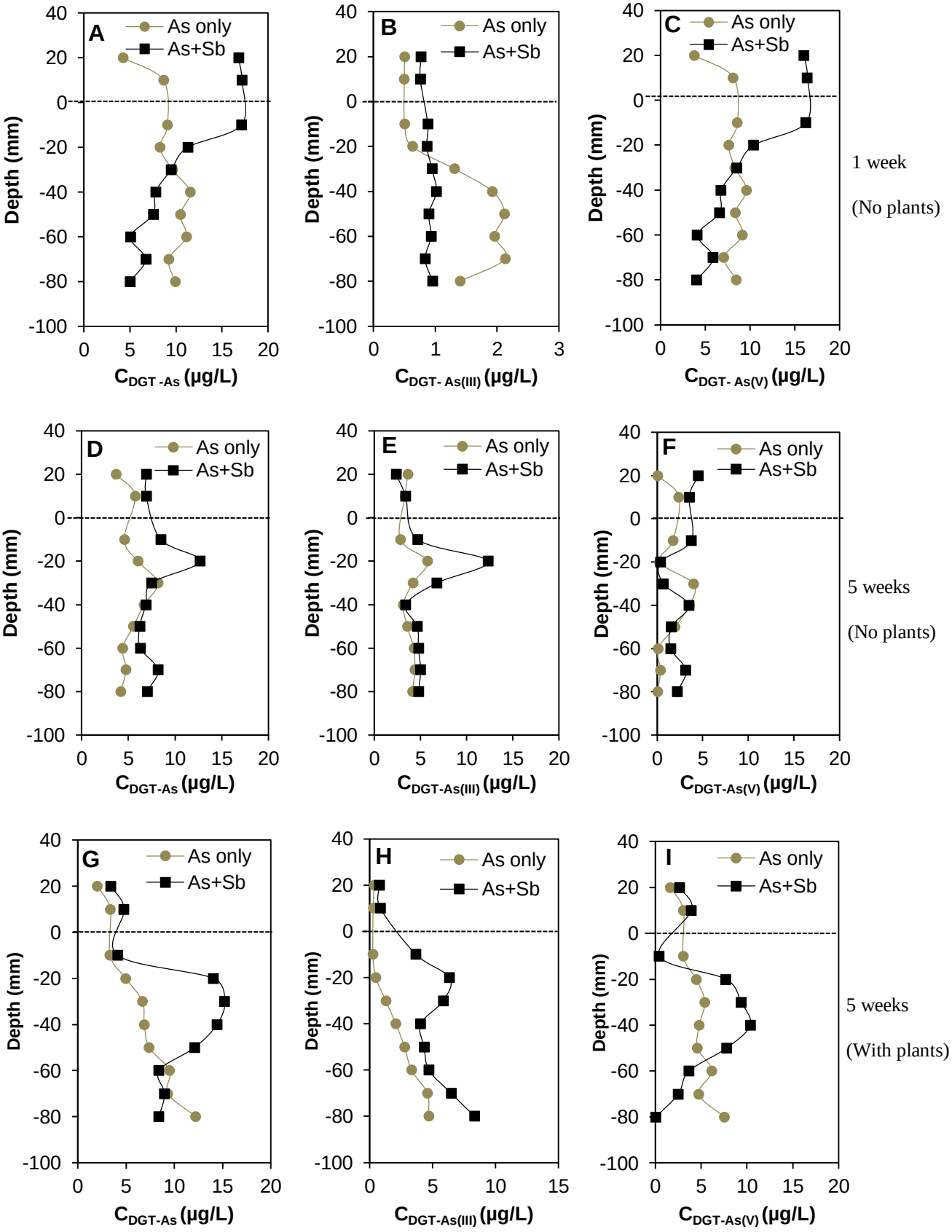

Figure 7.2 Depth profiles of As species concentrations in soil recently contaminated with As-only and with As+Sb soils after 1-week flooding without plants (A, B, C), 5-week flooding without plants (D, E, F) and with plants (G, H, I), measured by DGT. The dotted line represents the soil water interface.

After 5 weeks of flooding, $C_{DGT\text{-}As}$ in As-only soil exhibited the same pattern in 1-week flooded soil, with a peak at mid-profile (30-mm depth). In As+Sb soil, $C_{DGT\text{-}As}$ followed the same pattern as in As-only soil, but the visible peak at mid-profile and sub-peak at the bottom were larger (Figure 7.2D). For speciation, $C_{DGT\text{-}As(III)}$ in both soils had an excellent comparability, with maxima and constantly unchanged values occurring at the same positions. The noticeable result is that the peak of $C_{DGT\text{-}As(III)}$ at mid-profile was larger in As+Sb soil than in As-only soil (Figure 7.2E). As expected, $C_{DGT\text{-}As(III)}$ in As-only and As+Sb soils increased (a factor of 3.3 and 6.6, respectively) compared to that in 1-week flooded soils, with the average proportion of $C_{DGT\text{-}As(III)}$ in deep soil (below –10 mm) being ~75% of $C_{DGT\text{-}As}$. In contrast, $C_{DGT\text{-}As(V)}$ in both soils decreased in a fluctuating manner with depth, with minima and maxima at mid-profile corresponding with maxima and minima of As(III), Fe(II), and total labile As. Thus, it can be generalized that the zone around 20 to 30-mm depth (mid-profile) was the active zone for As mobilisation and Fe reduction. The relatively unchanged trends from -40 mm downwards indicate the possibly completely anoxic conditions in very deep soils and most of the newly formed trivalent species were immobilised by the precipitation or re-adsorption on the secondary Fe minerals. It seems that the presence of Sb led to the increase in As mobilisation in the flooded soil.

Overall, compared to $C_{DGT\text{-}As}$ in 1-week flooded soils, $C_{DGT\text{-}As}$ in As-only soil decreased by about half, while that in As+Sb soil increased. Thus, $C_{DGT\text{-}As}$ in As+Sb soil was higher than that in As-only soil, meaning that the presence of co-contaminant (Sb) enhanced the As lability in As+Sb soil. This can be explained by the adsorption strength of Sb(III), Sb(V), As(III), and As(V) on Fe minerals and organic matter and the precipitation capacity with sulphide resulting from the reduction of sulphate. Sb(III) binds to the soil much more strongly than As(III) because Sb(III) binds to Fe (hydr)oxides through stable bidentate inner-sphere complexes (Scheinost et al. 2006), whereas As(III) sorbs to Fe (hydr)oxides via multiple inner-sphere and outer-sphere complexes (Fendorf & Kocar 2009). In addition, Sb(V) has higher affinity to organic matter (OM) than As(V) (Dousova et al. 2015). Therefore, Sb could outcompete As in the adsorption on Fe (hydr)oxides and OM, resulting in lesser binding sites for As. Furthermore, given that sulphide was produced, Sb(III) would preferentially precipitate with sulphide before iron and arsenic due to the increasing solubility constant of Sb_2S_3 ($K_{sp} = 1.6 \times 10^{-93}$), FeS ($K_{sp} = 6.3 \times 10^{-18}$), and As_2S_3 ($K_{sp} = 8.4 \times 10^{-16}$). These suggest

that Sb should be involved in the adsorption and precipitation processes in priority in As+Sb soil, reserving As species in the solution phase. As a result, with the same given binding sites in two soils, As was more labile in As+Sb soil than in As-only soil. In As-only soil, due to Sb absence a proportion of As(III) and Fe(II) may have precipitated with sulphides, leading to the decrease in $C_{DGT\text{-}As}$ (Figure 7.2D) and $C_{DET\text{-}Fe}$ (Figure E.1) after 5-week flooding. This is supported by a previous study on the effect of sulphate reduction on the As mobility during waterlogging of As-contaminated soil by Burton et al. (2014). The authors stated that the microbial sulphate reduction lowered concentrations of pore water Fe(II) resulted from Fe(III) reduction as a result of the formation of FeS. The newly formed FeS subsequently sequestrated considerable amounts of As. The high-resolution transmission electron microscopy showed that the solid phase As was retained as an As_2S_3-like complex associated with FeS (mackinawite).

The dynamics of As and Sb in soils under flooding may be influenced by processes such as dissolution of Fe (hydr)oxides, precipitation of sulfide or by precipitation of authigenic minerals (María-Cervantes et al. 2010). The released As during the dissolution of Fe (hydr)oxides may be subsequently re-adsorbed to the secondary Fe minerals formed during the development of anoxic conditions (e.g. sederite, magnetite), which was observed by previous studies (Tufano et al. 2008; Yamaguchi et al. 2011). The formation of meta-stable metal sulfides is believed to be responsible for the sequestration of As, Sb, and metals (Du Laing et al. 2009; Otero et al. 2009). Moreover, green rust minerals with a structure of brucite-type layers of Fe^{3+} and Fe^{2+} sandwiched by anion layers and water molecules have been indicated for reducing contaminants in soil solutions (Génin et al. 2001; María-Cervantes et al. 2010). These processes seem to be reasonable in our study given that Fe(II), As, and Sb concentrations remained relatively unchanged or lower in deep soil (below 40-mm depth). It is possible that the formation of secondary Fe minerals or insoluble substance and complexes prevented the dissolution of Fe, As, and Sb, thereby resulting in the lower partitioning of As and Sb into soil solution phase.

With respect to mechanism of As mobilisation, it seems that the mobilisation of As in recently contaminated soils is different from that in historically contaminated soils. The results show that As(III) increased as anoxic environments increased over time (Figure 7.2); however, As concentrations displayed a relatively constant trend in deep soil (from

-40 mm downwards), while Fe concentrations exhibited fluctuating decreasing trends (Figure E.1), meaning that the reduction of Fe (hydr)oxides was unlikely to occur in this zone. The further relationship analysis on As and Fe concentration data shows that there was no or minor negative relationships between soluble As and Fe concentrations, indicating that the mobilisation of As in the recently contaminated soils was not associated with the reduction of Fe (hydr)oxides in deep soils. In addition, $C_{DGT\text{-}As(V)}$ decreased and $C_{DGT\text{-}As(III)}$ increased during flooding time, indicating that conversion of As(V) to As(III) did occur. Based on the As fractionation in As-amended soils (section 6.3.2, chapter 6), As in these soils was highly labile and present in the non-specifically sorbed and specifically sorbed (SO_4^{2-} and PO_4^{3-} extractable) fractions up to 35%. Thus, it can be speculated that the desorption of As(V) from the soil solid phase via the exchangeable process due to the presence of phosphate and sulphate as nutrients and the subsequent reduction of As(V) to As(III) into pore water were strongly involved in the mobilisation of As in recently contaminated soils.

7.3.2.2. Effect of plants on the speciation and solubility of As in flooded recently contaminated soils

In planted soils after 5-weeks of flooding, all C_{DGT} of As and its species in As-only soil exhibited an increasing trend with depth, which is different from those in unplanted soils (Figure 7.2.G). The C_{DGT} profiles of As and its species in As+Sb soil also displayed the increasing trend and shared the same feature which was a broad maxima at -20 to -40 mm. This can be considered as the active zone of chemistry of As and Fe as well as the plant root activity. Interestingly, these peaks increased with Sb added and the plants extended this process over a greater depth and caused As(V) mobilisation. Similar to unplanted soils after 5-week of flooding, the values of $C_{DGT\text{-}As}$, $C_{DGT\text{-}As(III)}$, and $C_{DGT\text{-}As(V)}$ in As+Sb soil were higher than those in As-only soil.

$C_{DGT\text{-}As(III)}$ and $C_{DGT\text{-}As(V)}$ in two soils generally increased with depth (Figures 7.2H, 7.2I); however, the proportions and DGT concentrations of As(III) generally decreased especially from the surface soil to 60-mm depth while As(V) increased compared to those in no plant treatments. The average $C_{DGT\text{-}As(V)}$ (from 20-mm depth downwards) in As-only and As+Sb planted soils was over 6 times higher than that in the unplanted soils, respectively. This may be due to possible narrow oxidative zones created around the roots resulting in the oxidation of As(III) to As(V) as discussed earlier in section 7.3.1.2, leading to the decrease in As(III) and increase in As(V). In addition, the oxygen

released from plant roots might have led to the oxidation of precipitates of sulphide (e.g. FeS, FeS_2, As_2S_3, Sb_2S_3), releasing As, Fe, Sb into the solution phase. Additionally, the root exudates (e.g. amino acids, organic acids) and protons might have contributed to the release of As and Sb bound to organic Fe complexes. These can be illustrated by the increase in Fe (Figure E.1) and As over depth (Figures 7.2G, H, I) in planted soils. The variation in As speciation in the planted recently contaminated soils were similar to that in planted historic soils. However, the net of these processes led to the higher $C_{DGT\text{-}As}$ in planted recently contaminated soils than in unplanted recently contaminated soils. The likely reason for this is unclear. This may be because plants were exposed to much greater labile As in recently contaminated soils, thus, the plants may use internal modifications to limit the As transport through root cell or there was more Fe plaque formed. This resulted in the restriction of As uptake, contributing to the increase in As concentrations in planted recently contaminated soils.

7.3.2.3. Dynamics of speciation and lability of Sb in flooded recently contaminated soils and the effect of As on Sb lability

The C_{DGT} profiles of Sb, Sb(III), and Sb(V) in Sb-only and As+Sb soils flooded for 1 and 5 weeks with and without plants are presented in Figure 7.3. In 1-week flooded recently contaminated soils, the profiles of C_{DGT} of Sb and its species displayed the same trend as those in flooded historic soils. $C_{DGT\text{-}Sb}$ in two soils gradually decreased with depth and had relatively identical profiles. For speciation, $C_{DGT\text{-}Sb(III)}$ in the two soils gradually increased with sub-peaks till the bottom of the probe, being <20% of $C_{DGT\text{-}Sb}$ in deep soils (below 10-mm depth) (Figure 7.3B), while $C_{DGT\text{-}Sb(V)}$ in two soils gradually decreased with depth, being the dominant species in soils (the average of Sb(V) below 20 mm >80%). The profiles of $C_{DGT\text{-}Sb(V)}$ were consistent with the profiles of $C_{DGT\text{-}Sb}$ (Figure 7.3A, 7.3C).

After 5-week flooding, the profiles of $C_{DGT\text{-}Sb}$ and $C_{DGT\text{-}Sb(V)}$ consistently displayed the same patterns, which sharply decreased from the OLW to 20-mm depth, after which they generally remained stable. $C_{DGT\text{-}Sb(III)}$ in two soils showed completely different trends with those in 1-week flooded soils. $C_{DGT\text{-}Sb(III)}$ in the two soils exhibited relatively unchanged trends, except a peak at 20-mm depth in As+Sb soil, corresponding with the maxima of As and Fe (Figures 7.2E and E.1).

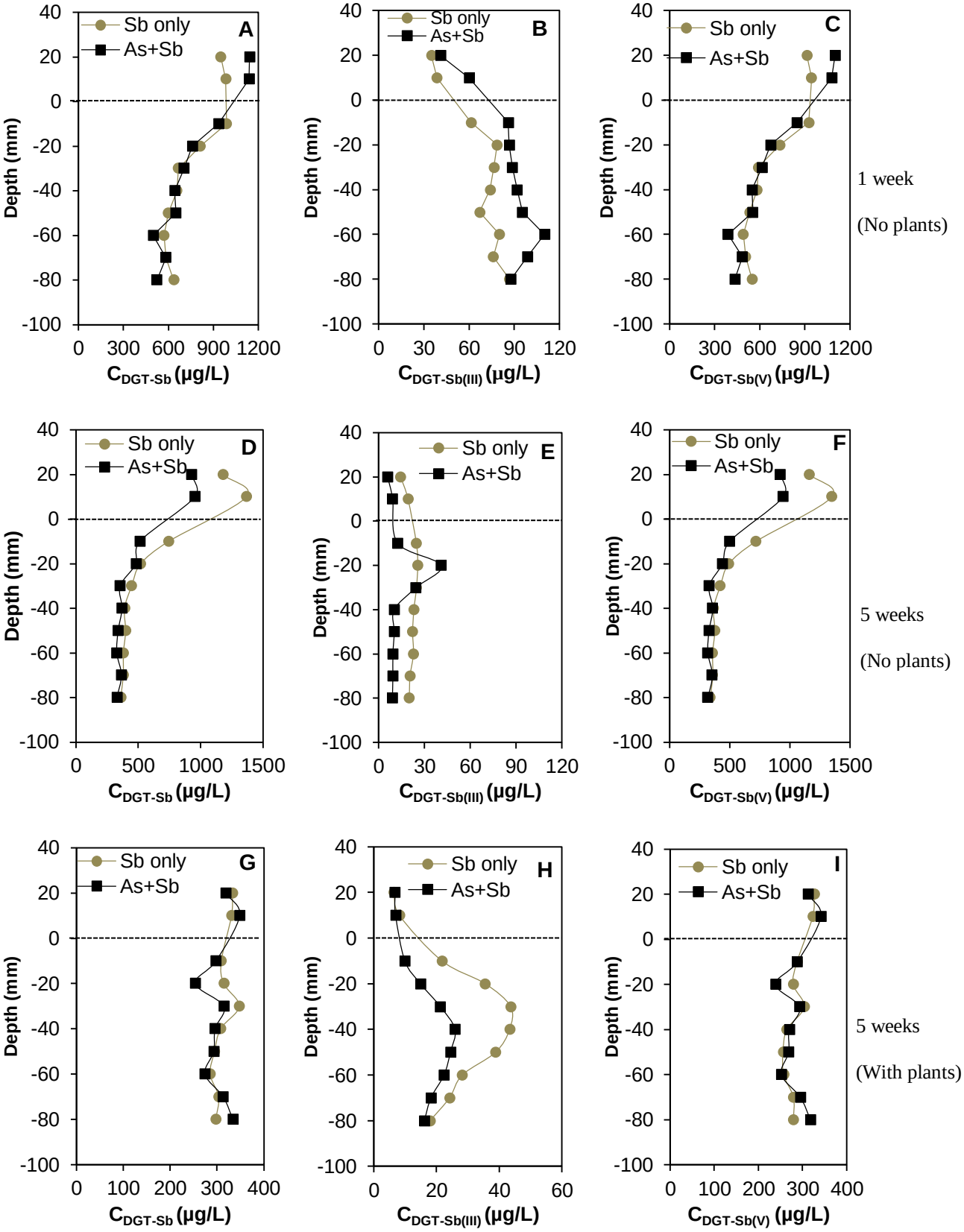

Figure 7.3 Depth profiles of Sb species concentrations in soil recently contaminated with Sb-only and with As+Sb soils after 1-week flooding without plants (A, B, C), 5-week flooding without plants (D, E, F) and with plants (G, H, I), measured by DGT. The dotted line represents the soil water interface.

Overall, $C_{DGT\text{-}Sb}$ in Sb-only soil was slightly higher than that in As+Sb soil, especially in OLW and 10-mm depth soil. It seems that there was little effect of As on the lability and solubility of Sb in the flooded soils studied. The values of $C_{DGT\text{-}Sb(III)}$, $C_{DGT\text{-}Sb(V)}$, and $C_{DGT\text{-}Sb}$ (below 20-mm depth) in 5-week flooded soils were much lower than those in soils flooded for 1 week. The lower $C_{DGT\text{-}Sb}$ in 5-week flooded soils was possibly due to a combination of the increased adsorption of Sb(V) on dissolved organic matter (DOM) via metal (e.g. Fe) bridges, the immobilisation of the newly reduced Sb(III) on Fe hydr(oxides) via adsorption, and the precipitation of the newly reduced Sb(III) with sulphide (as discussed in section 7.3.1.1). The possible large complexes and inner precipitates formed may also contribute to the decrease in $C_{DGT\text{-}Sb}$ in soil pore water because they were not measured by DGT.

The current results show that Sb and its species did not vary with soil depth (from 40-mm downwards), possibly indicating the completely anoxic conditions in deep soils. It seems that everything in the deep soil system was at equilibrium. Alternatively, the relatively unchanged trends of C_{DGT} of Sb and Sb species with depth and the decrease in $C_{DGT\text{-}Sb}$ under more reducing conditions may have resulted from the variation of Fe hydroxide properties known as the host phase of As and Sb. Under anaerobic conditions, the reductive dissolution of Fe(III) to Fe(II) and the reprecipitation due to the oxidation to Fe(III) hydroxide might be occurring in the soil system. Furthermore, the structural order of the host phase might decrease. According to Richmond et al. (2004), ferrihydrite with a decreased structural order had greater surface areas and stronger adsorptive capacity. It can be speculated that the greater surface areas of disordered host phase, Fe hydroxides, resulted in the higher partitioning of Sb to soil under anoxic conditions (Mitsunobu et al. 2006). Moreover, desorbed Sb at the initial stage may readsorb to the secondary Fe mineral (e.g. siderite, magnetite) and precipitate with sulphide resulted from sulphate reduction during the development of anaerobic conditions. In order to further understand the immobilisation of Sb during the anoxic environment, the analysis of solid phase speciation by XAFS is needed.

Regarding the mechanism of Sb mobilisation in flooded recently contaminated soils, the results show contrasting trends between Sb(III) and Fe(II), which is similar to that in historic soils, indicating that the mobilisation of Sb was not directly associated with the reduction of Fe (hydr)oxides. This is in agreement with the remobilisation of Sb in sediments collected by a core sampler and assessed by DGT (Gao et al. 2016). In Gao's

study the release of Sb was not associated with Fe and Mn in sediments. Like As mobilisation in recently contaminated soils, the mechanism of Sb mobilisation may include the desorption of Sb(V) from soil, the reduction of Sb(V) to Sb(III), and the subsequent immobilisation of the newly formed Sb(III).

7.3.2.4. Effect of plants on the speciation and solubility of Sb in flooded recently contaminated soils

In flooded planted soils, the profiles of $C_{DGT\text{-}Sb}$ in two soils consistently displayed the same patterns, with little variation in deep soils (Figure 7.3G). In contrast, $C_{DGT\text{-}Sb(III)}$ exhibited different manner with the presence of plants in soils. $C_{DGT\text{-}Sb(III)}$ in Sb-only and As+Sb soils displayed the same pattern which was a considerable increase reaching peaks at 40-mm depth, followed by a gradual decrease down the soil depth, but the profile of $C_{DGT\text{-}Sb(III)}$ in Sb-only soil had a much broader maxima than that in As+Sb soil (Figure 7.3H). Interestingly, the plants facilitated the release of Sb(III), especially in Sb-only soils. As a result, $C_{DGT\text{-}Sb(III)}$ in deep soils (below – 10 mm) increased by a factor of ~1.8 compared to unplanted soils, while $C_{DGT\text{-}Sb(V)}$ slightly decreased. The patterns of $C_{DGT\text{-}Sb(V)}$ in two soils were relatively identical and similar to that of $C_{DGT\text{-}Sb}$.

The increase in $C_{DGT\text{-}Sb(III)}$ in planted soils may be due to the oxygen supplied from the plant roots may have caused the sulphide oxidation, mobilising Sb, Fe, and As precipitated with sulphides, a process reported by Otero et al. (2009), María-Cervantes et al. (2010), and Williams et al. (2014). This is demonstrated by the increase in Sb(III), As(III), and Fe(II) with soil depth, especially large maxima at the zone -20 to -40 mm depth which may be the active zone of plant roots. In addition, organic exudates exerted in the rhizosphere may have facilitated the mobilisation of bound-Sb on the host phase. According to Ptak and McBride (2015), organic acid exudation can mobilise Sb bound by organic Fe complexes and the increase in maize rhizosphere Sb bioavailability and in plant tissues were observed. The decrease in $C_{DGT\text{-}Sb(V)}$ may be due to the uptake of Sb(V) by plant roots. Overall, the net of these processes led to the lower $C_{DGT\text{-}Sb}$ in planted soils than in unplanted soils. This may be because of plant uptake of Sb, especially Sb(V) and adsorption of Sb on the Fe plaque coated on plant roots, lowering the entire labile Sb. Fe plaque around plant roots has been recognised to be a scavenger of Sb and As by previous studies (Cui et al. 2015; Okkenhaug et al. 2012; Tripathi et al. 2014).

In summary, the presence of plants promoted the release of trivalent species from the solid phase and the oxidation of trivalent species to pentavalent species in flooded soils. The overall $C_{DGT\text{-}As}$ decreased, while $C_{DGT\text{-}Sb}$ increased in flooded historically contaminated soils with plants compared to unplanted soils. The opposite was observed for flooded recently contaminated soils with plants. It seems that the labile As in flooded recently contaminated soils was higher than the element acquisition of plants. As discussed in section 7.3.2.2, the plants were exposed to much greater labile As in recently contaminated soils, thus, the plants may use internal modifications to restrict the As transport through root cell. This led to the restriction of As uptake, contributing to the higher $C_{DGT\text{-}As}$ in planted recently contaminated soils. In contrast, Sb uptake by plants in recently contaminated soils may be determined by the mass flow due to increased plant biomass and plant stress, facilitating Sb transport to roots. This led to the decrease in $C_{DGT\text{-}As}$ in planted recently contaminated soils.

7.3.3. Accumulation of As and Sb in water spinach (*Ipomoea aquatica*)

I. aquatica is a very popular edible vegetable in Asia and can be grown in land and semiaquatic systems. The biomass of *I. aquatica* cultivated in flooded soils was higher than that in non-flooded soils. Of flooded plants, the biomass of *I. aquatica* exposed to flooded soil Aged-H was lowest. The plants looked healthy and exhibited no indicators of phytotoxicity (e.g. discoloration, stunting, wilting).

Interestingly, both roots and shoots of *I. aquatica* accumulated more As and Sb under flooded conditions than non-flooding (Figure 7.4). Arsenic concentrations in roots and shoots of *I. aquatica* were 2.7 – 3.1 and 2.2 – 3 times higher under flooded conditions than under non-flooded conditions, respectively, which is supported by the higher soluble As in flooded soils as discussed in sections 7.3.1 and 7.3.2. The translocation factors (TF) for As in flooded treatments ranged from 0.25 – 0.5 being similar to TF for As (0.24 – 0.53) in non-flooded soils (Table 7.1). It is clear that an increase in As bioavailability under flooded conditions resulted in the enhanced As accumulation. Xu et al. (2008) reported that growing rice in flooded paddy soils for 117 days markedly increased As in rice shoots and grains, which was >10 times higher than As in rice grown in aerobic soils.

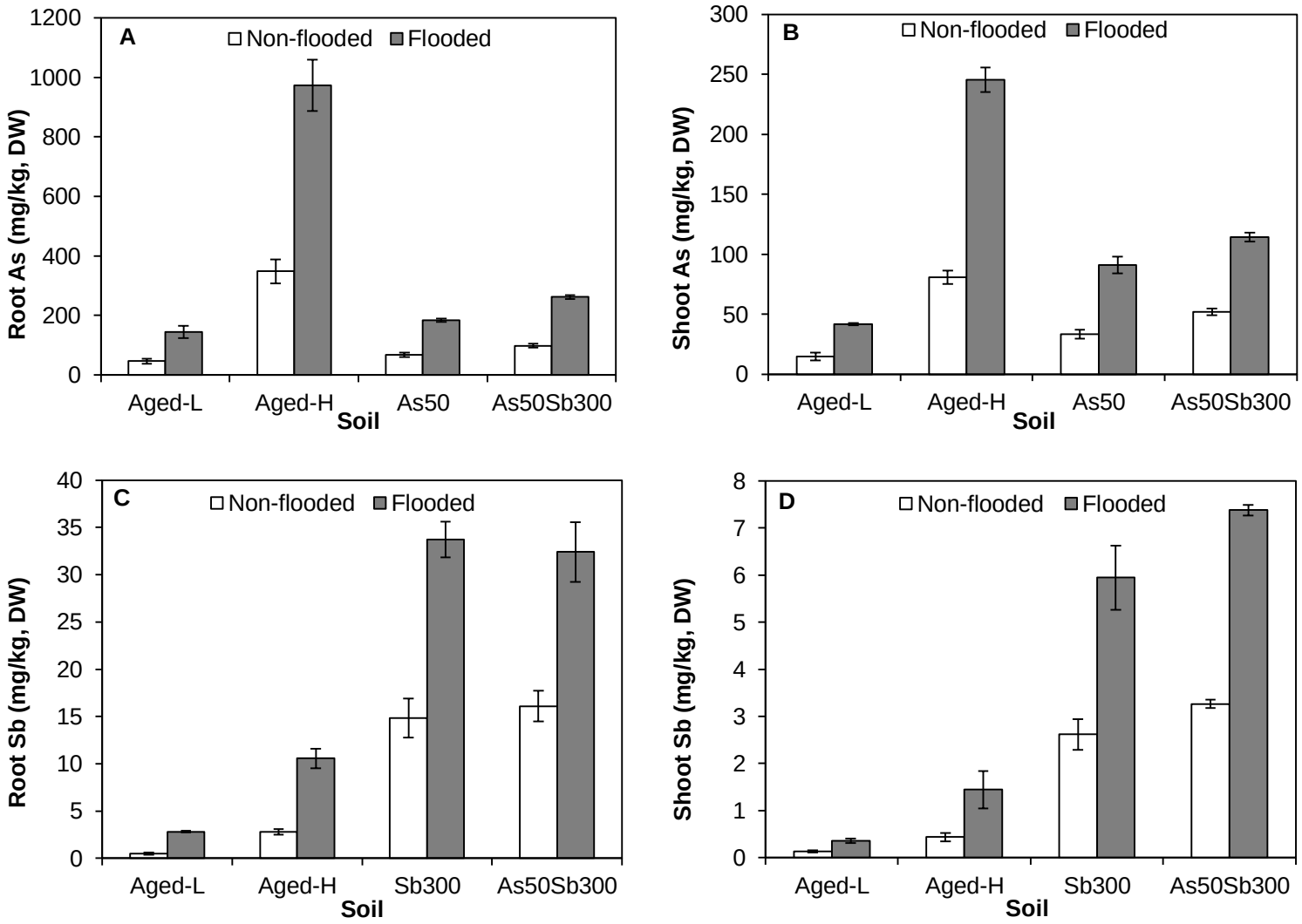

Figure 7.4 Concentrations of As in roots (A) and shoots (B), Sb in roots (C) and shoots (D) of *I. aquatica* cultivated in non-flooded and flooded soils. Data are presented as mean ± SE (n = 3).

Table 7.1 Total soil concentrations, bioaccumulation factor (BAF), and translocation factor (TF) of As and Sb in *I. aquatica* cultivated in soils under non-flooding and flooding. Data are presented as mean ± SE (n = 3).

Soil	As					
	Non-Flooded			Flooded		
	Total soil As (mg/kg)	BAF	TF	Total soil As (mg/kg)	BAF	TF
Aged-L	105 ± 10	0.44 ± 0.08	0.36 ± 0.1	105 ± 10	1.38 ± 0.2	0.30 ± 0.04
Aged-H	420 ± 10	0.82 ± 0.09	0.24 ± 0.03	420 ± 10	2.3 ± 0.2	0.25 ± 0.01
As50	52 ± 1	1.3 ± 0.2	0.5 ± 0.1	52 ± 1	3.5 ± 0.1	0.50 ± 0.04
As50Sb300	57 ± 1	1.7 ± 0.1	0.528 ± 0.004	57 ± 1	4.6 ± 0.1	0.44 ± 0.02
Soil	Sb					
	Non-Flooded			Flooded		
	Total soil Sb (mg/kg)	BAF	TF	Total soil Sb (mg/kg)	BAF	TF
Aged-L	120 ± 10	0.004 ± 0.001	0.29 ± 0.05	120 ± 10	0.029 ± 0.001	0.13 ± 0.02
Aged-H	620 ± 4	0.0045 ± 0.0005	0.16 ± 0.03	620 ± 4	0.017 ± 0.002	0.13 ± 0.02
Sb300	280 ± 10	0.0522 ± 0.0007	0.19 ± 0.04	280 ± 10	0.119 ± 0.007	0.18 ± 0.01
As50Sb300	280 ± 10	0.057 ± 0.006	0.21 ± 0.03	280 ± 10	0.12 ± 0.01	0.23 ± 0.02

Similar to As concentrations in shoots, *I. aquatica* shoots took up 2.3 – 3.3 times more Sb under flooded conditions than under non-flooded conditions. Sb accumulation in anaerobically grown roots was also enhanced compared to aerobic treatments, but to a greater extent (2.0 – 5.7 times). However, a close inspection on increase differences among soil treatments between two water regimes shows that *I. aquatica* cultivated in historically contaminated soils exhibited a higher increase of As and Sb concentrations in plant tissues compared to the plants exposed to recently contaminated soils. For example, As and Sb concentrations in flooded roots grown in soil Aged-L was 3.1 and 5.7 times higher than that in non-flooded roots, respectively, while the factor of 2.6 for As and 2.0 for Sb in roots grown in soil As50Sb300. This suggests that the release and resupply of As and Sb from dissolved complexes in soil solution and from the solid phase of historically contaminated soils contributed to the uptake by plants at a greater extent.

Sb bioaccumulation was higher under flooded conditions than non-flooded conditions, in spite of total DGT-labile Sb decreased at the fifth week of flooding. This may be because plants subjected to anoxic stress increased the apoplastic bypass (Wan et al. 2013b), which enabled more Sb to be accumulated and transported from roots to xylem. The mass flow was likely to be important for Sb accumulation by *I. aquatica*. In addition, the presence of plants enhanced the lability of Sb, which may contribute to the increase in Sb uptake by *I. aquatica*. Another possibility is that Sb(V), the dominant

species in soil systems was preferential for Sb uptake by *I. aquatica*. Wan et al. (2013a, 2013b) reported that waterlogging enhanced Sb concentrations in shoots of *L. perenne* by about 10 times. In the current study, the translocation factors for Sb in the flooded and non-flooded soils were in the range of 0.13 – 0.23 and 0.16 – 0.29, respectively (Table 7.1).

I. aquatica cultivated in As+Sb soils (As50Sb300, 57 mg As/kg) accumulated more As than in historically contaminated soils (Aged-L, 105 mg As/kg), by a factor of 1.8 – 2.1 for roots and 2.7 – 3.5 for shoots. Similarly, although total Sb concentration in As+Sb soils (As50Sb300, 280 mg Sb/kg) was approximately two times lower than that in the historically contaminated soil (Aged-H, 620 mg Sb/kg), Sb uptake by roots and shoots of *I. aquatica* cultivated in the As50Sb300 soil was 3.1 – 5.7 and 5.1 – 7.5 times higher than in the Aged-H soil. This indicates that total soil concentrations are not a good indicator for the prediction of plant uptake of metalloids and soil risk assessment. It also confirms that biogeochemical processes and bioavailability of metalloids plays an important role in predicting their accumulation by plants and accurate assessment of contaminated soils.

Arsenic concentrations in *I. aquatica* grown in As+Sb soil (As50Sb300) were approximately 1.5 times greater than that in As-only soil (As50), although total As concentrations in two soils were relatively similar. This indicates that the greater As bioaccumulation is likely due to the presence of Sb in the former soil, which is consistent with the higher DGT-labile As in soil pore water. In contrast, Sb concentrations in tissues of *I. aquatica* exposed to As+Sb soil (As50Sb300) and Sb-only soil (Sb300) with similar total soil Sb of 280 mg/kg were not significantly different, indicating that the presence of As was unlikely to affect Sb solubility and uptake by *I. aquatica* in this study. This is supported by the DGT-labile Sb being relatively similar in both soils.

In regards to the trend of As and Sb uptake by *I. aquatica*, As and Sb concentrations were significantly higher in roots than in shoots (Figure 7.4), which is in agreement with previous studies (chapters 5 and 6) on *I. aquatica* bioaccumulation in As and Sb contaminated soils under aerobic conditions, where As and Sb concentrations in roots were about 14 and 10 times higher than shoots, respectively. Since the Fe plaque coating on roots in this study was removed, the root As and Sb concentrations reflect the true accumulation by roots, suggesting the firm conclusion that As and Sb were

sequestrated in *I. aquatica* roots. This uptake tendency was also found for other plants (e.g. rice). Higher As and Sb concentrations in rice roots than shoots were reported by Okkenhaug et al. (2012) and Smith et al. (2008a). The average bioaccumulation factors (BAF) of As in non-flooded (0.63) and flooded historically contaminated soils (1.84) were 147 and 90 times higher than those of Sb (0.004 under non-flooding and 0.02 under flooding), respectively. This is consistent with the trend in chapter 5.

7.3.4. Arsenic and antimony speciation in water spinach (*Ipomoea aquatica*)

7.3.4.1. As speciation in non-flooded *I. aquatica*

The extraction efficiencies of As species in roots and shoots of *I. aquatica* grown in non-flooded soil were in the range of 86 – 102%, with higher proportions of As extracted from roots (Table 7.2). The high extraction efficiency is in agreement with published studies using the same extractant (dilute HNO_3) to extract As species from edible vegetables (Sadee et al. 2016), rice grains (Maher et al. 2013), and marine plants (Foster et al. 2007). These studies demonstrated that dilute HNO_3 returned quantitative extraction of As species in samples and no appreciable oxidation of As(III) and As(V) was observed.

Speciation analysis revealed that only As(III) and As(V) were identified in roots and shoots of non-flooded *I. aquatica*, with variation of As species between plant tissues. As(III) was the dominant species in roots (62 – 84%), followed by As(V) (14 – 31%). In contrast to As speciation in roots of *I. aquatica*, the proportion of As(III) and As(V) in shoots was not significantly different, ranging from 39 to 47% of total As in shoots, with the exception for Aged-L soil. It seems that there was an equilibrium between As(III) and As(V) in shoots.

The speciation in non-flooded *I. aquatica* roots is supported by (Yao et al. 2009) who reported that As(III) accounted for 71.3 – 81.1% of total As in roots of water spinach grown in soil amended with As-contaminated chicken manure, followed by As(V) (15.3 – 22%), and DMA (3.7 – 6.8%) which is derived from chicken manure. In the current study, MMA and DMA were not identified in *I. aquatica* tissues, suggesting that the methylation process was unlikely to occur in the studied soil and plant.

A study on As speciation in rice by Smith et al. (2008a) also showed the predominance of inorganic As species in rice roots, with As(III) comprising 57 – 78% of total As, whereas As(V) constituting 16 – 27%. Numerous studies on As speciation have reported

that inorganic As species are dominant species in terrestrial plants including edible vegetables (e.g. lettuce, chard, radish) (Muñoz et al. 2002; Smith et al. 2009; Tlustoš et al. 2002). Some As speciation studies identified the predominance of As(III) in tissues of *Pteris vittata* (Lombi et al. 2002; Webb et al. 2003), *Pityro-gramma calomelanos* (Francesconi et al. 2002), and mung bean roots accumulating up to 90% of As(III) (Smith et al. 2009).

High proportions of As(III) in roots may be due to the direct uptake of As(III) from the soil solution or the uptake of As(V) from soil solution, followed by the reduction of As(V) to As(III) in roots as a tolerant mechanism of plant to avoid As stress (Meharg 1994). In the studied aerobic soils, *I. aquatica* was likely to be exposed to As(V), the dominant species in oxic conditions (Sadiq 1997; Smith 1998; Wilson et al. 2010), thus the second pathway seems to be more reasonable for the non-flooded *I. aquatica*. The contribution of As(V) from soils to root uptake may be significant compared to As(III).

Several studies reported that once As(V) accumulated in roots, As(V) is quickly reduced to As(III) in root cells (Lombi et al. 2002; Pickering et al. 2000; Webb et al. 2003). Pickering et al. (2000) reported the main metabolic pathway of As in Indian mustard (*Brassica juncea*) involving processes of roots significantly accumulating As(V) which was rapidly reduced to As(III), and that the majority of As was restricted in roots, only a small amount of As(III) and As(V) was transferred to shoots of Indian mustard. This metabolic pathway of As seems to also occurred in *I. aquatica,* demonstrated by the much higher As concentrations in roots than shoots.

The reduction of As(V) to As(III) has been found to be a strategy for the detoxification of As in plants, which involves As(V) reduced to As(III) via enzyme arsenate reductase, then As(III) complexed with thiols and sequestrated in root vacuoles (Álvarez-Ayuso et al. 2016; Tripathi et al. 2007; Zhao et al. 2009). Álvarez-Ayuso et al. (2016) found a negative correlation between non-protein acid soluble thiol concentrations in rye roots and As concentrations in rye shoots, indicating that As complexation with thiols plays an important role to restrict As transport from rye roots to shoots.

Table 7.2 Total As and As species concentrations in roots and shoots of *I. aquatica* cultivated in non-flooded and flooded soils. Data are presented as mean ± SE (n = 3). Values in parentheses are the percentage of As species. There was no detectable MMA and DMA.

Treatment	Non-flooded									
	Root					Shoot				
	Total As (mg/kg)	As^{III} (mg/kg)	As^{V} (mg/kg)	Total As in extracts (mg/kg)	Extracted (%)	Total As (mg/kg)	As^{III} (mg/kg)	As^{V} (mg/kg)	Total As in extracts (mg/kg)	Extracted (%)
Aged-L	47 ± 8	33 ± 2 (74)	14 ± 7 (27)	48 ± 10	102 ± 2	15 ± 3	10 ± 4 (60)	5.2 ± 0.4 (38)	15 ±5	99 ± 9
Aged-H	348 ± 40	216 ± 30 (62)	108 ± 30 (31)	324 ± 40	93 ± 5	81 ± 6	33 ± 5 (41)	40 ± 3 (40)	73 ± 4	91 ± 2
As50	67 ± 8	57 ± 6 (84)	10 ± 2 (14)	66 ± 10	99 ± 1	33 ± 4	13 ± 1 (39)	16 ± 2 (47)	29 ± 3	86.1 ± 0.6
As50Sb300	99 ± 6	64 ± 20 (64)	29 ± 10 (30)	93 ± 10	94 ± 4	52 ± 3	22 ± 2 (42)	24.1 ± 0.7 (46)	46 ± 2	88.9 ± 0.7
Treatment	Flooded									
	Root					Shoot				
	Total As (mg/kg)	As^{III} (mg/kg)	As^{V} (mg/kg)	Total As in extracts (mg/kg)	Extracted (%)	Total As (mg/kg)	As^{III} (mg/kg)	As^{V} (mg/kg)	Total As in extracts (mg/kg)	Extracted (%)
Aged-L	144 ± 20	0.20 ± 0.07 (0.1)	140 ± 20 (99.9)	140 ± 20	96 ± 2	42 ± 1	-	41 ± 1 (99.9)	41 ± 1	98 ± 1
Aged-H	973 ± 90	0.73 ± 0.06 (0.08)	937 ± 80 (99.9)	938 ± 80	96.4 ± 0.9	245 ± 10	0.03 ± 0.03 (0.01)	248 ± 10 (99.99)	248 ± 10	101.1 ± 0.4
As50	183 ± 5	0.7 ± 0.5 (0.4)	183 ± 7 (99.6)	184 ± 6	100 ± 1	91 ± 7	0.01 ± 0.01 (0.01)	99 ± 6 (99.99)	99 ± 6	109 ± 2
As50Sb300	262 ± 7	0.6 ± 0.5 (0.2)	267 ± 10 (99.8)	267 ± 10	102 ± 2	114 ± 4	-	117 ± 3 (99.99)	117 ± 3	104 ± 2

-: not detected

Regarding how As species transported from roots to shoots, both As(III) and As(V) were transported to shoots or only As(V) was transported to shoots, followed by the reduction of As(V) to As(III) in shoots. The translocation pattern of As species from roots to shoots of *I. aquatica* is still unclear. For rice, the reduction of As(V) to As(III) and the complexation of As(III) with thiols occurred in rice shoots (Tripathi et al. 2007; Zhao et al. 2009).

7.3.4.2. As speciation in flooded *I. aquatica*

The extraction efficiencies of As species in roots and shoots of *I. aquatica* grown in flooded soil were in the range of 96 – 109%. For flooded *I. aquatica*, the As uptake and speciation was expected to be similar to non-flooded *I. aquatica*, but with higher proportion of total As and As(III) in plant tissues. This is because As was mobilised and soluble As increased in flooded soils as illustrated in sections 7.3.1 and 7.3.2, with As(III) as the dominant species in soil pore water. Thus, As(III) was expected to be present at higher proportions in flooded *I. aquatica*. However, the results of As speciation showed that As(V) was the main As species in flooded plant tissues, while As(III) was present in trace amounts (Table 7.2), which is opposite to both the expected results and the As speciation in non-flooded *I. aquatica*, and also opposite to the common species found in flooded paddy rice. These speciation results indicate differences between non-flooded and flooded *I. aquatica* in regards to the internal modifications of arsenic, perhaps due to different regulations of arsenic in the cell wall or different binding of arsenic to phytochelatins (Bergqvist & Greger 2012; Tripathi et al. 2007). The relative amount of As species in flooded roots and shoots were similar (Table 7.2), indicating that the same processes throughout the *I. aquatica* body may occur in response to arsenic or internal alteration of arsenic species did not occur within the *I. aquatica*.

The accumulation and speciation of As in tissues of flooded *I. aquatica* may involve (1) the uptake of As(V) by roots, followed by the translocation of As(V) from roots to shoots or (2) the uptake of As(III) or both As(III) and As(V), followed by the oxidation of As(III) to As(V) in root cells and the subsequent translocation of As(V) to shoots. A previous study on the accumulation and speciation of As in plants collected from different habitats (submerged, emergent, and terrestrial plants) reported that As(V) was the predominant species in submerged plants (Bergqvist & Greger 2012). The authors only investigated the patterns of As uptake by plants for As phytoremediation purposes.

The soil/sediment chemistry and plant characteristics were not presented and the mechanism of As accumulation and speciation in the plants were not explored.

As discussed in sections 7.3.1.2 and 7.3.2.2, the $C_{DGT\text{-}As(III)}$ decreased and $C_{DGT\text{-}As(V)}$ increased by a factor of 10 for flooded historically contaminated soils and 32 for flooded recently contaminated soils with *I. aquatica* compared to flooded soils without *I. aquatica*, indicating the diffusion of oxygen from above-ground parts to roots and the presence of oxygen in the rhizosphere. This supports the oxidation of As(III) to As(V) in rhizosphere and roots. In addition, *I. aquatica* is known as a semiaquatic plant (Shaibur et al. 2009) and grew healthily in the studied flooded soils. The roots of *I. aquatica* in flooded soils were better developed than those in non-flooded soils and no toxicity symptoms were observed. This indicates that *I. aquatica* oxygenated the rhizosphere to stimulate its growth under flooded conditions where oxygen was supposed to be microbially depleted and the oxidation of reduced species may be accompanied, the process supported by María-Cervantes et al. (2010). Taking all information into account, the oxidation of As(III) to As(V) in flooded planted soils and *I. aquatica* seems to be a reasonable hypothesis in this case. The current research is the first study on the As speciation in flooded water spinach (*I. aquatica*) in comparison with that in non-flooded soils. The mechanism of As uptake and speciation in *I. aquatica*, especially in flooded scenario, is still unknown. Further studies on this matter are warranted to explain this unusual trend.

7.3.4.3. Sb speciation in plants

In the current study, speciation of Sb in *I. aquatica* tissues was not determined because the extraction of Sb species in plant materials is yet to be successfully developed. The efficiency of published extraction procedures was low and varied greatly among extractants and materials. For example, citric acid extraction of rice roots gave a yield of 92 ± 11%, while only 55 ± 14% of Sb was extracted from total Sb in rice shoots (Okkenhaug et al. 2012). In addition, the change of Sb species during extraction was also problematic, counteracting the successful development of extraction of Sb species in plants. For instance, oxalic and ascorbic acid extraction was claimed to be suitable extractant for trimethylantimony (TMSb), but this new method did not completely discriminate Sb(III) and Sb(V) due to the reduction of Sb(V) to Sb(III) during extraction (Mestrot et al. 2016). The development of a reliable extraction method for Sb species in plants is warranted.

It is known that Sb(V) is the dominant species in oxic soils (Wilson et al. 2010; Wilson et al. 2014). Given that the proportion of Sb(III) increased in the anoxic soils illustrated by DGT-labile Sb(III) slightly increasing with flooded soil depth, especially at the initial stage of plant bioassay, Sb(V) was still dominant in soil pore water. Previous studies showed that Sb(III) was more strongly adsorbed on Fe (hydr)oxides than Sb(V) (Johnson et al. 2005; Leuz et al. 2006), thus the presence of iron plaque on plant roots might favour the influx of Sb(V) into roots. This is because Sb(V) less get trapped by the iron plaque. It can be speculated that uptake of Sb(V) by roots of *I. aquatica* cultivated in the studied soils would be a preferential pathway. Previous research by Okkenhaug et al. (2012) showed that Sb was mainly present as Sb(V) in roots and shoots of flooded paddy rice, partially supporting our speculation.

7.3.4.4. Practical implications

Arsenic concentrations in edible shoots of *I. aquatica* grown in non-flooded and flooded contaminated soils significantly exceeded the Australian maximum permissible value for food (1 mg As/kg, wet weight , WW) (FSANZ 2013); with an average water content of shoots of 90%, the value is equivalent to 10 mg As/kg, dry weight (DW). This indicates that the consumption of *I. aquatica* grown in the studied soils, even in soils with the lowest labile As, poses high risks to humans. Since flooding resulted in an increase of As accumulation in *I. aquatica* by >2 times, the intake of *I. aquatica* exposed to flooded contaminated soils is at higher risks.

In addition to exceeding the permissible value, the more alarming concerns are that *I. aquatica* grew healthily with no toxic signs observed and As was only present as inorganic As species. It is well known that the toxicity of inorganic As species is higher than organic species, in which As(III) is more toxic than As(V) (Mandal & Suzuki 2002). Taking these results into consideration, the consumption of the *I. aquatica* exposed to As-contaminated soils, especially under flooding poses severe risks to human health via the food chain. Based on the results of As risk assessment in section 5.3.6 (chapter 5) and 6.3.6 (chapter 6), it can be inferred that the ingestion of As-contaminated shoots of *I. aquatica* at concentrations in this study would greatly cause unacceptable non-carcinogenic and carcinogenic risks to both adults and children. Therefore, suitable strategies for the management of historically contaminated soils and suitable vegetable-growing practices to minimise cancer-related risks are required.

7.3.5. Evaluating the performance of DGT as a predictor of bioaccumulation and speciation in *I. aquatica*

As discussed in section 7.3.3, total soil concentration is not a good indicator for predicting metalloid uptake by plants and soil risk assessment. In addition, changes in the lability and speciation of As and Sb in planted soils occurred compared to unplanted soils. To better evaluate the performance of DGT in predicting As and Sb uptake by *I. aquatica* under different redox conditions, relationships between As and Sb bioaccumulation and their total soil concentrations and C_{DGT} measured at the initial stage and the end of bioassay were considered. As observed, the fibrous roots of *I. aquatica* developed healthily and occupied the entire deep soil layer; thus, relationships between bioaccumulation and C_{DGT} were examined using the average C_{DGT} below 10-mm soil depth (Figure 7.5).

The results also showed that the DGT measure in 5-week flooded soils with or without plants was a better predictor of As accumulation in *I. aquatica* (R^2 = 0.975 – 0.996 for roots, R^2 = 0.847 – 0.959 for shoots) than total soil As (R^2 = 0.944 for roots, R^2 = 0.785 for shoots), illustrated by higher correlation coefficients for both roots and shoots. The As concentrations in roots was more strongly correlated with $C_{DGT\text{-}As}$ in 5-week flooded soils without plants (R^2 = 0.975) than that in 1-week flooded soils without plants (R^2 = 0.746). This suggests that the strong increase in As mobility after 5 weeks contributed to As accumulation by *I. aquatica*, whereas after 1 week the system may have been in transition still. The correlation between As shoot concentration and $C_{DGT\text{-}As}$ in 5-week flooded unplanted soils (R^2 = 0.847) was relatively similar to that in 1-week flooded soils without plants (R^2 = 0.874). When $C_{DGT\text{-}As}$ in 5-week flooded soils with *I. aquatica* was considered, greater correlations between $C_{DGT\text{-}As}$ and As concentrations in both roots (R^2 = 0.996) and shoots (R^2 = 0.959) were observed. These suggests that the DGT measurements in planted soils captured changes in As mobility induced by *I. aquatica*, leading to the better reflection of As uptake by tissues of *I. aquatica*.

As with As, DGT outperformed total soil Sb in predicting Sb accumulation in tissues of *I. aquatica* grown in flooded soils (R^2 >0.92 for $C_{DGT\text{-}Sb}$ and Sb bioaccumulation, R^2 <0.01 for total soil Sb and Sb bioaccumulation). This is demonstrated by the very strong relationships between Sb concentrations in *I. aquatica* tissues and $C_{DGT\text{-}Sb}$ compared to the very weak relationships observed for total soil Sb (Figure 7.5). In contrast, $C_{DGT\text{-}Sb}$ measured in 5-week flooded soils with or without plants was as well correlated with Sb

bioaccumulation as with the 1-week flooded soils, which suggests that the Sb biogeochemistry responded to the waterlogged conditions more quickly than As. It seems that the Sb uptake by roots and the Sb translocation from roots to shoots were proportional to available concentrations. Sb in the soil systems was at equilibrium. As discussed in sections 7.3.1 and 7.3.2, the mobility of Sb decreased as flooding time increased, but Sb bioaccumulation in flooded plants increased compared to that in non-flooded plants. This suggests that the mass flow pathway and apoplastic bypass from roots to shoots may be important for Sb uptake and translocation due to *I. aquatica* subjected to physical damage and anaerobic stress.

The correlation analysis was also performed on $C_{DGT\text{-}As(III)}$, $C_{DGT\text{-}As(V)}$ and As(III) and As(V) in *I. aquatica* tissues to evaluate the possible preferential As species for uptake by flooded *I. aquatica* (Figure 7.6). The results showed that $C_{DGT\text{-}As(III)}$ measured in 1-week flooded soils, 5-week flooded unplanted soils, and 5-week flooded planted soils (from 20 mm below the soil surface downwards) were not correlated with As(III) concentrations in roots. In contrast, $C_{DGT\text{-}As(V)}$ measured in 5-week flooded soils with or without plants was strongly correlated with As(V) concentrations in tissues of *I. aquatica* (R^2 >0.88), and was better correlated with As(V) in *I. aquatica* tissues than that in 1-week flooded soils (R^2 <0.01). Similar to $C_{DGT\text{-}As}$, $C_{DGT\text{-}As(V)}$ measured in flooded soils with plants was the best predictor of As(V) concentrations in both roots (R^2 = 0.975) and shoots (R^2 = 0.985) of flooded *I. aquatica* in this study.

In addition, although As(III) concentrations in flooded soils were increased with the studied flooding time, As(III) concentrations were low in roots, and present at trace amounts or not-detected in shoots, while As(V) was the main species in tissues. Thus, the relationships between $C_{DGT\text{-}As(III)}$ in flooded soils and As(V) in *I. aquatica* tissues were also performed to identify if As(III) was taken up by roots and if there was redox reaction in flooded *I. aquatica*. Interestingly, the results showed that $C_{DGT\text{-}As(III)}$ in flooded soils were strongly correlated with As(V) in *I. aquatica* tissues (R^2 = 0.948 – 0.985 for roots and R^2 = 0.779 – 0.864 for shoots), even with $C_{DGT\text{-}As(III)}$ in 1-week of flooding (Figure 7.7). This suggests that As(III) may have been accumulated at the initial stage of plant growth. Similar to $C_{DGT\text{-}As(V)}$, $C_{DGT\text{-}As(III)}$ in 5-week flooded with plants was best correlated with As(V) in both roots and shoots of *I. aquatica*. This confirms that DGT measures included changes in As speciation induced by plants, resulting in the better reflection of plant uptake of As species.

In summary, after 1 week of flooding As(III) in flooded soil was not correlated with As(III) in plants, but strongly correlated with As(V) in plants. There was very weak relationship between As(V) in plants and in soils. These suggest that As(III) was preferentially accumulated by plant roots and subsequently oxidised to As(V). After 5 weeks of flooding, there was also a very week correlation between As(III) in soils and As(III) in plants and strong correlations between both As(III) and As(V) in flooded soils and As(V) in plants. These suggest that both As(III) and As(V) were accumulated by plant roots, followed by the oxidation of As(III) to As(V) in plants. Taking all into consideration, these correlations support the discussion in section 7.3.4 that roots accumulated As(III) or both As(III) and As(V), followed by the oxidation of As(III) to As(V) and the subsequent translocation of As(V) to shoots. This also explains the results of speciation analysis showing that As(V) was the main species in flooded *I. aquatica*.

Overall, the *in situ* sampling capability, which can be readily utilized in different environmental conditions, and the selective measurement capability for As(III) made DGT an effective technique for this study. Therefore, DGT is a very promising tool for measuring the bioavailability and speciation of As and Sb in soils, identifying differences in their biogeochemical behaviour, and predicting their uptake by plants under various conditions.

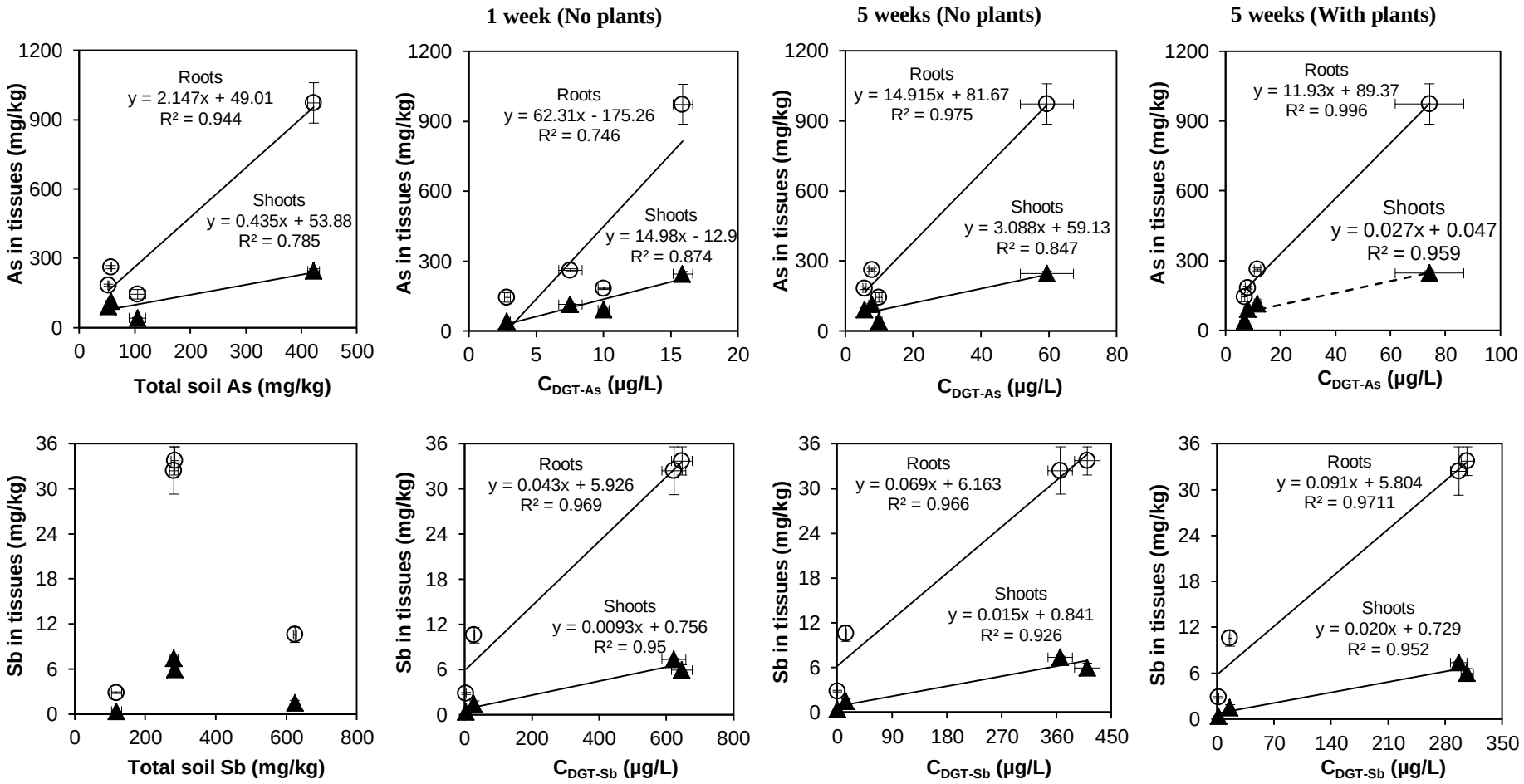

Figure 7.5 Relationships between As and Sb bioaccumulation and their average C_{DGT} measured in 1-week flooded soils without plants, 5-week flooded soils without plants, and 5-week flooded soils with plants (from -20 mm downwards). Roots (○), Shoots (▲). All data presented as mean ± SE (n = 3 for tissue concentrations, n = 7 for C_{DGT}) have error bars; many are smaller than symbols.

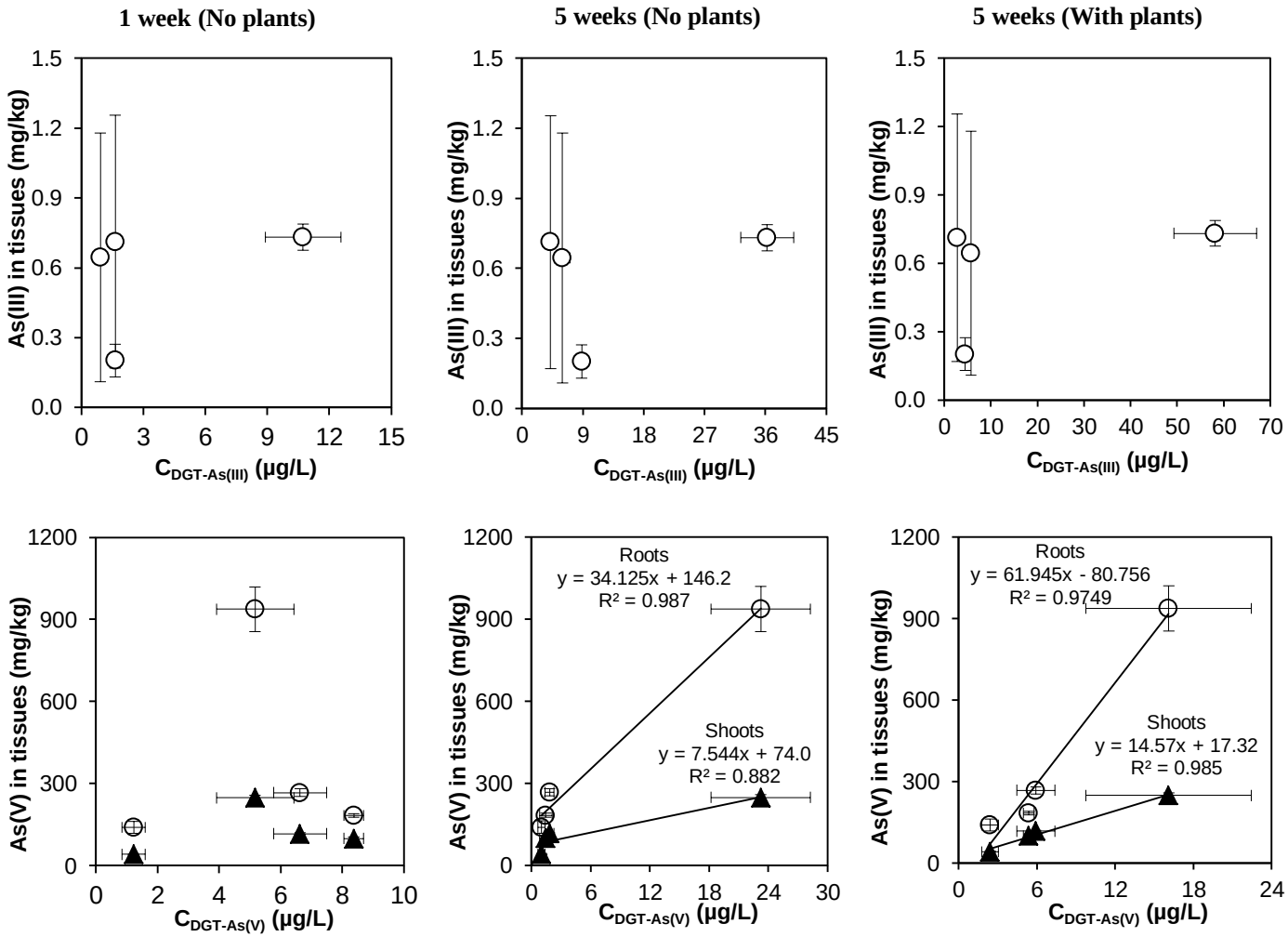

Figure 7.6 Relationships between As(III) and As(V) concentrations in plant tissues and $C_{DGT\text{-}As(III)}$, $C_{DGT\text{-}As(V)}$ measured in 1-week flooded soils without plants, 5-week flooded soils without plants, and 5-week flooded soils with plants (from -20 mm downwards). Roots (○), Shoots (▲). All data presented as mean ± SE (n = 3 for tissue concentrations, n = 7 for C_{DGT}) have error bars; many are smaller than symbols.

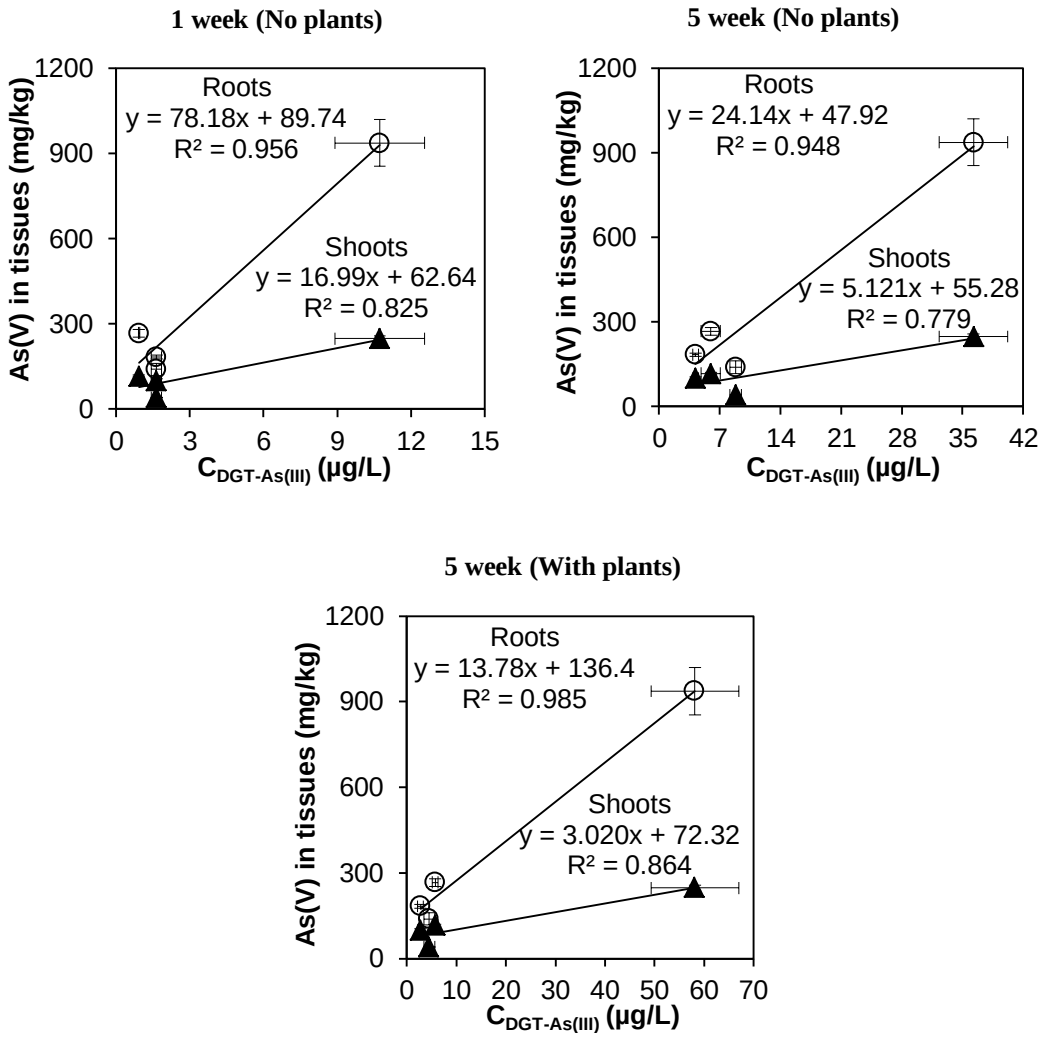

Figure 7.7 Relationships between As(V) concentrations in plant tissues and $C_{DGT\text{-}As(III)}$ measured in 1-week flooded soils without plants, 5-week flooded soils without plants, and 5-week flooded soils with plants (from -20 mm downwards). Roots (○), Shoots (▲). All data presented as mean ± SE (n = 3 for tissue concentrations, n = 7 for C_{DGT}) have error bars; many are smaller than symbols.

7.4. Conclusions

Our first use of the combined mercapto-silica-DGT, Metsorb-DGT, and DET for Fe successfully examined the dynamics of lability and speciation of As and Sb in flooded soils and differentiated the mechanisms of the mobilisation of As and Sb in soils under changes in redox conditions. The reductive dissolution of Fe (hydr)oxides was the main mechanism for As mobilisation in historically flooded soils, while the Sb mobilisation was not directly associated with Fe (hydr)oxide reduction and may involve the desorption of Sb(V) from soil, followed by the reduction of Sb(V) to Sb(III) and the immobilisation of Sb(III). During the flooding time of contaminated soils the lability of As increased, while that of Sb decreased, which may be due to the precipitation with sulphides. Thus, DGT probes for sulphide measurements need to be applied in future studies to fully explain the mechanism of Sb mobilisation.

The application of multi-DGT probes also demonstrated the differences in As and Sb behaviour in contaminated soils subjected to anoxic conditions in spatial resolution. The effect of *I. aquatica* on the changes in As and Sb speciation in flooded planted soils were also identified by DGTs. Due to the presence of Sb the lability of As in As+Sb soil was higher than that in As-only soil, leading to higher As bioaccumulation in *I. aquatica*. The relationships between As(III)/As(V) in soils measured by DGTs and As(III)/As(V) in plants determined by HPLC-ICP-MS allowed predicting the preferential As species uptake by flooded *I. aquatica* and explaining the proposed As speciation in flooded *I. aquatica*. This study has demonstrated that DGT is most useful under changing conditions and was a superior method in this study.

The waterlogging of contaminated soils resulted in much elevated concentrations of As and Sb in flooded *I. aquatica*, compared to those in non-flooded *I. aquatica*. The concentrations of As in edible shoots far exceeded the maximum permissible value for food (1 mg As/kg, WW). In addition, only inorganic As species were identified in *I. aquatica* tissues, with As(III) being the dominant species in non-flooded *I. aquatica*, while As(V) being the main species in flooded *I. aquatica*. As a result, the ingestion of edible shoots of *I. aquatica* exposed to soils contaminated with As poses dangerous risks for humans via the food chain. Therefore, the appropriate management of As and Sb-contaminated soils and agronomic practices are crucial to ensure environmental health and food safety.

Chapter 8. Concluding remarks and future directions

The aim of this study was to investigate the biogeochemical behaviour of Sb compared to that of As in contaminated soils in relation to their uptake by importantly agricultural plants identified by the diffusive gradients in thin films (DGT) technique in comparison to selective extractions. This was successfully obtained by achieving the following research objectives:

- Investigating the partitioning of As and Sb in various soil binding phases and their lability in different types of contaminated soils;
- Evaluating the performance of the DGT technique in measuring labile As and Sb in historically co-contaminated soils to two cultivars of radish (*Raphanus sativus*) compared to other techniques;
- Assessing the performance of the DGT technique in measuring the lability of As and Sb in historically co-contaminated soils with varying physicochemical soil properties and recently contaminated soils and predicting their accumulation by water spinach (*Ipomoea aquatica*) compared to other techniques;
- Investigating the fractionation and lability of As and Sb in single and multi-contaminant amended soils and their competitive interactions when they co-exist in soils in relation to their uptake by water spinach (*Ipomoea aquatica*);
- Investigating the mobilisation and speciation of As and Sb in soils induced by redox changes using multiple binding resins in DGT;
- Evaluating changes in As and Sb bioaccumulation and speciation in water spinach (*Ipomoea aquatica*) cultivated in contaminated soils subjected to different redox environmental conditions, which was linked to changes in As and Sb mobility and speciation in soils under such changing conditions predicted by DGTs.

This chapter draws together the research outcomes from this thesis. It will consider (1) the biogeochemical behaviour of As and Sb in various soil types under different redox conditions using DGT, SEP, and soil solution analysis; (2) the competitive interactions of As and Sb in soils under different redox conditions using DGTs; (3) uptake of As and Sb by a variety of edible vegetables under various growing conditions, focusing uptake

patterns, speciation transformation, and risk assessment of As and Sb-enriched vegetables to human health; (4) evaluation of the combination of multiple DGT to differentiate behavior of As and Sb in contaminated soils ; (5) evaluation of the performance of DGT in predicting As and Sb uptake by vegetables compared to other soil extraction methods.

8.1. The geochemical behaviour of As and Sb in contaminated soils

The partitioning and lability of As and Sb in contaminated soils determined by sequential extraction and DGT were deeply discussed in sections 3.3.2, 3.3.3, 4.3.2, 4.3.3, 5.3.2, 5.3.3, 7.3.1, and 7.3.2. The test soils were many series of historically co-contaminated soils (mining soils) with various soil physicochemical properties (e.g. Fe, organic matter, phosphorus), series of historically As-contaminated soil (cattle dip soil), and series of recently contaminated soils (As-only soils, Sb-only soils, and As+Sb soils) having comparable soil mineralogy.

8.1.1. The partitioning and lability of As and Sb in various soils determined by sequential extraction procedure (SEP)

In historically co-contaminated soils, irrespective of historic soil types with different soil physicochemical properties, As and Sb associated with non-specifically sorbed and specifically sorbed fractions were low (<10 and 1% of total soil As and Sb, respectively). The sum of As and Sb in these two fractions were defined as the bioavailable fraction which are readily mobilised and available for plant uptake (Wenzel et al. 2001). As was mainly associated with amorphous and crystalline Fe and Al oxide fractions (>60% of total soil As), while Sb predominantly bound to the residual phase (>60% of total soil Sb), followed by the crystalline phase (up to 29%). The lower proportions of Sb in the metal oxide phases and the higher percentage of Sb in the residual phase compared to As could be because of Sb association with organic matter, sulphides, or resistant minerals. Fe oxides and organic matter are phases that are somewhat (moderately) labile especially under certain biogeochemical conditions. Minerals associates with silicates and sulphides are non-labile. Thus, in historically co-contaminated soils Sb was biogeochemically much less mobile than As despite total soil Sb being higher.

In the recently contaminated soils studied, Sb was mainly found in amorphous and crystalline iron oxides fractions, whereas As was primarily partitioned in amorphous

iron oxide and specifically sorbed fractions. Regardless of singly or co-contaminated soils, the lability of Sb was less than that of As. The non-specifically sorbed and specifically sorbed Sb and As (the bioavailable fraction) in recently contaminated soils constituted 18% and 30% of total Sb and As, respectively, which were significantly higher than that in historically contaminated soils. This data suggests that As and Sb in recently contaminated soils have high lability, which is attributed to the soluble form of added As and Sb, and their shorter equilibration time in spiked soils compared to the long aging in historically contaminated soils. Irrespective of contamination sources and soil types, the Fe and Al oxides were the main sinks for these metalloids, especially As.

8.1.2. Labile As and Sb in various soils measured by the diffusive gradients in thin films (DGT) technique

The DGT technique successfully identified differences in the biogeochemical behaviour of As and Sb in contaminated soils with various soil physicochemical properties, illustrated by the variation in the R-value (C_{DGT}/C_{sol}) and distribution coefficients (K_d). The As and Sb soil solution and DGT-labile concentrations accounted for only a small proportion of total soil concentrations (<0.1% for historically contaminated soils, ≤2% for recently contaminated soils). Arsenic was partially resupplied from the solid phase to soil solution (R-value ranging from 0.21 – 0.65), while the resupply of Sb from the solid phase was consistently low (R-value being 0.12 – 0.19) indicating recharge of Sb in adjacent porewaters by diffusion only. The kinetic resupply of As varied with soil physicochemical properties, which was highest in the soil having the lowest iron oxides. Unlike As, the resupply rate of Sb was relatively unchanged across soil types with various soil physicochemical properties, demonstrated by the relatively constant R-value and K_d among soils, which confirms that Sb was biogeochemically immobilised in contaminated soils. DGT was sensitive to differentiate the lability, capacity, and kinetics of As and Sb release from the solid phase of various soils. DGT results were consistent with and complementary to the selective extraction data.

8.1.3. The dynamics of lability and speciation of As and Sb in contaminated soils induced by redox changes identified by DGT

Historically and recently contaminated soils were subjected to two water regimes: non-flooded and flooded. Mercapto-silica-DGT probes for sampling As(III) and Sb(III) and Metsorb-DGT probes for sampling total labile As and Sb were deployed into flooded

soils after 1 week (without plants) and 5 weeks of flooding (with and without plants). The diffusive gels of mercapto-silica DGT probes were used as diffusive equilibration in thin films (DET) for measuring Fe, giving pore water Fe concentrations in flooded soils.

The combined mercapto-silica-DGT, Metsorb-DGT, and DET for Fe allowed successful examination of the speciation and lability of As and Sb in flooded soils and differentiated the mechanisms of As and Sb mobilisation in soils under changing redox conditions. During extended flooding of historically contaminated soils the lability of As increased with soil depth and over time, while lability of Sb decreased, which may be due to Sb precipitation with sulphides. The reductive dissolution of Fe (hydr)oxides was the main mechanism for As mobilisation in flooded soils, while the Sb mobilisation was not directly associated with Fe (hydr)oxide reduction. In flooded soils As(III) was the dominant As species, whereas Sb(V) was the dominant Sb species.

The dynamics of As and Sb in recently contaminated soils exhibited the same patterns as in historically contaminated soils. However, their mobilisation mechanism may be involved the desorption of As and Sb from soil solid phases due to exchangeable processes, followed by the reduction of As(V)/Sb(V) to As(III)/Sb(III). The presence of *I. aquatica* in flooded soils lowered trivalent species and enhanced pentavalent species of As and Sb in flooded historically contaminated soils compared to flooded soils without plants, which was likely due to the oxygen released from the roots creating the narrow oxidising zone around roots. This study highlights a strong advantage of *in situ* DGT measurements, which able to be done in different redox conditions and combine with selective analysis.

8.2. Competitive interactions of As and Sb in soils under different redox conditions

The chemical fractionation, lability, and kinetic resupply of As and Sb in As+Sb soils were compared to those in singly contaminated (As-only, Sb-only) soils under aerobic conditions, assessed by SEP and DGT. The DGT profiles of As, Sb, and their species in these flooded soils were also examined by multiple *in situ* DGT samplers. These results provided a better understanding of the competitive interactions between As and Sb in soils under changes in redox conditions.

In comparison with As and Sb fractionation in singly contaminated (As-only and Sb-only) soils, the proportions of As and Sb in the non-specifically sorbed fraction of

As+Sb soils were higher, while those in the amorphous Fe oxide fraction tended to be lower. This is likely due to the simultaneous addition of As and Sb into soils, resulting in the decrease in binding sites and the redistribution of other substances. Thus, the sorbed and bound As and Sb decreased and more As and Sb were found in the available fractions. The competitive interaction of As and Sb for their preferred soil binding sites affected the distribution of As and Sb in various soil binding phases, which made them more labile and available in the As+Sb co-contaminated soils, compared to singly contaminated soils.

The average proportion of $C_{sol\text{-}As}$ was lower in As+Sb soils than in As-only soils under aerobic conditions, whereas that of $C_{sol\text{-}Sb}$ in As+Sb soils was ~2 times higher than in Sb-only soils. This phenomenon also occurred for $C_{DGT\text{-}As}$ and $C_{DGT\text{-}Sb}$, but to a lesser extent (as discussed in section 6.3.3). In contrast, when singly contaminated (As-only and Sb-only) and As+Sb contaminated soils were subject to flooding, the presence of As did not cause differences in the dynamics of Sb speciation and lability in Sb-only and As+Sb soils. In contrast, the presence of Sb enhanced the higher lability of As in As+Sb soil than As-only soil (as discussed in section 7.3.2).

The variation in competitive interactions of As and Sb is likely a result of the differences in their adsorption and precipitation capacity in the soil systems, which is dependent on their species present. The pentavalent and trivalent species were the dominant species in aerobic and anaerobic soils, respectively. As(V) has a higher affinity for Fe oxides compared to Sb(V) (Qi & Pichler 2017; Wilson et al. 2010), while Sb(V) has stronger binding strength with organic matter (OM) than As(V). The binding stability of As/Sb in soil types was also presented in order of As(V) – Fe(III) >> Sb(V) – OM > As(V) – OM > Sb(V) – Fe(III) (Dousova et al. 2015). Thus, when As and Sb were added together in soils under aerobic condition, Fe oxides gave preference to As, leading to more added As in solid phases and more added Sb in soil solutions. In anaerobic soils, Sb(III) has higher affinity for Fe oxides (Qi & Pichler 2017) and greater precipitation rate with sulphides than As(III) due to its lower solubility constant (as discussed in section 7.3.2). Hence, Sb would preferentially undergo adsorption and precipitation in the anoxic soil system, leading to higher amounts of As in As+Sb soil than in As-only soil where labile As decreased due to As involved in adsorption and precipitation processes.

It is clear that the speciation of As and Sb in the soil environment determines their lability and solubility, which in turn influences their accumulation by plants.

8.3. Uptake of As and Sb by a variety of vegetables cultivated in various soils under different redox conditions

8.3.1. Uptake trend

The bioaccumulation of As and Sb in the cherry bell radish (*Raphanus sativus*) cultivated in historically contaminated soils was very low due to the low lability of As and Sb in soils. There was more arsenic accumulated in roots and shoots than Sb; however, Sb had a higher ability to translocate from roots to shoots than As (a factor of 2.5), to the extent that much higher Sb concentrations were observed in shoots, suggesting a quite different pattern of transport within the plant. The white icicle radish displayed the same uptake trend as the cherry belle radish, but was less able to translocate As and Sb from roots to shoots than the cherry belle cultivar. These results suggest that the *R. sativus* uptake was clearly element and cultivar-specific.

In contrast, the leafy vegetable, water spinach (*Ipomoea aquatica*) cultivated in historically contaminated soils had a high capacity to accumulate As and Sb with concentrations in roots being 10 and 14 times higher than shoots, respectively. The bioaccumulation factor for As was >1, thus *I. aquatica* was considered as an As accumulator plant. The average bioaccumulation factor for As in *I. aquatica* grown in historically contaminated soils was 55.7-fold higher than that of Sb, while the translocation factor of Sb was 2.2-fold higher than that of As, indicating differences in uptake patterns. The same uptake patterns were also observed for As and Sb in *I. aquatica* grown in singly and co-amended soils. Compared with single-element soils under anaerobic conditions, the average bioaccumulation factor of Sb in As+Sb soils significantly increased, while that of As slightly decreased. The results demonstrated that the presence of a more toxic element (As) stimulated Sb uptake by *I. aquatica* cultivated in aerobic soils. The opposite phenomenon occurred for their interactive effects in *I. aquatica* grown in anaerobic soils. The results showed that the presence of As in flooded As+Sb soils was less likely to affect Sb uptake by *I. aquatica*, but the presence of Sb in flooded As+Sb soils led to the greater As bioaccumulation in *I. aquatica* exposure to As+Sb soil than As-only soil. This is consistent with the variation in DGT-labile As and Sb in soils induced by redox changes.

Concentrations of As and Sb in tissues of flooded *I. aquatica* were 2.2 – 3.1 and 2.0 – 5.7 times higher than those in non-flooded *I. aquatica*, respectively. Thus, the consumption of edible shoots of *I. aquatica* cultivated in flooded contaminated soils poses >2 times higher risks to humans. Irrespective of growing conditions and soil types, concentrations of As and Sb in roots of *I. aquatica* were significantly higher than in shoots.

8.3.2. Speciation transformation of As in *I. aquatica*

Inorganic As species were found in both roots and shoots of *I. aquatica* with both As(III) and As(V) present in non-flooded *I. aquatica*, which is supported by previous studies on As speciation in terrestrial plants. By contrast, mainly As(V) was present in flooded *I. aquatica*, which is opposite to the As speciation in flooded paddy rice. The predominance of As(V) in flooded *I. aquatica* was also opposite to the expected results that As(III) would be present in flooded *I. aquatica* at a greater extent than that in non-flooded *I. aquatica* because As(III) was the dominant species in flooded contaminated soils. This can be explained by the DGT data obtained in flooded planted soils showing that $C_{DGT\text{-}As(III)}$ decreased, but $C_{DGT\text{-}AS(V)}$ considerably increased compared to flooded soils without plants. This indicates the diffusion of oxygen from above-ground parts to roots, the presence of oxygen in the rhizosphere, and the oxidation of As(III) to As(V). The analyses of relationships between As(III)/As(V) in soils and As(III)/As(V) in plant tissues demonstrate that roots accumulated As(III) or both As(III) and As(V), followed by the oxidation of As(III) to As(V) and the subsequent translocation of As(V) to shoots (as discussed in section 7.3.5).

Sb species in *I. aquatica* tissues were not determined, but it was speculated that Sb(V) uptake by roots of *I. aquatica* grown in the studied soils would be favoured relative to Sb(III) (as discussed in section 7.3.4).

8.3.3. Risk assessment of As and Sb in *I. aquatica* to human health

Even for *I. aquatica* grown in the lowest treatment of historically contaminated soils under aerobic conditions, the shoots of *I. aquatica* accumulated As at very high concentrations which far exceeded the permissible limit of As in food set by the Food Standard Agency of China, Australia and New Zealand, and FAO/WHO, but no visible toxicity symptoms was observed in the plant, which is an alarming observation. *I. aquatica* presented direct risks to human health where $C_{sol\text{-}As} \geq 5.27$ µg/L and $C_{DGT\text{-}As}$

≥3.42 µg/L. The USEPA hazard quotient above 1, and cancer risk value above 10^{-4} for both adults and children, confirm high cancer risks to humans from consumption of As-contaminated *I. aquatica*.

Interestingly, the *I. aquatica* exposed to flooded soils grew healthily, had greater biomass compared to non-flooded *I. aquatica*, and did not exhibited visible toxicity symptoms. Inorganic As species were mainly found in *I. aquatica* tissues and known to be more toxic than organic As species. Consequently, the intake of *I. aquatica* cultivated in contaminated soils poses severe risks to humans via the food chain, especially when the semiaquatic growing practice was applied.

The ingestion of *I. aquatica* cultivated in Sb-only soils (≥650 mg Sb/kg) poses unacceptable non-carcinogenic risk to humans, but it looked healthy and there is no sign of toxicity symptoms. The Sb-enriched edible shoots of *I. aquatica* cultivated in Sb-only soils displayed the increasing trend of biomass with the increasing amounts of soluble Sb added into soils. As a result, Sb-contaminated shoots cannot be recognised and distinguished from uncontaminated shoots, compared to As-enriched shoots of *I. aquatica* cultivated in As-contaminated soils where shoot biomass decreased when the plant exposed to high soil concentrations. This implies that growing *I. aquatica* in Sb-contaminated soils should be avoided because the plant presents hidden risks and may pose non-carcinogenic risks to human health via the food chain.

The results suggest that the highly As-contaminated soil and underground water in some Asian regions may be dangerous and not suitable for cultivating water spinach (*I. aquatica*). Thus, cultivation of *I. aquatica* in As-contaminated soils or water or irrigation of *I. aquatica* with As-enriched water should be restricted in terms of food safety and human health. Appropriate strategies for the management of contaminated soils and suitable vegetable-growing practices to minimise As and Sb enrichment in soil and vegetables are crucial for the development of sustainable agriculture.

Overall, regardless of plant species, both As and Sb were restricted in roots and the translocation of Sb from roots to shoots was more efficient than As. The accumulation of As and Sb was higher in the leafy vegetable than in root vegetables. The uptake of As and Sb was clearly element, plant, and cultivar specific. In addition, the presented results indicate that total soil concentrations are not a good indicator for the prediction of plant uptake of metalloids. It also confirms that the biogeochemical processes and the

bioavailability of metalloids plays an important role in predicting their accumulation by plants.

8.4. Evaluation of the performance of DGT in predicting As and Sb uptake by vegetables compared with other soil extraction methods

The As and Sb concentrations in tissues of the tested vegetables were compared with labile As and Sb fractions in soils measured by DGT (as C_{DGT}), soil solution analysis (C_{sol}), and sequential extraction ($C_{SEP\text{-}labile}$). The concentrations of As and Sb in tissues of radishes were highly positively correlated with all labile As and Sb fractions (R^2 = 0.96 – 0.99 for cherry bell radish and R^2 = 0.92 – 0.99 for white icicle radish in historically co-contaminated soils, R^2 = 0.87 – 0.97 for As in white icicle radish grown in cattle dip soils). This indicates that tissue As and Sb concentrations in two cultivars of radish were well predicted by these labile measures.

The $C_{SEP\text{-}labile}$, C_{DGT}, and C_{sol} were better predictors for As and Sb in radish roots (greater correlation coefficients) than in shoots, with the exception of $C_{sol\text{-}As}$ and $C_{SEP\text{-}labile\ Sb}$. Arsenic and Sb accumulation by the white icicle radish from the test soils had a stronger relationship with C_{DGT} of As and Sb than their C_{sol}, especially from historically As-only-contaminated soils, which is illustrated by the higher correlation coefficients for C_{DGT}. From the predictive perspective, DGT exhibited better performance in predicting the phytoavailability of As and Sb in the studied soils, compared to soil solution concentration method.

Arsenic concentrations in tissues of *I. aquatica* cultivated in historically contaminated soils were better correlated with $C_{SEP\text{-}labile}$, C_{DGT}, and C_{sol} than Sb, illustrated by greater correlation coefficients for As. When the soil Sb associated with amorphous Fe and Al oxides were considered, a greater correlation between the extractable Sb in soil and Sb in roots was observed. This indicates that Sb bound to amorphous Fe and Al oxides may contribute to the Sb uptake by *I. aquatica* or that the plant induced the release and resupply of Sb from the soil solid phase, which was not included in labile measures for Sb in soils. The coupling of DGT and sequential extractions was useful in predicting potentially phytoavailable As and Sb in contaminated soils to *I. aquatica*, evident from the As and Sb high-uptake-capacity plant, and providing understanding about potential soil processes in the root zone.

In contrast, Sb concentrations in tissues of *I. aquatica* cultivated in recently contaminated soils were more strongly correlated with $C_{SEP\text{-}labile}$, C_{DGT}, and C_{sol} than for As. This may suggest that the reduction of *I. aquatica* yield grown in the freshly amended soils resulted from As than from Sb exposure. Arsenic concentrations in roots were significantly correlated with $C_{SEP\text{-}labile}$, C_{DGT}, and C_{sol} compared to shoot As concentrations. The weaker correlations for As concentrations in shoots and soils were likely due to the contrasting relationships between increased As concentrations in soils and decreased shoot As concentrations and plant yield. When the saturated bioaccumulation data point was excluded, shoot As concentrations were significantly correlated with all measures, C_{SEP}, C_{DGT}, and C_{sol}. The results demonstrated that all measures worked well in predicting As uptake by *I. aquatica* grown in the low range of labile As in soils. For *I. aquatica* cultivated in As+Sb soils, $C_{DGT\text{-}As}$ and $C_{SEP\text{-}As}$ predicted As concentrations in *I. aquatica* tissues better than $C_{sol\text{-}As}$.

Similar to radish (*R. sativus*), As and Sb concentrations in root of water spinach (*I. aquatica*) were better predicted than shoot As and Sb concentrations, which can be explained by the fact that roots directly contact soils, whereas bioaccumulation in shoots depends on the translocation from roots to shoots, usually governed by plant physiology. Overall, the prediction ability of these techniques varied with element, plant parts, plant species, and cultivars. DGT performed comparatively well with other measures, but DGT measurements are probably simpler than some of the other measurements which required single or multiple extractions of soil samples and soil characterisation. The combination of DGT, soil solution analysis, and SEP provided a better understanding of the biogeochemical behaviour of As and Sb in soils and useful mechanistic data (e.g. R, K_d). Compared to labile extractions, the dynamic DGT technique was sensitive to differentiate the lability, capacity, and kinetics of As and Sb release from the solid phase of various soils.

Interestingly, DGT outperformed total soil As and Sb in predicting As and Sb bioaccumulation in flooded *I. aquatica*. DGT was able to capture changes in As mobility and speciation induced by flooded *I. aquatica*, leading to the better reflection of As uptake by tissues of *I. aquatica*. Importantly, the relationships between As(III)/As(V) in soils measured by DGTs and As(III)/As(V) in plants measured by HPLC-ICP-MS allowed predicting the preferential As species uptake by flooded *I. aquatica* and explaining the proposed As speciation in flooded *I. aquatica*. On the long-

term basis of risk assessment, DGT could be a promising tool in assessing and predicting bioavailable As and Sb in contaminated soils to plants.

8.5. Evaluation of the combination of multiple DGT and DET to investigate behaviour of As and Sb in contaminated soils

Arsenic in soil solution was found to be partially resupplied from the solid phase. The resupply of Sb from the solid phase of historically contaminated soil is known to be slower than that of As. However, the biogeochemistry of As is strongly linked to that of Fe, thus the resupply of As from the solid phase is likely to be controlled by the redox reactions of Fe in sediment and soil. The strong adsorption of As on Fe oxides in aerobic conditions would limit the As resupply, while the reductive dissolution of Fe oxides in anaerobic conditions would partially resupply soluble As to DGT devices. DGT truly provides a useful technique for the interpretation of processes and mechanistic interactions occurring in sediment and soil and avoids the interferences and drawbacks of the traditional sampling techniques, which is supported by (Bennett et al. 2012a).

In this research the 3-mercapto-silica DGT was also used to selectively measure As(III) and Sb(III) in flooded soil pore water at high spatial resolution. The Metsorb DGT was simultaneously used to measure total labile inorganic As and Sb in flooded soil pore water at high spatial resolution. The difference between these two DGT measurements allows us to determine As(V) and Sb(V). The *in situ* characteristic of DGT sampling techniques coupled with the selectivity for redox states eliminates the potential changes in speciation that may occur during the collection of a sediment or soil core and subsequent processing steps such as extracting pore water samples, storing, and analysing them. This aspect is very important for the investigation of biogeochemistry of As and Sb which are very sensitive to environmental redox changes. The oxidation of As(III) to As(V), Sb(III) to Sb(V), and Fe(II) to Fe(III) during pore water processing steps confounds the redox chemistry and the relationships with their mobility.

The coupling of multiple DGT and DET techniques for Fe(II) allows us to measure co-distributions of As species, Sb species, and Fe(II) at the same spatial location within flooded soil, eliminating artifacts related to the heterogeneous distribution of solutes within flooded soils and their pore water and providing accurate comparison of As and Sb biogeochemical behaviour. The combined application of *in situ* DGT and DET

sampling techniques avoids changes in speciation and provides chemical profiles at high spatial resolution. Compared to the combined use of core-slicing, pore water sampler techniques and subsequent speciation analysis by HPLC-ICP-MS, DGT is likely the more reliable, economical and ease-for-use technique.

In addition, the dynamic DGT technique integrates soil properties and environmental conditions affecting soil processes, which determines the biogeochemical behaviour of As and Sb. DGT was non-destructive and far less laborious than some of the other measurements requiring single or multiple extractions of soil samples and soil characterisation. On the long-term basis of risk assessment, DGT provides a promise for assessing bioavailable As and Sb in contaminated soils routinely.

8.6. Future directions

8.6.1. Geochemical behaviour of As and Sb in soils

This research indicates the contrasting geochemical behaviour of As and Sb in contaminated soils. Irrespective of soil types, although both oxyanions are bound to the labile exchangeable fraction sufficiently to interact with changes in concentration of the other element and also to be taken up by plants, Sb was predominantly bound to the residual phase, whereas As mainly associated with Fe and Al oxides. The explanation was made that Sb was possibly associated with organic matter and sulphide fractions or other resistant minerals in soils, which was not included in the SEP used in this research. Thus, other extraction procedures targeting the organic matter and sulphide fractions should be used in future studies to have a better understanding of the distribution of Sb in various soil binding phases.

This research also demonstrates that regardless of soil types and contamination source, As was more labile and sustained than Sb. Arsenic was partially resupplied from the solid phase and its resupply varied with soil physicochemical properties. By contrast, the resupply of Sb was virtually minimal and relatively unchanged across soil types with various physicochemical properties. In addition, when contaminated soils were subject to flooding, labile As increased with soil depth and over time, while labile Sb decreased. The results showed that the mobilisation of As was associated with the reductive dissolution of Fe (hydr)oxides, but it was not the case for Sb. The explanation of the lower lability of Sb under various soil redox conditions was based on more Sb complexing with organic matter and in the inert forms (e.g. precipitation with

sulphides), which were non-DGT labile. The precipitation with sulphides would be also a reasonable explanation for the higher lability of As in As-only soil compared to As+Sb soil (as discussed in section 7.3.2.1). Hence, DGT probes for sulphide measurements and the analysis of solid phase speciation by XAFS need to be applied in future studies to fully interpret the mechanism of Sb immobilisation.

To understand their competitive adsorption by soil compositions, soils should have identical soil properties, mineralogy, and course of aging. Our first study on the competitive interactions between As and Sb in soils used a series of soils amended with As only, Sb only, and mixture of As and Sb which simultaneously added into soils in which amounts of Sb were equivalent and higher than As in soil treatments. The results presented in chapters 6 and 7 show that there were interactive effects of As and Sb in soils for their binding sites, which determined the changes in their lability in co-amended soils under anaerobic and aerobic conditions. The order and amounts of As and Sb added into soils would also be important in controlling their interactive effects on soil binding sites and in turn determining their lability in soils. This warrants further studies to fully understand the competitive interactions of As and Sb in soils and manage contaminated soils. To do this, a study could increase the concentration of one element while keeping the other constant and vice versa, which would be more intensive than anything attempted in this study.

8.6.2. Uptake of As and Sb by plants

The results in this thesis show that As bioaccumulation in all test vegetables grown in various soils under different conditions was much higher than Sb bioaccumulation, but the translocation of Sb from roots to shoots was consistently higher than that of As. In addition, the presence of As in soils enhanced the bioaccumulation factor of Sb in As+Sb soils compared to Sb-only soils. The possibility was made that the more toxic element (As) damaged the cell membrane and allowed the absorption and transportation of the less toxic Sb in plants. The mechanism of Sb uptake by plants remains relatively unknown. Thus, the determination of As and especially Sb in subcellular fractions of plant tissues needs to be done to enable a better understanding of their mechanisms.

The analysis of As species in flooded water spinach (*I. aquatica*) shows that the dominant As species in flooded *I. aquatica* was As(V). This is opposite to the expected results that As(III) would be present in flooded *I. aquatica* at higher concentrations than

that in non-flooded *I. aquatica* because As(III) was the dominant species in flooded contaminated soils. The dominance of As(V) in tissues of flooded *I. aquatica* was an unusual trend. This also warrants further studies to fully understand the mechanism behind this trend. Future studies on the speciation of As and Sb in fresh tissues of flooded *I. aquatica* using X-ray absorption spectroscopy would be expected to fully explain their uptake and metabolism in flooded *I. aquatica*.

A reliable procedure for the extraction and analysis of Sb species in plants has not been successfully developed in the literature. *I. aquatica* accumulated Sb at high concentrations, but the plant looked healthy and had great biomass, which poses chronic risks to humans consuming the contaminated *I. aquatica*. The Sb toxicity depends on its species. Thus, the development of a reliable protocol for the extraction and analysis of Sb species in plants is essential for the interpretation of Sb uptake and metabolism in plants and the development of sustainable agriculture.

The results in chapter 7 (sections 7.3.1.2, 7.3.2.2, and 7.3.2.4) show that the presence of *I. aquatica* in flooded contaminated soils altered the speciation of As and Sb in the soils identified by multiple profiles of $C_{DGT\text{-}As(III)}$, $C_{DGT\text{-}As(V)}$, $C_{DGT\text{-}Sb(III)}$, and $C_{DGT\text{-}Sb(V)}$, which influences the uptake and speciation of As and Sb in flooded *I. aquatica*. Hence, the application of DGT-planar optode sandwich sensor coupled with LA-ICP-MS for chemical imaging of labile As and Sb and *in situ* measurement of pH and oxygen in the rhizophere of flooded *I. aquatica* is warranted. This is supported by Williams et al. (2014) who applied DGT-planar optode sandwich sensor to evaluate the localised mobilisation of As, Fe(II), and Pb at the boundary of the aerobic rhizophere region of flooded rice roots, accompanying with the decrease in pH. These studies would enable a better understanding of the behaviour of As and Sb in such environments and contribute to the explanation of As and Sb speciation in flooded *I. aquatica.*

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Appendix A

Supplementary Information for Chapter 3

Table A.1 As and Sb concentrations in different fractions in soils (mg/kg, dry mass, mean ± SE, n = 3).

Soil	As concentrations in different fractions (mg/kg)					Sb concentrations in different fractions (mg/kg)				
	Non-specifically sorbed (A)	Specifically sorbed (B)	Amorphous Fe and Al oxides (C)	Crystalline Fe and Al oxides (D)	Residual (E)	Non-specifically sorbed (A)	Specifically sorbed (B)	Amorphous Fe and Al oxides (C)	Crystalline Fe and Al oxides (D)	Residual (E)
S1	0.048 ± 0.004	0.87 ± 0.01	4.4 ± 0.8	4.7 ± 0.3	3.3 ± 0.9	0.056 ± 0.001	0.084 ± 0.001	1.4 ± 0.1	2.3 ± 0.2	7.9 ± 0.7
S2	0.076 ± 0.003	1.43 ± 0.04	8.4 ± 0.7	7 ± 1	8 ± 3	0.102 ± 0.003	0.193 ± 0.004	4.14 ± 0.06	4.5 ± 0.4	17 ± 2
S3	0.096 ± 0.004	1.76 ± 0.09	90 ± 1	14 ± 1	5 ± 2	0.152 ± 0.003	0.25 ± 0.02	4.1 ± 0.6	9 ± 1	25 ± 2
S4	0.127 ± 0.003	2.39 ± 0.06	14.6 ± 0.6	10 ± 1	12 ± 3	0.212 ± 0.001	0.379 ± 0.008	7.7 ± 0.4	8.0 ± 0.3	39 ± 5
S5	0.150 ± 0.003	2.81 ± 0.06	19 ± 1	13.1 ± 0.4	14 ± 2	0.261 ± 0.002	0.466 ± 0.009	9.75 ± 0.09	10.6 ± 0.9	50 ± 2
S6	0.167 ± 0.007	3.14 ± 0.06	160 ± 2	19.8 ± 0.8	12 ± 2	0.293 ± 0.008	0.539 ± 0.009	7.8 ± 0.8	13.7 ± 0.5	58 ± 4
S7	0.207 ± 0.005	3.76 ± 0.01	27.9 ± 0.8	20.3 ± 0.9	25 ± 3	0.212 ± 0.001	0.641 ± 0.008	14.4 ± 0.8	15 ± 2	82 ± 10
S8	0.230 ± 0.008	4.00 ± 0.06	30.6 ± 0.6	19.70 ± 0.08	25 ± 3	0.261 ± 0.002	0.70 ± 0.03	18 ± 2	16.5 ± 0.4	92 ± 6
S9	0.25 ± 0.01	4.96 ± 0.08	25 ± 5	40 ± 3	23 ± 6	0.482 ± 0.007	0.86 ± 0.01	12 ± 1	25 ± 2	110 ± 4
S10	0.49 ± 0.02	7.58 ± 0.06	53.4 ± 0.8	36.2 ± 0.8	87 ± 10	0.78 ± 0.03	1.36 ± 0.03	28 ± 1	28.8 ± 0.9	220 ± 10
S11	0.55 ± 0.03	9.2 ± 0.1	57.3 ± 0.8	63 ± 9	77 ± 20	0.99 ± 0.04	1.61 ± 0.03	28 ± 2	43 ± 6	290 ± 30
S12	1.49 ± 0.05	17.0 ± 0.4	115 ± 2	110 ± 5	160 ± 20	1.75 ± 0.01	3.0 ± 0.1	45 ± 7	79 ± 3	590 ± 30

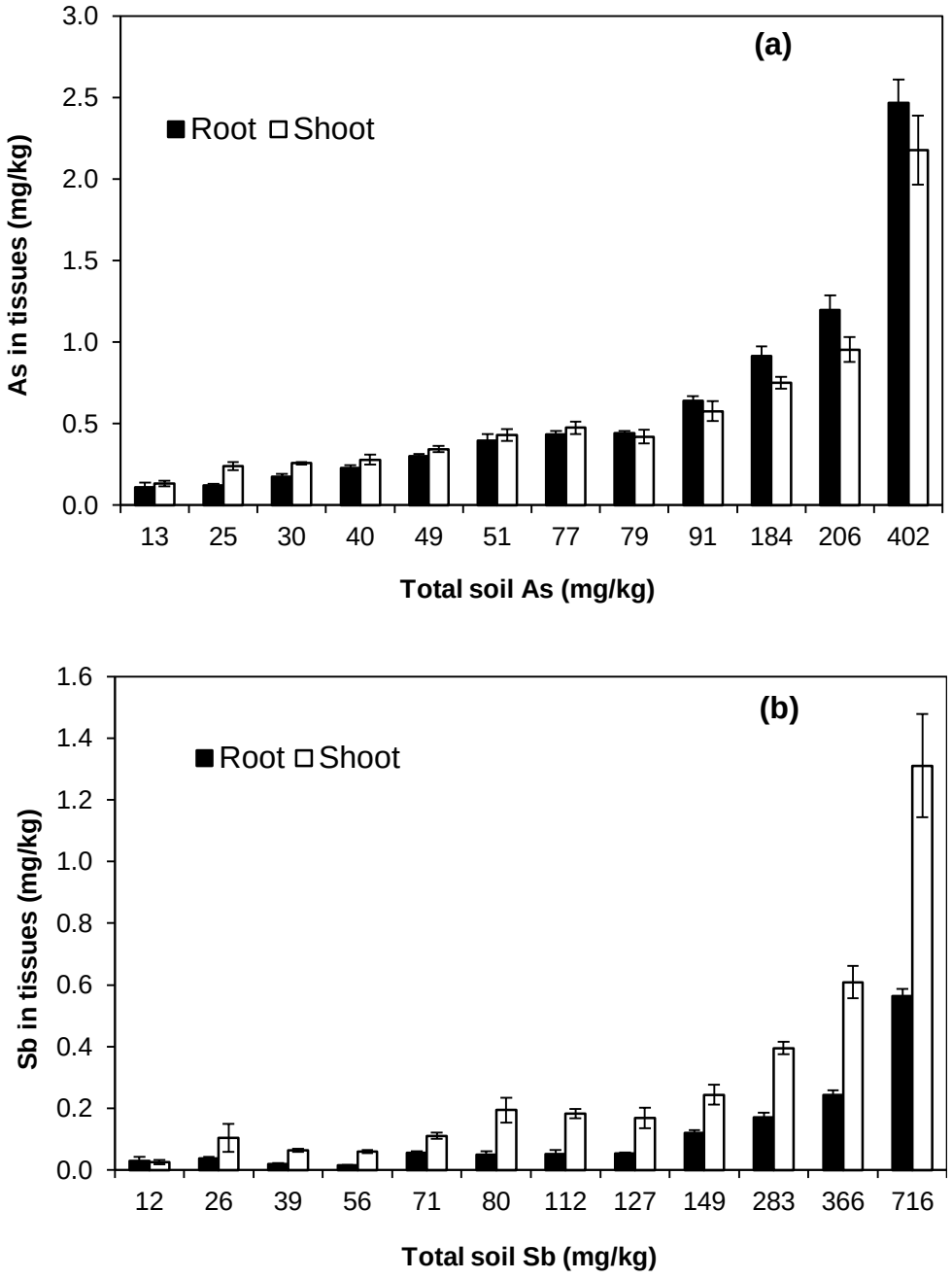

Figure A.1 Arsenic (a) and antimony (b) concentrations in *R. sativus* roots and shoots (mg/kg dry mass, mean ± SE, n = 4).

Appendix B

Supplementary Information for Chapter 4

Table B.1 As concentrations in individual different fractions in cattle dip soils (mg/kg dry mass, mean ± SE, n = 3).

Soil	As concentrations in different fractions (mg/kg)				
	Non-specifically sorbed	Specifically sorbed	Amorphous Fe and Al oxides	Crystalline Fe and Al oxides	Residual
Q1	0.228 ± 0.004	1.76 ± 0.03	9.62 ± 0.06	4.0 ± 0.4	1.7 ± 0.5
Q2	0.34 ± 0.02	2.8 ± 0.1	15.8 ± 0.5	4.5 ± 0.2	2 ± 1
Q3	0.68 ± 0.03	4.2 ± 0.2	23 ± 1	5.8 ± 0.7	2 ± 2
Q4	0.88 ± 0.02	5.1 ± 0.1	34.3 ± 0.9	7.7 ± 0.9	3 ± 1
Q5	1.183 ± 0.005	6.78 ± 0.03	41.1 ± 0.6	8.8 ± 0.5	4.1 ± 0.9
Q6	1.40 ± 0.02	7.9 ± 0.2	47 ± 1	8.1 ± 0.1	3 ± 1
Q7	1.58 ± 0.03	9.37 ± 0.06	55 ± 1	10.6 ± 0.8	4 ± 2
Q8	1.73 ± 0.04	10.11 ± 0.06	61.3 ± 0.8	10.2 ± 0.4	8 ± 2
Q9	2.03 ± 0.05	13.0 ± 0.6	77.3 ± 0.8	14.2 ± 0.6	9 ± 1
Q10	3.013 ± 0.004	19.4 ± 0.2	120 ± 1	20.5 ± 0.9	9 ± 4
Q11	3.55 ± 0.01	27 ± 1	155.6 ± 0.7	28.0 ± 0.3	22 ± 9
Q12	6.7 ± 0.1	55.2 ± 0.6	370 ± 10	71 ± 1	35 ± 10

Table B.2 As and Sb concentrations in individual different fractions in mining soils (mg/kg dry mass, mean ± SE, n = 3).

Soil	As concentrations in different fractions (mg/kg)					Sb concentrations in different fractions (mg/kg)				
	Non-specifically sorbed	Specifically sorbed	Amorphous Fe and Al oxides	Crystalline Fe and Al oxides	Residual	Non-specifically sorbed	Specifically sorbed	Amorphous Fe and Al oxides	Crystalline Fe and Al oxides	Residual
S1	0.041 ± 0.002	0.822 ± 0.005	4.6 ± 0.3	4.9 ± 0.2	2.7 ± 0.4	0.051 ± 0.001	0.074 ± 0.001	1.52 ± 0.01	2.42 ± 0.06	6.9 ± 0.6
S2	0.0476 ± 0.0008	1.01 ± 0.07	6.7 ± 0.5	5.7 ± 0.3	5 ± 1	0.088 ± 0.002	0.167 ± 0.006	4.17 ± 0.04	4.72 ± 0.02	14.1 ± 0.6
S3	0.103 ± 0.002	2.12 ± 0.03	12.3 ± 0.7	19 ± 1	5 ± 3	0.158 ± 0.004	0.261 ± 0.004	5.3 ± 0.2	10.9 ± 0.2	26 ± 7
S4	0.119 ± 0.002	2.53 ± 0.06	17.4 ± 0.6	12.6 ± 0.4	11.5 ± 0.8	0.198 ± 0.009	0.350 ± 0.009	8.5 ± 0.3	9.26 ± 0.04	36 ± 7
S5	0.135 ± 0.002	2.80 ± 0.05	21.5 ± 0.7	14.8 ± 0.4	13 ± 6	0.240 ± 0.004	0.426 ± 0.006	11 ± 1	12.1 ± 0.4	46 ± 2
S6	0.169 ± 0.002	3.53 ± 0.09	20 ± 1	25 ± 1	11 ± 10	0.261 ± 0.004	0.477 ± 0.007	8.7 ± 0.3	14.8 ± 0.7	51 ± 3
S7	0.175 ± 0.002	3.67 ± 0.04	30.9 ± 0.7	22.7 ± 0.8	22 ± 9	0.212 ± 0.005	0.67 ± 0.01	18.4 ± 0.6	20.2 ± 0.5	86 ± 20
S8	0.198 ± 0.006	3.93 ± 0.05	34 ± 1	22.2 ± 0.1	22 ± 2	0.246 ± 0.009	0.68 ± 0.006	21 ± 1	20.6 ± 0.7	91 ± 20
S9	0.216 ± 0.004	4.86 ± 0.04	28 ± 2	40.7 ± 0.7	20 ± 5	0.57 ± 0.03	1.00 ± 0.03	18.24 ± 0.09	36 ± 2	130 ± 20
S10	0.298 ± 0.008	5.41 ± 0.07	45 ± 2	31 ± 2	61 ± 3	0.66 ± 0.03	1.14 ± 0.05	30.4 ± 0.9	33 ± 1	190 ± 4
S11	0.491 ± 0.005	9.3 ± 0.1	68 ± 1	72 ± 2	73 ± 30	1.00 ± 0.04	1.59 ± 0.02	37 ± 2	57 ± 2	300 ± 20
S12	1.36 ± 0.02	16.7 ± 0.2	130 ± 4	120 ± 4	150 ± 30	1.51 ± 0.05	2.6 ± 0.1	55 ± 4	92 ± 3	520 ± 50

Table B.3 Bioaccumulation factor (BAF) and translocation factor (TF) for As and Sb in white icicle radish (mean ± SE, n = 4).

Soil	As - Cattle dip		Soil	As - Mining		Sb - Mining	
	BAF	TF		BAF	TF	BAF	TF
Q1	0.0085 ± 0.0003	0.80 ± 0.03	S1	0.010 ± 0.001	0.77 ± 0.06	0.0030 ± 0.0004	1.0 ± 0.2
Q2	0.011 ± 0.003	0.5 ± 0.1	S2	0.011 ± 0.002	0.5 ± 0.1	0.0020 ± 0.0002	1.0 ± 0.3
Q3	0.0099 ± 0.0009	0.57 ± 0.05	S3	0.0054 ± 0.0001	0.5 ± 0.1	0.0011 ± 0.0001	1.7 ± 0.6
Q4	0.013 ± 0.002	0.37 ± 0.06	S4	0.0060 ± 0.0006	0.37 ± 0.02	0.0015 ± 0.0002	0.9 ± 0.1
Q5	0.0082 ± 0.0005	0.49 ± 0.05	S5	0.006 ± 0.001	0.46 ± 0.1	0.0013 ± 0.0002	1.2 ± 0.3
Q6	0.009 ± 0.001	0.43 ± 0.07	S6	0.0051 ± 0.0004	0.43 ± 0.07	0.0014 ± 0.0004	1.1 ± 0.2
Q7	0.0083 ± 0.0005	0.8 ± 0.3	S7	0.0054 ± 0.0007	0.39 ± 0.02	0.0010 ± 0.0003	1.2 ± 0.3
Q8	0.0079 ± 0.0006	0.6 ± 0.1	S8	0.0047 ± 0.0003	0.35 ± 0.01	0.0008 ± 0.0001	1.0 ± 0.03
Q9	0.009 ± 0.001	0.5 ± 0.1	S9	0.0046 ± 0.0003	0.37 ± 0.01	0.0010 ± 0.0004	0.9 ± 0.2
Q10	0.0068 ± 0.0004	0.53 ± 0.02	S10	0.0037 ± 0.0002	0.46 ± 0.06	0.0008 ± 0.0003	1.2 ± 0.3
Q11	0.008 ± 0.002	0.62 ± 0.07	S11	0.0039 ± 0.0004	0.46 ± 0.06	0.0009 ± 0.0002	0.9 ± 0.1
Q12	0.009 ± 0.001	0.9 ± 0.2	S12	0.0033 ± 0.0003	0.44 ± 0.08	0.0007 ± 0.0001	1.4 ± 0.2

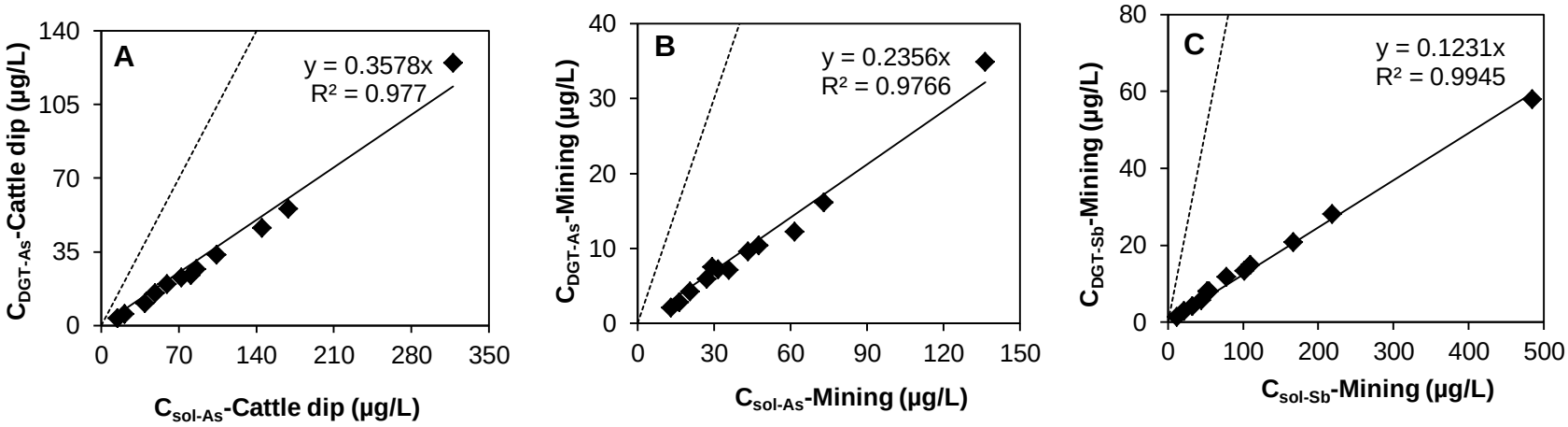

Figure B.1 The relationship between DGT-labile (C_{DGT}) As and Sb after 24-h deployment and concentrations in extracted soil solutions (mean ± SE, n = 3). Dotted line represents a 1:1 gradient, regression set through zero. Note that total Sb soil concentrations were higher than total As concentrations in the same soils. Arsenic in bioassay cattle dip soils (A), As in bioassay mining soils (B), and Sb in bioassay mining soils (C).

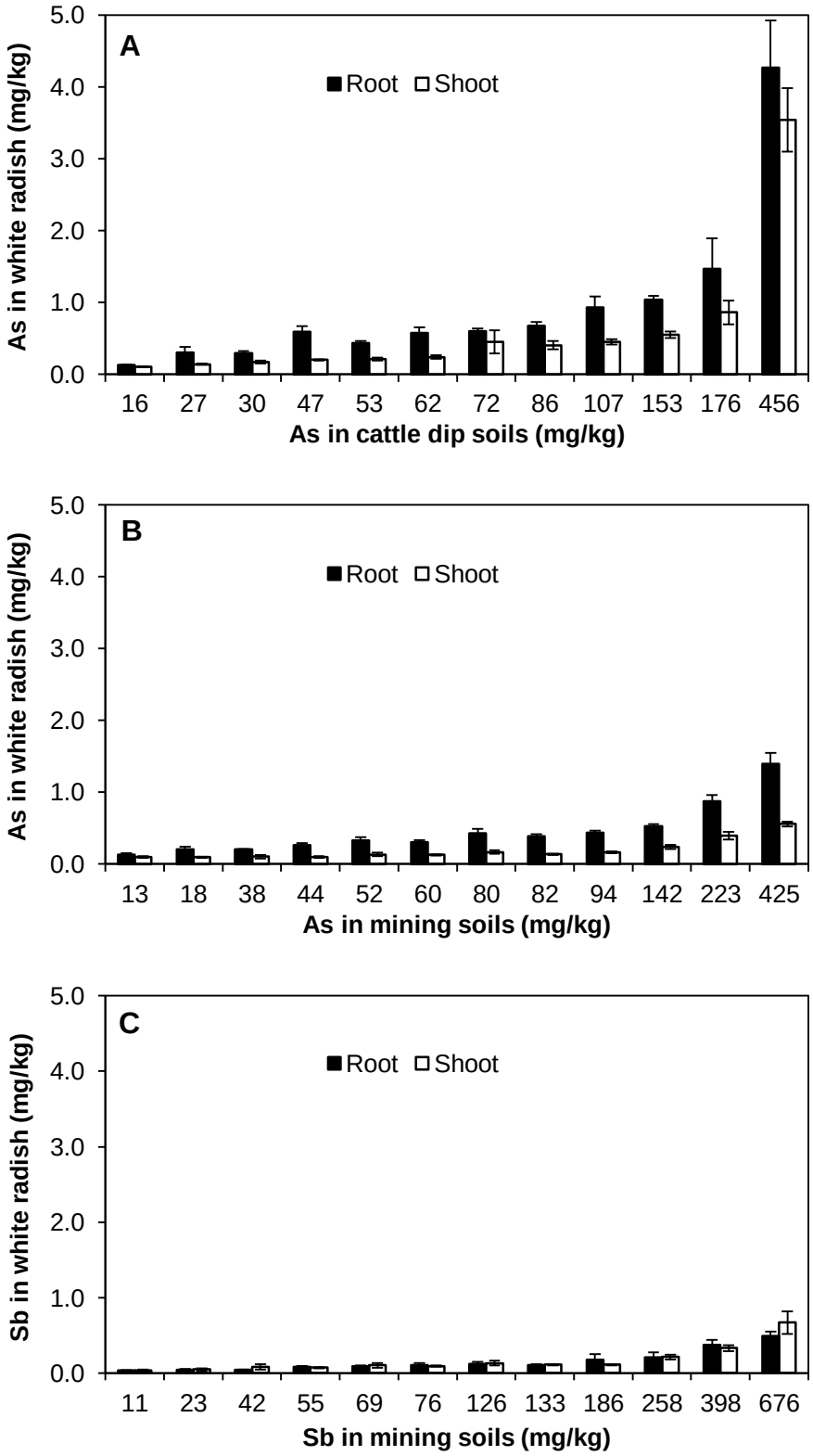

Figure B.2 Arsenic and antimony concentrations in white icicle radish roots and shoots (mg/kg dry mass, mean ± SE, n = 4). Arsenic in cattle dip soils (A), As in mining soils (B), and Sb in mining soils (C).

Appendix C

Supplementary Information for Chapter 5

Table C.1 As and Sb concentrations in different fractions in soils (mg/kg, dry mass, mean ± SE, n = 3).

Soil	Treatment	As concentrations in different fractions (mg/kg)					Sb concentrations in different fractions (mg/kg)				
		Non-specifically sorbed	Specifically sorbed	Amorphous Fe and Al oxides	Crystalline Fe and Al oxides	Residual	Non-specifically sorbed	Specifically sorbed	Amorphous Fe and Al oxides	Crystalline Fe and Al oxides	Residual
L	S1	0.059 ± 0.007	3.41 ± 0.05	18.38 ± 0.2	14 ± 1	7 ± 1	0.130 ± 0.005	0.195 ± 0.001	5.8 ± 0.2	14.6 ± 0.8	31 ± 6
	S2	0.38 ± 0.01	12.72 ± 0.06	67 ± 2	49 ± 8	33	0.657 ± 0.007	1.02 ± 0.01	20.7 ± 0.6	55 ± 7	124 ± 30
	S3	0.541 ± 0.002	25.8 ± 0.2	139 ± 3	102 ± 4	1	1.30 ± 0.02	2.47 ± 0.01	46 ± 1	109 ± 3	169 ± 30
	S4	2.06 ± 0.03	48.9 ± 0.5	294 ± 10	220 ± 2	110	3.34 ± 0.02	5.21 ± 0.08	94 ± 2	240 ± 20	530 ± 200
	S5	8.3 ± 0.1	113.6 ± 0.3	627 ± 7	535 ± 10	130 ± 50	7.93 ± 0.05	12.6 ± 0.1	270 ± 3	620 ± 20	1360 ± 100
M	S6	0.169 ± 0.003	3.95 ± 0.03	32.0 ± 0.9	27 ± 6	3	0.176 ± 0.005	0.231 ± 0.007	9.8 ± 0.4	27 ± 6	54 ± 6
	S7	0.54 ± 0.02	12.2 ± 0.2	76 ± 5	83 ± 6	5	0.48 ± 0.01	0.82 ± 0.02	23.2 ± 0.6	73 ± 8	120 ± 30
	S8	1.31 ± 0.05	23.8 ± 0.9	168 ± 6	130 ± 10	8	1.02 ± 0.03	1.79 ± 0.06	50 ± 3	127 ± 10	350 ± 200
	S9	4.7 ± 0.1	58.9 ± 0.3	413 ± 7	340 ± 10	130 ± 60	2.68 ± 0.01	4.39 ± 0.06	122 ± 2	330 ± 20	730 ± 60
	S10	20.56 ± 0.04	126 ± 4	740 ± 300	960 ± 60	780 ± 300	5.03 ± 0.02	11 ± 1	300 ± 100	1100 ± 5	2380 ± 200
H	S11	0.28 ± 0.01	6.3 ± 0.1	44 ± 2	38.6 ± 0.2	12 ± 9	0.236 ± 0.006	0.330 ± 0.005	13.5 ± 0.5	38.6 ± 0.2	91 ± 7
	S12	0.55 ± 0.01	11.2 ± 0.2	73 ± 2	53 ± 1	65 ± 30	0.42 ± 0.01	0.65 ± 0.01	22.8 ± 0.6	52 ± 1	150 ± 30
	S13	1.77 ± 0.01	28.8 ± 0.3	188 ± 2	169 ± 6	130 ± 30	1.022 ± 0.006	1.75 ± 0.02	58 ± 1	152 ± 8	450 ± 70
	S14	6.58 ± 0.09	77 ± 1	490 ± 20	433 ± 4	270 ± 50	2.70 ± 0.06	4.55 ± 0.07	180 ± 20	480 ± 10	940 ± 60
	S15	44.4 ± 0.7	163 ± 1	1620 ± 10	1400 ± 20	950 ± 200	5.9 ± 0.1	14.8 ± 0.1	590 ± 10	1740 ± 30	4260 ±500

Table C.2 Total and labile concentrations measured by SEP, DGT, soil solution in a concentration gradient obtained from historically contaminated soils, and R values of As and Sb (mean ± SE, n = 3). $C_{SEP\text{-}labile}$ represents sum of the non-specifically sorbed and specifically sorbed fractions obtained from SEP.

Soil	Treatment	As					Sb				
		$Total_{As}$ (mg/kg)	$C_{SEP\text{-}As}$ (mg/kg)	$C_{sol\text{-}As}$ (µg/L)	$C_{DGT\text{-}As}$ (µg/L)	Ratio $R_{As}=C_{DGT}/C_{sol}$	$Total_{Sb}$ (mg/kg)	$C_{SEP\text{-}Sb}$ (mg/kg)	$C_{sol\text{-}Sb}$ (µg/L)	$C_{DGT\text{-}Sb}$ (µg/L)	Ratio $R_{Sb}=C_{DGT}/C_{sol}$
L	S1	42.1 ± 0.2	3.47 ± 0.05	5.27 ± 0.01	3.42 ± 0.08	0.65 ± 0.01	52 ± 6	0.325 ± 0.005	18.8 ± 0.1	2.20 ± 0.02	0.1173 ± 0.0003
	S2	160 ± 30	13.09 ± 0.07	18.5 ± 0.5	10.4 ± 0.9	0.57 ± 0.06	200 ± 30	1.68 ± 0.02	71 ± 1	14 ± 2	0.20 ± 0.02
	S3	270 ± 9	26.4 ± 0.2	26.1 ± 0.1	15.6 ± 0.4	0.60 ± 0.02	330 ± 30	3.77 ± 0.02	133.8 ± 0.8	24.7 ± 0.2	0.189 ± 0.001
	S4	670 ± 100	51.0 ± 0.5	61 ± 1	41.9 ± 0.6	0.682 ± 0.001	870 ± 170	8.56 ± 0.08	268.0 ± 0.6	56 ± 4	0.21 ± 0.01
	S5	1400 ± 50	121.9 ± 0.3	290.7 ± 0.5	176.9 ± 0.8	0.609 ± 0.002	2277 ± 130	20.6 ± 0.1	580 ± 2	122 ± 1	0.214 ± 0.001
M	S6	65 ± 3	4.12 ± 0.03	8.48 ± 0.09	3.1 ± 0.3	0.36 ± 0.04	90.7 ± 0.6	0.41 ± 0.01	20.4 ± 0.3	2.74 ± 0.08	0.137 ± 0.006
	S7	176 ± 2	12.8 ± 0.2	22 ± 1	10.3 ± 0.2	0.46 ± 0.03	220 ± 30	1.30 ± 0.03	47 ± 2	7.54 ± 0.09	0.166 ± 0.006
	S8	330 ± 20	25.1 ± 0.9	77 ± 3	24.2 ± 0.7	0.32 ± 0.01	530 ± 180	2.80 ± 0.06	85 ± 1	15.6 ± 0.2	0.187 ± 0.004
	S9	950 ± 50	63.6 ± 0.4	215 ± 10	85 ± 3	0.40 ± 0.03	1190 ± 60	7.07 ± 0.07	178 ± 1	33 ± 1	0.189 ± 0.008
	S10	2630 ± 90	147 ± 4	-	-	-	3790 ± 180	16 ± 1	-	-	-
H	S11	101 ± 8	6.6 ± 0.1	10.2 ± 0.1	4.0 ± 0.2	0.39 ± 0.03	140 ± 8	0.57 ± 0.01	23.7 ± 0.2	3.3 ± 0.2	0.143 ± 0.008
	S12	200 ± 30	11.8 ± 0.2	24 ± 1	9.5 ± 0.4	0.41 ± 0.03	230 ± 30	1.06 ± 0.02	42 ± 2	7.0 ± 0.2	0.172 ± 0.007
	S13	510 ± 30	30.5 ± 0.3	63.8 ± 0.7	24.5 ± 0.8	0.38 ± 0.01	670 ± 70	2.77 ± 0.02	71.1 ± 0.7	12.8 ± 0.5	0.183 ± 0.005
	S14	1280 ± 40	83 ± 1	237 ± 8	114 ± 4	0.48 ± 0.01	1600 ± 60	7.26 ± 0.09	177.4 ± 0.7	38 ± 2	0.22 ± 0.01
	S15	4180 ± 160	207 ± 2	-	-	-	6610 ± 460	20.7 ± 0.2	-	-	-

-: not measured due to analysis problems

Appendix D

Supplementary Information for Chapter 6

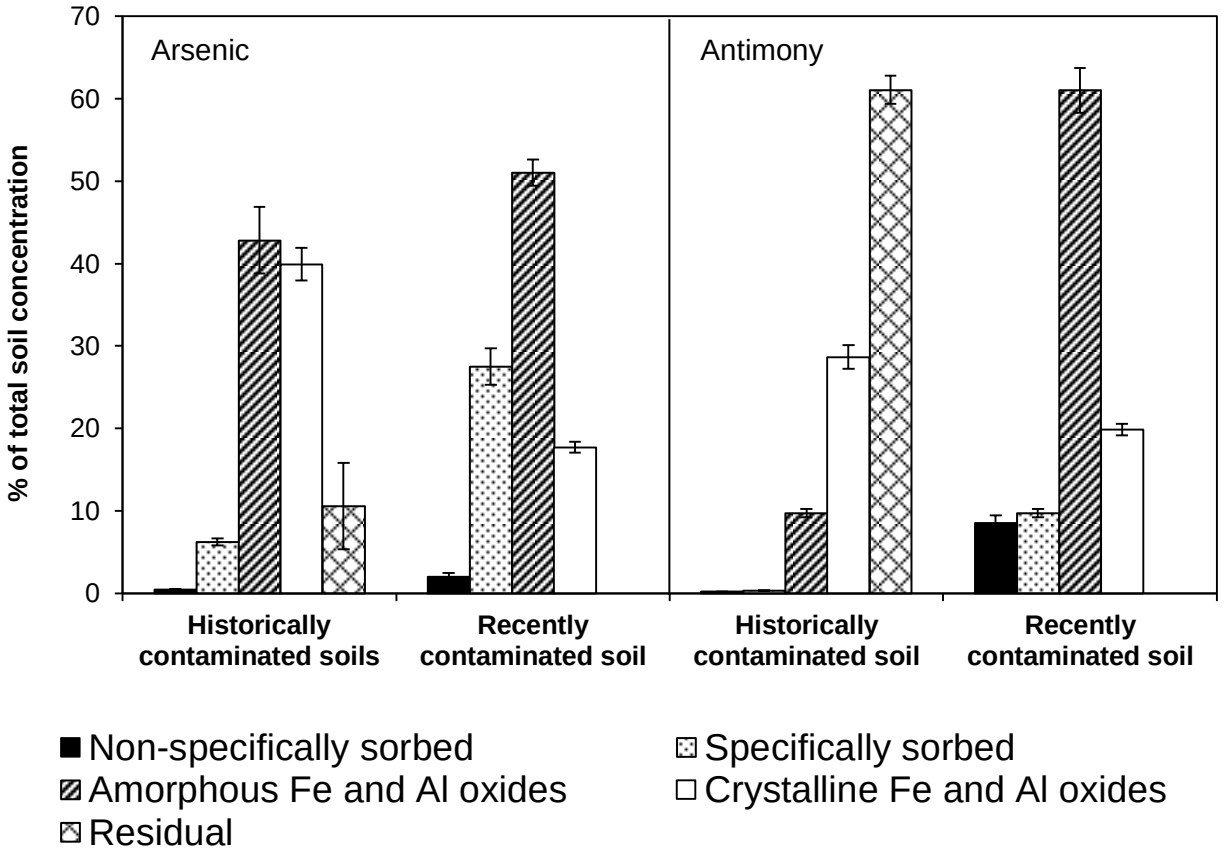

Figure D.1 As and Sb associated with different binding fractions in historically and recently contaminated soils, each fraction as a percentage of the total soil concentration (mean ± SE, n = 15 for historically contaminated soils (chapter 5), n = 21 for recently contaminated soils (chapter 6)).

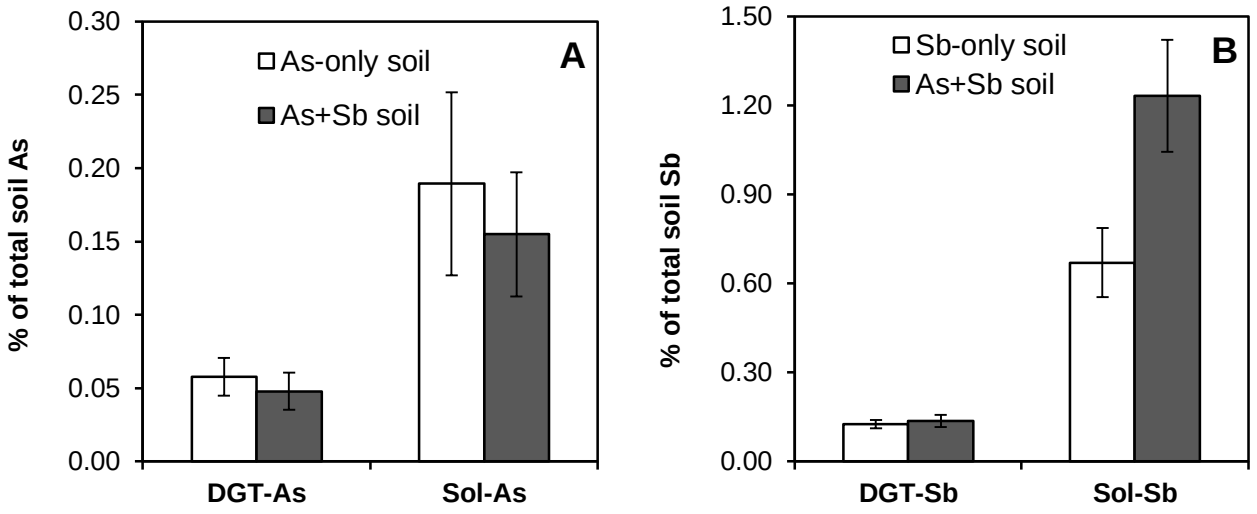

Figure D.2 The average percentage of $C_{DGT\text{-}As}$ and $C_{sol\text{-}As}$ of total soil As in As-only and As+Sb amended soils (A); $C_{DGT\text{-}Sb}$ and $C_{sol\text{-}Sb}$ of total soil Sb in Sb-only and As+Sb amended soils (B).

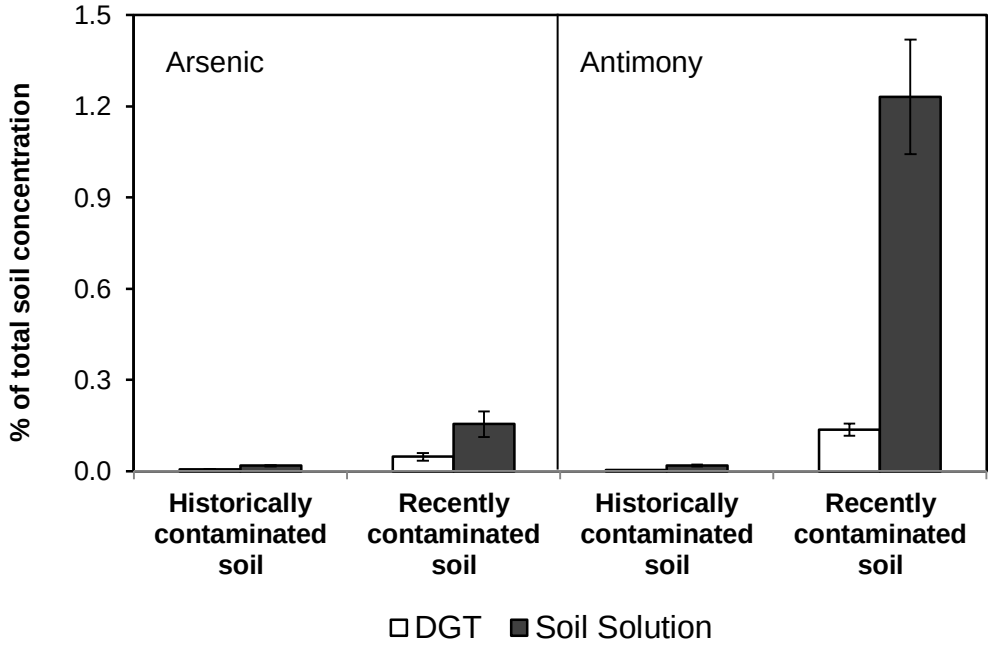

Figure D.3 The average percentage of $C_{DGT\text{-}As}$ and $C_{sol\text{-}As}$; $C_{DGT\text{-}Sb}$ and $C_{sol\text{-}Sb}$ of total soil As and Sb in historically and recently contaminated soils.

Appendix E

Supplementary Information for Chapter 7

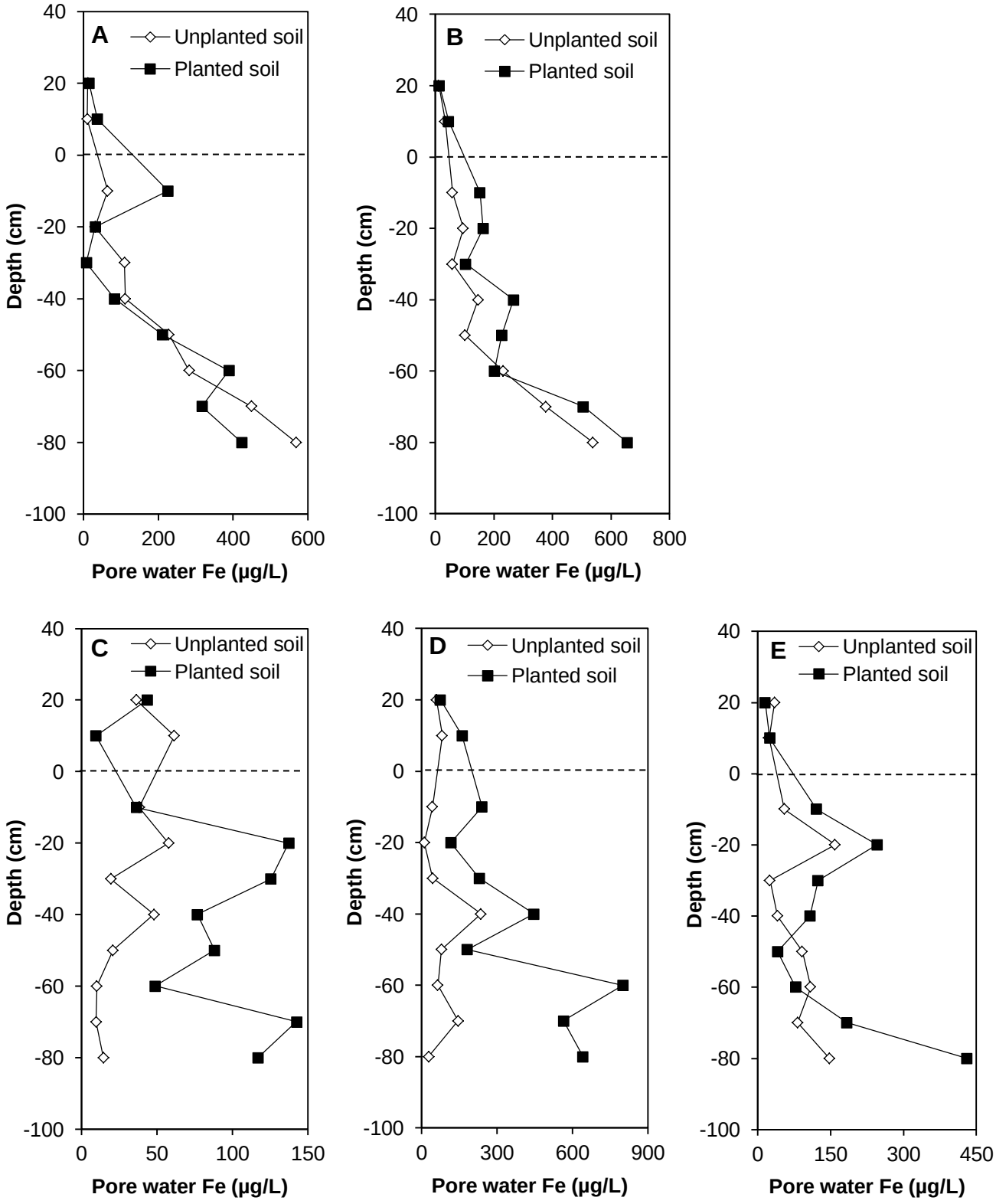

Figure E.1 Depth profiles of pore water Fe concentrations in soils after 5 weeks of flooding with and without plants, measured by DET. Unplanted soils (◊), Planted soils (■). Soil Aged-L (A), Soil Aged-H (B), Soil 50As (C), Soil 300Sb (D), Soil 50As300Sb (D). The dotted line represents the soil water interface.

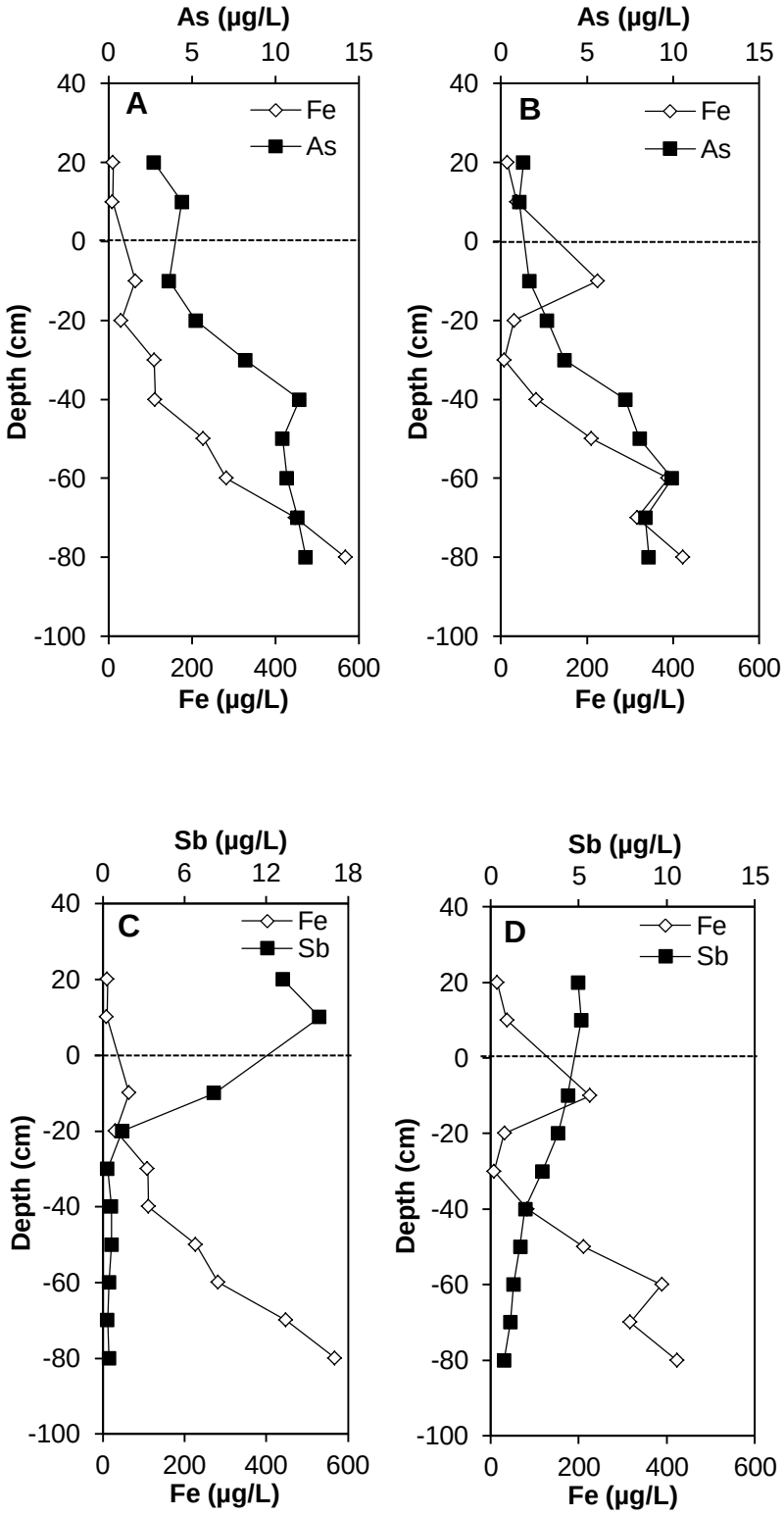

Figure E.2 Depth profiles of pore water inorganic arsenic and antimony concentrations measured by DGT, and iron(II) concentrations measured by DET in a historically contaminated soil (Aged-L). Unplanted soil (A, C) and Planted soil (B, D). The dotted line represents the soil water interface.

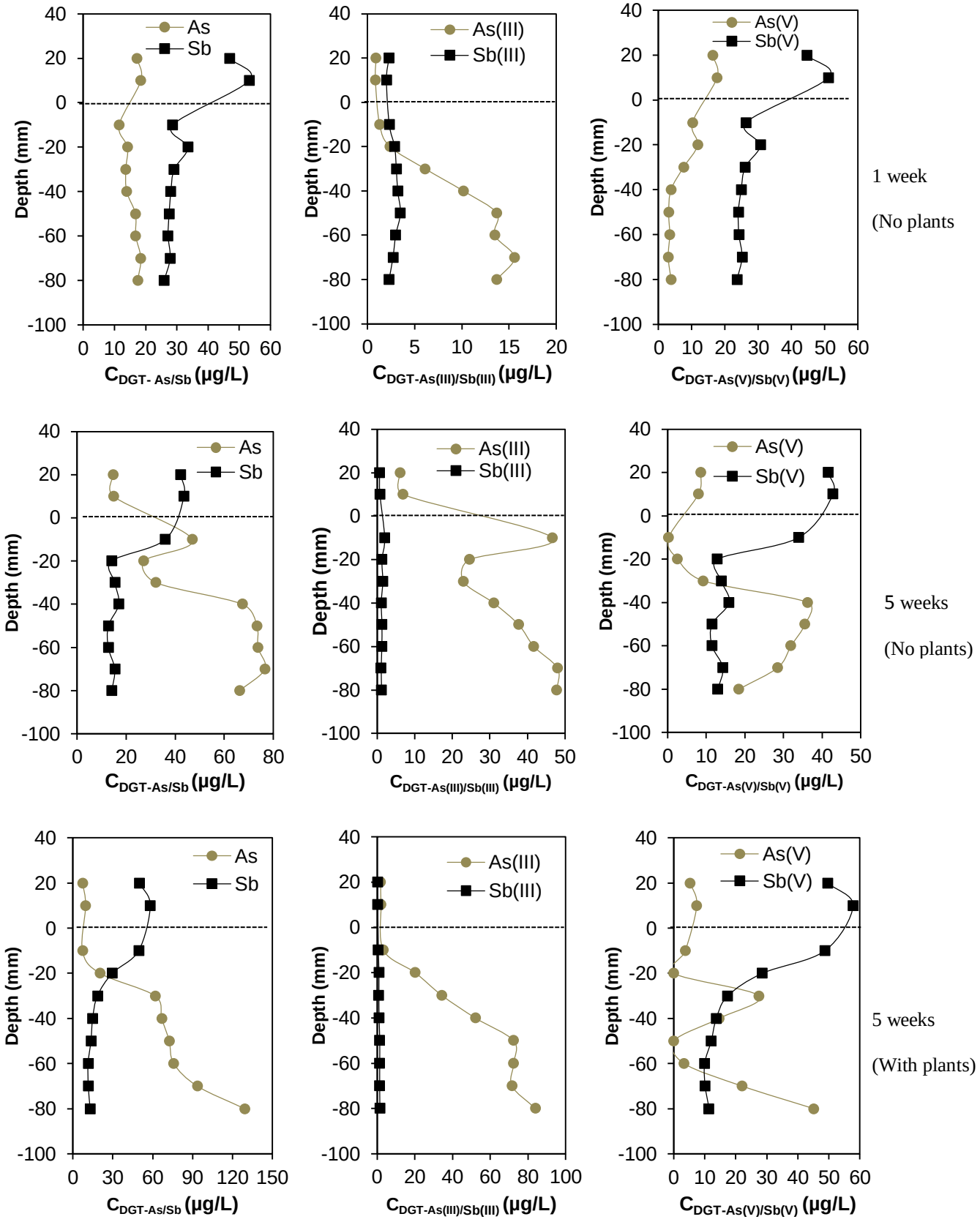

Figure E.3 Depth profiles of As and Sb species in soil Aged-H after 1-week flooding and 5-week flooding with and without plants. The dotted line represents the soil water interface.